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Proteomic Profiling to Identify Prognostic Biomarkers in Heart Failure

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Abstract. *Background: The ability to predict mode, as well as risk, of death in left ventricular systolic dysfunction (LVSD) is important, as the clinical and cost-effectiveness of implantable cardioverter defibrillators (ICD) therapy depends on its use in appropriately selected patient populations. The value of a proteomic approach in identifying prognostic biomarkers in LVSD is unknown. The aims of this pilot study were to use proteomic techniques to identify serum biomarkers associated with LVSD and to prospectively explore their association with prognosis. Patients and Methods: Serum was analysed by surface-enhanced laser desorption ionisation time-of-flight mass spectrometry (SELDI-TOF MS) in patients with (n=78) and without (n=45) systolic heart failure (SHF). Spectra were compared to identify differentially expressed signal peaks as potential biomarker indicators. The ability of these peaks to predict all-cause mortality and survival with appropriate ICD therapy was then tested prospectively in patients with ICDs, on the background of LVSD (n=141). Results: For the identification stage spectra (2-200 kDa) from SHF and control patients were randomly separated into two equally sized discovery and validation sets. Six protein peaks were identified that were differentially expressed in SHF in both sets. In the prospective phase, during a mean follow-up of 15±3 months, 11 patients died and 39 survived with appropriate ICD therapy. Five out of the six proteomic biomarkers predicted all-cause mortality but none predicted appropriate ICD therapy. Conclusion: These results provide proof-of-principle and are supportive of the SELDI proteomic approach as a high-throughput screening tool in identifying potentially prognostic protein peaks in patients with LVSD.*

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Heart failure is a major healthcare problem, effecting over 10 million people in Europe and America alone (1). Although contemporary medical therapy has significantly improved prognosis in patients with heart failure and asymptomatic left ventricular systolic dysfunction (LVSD), mortality remains high (2). However, there is a wide variation in mortality rates and mode of death among patient groups with LVSD (2). Though overall sudden cardiac death (SCD) is the commonest cardiac mode of death, in patients with more advanced disease death due to pump failure predominates (2). The ability to predict mode, as well as risk, of death in patients with LVSD is important, as the clinical and cost-effectiveness of implantable cardioverter defibrillator (ICD) therapy depends on its use in appropriately selected patient populations (3).

Multiple studies have demonstrated that individual biomarkers are powerful predictors of prognosis in LVSD (4). However, such studies are limited by their evaluation of a small number of pre-determined biomarkers. Proteomic techniques enable the simultaneous and unbiased assessment of a large number of proteins in a sample and allow for the identification of potential candidate biomarkers by comparison of protein expression patterns between patient groups (5). Such techniques have been successfully used to identify potential biomarkers in a range of cardiovascular

conditions (6-9). We hypothesised that an approach using untargeted proteomic profiling techniques may identify prognostic biomarkers in patients with LVSD.

The aims of this pilot study were two-fold: 1) To use surface-enhanced laser desorption ionisation time-of-flight mass spectrometry (SELDI-TOF/MS) to identify potential serum biomarkers associated with systolic heart failure (SHF). 2) To prospectively explore the association of these biomarkers with mortality and the occurrence of ventricular arrhythmias in a cohort of patients with ICDs on the background of LVSD.

Patients and Methods

Patient population. All patients were recruited from those attending the Southampton University Hospital device service. Two different patient populations were enrolled: 1) Control patients. This group comprised of consecutive patients with a permanent pacemaker and preserved LVEF. Patients with a high percentage of right ventricular pacing (>30%), or history, signs or symptoms of heart failure, were excluded. This group were chosen as controls to avoid the introduction of potential bias related to pre-analytical factors due to sample collection and patient preparation; they attended the same hospital clinic, in the same fashion (same time of day, no specific food requirements *i.e.* not nil by mouth), as patients with ICDs (the LVSD group). 2) Left ventricular systolic dysfunction patients. This group comprised of patients with LVSD, and an ICD or cardiac resynchronisation defibrillator (CRT-D). All patients were on optimal medical therapy. None had heart failure admissions or therapy changes in the six weeks prior to enrolment. Based on New York Heart Association (NYHA) functional class and left ventricular ejection fraction (LVEF), two further subgroups of patients with LVSD were identified: (i) Patients with SHF, defined as LVEF ≤40% and NYHA ≥II. (ii) Patients with asymptomatic LVSD, defined as LVEF ≤40% and NYHA I.

