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Clinical Research

Ensembling Electrical and Proteogenomics Biomarkers for Improved Prediction of Cardiac-Related 3-Month Hospitalizations: A Pilot Study

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ABSTRACT

Background: Many risk models for predicting mortality, hospitalizations, or both in patients with heart failure have been developed but do not have sufficient discriminatory ability. The purpose of this study was to identify predictive biomarkers of hospitalizations in heart failure patients using omics-based technologies applied to blood and electrical monitoring of the heart.

Methods: Blood samples were collected from 58 heart failure patients during enrollment into this study. Each patient wore a 48-hour Holter monitor that recorded the electrical activity of their heart. The blood samples were profiled for gene expression using microarrays and protein

RÉSUMÉ

Contexte : Beaucoup de modèles de risque visant à prédire le décès ou l'hospitalisation, ou les deux, chez les patients atteints d'insuffisance cardiaque ont été créés, mais ils ne sont pas dotés d'une puissance discriminatoire suffisante. Cette étude visait à recenser des biomarqueurs permettant de prédire l'hospitalisation de patients atteints d'insuffisance cardiaque à l'aide de technologies fondées sur les sciences omiques et appliquées à la surveillance hématologique et électrique du cœur.

Méthodologie : Pendant la période de recrutement de cette étude, des échantillons de sang ont été prélevés chez 58 patients atteints

Heart failure (HF) remains a leading cause of hospitalizations with unchanged readmission rates in the past 20 years.[1] The prevalence of HF continues to increase in the ageing population, because of improved care of ischemic disease and advancements in treatment and wearable technologies. Although survival after onset of HF has improved, half of the patients die within 5 years after diagnosis.[2] Predicting prognosis in HF patients might help identify high-risk patients who can be closely monitored with different treatment regimens enabling a precision approach to care.[3]

Prediction risk models for incident HF have shown good discriminative ability (C-statistics between 0.70 and 0.89) using traditional and sophisticated machine learning algorithms.[4-6] Unfortunately, multivariable statistical models that have been developed to predict hospitalizations and mortality, or both, in patients with HF[7,8] have shown modest to moderate discriminative ability (average C-statistics between 0.63 and 0.71). In patients hospitalized for HF, neither traditional or machine learning approaches were useful in predicting 30-day all-cause readmissions.[9] To date, these

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levels using multiple reaction monitoring. Statistical deconvolution was used to estimate cellular frequencies of common blood cells. Classification models were developed using clinical variables, Holter variables, cell types, gene transcripts, and proteins to predict hospitalization status.

Results: Of the 58 patients recruited, 13 were hospitalized within 3 months after enrollment. These patients had lower diastolic and systolic blood pressures, higher brain natriuretic peptide levels, most had higher blood creatinine levels, and had been diagnosed with heart failure for a longer time period. The best-performing clinical model had an area under the receiver operating characteristic curve of 0.76. An ensemble biomarker panel consisting of Holter variables, cell types, gene transcripts, and proteins had an area under the receiver operating characteristic curve of 0.88.

Conclusions: Molecular-based analyses as well as sensory data might provide sensitive biomarkers for the prediction of hospitalizations in heart failure patients. These approaches may be combined with traditional clinical models for the development of improved risk prediction models for heart failure.

d'insuffisance cardiaque, qui ont tous par la suite porté un moniteur Holter pour enregistrer leur activité cardiaque pendant 48 heures. Les échantillons de sang ont été analysés afin de dresser leur profil d'expression génique à l'aide de microréseaux et de la protéinémie en faisant appel à la surveillance des réactions multiples (MRM; *Multiple Reaction Monitoring*). Une méthode de déconvolution statistique a permis d'estimer la fréquence totale des cellules sanguines communes. Des modèles de classification ont été mis au point à l'aide de variables cliniques, de variables mesurées par Holter, des types de cellules, des transcriptions géniques et des protéines aux fins de prévision de l'hospitalisation ou non des patients.

Résultats : Sur les 58 patients recrutés, 13 ont été hospitalisés dans les 3 mois ayant suivi leur recrutement. Ces patients avaient une pression artérielle diastolique et une pression artérielle systolique plus faibles, des taux de peptide natriurétique de type B plus élevés, la plupart d'entre eux affichaient une créatininémie sanguine plus élevée et leur insuffisance cardiaque avait été diagnostiquée depuis plus longtemps. Le modèle clinique le plus performant a donné une aire sous la courbe ROC (*Receiver Operating Characteristic*) de 0,76. L'aire sous la courbe ROC d'un panel de marqueurs composés de variables mesurées par Holter, des types de cellules, des transcriptions géniques et des protéines se chiffrait à 0,88.

