Fundació La Marató de TV3
21st SYMPOSIUM
Heart diseases

EARLY DIAGNOSIS OF RENAL INSUFFICIENCY IN ACUTE HEART FAILURE (EDRIAHF)

Teresa Padró Capmany
Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau

1. Summary of the project

Background

Acute decompensated heart failure (ADHF) is one of the main causes of hospitalisation in industrialised countries and is the main determinant of healthcare expenditure. Several factors including cardiovascular and non-cardiovascular conditions as well as patient related and iatrogenic[1] factors may facilitate the rapid development or deterioration of signs and symptoms of heart failure, thus leading to ADHF. Patients with ADHF often present with renal insufficiency when admitted to hospital or develop acute kidney injury (AKI) due to acutely worsening HF, its treatment, or both during hospitalisation. The co-occurrence of AKI in patients with ADHF, also known as cardiorenal syndrome, complicates patient management and is consistently associated with longer hospital and intensive care unit stays, increased rates of rehospitalisation, worse prognosis (increased mortality) and higher associated costs. Thus, the evaluation of renal function in patients with heart failure before AKI develops is important in order to provide a better prognosis in these patients.

In daily cardiologic practice, renal injury is conventionally defined by a reduced glomerular filtration rate calculated from blood creatinine (gold marker) and creatinine-based equations such as MDRD-4 that also consider variables such as age, gender, and race that influence creatinine levels in blood. It is important to notice that increased creatinine levels imply that the kidney is already failing, therefore it is too late. Thus, to have biomarkers[2] that serve as diagnostic tool for early (before creatinine increase) and accurate detection of renal damage represents a medical benefit in the risk-stratification and treatment of patients hospitalised for acute decompensated heart failure. Different molecules (cystatin-C, neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule 1) have been proposed as possible biomarkers. However, their usefulness to detect deterioration of renal function earlier than creatinine in hospitalised ADHF patients has not been consistently proven, nor has their value to gain prognostic accuracy in these patients.

---

[1] Induced in a patient as the result of a physician's words or actions.

[2] A substance, physiological characteristic, gene, etc that indicates, or may indicate, the presence of disease, a physiological abnormality or a psychological condition.

Therefore, identification of new biomarkers that individually or in combination improve detection of renal dysfunction and prognostic stratification in ADHF remains a medical challenge.

Objectives

To meet these needs we designed EDRIAHF, a project using a proteomic[3] approach to identify and validate new biomarkers for early detection of renal function deterioration in hospitalised ADHF patients before creatinine and urea levels rise. Moreover, these molecular targets might provide pathophysiological information on acute cardiorenal syndrome, enabling development of new treatment options in ADHF.

Study design and methodology

This project was based on a longitudinal study consisting of patients (68% men, 69±12 years old) hospitalised due to ADHF in the cardiology department of the Hospital de la Sant Creu i Sant Pau (Barcelona). ADHF patients (N=66) were distributed in three different groups based on presence or absence of renal dysfunction at admission (ADHF-RD+ / ADHF-RD-) and the development of kidney injury (ADHF-DKI) after day 5 hospitalisation. Kidney injury was defined using the creatinine-dependent MDRD-4 formula, with levels below 60 mL/min/1,73 m^2 considered as pathological. A group of healthy subjects (HS, N=15, 49±9 years) served to establish the normal range for the identified protein biomarkers.

Urine and blood samples from patients were collected at hospital admission and different time points during the hospitalisation stay (24, 48, and 72 hours, day 5), and at patient's discharge. Demographic data, clinical history and background treatments were collected for all the patients included in the study.

Discovery Phase studies were based on identification of differential protein profiles in urine of ADHF patients by two-dimensional polyacrylamide gel electrophoresis and mass spectrometry (MS/MS) analysis and liquid chromatography and MS/MS. In addition, Validation of specific selected proteins was performed using quantitative immunoassays (ELISA).

[3] The branch of biochemistry concerned with the structure and analysis of the proteins occurring in living organisms.

2. Results

Upon admission, all ADHF patients had very high plasma levels of the heart failure biomarker NT-proBNP. In addition, 45% of the ADHF patients presented a reduced left ventricular ejection fraction (43.6±16%). Regarding disease comorbidities, 75% of the patients were under treatment due to hypertension, 44% had type 2 diabetes mellitus, and 70% dyslipidaemia.

