Using matrix assisted laser desorption ionisation mass spectrometry (MALDI-MS) profiling in order to predict clinical outcomes of patients with heart failure

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Abstract

Background: Current risk prediction models in heart failure (HF) including clinical characteristics and biomarkers only have moderate predictive value. The aim of this study was to use matrix assisted laser desorption ionisation mass spectrometry (MALDI-MS) profiling to determine if a combination of peptides identified with MALDI-MS will better predict clinical outcomes of patients with HF.

Methods: A cohort of 100 patients with HF were recruited in the biomarker discovery phase (50 patients who died or had a HF hospital admission vs. 50 patients who did not have an event). The peptide extraction from plasma samples was performed using reversed phase C18. Then samples were analysed using MALDI-MS. A multiple peptide biomarker model was discovered that was able to predict clinical outcomes for patients with HF. Finally, this model was validated in an independent cohort with 100 HF patients.

Results: After normalisation and alignment of all the processed spectra, a total of 11,389 peptides (m/z) were detected using MALDI-MS. A multiple biomarker model was developed from 14 plasma peptides that was able to predict clinical outcomes in HF patients with an area under the receiver operating characteristic curve (AUC) of 1.000 (p = 0.0005). This model was validated in an independent cohort with 100 HF patients that yielded an AUC of 0.817 (p = 0.0005) in the biomarker validation phase. Addition of this model to the BIOSTAT risk prediction model increased the predictive probability for clinical outcomes of HF from an AUC value of 0.643 to an AUC of 0.823 (p = 0.0021). Moreover, using the prediction model of fourteen peptides and the composite model of the multiple biomarker of fourteen peptides with the BIOSTAT risk prediction model achieved a better predictive probability of time-to-event in prediction of clinical events in patients with HF (p = 0.0005).

Conclusions: The results obtained in this study suggest that a cluster of plasma peptides using MALDI-MS can reliably predict clinical outcomes in HF that may help enable precision medicine in HF.
Background

Biomarkers play a major role in the management of patients with heart failure (HF) with established roles in diagnosis, prognosis, risk stratification and guiding therapy. In addition, biomarkers have been shown to be useful in understanding the pathophysiology of HF, particularly in specific phenotypes. Therefore, finding novel biomarkers might further improve our understanding and management of HF [1].

Matrix assisted laser desorption ionisation mass spectrometry (MALDI-MS) has emerged into an important proteomic technology, which has been used for analysing plasma proteomic spectra [2–9]. MALDI-MS analysis offers a highly sensitive method for discovery of biomarkers directly from complex biological fluids such as plasma.

To the best of our knowledge, there has not been any study using MALDI-MS technology that enables the detection of novel biomarkers predicting clinical outcomes in patients with HF. The main aim of this study was to develop a plasma peptide model that would enable better prediction of clinical outcomes in patients with HF. In this turn, this may help increase our understanding of the pathophysiology of HF.

Methods

Patient population

Patients were selected from the BIOSTAT-CHF (A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure) project which was an investigator-driven multicentre clinical study [10]. The main aim of this project was to identify poor outcomes in HF patients with a standard treatment using a systems biology approach which includes demographics, biomarkers, genetics and proteomics [11, 12]. The BIOSTAT-CHF project was conducted according to the declaration of Helsinki which was approved by national and local ethics committees. All patients provided written informed consent. Participating subjects who met inclusion and exclusion criteria according to the European Society of Cardiology (ESC) guideline were collected [13]. In brief, 2516 patients were more than 18 years old, presented symptoms of HF and had left ventricular ejection fraction (LVEF) ≤ 40% and/or B-type natriuretic peptide (BNP) > 400 pg/mL or N-terminal pro B-type natriuretic peptide (NT-proBNP) > 2000 pg/mL who were recruited into the BIOSTAT-CHF project. At the beginning of the study, blood samples were collected for proteomic analysis. Blood was drawn by venepuncture that were obtained from supine patients after at least 15 min bed rest. Blood was collected in 10 mL EDTA vacutainer tubes, inverted 8 times and put on ice immediately. Plasma was obtained after centrifugation at 1000 \( \times \) g for 15 min at 4 °C, transferred to small aliquots and stored at −80 °C until further analysis. Then the patients received a standard therapy for HF which included up-titration with angiotensin converting enzyme inhibitors and beta blockers from 0 to 6 months to optimise the treatment. Clinical events such as death and HF hospitalisation were followed. The plasma sample groups were sex and age matched. In the biomarker discovery phase, one group consisted of 50 patients with HF (25 male and 25 female) who died or were rehospitalised