Conclusion : Les analyses moléculaires et les données sensorielles peuvent fournir des biomarqueurs sensibles permettant de prédire l'hospitalisation chez les patients atteints d'insuffisance cardiaque. Il est possible de combiner ces méthodes aux modèles cliniques classiques pour mettre au point de meilleurs modèles de prévision des risques dans les cas d'insuffisance cardiaque.

models have focused on easily obtainable data such as patient demographic characteristics, comorbidities, medication use, and cardiac function parameters (eg, electronic medical records). Commonly used variables have included age, sex, systolic blood pressure, sodium, diabetes status, creatinine, New York Heart Association functional class, blood urea nitrogen, left ventricular ejection fraction, hemoglobin, and (N-terminal pro) brain natriuretic peptide (BNP) levels. Although many risk models have been proposed, only a few have been routinely used in clinical practice.[8] Therefore, the use of novel methodologies such as wearable devices and high throughput omics technologies might have the potential to provide more sensitive markers of disease progression, which might help improve prediction of HF hospitalizations.

Systems medicine represents a promising approach to delineating the complexity of HF by understanding the molecular networks that span multiple omics domains (eg, transcriptomics, proteomics).[10,11] Therefore, we designed a pilot study to determine the utility of blood-based omic biomarkers in combination with the electrical activity of the heart (via Holter monitors) to predict hospitalizations within 3 months of enrollment. We hypothesize that a combinatorial approach that incorporates molecular and sensory data will provide predictive biomarkers of hospitalizations in HF patients.

Methods

Study cohort

A total of 58 patients were included in this study after written informed consent. Blood samples were collected, in EDTA (BD, Franklin Lake, NJ) and PAXgene (PreAnalytiX, Hombrechtikon, Switzerland) tubes at enrollment (baseline). Clinical data were collected at enrollment, at the day 30 visit, and at other follow-up visits through 1 year post enrollment. This study was approved by the Providence Health Care Research Ethics Board and conforms to the principles outlined in the Declaration of Helsinki.

Holter data

Holter monitors (SEER Light Extend; GE Healthcare, Little Chalfont, UK) were worn by patients for 14 days (48-hour Holter monitor repeated for a total of 7 times). Holter monitor data were then processed using MARS (version 6; GE Medical Systems Inc, Milwaukee, WI) and analyzed by a cardiovascular technologist at the St Paul's Hospital, Electrocardiography Laboratory. For the purpose of the current study, each of the 3 channels were reviewed. All QT analysis data were over-read by 2 cardiologists (K.I. and M.B.). Only Holter data obtained at the first visit were used for downstream analysis. Using the first visit hourly data (over a 48-hour time period) for average heart rate (HR_AVE) and isolated ventricular beats variables (V_ISO), 5 composite variables were constructed. Briefly, the average daytime (9:00 AM to 8:00 PM) and night-time (midnight to 5:00 AM) values were computed (hourly summary [HS]_HR_AVE_daytime, HS_HR_AVE_night-time, HS_V_ISO_daytime, and HS_V_ISO_night-time) as well as the ratio between daytime and night-time variables (HS_HR_AVE_DayToNight and HS_V_ISO_DayToNight). The Holter data were log2-transformed before statistical analysis.

Transcriptomics analysis

Total RNA was extracted using QIAcube (Qiagen Inc, Valencia, CA) from the baseline PAXgene blood using the PAXgene Blood miRNA kit from PreAnalytix (Cat #763134) according to the manufacturer's instructions. RNA was amplified and hybridized overnight to the Affymetrix Human Gene 1.1 ST array plates at the TSRI DNA Array Core Facility, Scripps Research Institute (Affymetrix Inc, La Jolla, CA). Array plates were scanned using the Affymetrix GeneTitan MC Scanner (Affymetrix Inc) with default settings.

The microarray data were checked for quality problems using the oligo Bioconductor package, and samples that did not pass the quality check were repeated using RNA from the same PAXgene tube (PreAnalytix). Arrays were background corrected, normalized, and summarized using the robust multiarray average method.

Estimated cell type frequencies

CIBERSORT[12] was used to estimate the frequencies of 22 cell types using the expression values of 547 genes. These genes were removed from the transcriptomics data set before downstream analyses. Removal of cells with 0 or near 0 variation resulted in 14 cell types that were used for downstream analyses. A public gene expression dataset (GSE77343) on chronic HF patients was used to assess the association of predicted cellular frequencies using CIBERSORT[12] and white blood cell counts assessed using a hematology analyzer.[13,14]

Proteomics analysis

Blood was collected at the time of enrollment in EDTA (BD) and PAXgene (PreAnalytiX) tubes. The EDTA tubes were placed on dry ice and centrifuged within 2 hours of collection. Plasma aliquots and the PAXgene tubes (BD) were stored at −80°C until selected for omics profiling. Plasma samples were trypsin digested and analyzed with multiple reaction monitoring (MRM) mass spectrometry at the UVic Genome BC Proteomics Centre, Victoria, Canada. A total of 117 peptides, corresponding to 65 proteins, were measured.