Additional exclusion criteria for both groups were pregnancy, an acute coronary syndrome or surgery of any type within the preceding 6 weeks.

At enrolment, baseline demographic and clinical data were recorded, a 12-lead resting ECG performed, and NYHA functional class assessed. All patients had a transthoracic echocardiogram prior to study entry. Blood was drawn from a forearm vein and collected in serum separator (serum) and EDTA tubes (plasma). The collection and handling of all sera samples was applied in accordance to the recommendations of the Standard Operating Procedure Integration Working Group (SOPIWG) (10). Briefly, serum samples were allowed to clot for 30 minutes and then centrifuged at 3000 rpm for 10 minutes. Samples were then divided into aliquots and frozen within 1 hour of sampling. Samples were finally stored at –80˚C prior to analysis and underwent no more than two freeze-thaw cycles. The study complied with the Declaration of Helsinki and was approved by the local research ethics committee. Written informed consent was obtained from all patients.

Study end points and follow-up. Control patients were not followed-up. LVSD patients were followed up at 3-6 months with a hospital visit or *via* a remote patient management system. Patients under remote follow-up also attended the hospital every 6 months. At each hospital visit the patient was clinically assessed and the device interrogated. The occurrence of any ICD therapy was recorded.

Appropriate ICD therapy was defined as: (i) Antitachycardia pacing therapy (ATP) for ventricular tachycardia (VT). (ii) Shock therapy for VT or ventricular fibrillation (VF).

Correct arrhythmia detection/discrimination was confirmed by analysis of stored electrograms by two electrophysiologists blinded to the biomarker analysis.

For the prospective part of the study two study end-points were chosen to enable exploration of the utility of the serum proteomic biomarkers in defining outcomes. These were: (i) All-cause mortality (as an approximate surrogate for non-sudden cardiac death). (ii) Patient survival with appropriate ICD therapy (as an approximate surrogate for preventable sudden cardiac death).

NT-proBNP analysis. In view of its established diagnostic and prognostic role in LVSD, N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured as a comparator to proteomic biomarkers. The NT-proBNP assay was based on a two-site non-competitive assay format, using in-house antibodies, as previously described (11).

SELDI-TOF MS analysis. Serum samples were analysed on the weak cationic exchange (CM10) ProteinChip array (BioRad, California, USA), chosen on the basis of greater peak abundance relative to other surface chemistries. To aid reproducibility, a BioMek3000 (Beckman Coulter, California, USA) liquid handling robot was used. Samples were analysed in duplicate on a bioprocessor (BioRad). Samples were randomly assigned to bioprocessor wells to minimise bias. All samples were run over a 1 week period to reduce potential error due to variation in instrument performance.

Serum samples (10 μL) were denatured at pH 9 for 60 min on ice in 90 μl of U9 buffer (9 mol/L urea, 2% CHAPS, 50 mM Tris-HCl, pH 9) (Bio-Rad). Wells were pre-incubated with 5 μl of buffer (CM10 low-stringency, Bio-Rad), and then incubated twice with 200 μl of buffer at room temperature for 10 min. A 10-μl aliquot of the resulting serum mixture was further diluted in 90 μl sample buffer and applied to the wells. Samples were further incubated for 60 min at room temperature (23˚C) on a Micromix5 platform shaker (Diagnostic Products Corporation, California, USA), using a form of 20 and amplitude of 7. Following incubation, unbound proteins were removed by washing with two volumes of 200 μl of each buffer, and di-ionised water. Each washing step was performed with horizontal shaking for 10 min. The ProteinChips were removed from the bio-processor and allowed to air-dry for 45 min. Two applications of 1 μl of 50% sinapinic acid in 50% acetonitrile/0.5% trifluoroacetic acid were delivered to each spot and allowed to air-dry for 15 min.