Table 1 Patient characteristics of biomarker discovery HF patient cohort

| Characteristics                      | HF hospitalisation or death (n = 50) | No event (n = 50) | p value |
|--------------------------------------|-------------------------------------|------------------|---------|
| Age (years)                          | 76.64 ± 8.14                        | 76.64 ± 8.14     | 1.000   |
| Male sex, n (%)                      | 25 (50)                             | 25 (50)          | 1.000   |
| BMI (kg/m²)                          | 30.01 ± 6.17                        | 28.94 ± 6.66     | 0.471   |
| NYHA class III/IV, n (%)             | 38 (76)                             | 27 (54)          | 0.21    |
| Systolic blood pressure (mmHg)       | 126.38 ± 20.63                      | 130.94 ± 21.12   | 0.247   |
| Diastolic blood pressure (mmHg)      | 66.92 ± 11.92                       | 69.22 ± 12.24    | 0.324   |
| Heart rate (bpm)                     | 75.69 ± 19.91                       | 73.94 ± 18.28    | 0.848   |
| HF hospitalisation/Death             | 32/18                               | 0/0              |         |
| Serum creatinine (µmol/L)            | 126.88 ± 58.56                      | 107.16 ± 34.27   | 0.076   |
| eGFR (mL/min·m²⁻¹)                   | 45.76 ± 14.23                       | 51.34 ± 11.19    | 0.037   |
| Primary aetiology                    |                                     |                  |         |
| Ischemic heart disease               | 40 (80)                             | 32 (64)          | 0.118   |
| Non ischemic heart disease           | 10 (20)                             | 18 (36)          | 0.118   |

Italic values indicate significance of p value (p < 0.05)  
BMI body mass index, eGFR estimated glomerular filtration rate, NYHA New York Heart Association
for HF, and they were compared with the group of 50 HF patients who did not have such an event (Table 1). A separate cohort of a hundred HF patient plasma samples from the BIOSTAT-CHF project [10] that was employed for verification in the biomarker validation phase in this study (Table 2).

Sample preparation
Peptide extraction
Reversed phase C18 (C18 extra wide pore solid phase extraction cartridges) was used to capture peptides in plasma samples. C18 EWP SPE cartridges were primed with 1 column volume (3 mL) of methanol and then washed with 2 column volumes (6 mL) of 18.2-MΩ-cm deionised water before washing with 2 column volumes of 0.1% formic acid (FA). 100 μL of each plasma sample were mixed with 1 mL of 1% trifluoroacetic acid (TFA) and left on ice for 20 min to allow precipitation. Then, the sample was centrifuged at 14,000 g for 10 min at 4 °C. 950 μL of the dissolved sample was applied on a C18 EWP SPE cartridge. Each cartridge was washed with 2 column volumes of 0.1% formic acid and then 2 column volumes of water. Peptides were eluted by adding 1.2 mL elution solution of 60% acetonitrile (ACN) + 0.1% formic acid (FA) in water and then 1.2 mL of 90% acetonitrile + 0.1% formic acid in water. Finally, the eluates were dried by using a Speed-Vac (Jouan, Thermo Scientific, USA) for 2 h and followed by freeze-drying overnight (Edwards, Modulyo, BPS, UK). The samples were stored at −80 °C until MALDI-MS analysis.

MALDI spot preparation
The dried samples were reconstituted in 0.1% trifluoroacetic acid (TFA). 10 μL of each sample were mixed with 990 μL of α-CHCA matrix solution (5 mg α-cyano-4-hydroxycinnamic acid in 1 mL of 50% acetonitrile + 50% water with 0.1% trifluoroacetic acid). Then, 1 μL of this mixture was spotted in triplicate directly onto a 96 well MALDI target plate (Waters Corporation, Manchester, UK). The target plates were dried at room temperature for 45 min and immediately transferred into the MALDI-MS for analysis.