The quality of the MRM data was evaluated and peptides with median relative ratio < 0.0005, median response < 100, and more than 2 standards' accuracy being out of the 80-120 range were eliminated from further analyses. Peptides present in $< 75\%$ of the patients were eliminated from analysis. At the next step, the levels of the peptides not detected in a sample were replaced with half of the minimum peptide level detected in the rest of the patients. After this, the MRM data were log2 transformed. Protein-level data were computed by averaging across peptides mapping to the same protein (Supplemental Table S1).

Statistical analyses

Hypothesis testing. Patient characteristics including demographic characteristics, measures of cardiac and kidney function, comorbidities, and medications were recorded for all patients. The *t* test was used to compare hospitalized and not hospitalized patients when the linear model assumptions (linearity, homoscedasticity [constant variance], independence [uncorrelatedness] and normality) were met.[15] If linear model assumptions were not met, then a log2 transformation was applied to the response variable. If the assumptions were still not met, then a Wilcoxon rank sum test (nonparametric) was used. A χ^2 test of independence was used to test the association between each categorical variable and outcome variable (hospitalized vs not hospitalized). A P value < 0.05 was deemed significant for all clinical variables.

Exploratory data analysis. Exploratory data analysis was performed on each molecular data set using principal component analysis. Differential expression analysis for cells, gene transcripts, and proteins, comparing hospitalized and not hospitalized patients was performed using linear models (or moderated *t* tests) using the limma R-library (version 3.38.3).[16] Pathway analysis of the gene transcripts data was performed using CAMERA, a competitive gene set test,[17] using the KEGG and WikiPathway gene set collections (Supplemental Table S2) as part of the limma R-library. The Benjamini-Hochberg false discovery rate (FDR) of 5% was used for all comparisons.[18]

Biomarker discovery. Classification algorithms were on the basis of elastic-net regularized logistic regression models, which select a subset of variables that are relevant predictors of a binary outcome of interest.[19] The elastic net penalty was optimized using a grid of lambda and alpha values as implemented in the glmnet R-library (version 2.0-16). Lambda is a parameter that controls the strength of penalty, whereas alpha controls the tradeoff between the least absolute shrinkage and selection operator (which retains the fewest number of predictors) and ridge (which retains all predictors) penalties. A grid of alpha values (0.7, 0.775, 0.850, 0.925, 1) and a grid of lambda values (ranging from 0.001 to 0.1 with a step of 0.01) was used for all models. The caret R-library (version 6.0-81) which contains $>$ 200 classification and regression algorithms was used to build these classification models (biomarker panels) as well as implement the fivefold cross-validation scheme, repeated 5 times (the same split of samples during cross-validation was used when constructing all models). Classification performance was on the basis of the area under the receiver operating characteristic curve (AUC), and AUCs between 2 receiver operating characteristic curves were compared using the De Long test.

Gene set enrichment analysis. Enrichr was used to perform pathway enrichment analysis (on the basis of the Fisher exact test) of the gene transcripts and proteins in the ensemble biomarker panel.[20] The databases used included BioCarta (2016), Reactome (2016), KEGG (2017), and Jensen Diseases. An FDR cutoff of 5% was used to determine significantly enriched pathways/diseases.

Readers are encouraged to reproduce the results at https://amritsingh.shinyapps.io/multiomics_HFhospitalizations/.

Results

Clinical variables as potential predictors of hospitalization status

Of the 58 patients with advanced HF, 13 were hospitalized within 3 months of enrollment into the study (Table 1). Hospitalized patients were all male, and had a significantly

Table 1. Patient characteristics

Clinical variable	Hospitalized (n = 13)	Not hospitalized (n = 45)	*P*
Mean age, years	69.4 ± 11.7	62.7 ± 13.1	0.10
Male sex, n (%)	13 (100)	30 (67)	0.04
NYHA classification, n (%)			0.17
I	1 (8)	13 (29)	
II	6 (46)	21 (47)	
III	6 (46)	11 (24)	
Mean systolic blood pressure, mm Hg	105 ± 12.8	118 ± 20.2	0.03
Mean diastolic blood pressure, mm Hg	59.8 ± 6.4	66.5 ± 9.5	0.02
Mean BNP, pg/mL	388 ± 304	183 ± 275	0.003
Mean left ventricular ejection fraction, %	30.4 ± 9.9	37.4 ± 12.4	0.07
Ischemia, n (%)	11 (85)	23 (51)	0.07
Mean time since HF onset, years	12.5 ± 11.8	5.3 ± 6.4	0.02
Diabetes mellitus, n (%)	8 (62)	16 (36)	0.18
Current smoker, n (%)	0 (0)	7 (16)	0.30
Former smoker, n (%)	8 (62)	22 (49)	0.62
Hypertension, n (%)	10 (77)	26 (58)	0.35
Peripheral vascular disease, n (%)	2 (15)	2 (4)	0.45
Mean heart rate, beats per minute	63.3 ± 8.8	67.8 ± 13.5	0.27
Mean glomerular filtration rate, mL/min	53.8 ± 24.3	69.3 ± 25.6	0.07
Median creatinine (range), μmol/L	128 (79-866)	85.5 (62-168)	0.006
Medications, n (%)			
Spironolactone	5 (38)	22 (49)	0.73
Digoxin	0 (0)	3 (7)	0.81
Aspirin	9 (69)	29 (64)	1
Warfarin	3 (23)	2 (4)	0.12
Clopidogrel	2 (15)	5 (11)	1
Amiodarone	1 (8)	4 (9)	1
β-Blocker	12 (92)	41 (91)	1
ACEI/ARB	9 (69)	39 (87)	0.29
Statins	9 (69)	25 (56)	0.57
Diuretics	11 (85)	29 (64)	0.30
Calcium channel blockers	2 (15)	8 (18)	1

