Appendix 2 Description of methods used to identify biomarkers of heart failure in plasma and urine.   
To search for biomarkers of heart failure and other cardiovascular disease, plasma and urine aminoacid and steroid profile was analysed in patients with previous diagnosis of CHF and in representative study sample. Our aim was not to look for single substances as biomarkers, but rather to consider a whole set of several compounds to be evaluated simultaneously.

Selective assaying of corticosteroids and aminoacids was performed using liquid chromatography combined with a quadrupole mass analyser which allowed analyte identification with an adequate sensitivity. As the next step, the analytic findings were subjected to a statistical and chemometric analysis to identify differences in the levels of the evaluated analytes between healthy subjects and patients with cardiovascular disease. Aminoacids and steroid hormones were assayed by liquid chromatography-mass spectrometry (LC-MS, Agilent Technologies, Santa Clara, CA, USA). The mass detector was equipped with an electrospray ionization (ESI) source and a quadrupole analyser. Chromatographic data were collected and processed using the Chemstation software, Rev. B.04.02 SP1.

The optimized LC-MS method for both groups of compounds has been validated for specificity, linearity, detection thresholds and limits of quantification in accordance to the requirements for bioanalytical methods.