Sample analysis
Samples were analysed using a Synapt G2 MALDI mass spectrometer (Waters Corporation, Manchester, UK) tuned to 10,000 mass resolution (full width at half height). The MALDI-MS instrument and mass spectra were automatically acquired in positive mode. Peptides were detected in a mass range of m/z from 700 to 10,000 using instrument settings optimised for plasma analysis with the following acquisition settings: plate speed: 15, laser firing rate: 200, laser energy: 300, mass threshold: 10. Ionisation was performed with a laser operating at a frequency of 1000 Hz. For each MALDI spot, spectra were recorded from vertical spot positions.

Data analysis
Raw data files were converted to txt files using MassLynx version 4.1 software (Waters Corporation, Manchester, UK) before they were imported into Progenesis MALDI version 1.4 software (Nonlinear Dynamic, UK). Spectra were pre-processed to remove noise and background across all spectra: a noise filter size of 5 was applied and background subtracted using a top hat filter size of 60. All the features in spectra were aligned using a search area of 5 before analysis. The data obtained was exported to Excel for further analysis.

Table 2 Patient characteristics of the biomarker validation HF patient cohort

| Characteristics | HF hospitalisation or death (n = 58) | No event (n = 42) | p value |
|-----------------|-------------------------------------|------------------|--------|
| Age (years)     | 69.52 ± 12.15                      | 68.86 ± 11.95    | 0.696  |
| Male sex, n (%) | 29 (50.0)                           | 20 (47.6)        | 0.814  |
| BMI (kg/m²)     | 27.76 ± 6.20                       | 29.27 ± 5.85     | 0.125  |
| NYHA class III/IV, n (%) | 36 (65.5) | 27 (64.3) | 0.905 |
| Systolic blood pressure (mmHg) | 125.10 ± 25.20 | 123.52 ± 17.07 | 0.936 |
| Diastolic blood pressure (mmHg) | 72.00 ± 14.41 | 75.50 ± 11.48 | 0.054 |
| Heart rate (bpm) | 82.53 ± 22.37 | 83.55 ± 24.52 | 0.975 |
| BNP (pg/mL)     | 467.45 ± 433.66                     | 288.49 ± 390.02  | 0.004  |
| Serum creatinine (µmol/L) | 123.72 ± 47.06 | 101.32 ± 46.09 | 0.004 |
| eGFR (mL/min−1) | 53.74 ± 20.03                       | 67.63 ± 27.72    | 0.013  |
| Primary aetiology |                                      |                  |        |
| Ischaemic heart disease | 27 (47.4) | 15 (36.6) | 0.287 |
| Non ischaemic heart disease | 30 (52.6) | 26 (63.4) | 0.287 |

Italic values indicate significance of p value (p < 0.05)

BMI body mass index, BNP brain natriuretic peptide, eGFR estimated glomerular filtration rate, NYHA New York Heart Association
Statistical analysis

All data for continuous variables are reported as mean ± SD. After testing for normal distribution, values were compared by unpaired Student’s t tests or Mann–Whitney U test, as appropriate. All statistical tests were performed 2-tailed, and a significance level of p value < 0.05 was considered to indicate statistical significance. To evaluate test performance of candidate biomarkers as predictors for outcomes in patients with HF, the area under the receiver operating characteristic curves (AUC) were plotted. The multiple biomarker model were built using a logistic regression with candidate peptides (m/z) which were entered simultaneously in order to improve the predictive probability of outcomes in patients with HF. The SPSS statistics software version 24.0 (Statistical Package for the Social Sciences, Chicago, USA) for Windows was employed for all statistical analyses in this study.

Results

Patient characteristics

Patient characteristics of the biomarker discovery HF cohort are described in Table 1. In the biomarker discovery HF patient cohort, the groups were matched in age (average age: 76.6 ± 8.1 years old) and gender between both HF groups. The age and gender distribution of both groups of patients with HF was not statistically different (p = 1.000). Therefore, age and gender bias was completely excluded.