The *t* test was used for age, baseline LVEF, heart rate, diastolic blood pressure, systolic blood pressure, BNP, glomerular filtration rate, and days since HF onset. The Wilcoxon rank sum test was used for creatinine. Systolic blood pressure, BNP, and days since HF onset were log2-transformed to satisfy assumptions of the *t* test. Glomerular filtration rate has 6 missing values and creatinine had 3 missing values, thus were not used in the classification models.

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, brain natriuretic peptide; HF, heart failure; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction.

lower systolic and diastolic blood pressure. The time since the HF onset in hospitalized patients was more than double of that for patients who were not hospitalized (5 vs 12 years). The levels of BNP and creatinine were significantly elevated in baseline blood samples of hospitalized patients compared with patients who were not hospitalized.

Principal component analysis was performed using the clinical data presented in Table 1 (all continuous variables underwent a log2-transformation). Figure 1 shows some separation between patients who were hospitalized vs those who were not hospitalized within 3 months after enrollment (along dimension; Dim 1). Variables contributing to this separation included variable axes (depicted by arrows) that aligned with the x-axis (in the direction of Dim 1). Each arrow points in the direction of increasing values for that given variable, and the length of the arrow is indicative of its contribution to Dim 1. For example, variables such as BNP, age, ischemia, and diabetes pointed to the left side of the graph, therefore these patients (depicted mostly by green triangles) had higher BNP levels, were older, and had ischemia and diabetes (ie, mostly hospitalized patients) compared with patients on the right side of the graph. Diastolic blood pressure was parallel to Dim 1 and pointed to the right suggesting that these patients (depicted mostly by orange circles) had higher diastolic blood pressure and left ventricular ejection fraction (ie, mostly patients who were not hospitalized).

Systemic inflammation is upregulated in baseline blood samples in patients who were hospitalized within 3 months after enrollment

Differential expression analysis identified 1 cell type (monocytes), 6 gene transcripts (Toll-Like Receptor 7, Feline Leukemia Virus subgroup c Cellular Receptor family member 2, Family with sequence similarity 198 member B, Plexin B2, Prolactin Receptor, FYVE RhoGEF and PH domain containing 6) and 5 proteins (β-2 microglobulin [B2M], cystatin C [CST3], paraoxonase 3, apolipoprotein M, and apolipoprotein A2) with levels that were statistically different between patients who were hospitalized compared with those who were not at an FDR of 5% (see volcano plots in Fig. 2). Although no Holter variables were significant at the FDR cutoff of 5%, the ratio between the average daytime to night-time heart rates (HS_HR_AVE_DayToNight) was marginally significant with an FDR of 6%. Pathway enrichment analysis identified 38 significant pathways from the KEGG and WikiPathways databases, at an FDR of 5% (Supplemental Table S3). In total, 36 pathways were upregulated whereas 2 were downregulated in hospitalized patients compared with not hospitalized patients. Examples of upregulated pathways in hospitalized patients included type II interferon signalling, oxidative stress, toll-like receptor signalling pathway, complement and coagulation cascades, and interleukin (IL)-1 signalling pathway

