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HIGH RESOLUTION MULTIDIMENSIONAL PROTEOMICS DETECTS CANDIDATE ARRHYTHMIA BIOMARKERS

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Introduction The ability to predict arrhythmia risk 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. Individual biomarkers can be powerful predictors of prognosis, but studies are limited to small marker panels, chosen a priori. Mass spectroscopy based proteomic techniques can powerfully and simultaneously demonstrate differential candidate proteins in an unbiased fashion but resolving power can be limited by high serum abundant proteins. Depletion methods can overcome this, but often result in co-removal of potential biomarkers too. The aim of the study was to utilise a novel wide spectrum technique to identify candidate biomarkers from the whole proteome associated with arrhythmia outcomes.

Methods Consecutive patients attending device follow up clinic were recruited to the study. Serum was collected and stored at −80°C. The occurrence of prespecified arrhythmia end points and death was recorded. Sera were then pooled according to clinical categories occurring during follow up. Grouped sera underwent stable isotope iTRAC labelling, and following protein fractionation

Table 1 Proteins differentially expressed in arrhythmia outcomes. Values represent ratio of protein expression in clinical group compared to control (no VT/VF).

Protein	Death	VT/VF	No VT/VF
Semaphorin-6B	0.728	7.064	1
Tropomyosin α-3 chain	1.354	3.367	1
Heat shock cognate	1.849	3.048	1
Ubiquitin carboxyl-terminal hydrolase	1.686	2.175	1
F-box only protein 36	1.751	2.132	1
Apolipoprotein C-III	1.629	2.095	1
Histone H2A type 1-H	1.928	2.091	1
Hepatocyte nuclear factor 6	1.179	0.333	1
Proteasome subunit α type-1	0.859	0.344	1
Dynein heavy chain 17	0.726	0.355	1
Keratin, type I cytoskeletal	1.981	0.437	1
Collagen α-1(XVIII) chain	1.741	0.440	1
Ig heavy chain V-II region OU	0.706	0.489	1
DnaJ homologue subfamily C member 1	1.177	0.496	1
TIR domain-containing adapter molecule 1	0.811	0.498	1
Natriuretic peptides B	4.374	1.764	1

and immunodepletion, samples underwent simultaneous tandem mass spectroscopy followed by data processing and identification of differentially expressed protein peaks $p<0.05$. Technical validation of the technique was achieved through detection of B type natriuretic peptide using mass spectroscopy and in unfractionated serum using standard ELISA techniques.

Results 243 patients (54% male, age 71±6) were included in the analysis. During follow up of 40 months, there were eight cardiovascular deaths. 25 experienced VT>182 bpm or VF, whilst 48 never experienced VT at any rate/VF (controls).

634 proteins were identified by this method. When compared to the control group, proteins had significant differential expression if twofold up- or down-regulated. Overall, 94 proteins were differentially expressed in those who died or experienced VT>182 or VF. 15 proteins were associated with the arrhythmia endpoint but not death (table 1). BNP was detected by both MS and ELISA, and had greater up-regulation in patients who died, but was not discriminative for arrhythmia occurrence.

Conclusions This study provides proof of principle that proteomic techniques can identify candidate proteins for use as biomarkers of arrhythmia risk. Further investigation is needed to select proteins with potential for clinical application before testing in a prospective setting.