Patient characteristics of the biomarker validation HF patient cohort are displayed in Table 2. Mean age was 69.5 ± 12.2 years in the patients who died or were rehospitalised and 68.9 ± 12.0 years in the patients who did not have an event (p = 0.696). In the patients with an event, eGFR (mL/min⁻¹) was lower (53.74 ± 20.03 vs. 67.63 ± 27.72, p = 0.013 and BNP levels (pg/mL) were higher (467.45 ± 433.66 vs. 288.49 ± 390.02, p = 0.004). All other patient characteristics were not significantly different between the two HF groups.

Identification of plasma peptide spectra in patients with heart failure

We analysed the plasma peptide profiles of a hundred patients with HF in the biomarker discovery cohort and a hundred patients with HF in the biomarker validation cohort. After normalisation and alignment of all the processed spectra, a total of 11,389 peptides (m/z) were detected using MALDI-MS combined with C₁₈ SPE. From the 11,389 peptides, expression of 53 peptides (m/z) were significantly different in both cohorts in HF.

| m/z   | Fold change | p value |
|-------|-------------|---------|
| 1724.22 | 0.97        | 0.034 |
| 2279.24 | 0.94        | 0.028 |
| 2290.24 | 0.95        | 0.043 |
| 2300.24 | 0.95        | 0.029 |
| 2410.29 | 0.95        | 0.028 |
| 2472.34 | 0.94        | 0.028 |
| 2646.44 | 1.06        | 0.019 |
| 2691.47 | 0.93        | 0.007 |
| 2729.47 | 0.94        | 0.037 |
| 2868.59 | 1.08        | 0.018 |
| 3113.71 | 1.07        | 0.042 |
| 5636.08 | 1.43        | 0.041 |
| 5660.99 | 1.31        | 0.049 |
| 5855.33 | 0.82        | 0.030 |
| 5953.32 | 1.58        | 0.009 |
| 6165.30 | 1.60        | 0.036 |
| 6279.13 | 2.26        | 0.023 |
| 6283.58 | 1.45        | 0.014 |
| 6314.83 | 1.49        | 0.031 |
| 6446.94 | 1.24        | 0.043 |
| 6460.55 | 2.00        | 0.027 |
| 6465.03 | 2.98        | 0.004 |
| 6515.90 | 0.38        | 0.001 |
| 6551.62 | 1.52        | 0.041 |
| 6576.58 | 1.99        | 0.004 |
| 6576.99 | 1.55        | 0.010 |
| 6601.97 | 1.63        | 0.045 |
| 6609.77 | 1.52        | 0.047 |
| 6722.04 | 1.61        | 0.009 |
| 6764.13 | 1.60        | 0.025 |
| 6918.14 | 1.63        | 0.028 |
| 7061.32 | 2.99        | 0.040 |
| 7100.13 | 3.22        | 0.027 |
| 7118.44 | 1.83        | 0.037 |
| 7121.74 | 1.69        | 0.048 |
| 7158.59 | 2.77        | 0.045 |
| 7185.63 | 2.10        | 0.028 |
| 7213.01 | 2.17        | 0.028 |
| 7358.59 | 3.76        | 0.011 |
| 7409.39 | 0.92        | 0.002 |
| 7463.58 | 1.74        | 0.013 |
| 7479.14 | 0.44        | 0.003 |
| 7492.90 | 0.58        | 0.027 |
| 7526.71 | 1.60        | 0.048 |
| 7572.41 | 1.84        | 0.036 |
patients with and without an event at a p value $< 0.05$ (Table 3).

**Selection of candidate peptide (m/z) biomarkers for prediction of clinical outcomes in the biomarker discovery phase**

To determine if peptides (m/z) could help to discriminate clinical outcomes between the HF patients with or without an event, receiver operating characteristic (ROC) curves were generated. Additional file 1: Table S1 shows the values of area under the receiver operating characteristic curves (AUC) for 53 peptides (m/z). The best AUC was peptide m/z 6515.90 with AUC of 0.688 at $p = 0.001$ (Asymptotic 95% confidence interval [CI], 0.583—0.793) that is presented in Fig. 1.