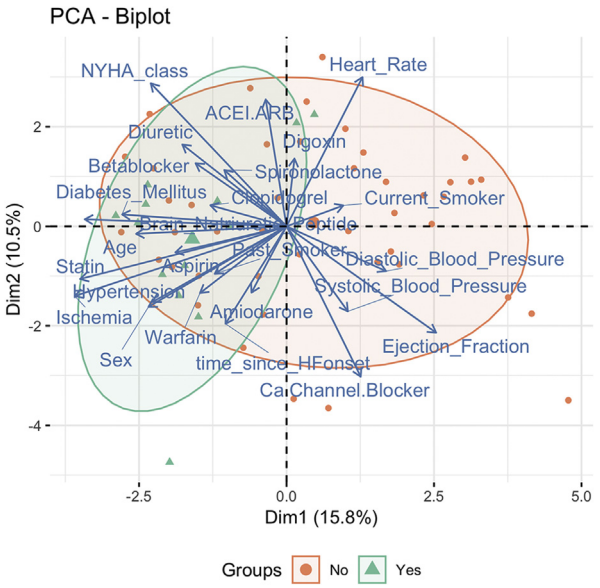


Figure 1. Exploratory data analysis of clinical data using principal component analysis (PCA). This biplot simultaneously depicts all the dimensions (variables) of the data, to identify the key variables that drive the spread of the data. Variable axes point in the direction (**arrow head**) of increasing values whereas the origin represents the average value of each clinical variable. The length of a given arrow refers to the effect size (magnitude) of that given variable (strength in separating patients). The clustering of variables (**arrow heads**) indicate a positive correlation between these variables, whereas arrows pointing in opposite directions (angle between 2 arrows $> 90°$ and $< 180°$) depicts a negative correlation. Overall, a separation between patients who were hospitalized compared with those who were not can be observed. This separation is mostly driven by variables such as ischemia, age, brain natriuretic peptide (BNP), and diabetes to name a few. A positive correlation can be observed between variables such as diabetes, BNP, age, peripheral vascular disease, and ischemia (acute angle between variable vectors). A negative correlation can be observed between left ventricular ejection fraction and BNP (obtuse angle) whereas a stronger negative correlation can be observed between left ventricular ejection fraction and New York Heart Association class (approximately 180°).

whereas downregulated pathways included cytoplasmic ribosomal proteins, and primary immunodeficiency.

The ensemble of omics and electrical biomarkers outperformed clinical biomarkers in predicting cardiac-related 3-month hospitalizations

Models were independently developed using clinical, Holter, cellular, gene transcripts, and proteins to assess their predictive ability in discriminating hospitalized from not hospitalized patients (Fig. 3). The best performing clinical panel (mean AUC, 0.76; SD, 0.035) consisted of New York Heart Association classification, angiotensin-converting enzyme inhibitor/angiotensin receptor blockers, diuretic, time since HF onset, BNP, current smoker, systolic blood pressure, sex, and diastolic blood pressure (Fig. 3A and B). Monocytes were significant predictors of hospitalization status (AUC, 0.70; SD, 0.015). Although actual monocyte counts were not available for this study, a reasonable concordance was observed between predicted and actual monocyte counts (Pearson $r = 0.63$ and a root mean square error $= 0.03$) in a chronic HF cohort of 197 patients[13,14] (Supplemental Fig. S1). The Holter panel achieved a similar performance with an AUC of 0.70 (SD, 0.032). The transcriptomics and proteomics panels achieved a classification performance of 0.72 (SD, 0.041) and 0.82 (SD, 0.041), respectively.

The ensemble panel consisting of 2 cell types, 2 Holter variables, 29 gene transcripts, and 8 proteins, strongly outperformed the clinical panel (mean AUC, 0.88; SD, 0.024; De Long test $P = 0.03$). The performance of the ensemble panel remained unchanged when clinical variables were also selected as part of the ensemble biomarker panel. The individual biomarkers that were identified and their intersection with the ensemble biomarker panel are depicted in Figure 3C. Most biomarkers in the ensemble biomarker panel overlapped with the individual biomarker panels with the exception of the maximum R-R interval Holter variable, the apolipoprotein A2 protein, and activated dendritic cells.

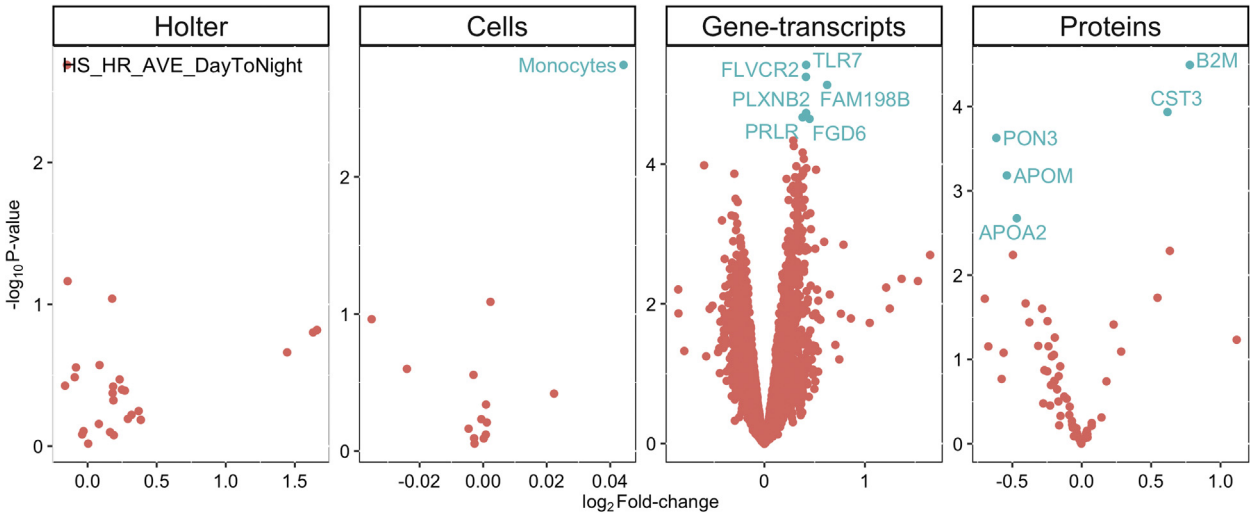
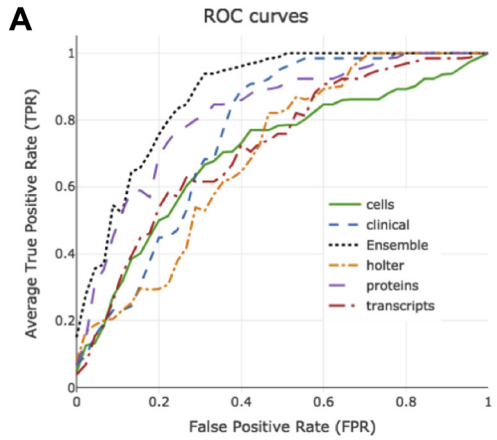