Time-of-flight (TOF) spectra were generated using an Enterprise 4011 mass spectrometer (Bio-Rad). Each spot was analysed using laser settings optimised for both low- and high-mass proteins. For the low-mass range (mass range 0-20 kDa, focus mass 10 kDa, matrix attenuation 1 kDa) spectra were generated with 17 shots per position, at laser intensities of 2000, 3000 and 4000, preceded by 2 warming shots at 2200, 3300 and 4400, respectively. For the high mass range (mass range 18-200 kDa, focus mass 20 kDa, matrix attenuation 1 kDa), TOF spectra were generated with 17 shots at a laser intensity of 4000, preceded by 2 shots at 4400.

Spectra were externally calibrated using a protein standard calibration kit composed of recombinant hirudin (6.96 kDa), equine cytochrome *C* (12.23 kDa), equine myoglobin (16.95 kDa) and carbonic anhydrase (29.0 kDa) (Bio-Rad). Following baseline

subtraction, spectra were normalised against the total ion current at the 2-20kDa region for the low mass range and the 18-200 kDa region for the high mass range. Spectra were visually inspected and those exhibiting a poor signal-to-noise (S/N) ratio (approx. <5:1) were excluded for further processing. For each sample, one spectrum for each mass range was used, so as to minimise deviation in total ion current to within 0.4-2.5 times the mean of all patients.

The PCDM software (Biomarker Wizard version 3.1, Bio-Rad) was used to identify peaks. The low *m/z* range between 0 and 2000 was excluded from the analysis to avoid chemical noise interference from the UV matrix species. Peaks with a S/N ratio of ≥5 for the first pass and ≥2 for the second pass, and a valley depth greater than or equal to 3, were considered for clustering if present in ≥10% of spectra. The mass window for each cluster was 0.3% of the peak mass. To avoid analysis of artifact signals all peaks were visually inspected and relabelled as required prior to statistical analysis. The high mass range was run in parallel.

Every other ProteinChip contained a control sample taken from a single pooled mixture of 50 case and control patients to assess assay variability. Co-efficient of variation (CV) for peak intensity for these spectra, derived from the pooled sample, run 34 times (five assays) was 34%. These data were obtained by averaging values for 17 randomly selected peaks spread across the analysed mass range (2-200kDa), using the formula $CV=\sqrt{((CV_1^2+CV_2^2+CV_3^2...+CV_n^2)/n)}$, where n represents the number of included peaks.

Statistics. Categorical variables are expressed as percentages (numbers). Normally-distributed continuous variables are expressed as mean±standard deviation. Variables not normally distributed are expressed as median (lower quartile to upper quartile) and compared using the Mann-Whitney *U*-test.

To identify potential protein biomarker peaks associated with LVSD, the serum proteomic profiles were compared between patients with SHF and controls. To achieve this analysis objective, patients were randomly separated into two equal-sized discovery and validation sets, each containing equal numbers of SHF and control patients. For each proteomic peak, the *p* value was calculated, using the Mann-Whitney *U*-test, to indicate its ability to differentiate SHF patients from controls. Peaks differentially expressed ($p<0.05$) in both sets were considered significant. For each of the significant peaks, Receiver Operating Characteristic (ROC) curves were constructed, to assess accuracy in distinguishing SHF from control patients. The Jonckheere-Terpstra test was used to assess the trend in peak intensity levels across patient groups categorised by functional class.

The 6 peaks that demonstrated an association with SHF were then further evaluated in a prospective study of ICD recipients to assess their ability to predict prognosis in patients with LVSD. Univariate Cox proportional hazards analyses were used to investigate biomarker predictors of the two prospective end-points (all-cause mortality and survival with appropriate ICD therapy). For the end-point of all-cause mortality, in view of the small number of patients reaching the end-point (n=11), multivariable analysis was not performed. As NT-proBNP was not normally-distributed and its normality improved with log transformation the log transformed values were used for analysis. The proportional hazards assumption was checked using Schoenfeld residuals (12).

For the end-point of all-cause mortality, for selected biomarker peaks Kaplan-Meier survival analysis was performed to compare survival between patient groups stratified according to ROC curve-derived cut-off points. Survival between groups was compared using the log-rank test.

Table I. *Patients' characteristics*.