However, no individual peptide (m/z) was an excellent classifier for prediction of clinical outcomes in patients with HF. Therefore, the development of a multiple peptide biomarker approach would be useful to provide more pathophysiological information about patients with HF and able to predict clinical outcomes. We developed a multiple biomarker model with fourteen peptides (m/z 2646.44, 2729.47, 3113.71, 5636.08, 5855.33, 5953.32, 6314.83, 6465.03, 6515.90, 7061.32, 7358.59, 7492.90, 7582.00 and 7929.78) by using a logistic regression in which all these peptides were entered simultaneously (Additional file 1: Table S1 and Additional file 2: Figure S1). The AUC value in the multiple biomarker model of fourteen peptides showed an excellent improvement in the performance of predictive probability for clinical outcomes in patients with HF with an AUC of 1.000 (Asymptotic 95% CI, 1.000–1.000) at $p = 0.0005$. The prediction capability of this model achieved 100% sensitivity and 100% specificity (Fig. 1). There was a very good separation between the HF patients who responded to treatment and HF hospitalisation or death which is displayed in a scatter 3D plot of fourteen peptide model (Additional file 3: Figure S2).

**Validation of candidate peptide (m/z) biomarkers for prediction of clinical outcomes in the biomarker validation phase**

To confirm the result achieved in the biomarker discovery phase, the multiple biomarker model with a combination of fourteen peptides discovered from the biomarker discovery HF patient cohort was tested in the biomarker validation HF patient cohort with another hundred patients with HF. The AUC value of this multiple biomarker model with the fourteen peptides yielded an AUC of 0.817 at the $p$ value of 0.0005 (Asymptotic 95% CI, 0.734—0.900) that is shown in Fig. 2 and Table 4.

The added value of the multiple peptide biomarker model on top of the BIOSTAT risk prediction model

Recently, we developed a risk prediction model for patients with HF from the BIOSTAT-CHF cohort [14] which risk scores can be calculated using the online calculator available at: http://www.biostat-chf.eu (including age, HF hospitalisation last year, peripheral oedema,

### Table 3 (continued)

| m/z        | Fold change | p value |
|------------|-------------|---------|
| 7582.00    | 5.29        | 0.016   |
| 7600.74    | 2.25        | 0.018   |
| 7634.93    | 1.81        | 0.013   |
| 7649.22    | 3.10        | 0.033   |
| 7889.48    | 1.66        | 0.033   |
| 7914.92    | 2.78        | 0.005   |
| 7928.13    | 0.31        | 0.006   |
| 7929.78    | 3.25        | 0.028   |
systolic blood pressure, NT-proBNP, haemoglobin, high-density lipoprotein, serum sodium and beta-blocker use at baseline). Using the BIOSTAT risk prediction model generated an AUC value of 0.643 (Asymptotic 95% CI 0.530–0.757, p = 0.015) that risk scores were calculated using the online calculator available at: http://www.biostat-chf.eu (including age, HF hospitalisation last year, peripheral oedema, systolic blood pressure, NT-proBNP, haemoglobin, high-density lipoprotein, serum sodium and beta-blocker use at baseline). The green curve describes the multiple biomarker model with fourteen peptides with an AUC of 0.817 (Asymptotic 95% CI 0.734–0.900, p = 0.0005). The blue curve displays the prediction model of fourteen peptides on top of the BIOSTAT risk prediction model with an AUC of 0.823 (Asymptotic 95% CI 0.743–0.904, p = 0.0005) that is displayed in Fig. 2 and Table 4. The increase in the AUC value of the composite model of the BIOSTAT risk prediction model with the multiple peptide model as compared to the BIOSTAT risk prediction model had a statistically significant p value of 0.0021. In addition, using the prediction model of fourteen peptides and the composite model of the multiple biomarker of fourteen peptides with the BIOSTAT risk prediction model gave a better predictive probability of time-to-event in prediction of clinical events in patients with HF (p = 0.0005, Additional file 4: Figure S3).