Figure 2. Univariate analysis; comparison of patients who were hospitalized compared with those who were not hospitalized. Volcano plots depict the $\log_2$ fold-change (hospitalized/not hospitalized patients) and significance ($-\log_{10}$P-value) for variables in each data set (Holter, cell types, gene transcripts, proteins).



B

Panels	Penalties (alpha/lambda)	Number of predictors	AUC Mean (SD)
clinical	0.70/0.061	9	0.76 (0.035)
cells	0.92/0.091	1	0.70 (0.015)
Holter	1/0.091	1	0.70 (0.032)
transcripts	0.70/0.091	32	0.72 (0.041)
proteins	0.85/0.001	24	0.82 (0.041)
Ensemble*	0.77/0.091	41	0.88 (0.024)

*cells + Holter + transcripts + proteins

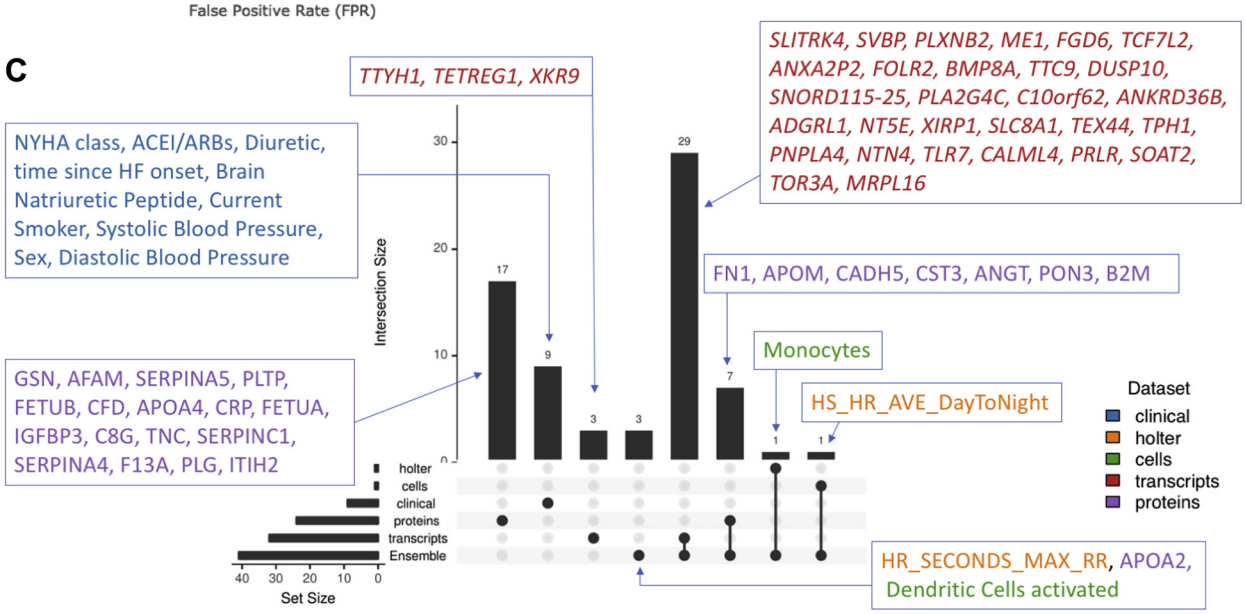


Figure 3. Classification performance of individual and ensemble biomarker panels. (**A**) Receiver operating characteristic (ROC) curves for each individual model (classifier) developed on each data set separately, as well as an ensemble panel consisting of Holter variables, cell types, gene transcripts, and proteins. (**B**) The optimal parameters (alpha, and lambda) as well as the average (mean) and SD area under the ROC curve (AUC) for each model in (**A**). (**C**) An intersection plot depicting the number of distinct and overlapping biomarkers in the single biomarker panels compared with the ensemble biomarker panel. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; HF, heart failure.