	Controls (n=45)	ICD patients		
		Asymptomatic LVSD (n=47)	SHF (n=78)	Overall (n=141)
Age (years)	66±14	66±11	70±10	69±10
Gender (% male)	53 (24)	87 (41)	77 (59)	85 (120)
Diabetes (%)	9 (4)	19 (9)	20 (15)	24 (34)
Atrial fibrillation (%)	20 (9)	30 (14)	39 (30)	36 (51)
History of hypertension (%)	40 (18)	36 (17)	44 (34)	46 (65)
NYHA Class (%)				
I	100 (45)	100 (47)	-	37 (52)
II	-	-	64 (50)	40 (57)
III	-	-	33 (26)	21 (30)
IV	-	-	3 (2)	1 (2)
Device Type (%)				
ICD	-	96 (45)	46 (36)	66 (93)
CRT-D	-	4 (2)	54 (42)	34 (48)
PPM single-chamber	27 (12)	-	-	-
PPM dual-chamber	73 (33)	-	-	-
Heart disease type (%)				
Ischaemic		72 (34)	80 (62)	77 (108)
NICM		17 (8)	18 (14)	18 (25)
Other		11 (5)	2 (2)	6 (8)
LVEF	-	23±6	26±6	29±9
ICD indication (%)				
Primary prevention	-	23 (11)	46 (36)	37 (52)
Secondary prevention	-	77 (36)	54 (42)	63 (89)
Beta-blocker (%)	18 (8)	83 (39)	78 (61)	79 (111)
ACE-I/ARB (%)	20 (9)	87 (41)	92 (72)	91 (129)
Amiodarone (%)	2 (1)	36 (17)	28 (22)	29 (41)

NYHA, New York Heart Association functional class; NICM, non-ischaemic cardiomyopathy; CRT-D, cardiac resynchronisation therapy defibrillator; PPM, permanent pacemaker; ACE-I, angiotensin-converting enzyme inhibitor; ARBs, angiotensin II receptor blockers.

Statistical analyses were performed on SPSS Version 17 (SPSS Inc., Chicago, IL, USA). In all analyses $p<0.05$ was considered significant.

Results

Patients' characteristics. One hundred and eighty six patients were enrolled (Table I). These comprised of 45 patients with permanent pacemakers, preserved LVEF, and no signs/symptoms of heart failure (control group), and 141 patients with ICDs on the background of LVSD. Out of the 141 patients with ICDs, 78 had SHF and 47 asymptomatic LVSD.

Proteomic biomarkers associated with SHF. Serum proteomic profiles were compared between 78 SHF patients and 45 controls. Patients were randomly assigned to two sets which were separately analysed (see Methods). Biomarker wizard identified 94 analysable protein peaks.

Table II. *Association of proteomic biomarkers with systolic heart failure and asymptomatic left ventricular dysfunction.*

Biomarker	Peak intensity			*p*-value		
	Controls (n=45)	Asymptomatic LVSD (n=47)	SHF (n=78)	Controls *vs.* Asymptomatic LVSD	Controls *vs.* SHF	For trend (controls-asymptomatic LVSD-SHF)
m/z 4221	67.4 (47.7-90.6)	44.0 (25.4-78.5)	35.0 (19.7-73.5)	0.01	<0.001	<0.001
m/z 5351	22.1 (12.3-36.6)	15.1 (5.8-28.9)	7.6 (4.6-18.9)	0.08	<0.001	<0.001
m/z 5921	164.6 (94.3-234.0)	152.0 (82.9-152.0)	95.9 (60.9-160.1)	0.72	0.001	0.003
m/z 6125	14.2 (8.4-20.3)	12.9 (6.3-21.6)	8.2 (5.7-13.9)	0.85	0.001	0.002
m/z 11834	4.1 (3.2-5.9)	5.2 (3.8-7.5)	7.4 (5.3-10.9)	0.02	<0.001	<0.001
m/z 14766	2.5 (2.1-3.1)	2.8 (2.3-3.6)	3.5 (2.6-4.5)	0.06	<0.001	<0.001
NT-proBNP (pmol/L)	78 (20-223)	757 (247-1118)	811 (278-1440)	<0.001	<0.001	<0.001

Peak intensity data are expressed as median (Q1 to Q3). Peak intensities were compared using the Mann-Whitney *U*-test for individual group comparisons and the Jonckheere-Terpstra test for trend. *m/z*, mass/charge.