**Discussion**

There is no single effective parameter to predict clinical outcomes in patients with HF. Therefore, several models have been applied to predict mortality and HF hospitalization in patients with HF. In a meta-analysis, the mean c-statistics of all of these models to predict mortality and/or HF admission was only 0.63 [15]. Recently, we developed a risk prediction model from the BIOSTAT-CHF cohorts, which yielded a c-statistics of 0.71 to predict death or HF admission [14]. Therefore, a method to enable clinicians to better predict clinical outcomes in HF would be important and useful for improving prediction and in stratifying patients with HF. Using the MALDI-MS technique for proteomic analysis is one of the most promising approaches for the discovery and identification of peptides and proteins in many diseases. Based on this technology, some biomarkers of several diseases have been discovered, particularly in cancer [2–9]. Thus we sought to see if we could devise a strategy to combine MALDI-MS and C18 SPE technique and employ statistical tools to establishing a model that could discriminate between HF patients who respond to treatment and HF hospitalisation or death.

In this study, a total of 11,389 peptides (m/z) were detected using MALDI-MS combined with C18 SPE in both biomarker discovery and validation HF patient cohort. Moreover, 53 peptides showed a significantly different expression between patients who died or had a

### Table 4 AUC values of the multiple biomarker model of fourteen peptides for prediction of clinical outcomes in the biomarker validation HF patient cohort in comparison with the BIOSTAT risk prediction model and the added value of the prediction model of fourteen peptides on top of the BIOSTAT risk prediction model

| m/z                          | AUC       | Standard error | p value | Asymptotic 95% confidence interval |
|------------------------------|-----------|----------------|---------|-----------------------------------|
|                              |           |                |         | Lower bound | Upper bound |
| BIOSTAT risk prediction model | 0.643     | 0.058          | 0.015   | 0.530             | 0.757       |
| Prediction model of 14 peptides | 0.817     | 0.042          | 0.0005  | 0.734             | 0.900       |
| Prediction model of 14 peptides tested on top of the BIOSTAT risk prediction model | 0.823 | 0.041 | 0.0005 | 0.743 | 0.904 |
HF admission and those who did not have such an event. These results demonstrated that MALDI-MS profiling could be used to discriminate between HF patients with and without clinical events. These peptides correspond to small proteins or fragments of proteins in plasma that might have important roles in the pathogenesis of the HF.

The change in expression of peptides reflects changes in plasma which could potentially be due to pathophysiological processes in HF. Thus, it is unlikely that there would be a single peptide which could be able to identify clinical outcomes in patients with HF. With a single biomarker, peptide m/z 6515.90 gave the best AUC value of 0.688 (p = 0.001) in discriminating the HF patients who respond to treatment from HF patients with death/rehospitalisation (Fig. 1). However, due to the heterogeneity of clinical populations (age, sex, ethnicity and comorbidity) an ideal single biomarker may not exist for each disease [16]. Some reports have demonstrated that a panel of multiple potential biomarkers in a specific model could improve precision and be more robust [17–19]. Therefore, we developed a multiple biomarker model with a cluster of peptides (m/z) that would provide better prediction of clinical outcomes for patients with HF. The performance of this multiple biomarker model was much better as compared to each single peptide biomarker for prediction of clinical outcomes in patients with HF (Fig. 1 and Table 4). The multiple biomarker model with fourteen peptides (m/z 2646.44, 2729.47, 3113.71, 5636.08, 5855.33, 5953.32, 6314.83, 6465.03, 6515.90, 7061.32, 7358.59, 7492.90, 7582.00 and 7929.78) gave an excellent area under the ROC curve of 1.000, p = 0.0005 (Fig. 1). This discrimination value was maintained with an AUC of 0.817 (p = 0.0005) in the biomarker validation HF patient cohort (Fig. 2 and Table 4). In addition, this multiple biomarker model added a statistically significant increase in the predictive probability for clinical outcomes in patients with HF (AUC = 0.823, p = 0.0005) when it was tested on top of the BIOSTAT risk prediction model or as compared to the BIOSTAT risk prediction model alone (AUC = 0.643), respectively (Fig. 2 and Table 4). The increase in the AUC value between the BIOSTAT risk prediction model and the composite model of the BIOSTAT risk prediction model with the multiple peptide model was statistically significant.