The principal component analysis plot on the basis of the biomarkers in the ensemble biomarker panel depicted a clear separation between the patients who were hospitalized compared with those who were not hospitalized (Fig. 4A). Similarly, these ensemble biomarkers depicted distinct expression patterns between hospitalization groups (Fig. 4B). Biological enrichment and correlation analyses identified groups of interconnected biomarkers attributed to pathways/ontologies such as antigen processing and presentation, amyloidosis, mechanism of gene regulation by peroxisome proliferations via peroxisome proliferator-activated receptor alpha (PPARα) and coronary artery disease (Fig. 4C).

Discussion

In this pilot study, we demonstrated the utility of using multiple modalities (electrical monitoring, and omics technologies) to improve on the discriminatory ability of clinical risk models in predicting hospitalizations in HF patients. Similar to previously published studies, the best performing clinical model achieved a modest classification performance (AUC, 0.76).[7,8] The clinical model included a mix of demographic characteristics, cardiac function parameters, comorbidities, and time since the onset of HF. However, an ensemble biomarker panel, which consisted of cell types, Holter variables, gene transcripts, and proteins outperformed the clinical model (AUC, 0.88).

Predictive biomarkers were identified from each omic data set. For example, the ratio between the daytime and night-time HR_AVEs was significantly higher in patients who were not hospitalized compared with patients who were hospitalized. The heart rate is usually lower during the night compared with during the day, however, in hospitalized patients the day- and night-time HR_AVEs were similar. These findings suggest either a lower daytime or higher night-time HR_AVE in hospitalized patients. The lower daytime HR_AVE can be because of decreased activity as a result of HF, whereas a higher night-time HR_AVE can be a result of