Twelve peaks in the first set and 15 in the second set were differentially expressed ($p<0.05$), between SHF patients and controls. Six of these peaks were significantly different in both sets (Table II). Four of these peaks were observed in the lower region (*m/z* 4221, 5351, 5921 and 6125) and the two other peaks in the higher mass region (*m/z* 11834 and 14766) in SHF patient samples against those of the control samples.

The proteomic profiles were also compared between the 47 patients with asymptomatic LVSD and 45 controls. Only 2 of the 6 previously identified protein peaks (*m/z* 4221 and 11834) were significantly different between the groups (Table II). However all 6 peaks had an intermediate value between that of SHF and control patients, and for each peak there was a trend in peak intensity from controls through asymptomatic LVSD and SHF (Table II). Furthermore, there was a significant association between peak intensity and functional class (Figure 1). For each of the six proteomic peaks there was a trend in peak intensity from controls, through asymptomatic LVSD, NYHA class II SHF, and NYHA class III/IV SHF: *m/z* 4221 ($p=0.001$), *m/z* 5351 ($p<0.001$), *m/z* 5921 ($p=0.004$), *m/z* 6125 ($p=0.03$), *m/z* 11834 ($p<0.001$) and *m/z* 14766 ($p<0.001$).

ROC curves were constructed to evaluate the ability of the 6 protein peaks to differentiate between SHF and control patients. All peaks significantly distinguished between SHF patients and controls with areas under the ROC curve (AUC) ranging from 0.68-0.80: *m/z* 4221 (AUC 0.69; 95% CI 0.60-0.79; $p<0.001$), *m/z* 5351 (AUC 0.75; 95% CI 0.66-0.84; $p<0.001$), *m/z* 5921 (AUC 0.68; 95% CI 0.58-0.77; $p=0.001$), *m/z* 6125 (AUC 0.68; 95% CI 0.58-0.78; $p=0.001$), *m/z* 11834 (AUC 0.80; 95% CI 0.72-0.88; $p<0.001$), and *m/z* 14766 (AUC 0.77; 95% CI 0.68-0.85; $p<0.001$). However, none of the proteomic peaks out-performed NT-proBNP (AUC 0.88; 95% CI 0.82-0.94; $p<0.001$).

Relationship of proteomic biomarkers to outcomes in patients with LVSD. The ICD patients (n=141) were followed-up for a mean of 15±3 months. During this time there were 11 deaths (8%) and 43 patients (30%) experienced appropriate ICD therapy. Four patients (3%) who experienced appropriate ICD therapy subsequently died, leaving 39 patients (28%) who survived with appropriate ICD therapy. Out of these, 21 experienced appropriate shock therapy while the remainder experienced only appropriate ATP.

The median values for all 6 proteomic peaks were higher in patients that died (n=11) compared to patients with event-free survival (n=91) (Table III). In contrast, the levels were similar between patients that survived with appropriate ICD therapy (n=39) and those with event-free survival (n=91). An example of peak *m/z* 11834 is shown in Figure 2.

In univariate analyses 5 out of the 6 peaks were significant predictors of death (Table IV). However none predicted survival with appropriate ICD therapy. In comparison, Log-transformed NT-proBNP predicted both mortality ($p=0.001$) and survival with appropriate ICD therapy ($p=0.01$).

Survival analysis and proteomic biomarkers. For the ICD patients (n=141), using two of the prognostic peaks (*m/z* 11834 and *m/z* 14766), survival curves were compared between groups stratified according to biomarker levels using ROC-derived cut-off points. For peak *m/z* 11834, 6 of 15 patients with a signal intensity ≥17.5 died, compared to only 5 of 126 below this level ($p<0.001$). For peak *m/z* 14766, 6 of 15 patients with a signal intensity ≥5.6 died, compared to only 5 of 126 below this level ($p<0.001$).