Whilst some of these peptides could be derived from just one protein, it is likely that these fourteen peptides belong to several proteins. Identification of the peptides could provide more information about the pathogenesis in patients with HF in order to guide therapy. The multiple biomarker model of fourteen peptides may be useful if it could be applied for clinical practice. The prediction of clinical outcomes in patients with HF would be significantly improved using this multiple peptide biomarker model. Furthermore, the findings in this study demonstrated that there is a lot of predictive information in the proteomics which are not represented by the clinical factors and well-known biomarkers in the BIOSTAT risk prediction model. Therefore, proteomics mechanisms may improve our insight into the pathophysiological processes in HF that opens new perspectives for translational research in HF.
This is the first study using MALDI-MS profiling in order to predict clinical outcomes of patients with HF. The results obtained in this study demonstrated that MALDI-MS combined with C18 SPE technique is a good approach for discovery of potential biomarkers in plasma of patients with HF (Fig. 3: Central Illustration). This method also has the potential to provide insight into the pathophysiological processes in HF. MALDI is already established in some microbiology sections of clinical laboratories and consequently the expertise is already present to incorporate this kind of testing in the future [20, 21]. In addition, identification of clinical outcomes in HF that allow measurement of the disease on a peptide level. Therefore, this may result in their use in prognostication and selection of appropriate treatment in order to tailor therapeutics in HF [22].

A limitation of this study is that the mass spectrometer in our laboratory for MALDI technique only provides the m/z peptide peaks and their intensities to generate a profile for prediction of clinical outcomes in patients with HF, rather than identifying the underlying peptides or proteins. Another limitation of this study is that BIOSTAT-CHF project was exclusively Caucasian due to the study design. Therefore, the results of this study may only apply to patients of Caucasian ethnicity.

Conclusions
In conclusion, to the best of our knowledge, this is the first study which discovered potential peptide biomarkers and a multiple peptide biomarker model for predicting clinical outcomes in patients with HF by using MALDI-MS combined with C18 SPE. The multiple peptide model in this study provided significant additional predictive information to the existing BIOSTAT risk prediction model. Further identification of these peptides may have important therapeutic implications for patients with HF in order to improve poor outcomes.

Additional files

Additional file 1: Table S1. AUC values of 53 peptides (m/z) and the multiple biomarker model of fourteen peptides for prediction of clinical outcomes in the biomarker discovery HF patient cohort.

Additional file 2: Figure S1. Representative mass spectra of fourteen peptides (m/z) in the multiple biomarker model for prediction of clinical outcomes in patients with HF. There are m/z 2646.44, 2729.47, 3113.71, 5636.08, 5855.33, 5953.32, 6514.83, 6465.03, 6515.90, 7061.32, 7358.59, 7492.90, 7582.00 and 7929.78.

Additional file 3: Figure S2. Scatter 3D plot of fourteen peptides for predicting clinical outcomes in the biomarker discovery HF patient cohort. Each data sphere in the 3D plot corresponds to a patient with X-axis for treatment response, peptide (m/z) peak for the Y-axis, and Z-axis for the patient samples. This plot shows a very good separation between the HF patients who responded to treatment (green sphere) and HF hospitalisation or death (blue sphere).

Additional file 4: Figure S3. Predictive probability of time-to-event in patients with HF using the BIOSTAT prediction model, the prediction model of fourteen peptides and the combination model of prediction model of fourteen peptides and the BIOSTAT risk prediction model.

Abbreviations
AUC: area under the receiver operating characteristic curve; CI: confidence interval; EWP: extra wide pore; HF: heart failure; MALDI: matrix assisted laser desorption ionisation; MS: mass spectrometry; m/z: mass-to-charge ratio; ROC: receiver operating characteristic; SPE: solid phase extraction.

Authors’ contributions
THC designed the study, did experiments, analysed the data, and wrote the manuscript. DJLJ designed experiments and gave support for data analysis. PAQ and JKS did experiments and gave technical support. DCSC and NH gave support in data analysis. HMP and MM recruited patients and collected plasma samples. CCL, SDA, JGCC, KD, GF, HLH, MM, PP, NJS, DJJV, FZ and AHZ recruited patients, collected plasma samples and revised the manuscript. AAN developed the concept, designed experiments, supervised the project, analysed the data and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests
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