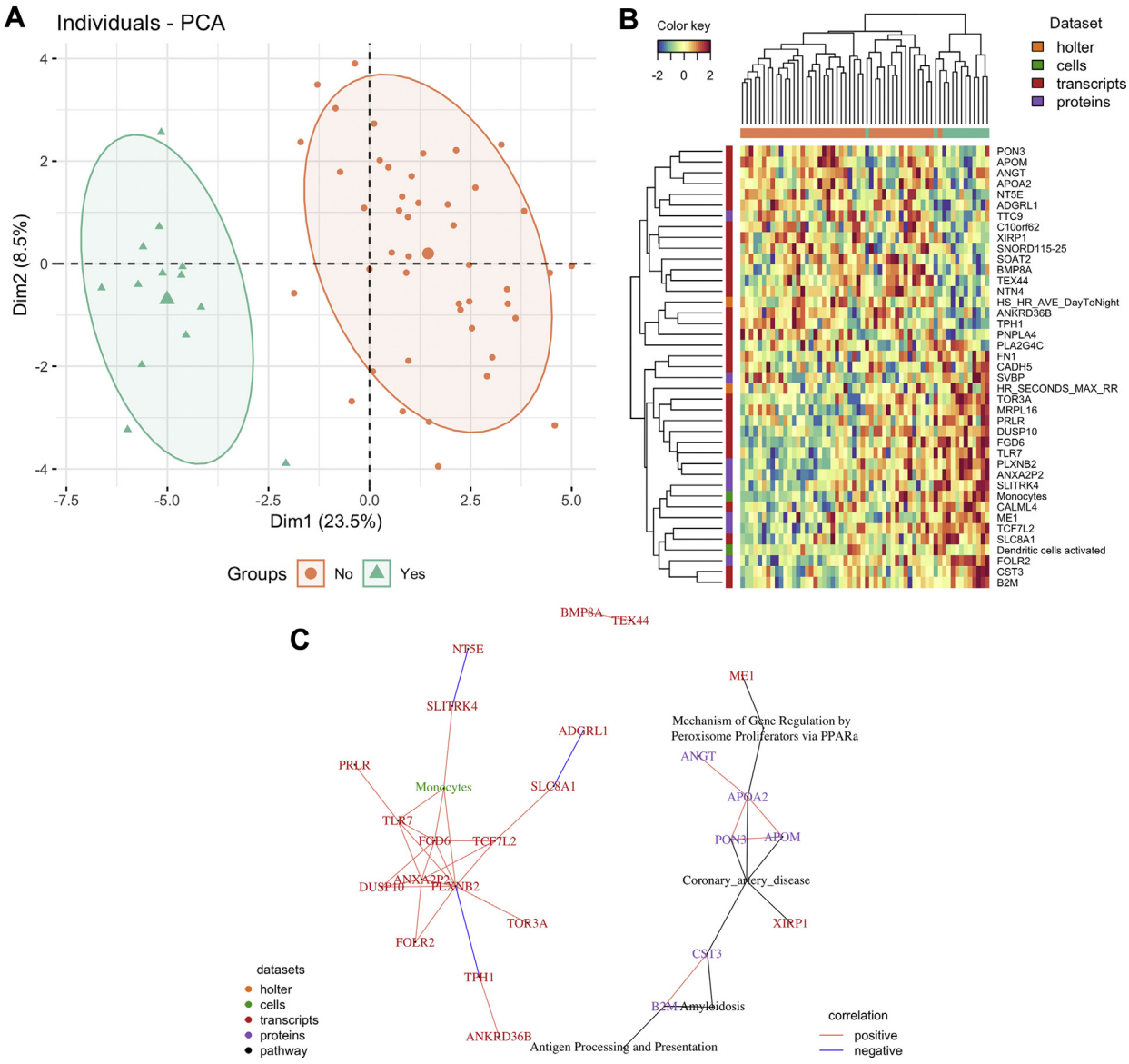


Figure 4. The ensemble biomarker panel. (**A**) A principal component analysis (PCA) plot that depicts the clustering of subjects with respect to the biomarkers in the ensemble biomarker panel. (**B**) A heat map using the scaled data for all patients (columns) and biomarkers (rows) that are reordered on the basis of hierarchical clustering. (**C**) A network of biomarkers as part of the ensemble biomarker panel with a correlation (absolute value) $>$ 0.5, or connected via significantly enriched pathways/ontologies (FDR, 5%). FDR, false discovery rate.

the increased venous return when people lie flat at night because of the mobilization of pedal edema resulting in paroxysmal nocturnal dyspnea. Further, it is well known that the autonomic nervous system is dysfunctional in patients with HF and might be contributing to some degree. Another potential explanation could be that hospitalized patients might be hemodynamically decompensated with lower stroke volume such that the usual dip in night-time heart rate was not observed. Unlike this day-to-night-time HR_AVE ratio, the heart rate at the time of enrollment was not selected as a predictor in the clinical model and neither was it statistically significant (Table 1). This result speaks to the importance of serial monitoring of patients with HF, in whom dynamic changes in cardiac function might be better associated with patient outcomes.[21,22]

The transcriptomics data indicated upregulation of many proinflammatory mechanisms such as complement activation, toll-like receptor signalling and IL-1/type II interferon signalling, in patients who were subsequently hospitalized. Proinflammatory cytokines such as tumour necrosis factor α, IL-1β, and IL-6 are known to increase in patients with HF[23] and lead to various deleterious effects such as myocardial remodelling, cardiomyocyte stiffness, and apoptosis.[24] Inflammatory processes can be modulated by cell types such as monocytes, which were also identified as predictive biomarkers in this study. Blood monocyte levels have been shown to predict cardiovascular events independent of other risk factors such as age, sex, smoking status, high-density lipoprotein cholesterol, and presence of diabetes and hypertension.[25] Last, various proteins that were identified as predictive

biomarkers in this study have also been previously implicated in HF. Paraoxonase 3, which was decreased in hospitalized patients is known to exert antiatherogenic effects by oxidative stress.[26] B2M and CST3 were upregulated in hospitalized patients. Higher levels of B2M and CST3 have been associated with adverse cardiovascular outcomes in patients with coronary artery disease.[27] These different omics domains might be capturing different aspects of the HF etiology and therefore their combination resulted in improved classification performance compared with the usual clinical biomarkers.

Several limiting factors such as small sample size, imbalanced class sizes, lack of an external cohort for model calibration and validation might affect the results of this pilot study. Although a validation cohort was not present for this study, the main purpose of this study was to compare the performance between the clinical and ensemble (Holter with omics) models in predicting 3-month hospitalizations. The statistical deconvolution approach used to estimate cellular frequencies was originally validated using cancer samples, therefore it is difficult to determine its accuracy in our patient cohort.[12] However, in a similar cohort of chronic HF patients, a reasonable concordance was observed between predicted and actual monocyte counts. Further, unlike many biomarker studies that select significant biomarkers before developing biomarker panels, the cross-validation scheme used in this study was independent of any previous filtering of features on the basis of class labels.[28] Last, very few patients had an ejection fraction $\geq$ 50%, therefore whether these results can be extrapolated to patients with HF with persevered ejection fraction remains a topic for further exploration. Nonetheless, the results of this pilot experiment provide evidence that incorporation of data other than common clinical variables might be fruitful in identifying predictive risk models for short-term hospitalization in patients with HF.

We showed that molecular profiling of blood and electrical monitoring of the heart might provide more sensitive markers of risk prediction compared with commonly used clinical variables such as those obtained from electronic medical records. We anticipate that such techniques and approaches might become mainstream in the future as biobanking of specimens, and molecular and sensory data become routinely obtained and used.

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Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Cardiology* at www.onlinecjc.ca and at https://doi.org/10.1016/j.cjca.2018.12.039.