Discussion

The main findings of this pilot study indicate that using the SELDI proteomic technique, serum-derived protein peaks were

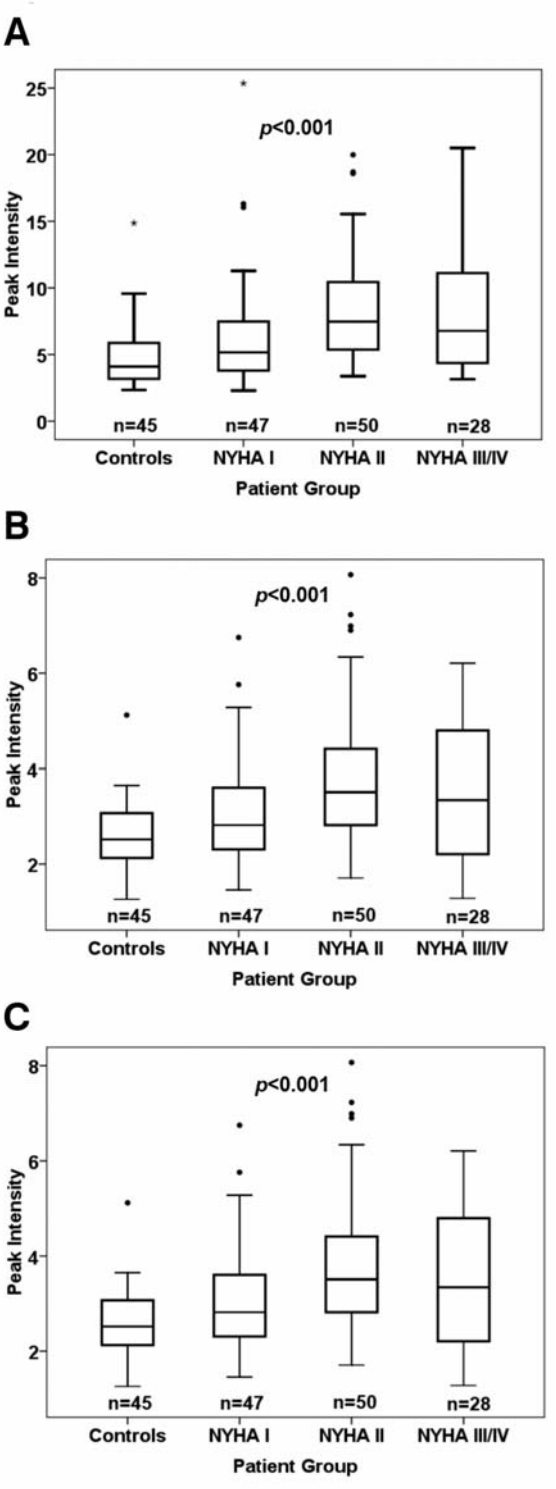

Figure 1. *Relationship between clinical status and biomarker peak intensity for biomarker peaks at: (A) m/z 11834 , (B) m/z 14766, and (C) m/z 5351. Patients are grouped into controls, and for patients with LVEF ≤40%, by NYHA class (III/IV combined). The peak intensity levels are presented as box (25th percentile, median, 75th percentile) and whisker (10th and 90th percentiles) plots. Patient numbers are indicated. The Jonckheere-Terpstra test was used to assess the trend in peak intensity levels across patient groups.*

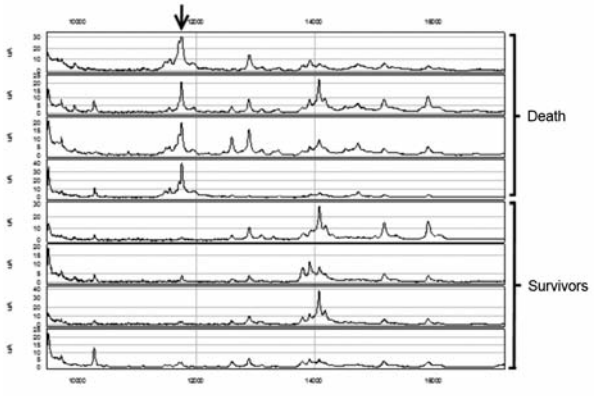

Figure 2. *Increased expression of peak m/z 11834 in patients with ICDs that died compared to those that survived. A region of mass spectra from 10 to 16 kDa has been expanded and aligned for 4 patients that died during follow-up and 4 patients that survived. Peak intensity for biomarker peak m/z 11834 (arrow) is higher in patients that died versus those that survived. The x-axis is the ratio of mass-to-charge (m/z) and the y-axis represents peak intensity.*

identified to be differentially expressed in patients with SHF compared to controls, and that these peaks exhibited predictive potential for all-cause mortality in patients with ICDs on the background of LVSD. Furthermore, although these protein peaks were associated with all-cause mortality, they did not predict the occurrence of appropriate ICD therapy. These findings constitute a proof-of-principle and suggest that the SELDI proteomic approach may be useful in the screening of potential predictive protein markers of overall mortality, as well as mode of death, in patients with LVSD. However, this pilot study does not warrant firm conclusions to be drawn. Additionally, none of the protein peaks or combination thereof, outperformed the NT-proBNP protein biomarker in either a diagnostic or prognostic role. Lastly, the identity of the protein peaks will need to be further analyzed with electrospray ionization tandem mass spectrometry (ESI MS-MS)-based methods, using either high resolution top-down or bottom-up LC-MS techniques. The use of these low-throughput and in-depth proteomic approaches was deemed beyond the scope of this proof-of-principle SELDI proteomic screening study.

Our finding that the SELDI proteomic technique can identify potential protein peaks with diagnostic accuracy in SHF was consistent with the results of other studies (7). Jones *et al*. evaluated the diagnostic value of plasma protein peaks in 100 SHF patients and 100 controls (7). Using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS), a complementary proteomic technique, they identified 67 protein peaks that were differentially expressed between SHF and control patients, out of which 6 had predictive value independent of NT-proBNP.

Table III. *Baseline biomarker levels in relation to outcome in ICD recipients.*

Biomarker	Peak intensity		
	Event-free survival (n=91)	Survival with appropriate ICD therapy (n=39)	All-cause mortality (n=11)
m/z 4221	37.1 (21.6-73.4)	39.0 (34.5-68.5)	56.1 (26.9-78.4)
m/z 5351	9.4 (4.7-22.6)	10.0 (5.8-22.3)	10.1 (6.8-36.6)
m/z 5921	111.0 (59.4-191.9)	102.5 (73.5-182.7)	159.6 (94.5-252.7)
m/z 6125	9.9 (5.9-16.9)	9.8 (6.3-15.2)	14.3 (8.2-23.7)
m/z 11834	5.8 (4.4-9.3)	5.8 (4.4-9.3)	18.6 (6.0-29.9)
m/z 14766	3.0 (2.3-3.8)	3.0 (2.3-3.8)	5.7 (2.6-6.8)
NT-proBNP (pmol/L)	412.1 (124.6-1144.3)	820.6 (399.3-1118.1)	2207.1 (611.3-2883.2)

Peak intensity data are expressed as median (Q1 to Q3). *m/z*: Mass/charge.

Table IV. *Biomarker univariate predictors of all-cause mortality and survival with appropriate ICD therapy.*

Biomarker	All-cause mortality		Survival with appropriate ICD therapy	
	P-value	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)
m/z 4221	0.51	1.00 (0.99-1.02)	0.85	1.00 (0.99-1.01)
m/z 5351	0.01	1.02 (1.00-1.03)	0.89	1.00 (0.98-1.02)
m/z 5921	0.02	1.01 (1.00-1.01)	0.50	1.00 (0.99-1.00)
m/z 6125	0.002	1.03 (1.01-1.05)	0.45	0.99 (0.95-1.02)
m/z 11834	0.002	1.04 (1.02-1.06)	0.48	1.01 (0.98-1.04)
m/z 14766	0.007	1.40 (1.10-1.80)	0.36	0.89 (0.70-1.14)
Log NT-proBNP	0.001	53.08 (4.96-568.4)	0.01	2.33 (1.20-4.53)

m/z: Mass/charge.

To our knowledge, this was the first study to evaluate the potential use of the SELDI proteomic technique to screen protein peaks that may predict mortality in patients with LVSD. The ability to predict mode of death in patients with LVSD is important, as the efficacy and cost-effectiveness of ICD therapy depends on the relative contribution of SCD and non-sudden death. ICD therapy improves overall survival in patients at high SCD risk by terminating life-threatening arrhythmias. However it does not prevent non-SCD, which in patients with LVSD is predominantly due to pump failure (2). Furthermore, even in patients at high SCD risk, ICD therapy does not improve overall survival if the risk of non-SCD is significantly elevated (3). There is therefore significant clinical value in identifying novel markers of non-SCD risk that may identify patients with LVSD who are unlikely to benefit from ICD therapy.

In our study 5 of the 6 proteomic peaks differentially expressed in SHF predicted all-cause mortality in patients with LVSD, but none predicted the occurrence of ventricular arrhythmias. It is interesting to speculate that this may reflect the different pathophysiological mechanisms underlying pump failure death and SCD in patients with LVSD. Although a range of potentially pro-arrhythmic structural and functional changes have been described in LVSD, the precise pathophysiological processes that lead to SCD are not clearly understood (13). The identification of potential protein markers associated with mode of death may help to better-understand the pathological processes underlying mortality in patients with LVSD.

The levels of all 5 protein peaks associated with mortality were higher in patients that died compared to those that survived. However, in the identification phase of the study, the levels of 3 of these peaks (*m/z* 5351, 5921 and 6125) were actually lower in patients with SHF compared to controls. Although a non-linear relationship between biomarkers and disease severity in SHF has been described, our findings are new and require confirmation (14).

Our results suggest that the SELDI proteomic approach may be useful in the screening and identification of protein peaks that predict mode of death. However, to be of clinical value such protein peaks would need to add incremental prognostic accuracy in addition to the currently available clinical risk models, and there are currently no data to support this possibility (3, 15).

The aim of this study was to demonstrate proof-of-principle, that the SELDI proteomic approach may be able to identify prognostic biomarkers in patients with LVSD. As such, we performed only limited proteomic profiling, using one-surface chemistry (CM10) and a single set of binding conditions, an approach that has been used successfully by other investigators, in a small group of patients with a relatively short follow-up (5). We have not established the identities of the proteomic peaks, as we feel that the next step would be to perform more exhaustive proteomic profiling in an appropriately-powered study, and only then identify the most discriminative peaks. Our limited profiling yielded approximately 100 analysable protein peaks. However, it has been estimated that up to 900,000 plasma proteins exist, and therefore we have only analysed a very small subset of the serum proteome (16). It is likely that using a range of surface chemistries and binding conditions would identify a significantly greater number of potential biomarkers (8).

Although our co-efficient of variation of the normalised peak intensities is consistent with published data, it is significantly higher than that of more conventional measurement methods, such as those using antibodies (5, 17). Thus the biomarker peaks we identified are likely to have significantly better diagnostic and prognostic accuracy, once identified and measured using more conventional methods.

Limitations

The study sample is small and the number of patients reaching the study end-points low. Furthermore, the follow-up in the prognostic part of the study was relatively short. Our findings need repeating in a significantly larger cohort with a longer follow-up.

Although we have found that specific proteomic peaks have diagnostic and prognostic value in patients with LVSD the identity of these proteins was not known. This is an important issue in proteomic research as there is a significant risk of false-positive results with the multiple biomarker peaks being analysed (18). Peak identification enables demonstration of biological plausibility, thereby strengthening the conclusions that can be drawn as well as potentially enabling insight to be gained into the underlying pathophysiology. However, the protein peaks were identified in two separate randomly selected sets of SHF patients and controls, and then evaluated in a separate prospective study using a separate prognostic end-point (all cause mortality). These factors suggest that, despite the study limitations, the protein peaks identified were less likely to be false-positive findings.

Delivery of appropriate ICD therapy is not always a surrogate for preventable SCD. However, with current guidelines widening the recipient population for ICDs, the investigation of predictors of SCD in higher risk patients is difficult, as most such patients are indicated for an ICD.

Conclusion

In this pilot study we have used the SELDI proteomic technique to identify distinct serum protein patterns, traceable to patients with SHF that predict all-cause mortality but not appropriate ICD therapy in patients with ICDs on the background of LVSD. These results provide proof-of-principle and were suggestive of the SELDI proteomic approach as a high-throughput screening tool in identifying potential protein peaks in patients with LVSD.

Disclosures

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