At hospital admission 47% of the ADHF patients already had pathological levels of MDRD-4, signalling a deficient renal function (ADHF-RD+ group, MDRD-4: 41.1±11). In contrast, MDRD-4 median value was within the healthy range (82.4±19) in the ADHF-RD- group. In this latter ADHF group, **42% of the patients developed acute kidney injury during hospitalisation** (**ADHF-DKI**), **whereas the other 58% maintained a normal renal function during hospital stay (ADHF-RD-).** The median length of hospital stay was 9-10 days for all patients, being longer in the subgroup of patients developing kidney injury compared with those who maintained normal renal function.

Among all the proteins detected in urine, albumin entering by filtration and alpha-1-microglobulin (AMBP), derived from the glomerular and tubular cells were the most abundant, independently of kidney condition of the ADHF patients.

Analysis of the differential urine protein pattern in ADHF at admission showed 33 proteins with consistently detected changes in their detection levels compared to healthy subjects. These proteins, with molecular weights in the range from 15 to 200KDa belong to different function groups, such as cell metabolism, cell signalling, coagulation or vitamin metabolism. Eighteen of these proteins are associated to heart failure condition, independently of the kidney function (proteins with altered urine levels in ADHF patients compared to healthy subjects), whilst ten of the proteins differentially detected in urine are specifically associated to renal insufficiency (differences in protein levels between ADHF-RD+ and ADHF-RD- groups).

Only two of the proteins showing changes in the urine levels of ADHF patients are directly produced in the cells of the kidney (glomeruli), whereas the other proteins are expressed in different organs and are filtered through the kidney into the urine. Within

this latter group, seven proteins are components of the coagulation cascade, their urinary loss in ADHF patients being up to 2.5 (coagulation inhibitor protein) above the level in healthy subjects, suggesting alteration in the coagulation cascade in ADHF patients.

In addition, we detected increased urinary loss of three proteins involved in vitamin metabolism in urine of ADHF patients. Thus, anomalously high urinary loss of one of these proteins (codified as protein-X) due to impaired proximal tubular reabsorption has been reported in the setting of renal damage in preclinical studies and metabolic-disease related nephropathies in humans. In our study, urinary protein-X was more than 3 times higher in ADHF patients at hospitalisation than in healthy subjects, with ADHF-RD+ patients showing the highest levels. When analysed by western blot, protein-X in urine appears as a single band of 50KDa.

Quantitative analysis of protein-X levels by immunoassay (ELISA) depicted a similar pattern as found by 2D-electrophoresis in the urine samples, which validates the findings obtained in the Discovery Phase when ADHF patients with and without kidney dysfunction were analysed in relation to healthy subjects.

In our study, we also investigated changes in urine protein-X at three days of hospitalisation. At this time, protein-X levels dropped back to normal range in the ADHF-RD- patients, which maintained normal renal function during hospitalisation. In contrast, urine protein-X remained anomalously high, with values twice the normal range, in those ADHF patients that had normal renal function at admission, but developed kidney injury after day 5 of hospitalisation (ADHF-DKI). Similarly, protein-X remained high at day 3 in the ADHF patients with kidney dysfunction since admission (ADHF-RD+).

It is noteworthy that this pattern at day 3 was maintained at days 5-8 hospitalisation, when renal function decline to pathological levels in the group ADHF-DKI, which strongly suggests the value of protein-X as an early marker of kidney injury in ADHF patients during hospitalisation.

For comparative reasons, we also studied urine values of proteins proposed in the literature as possible biomarkers of kidney injury, such as cystatin-C, and NGAL. Our

results showed that when taken as a whole group ADHF has similar urinary cystatin-C and NGAL loss to the healthy group. However, ADHF-RD+ patients present higher urinary levels of both proteins than those ADHF-RD- at hospital admission. At three days of hospitalisation, ADHF-RD- and ADHF-DKI patients did not differ in cystatin-C or NGAL levels. In addition, there were no visible changes in cystatin-C or NGAL levels at day 5 of hospitalisation. Thus, our results support findings of previous studies about the value of cystatin-C and NGAL as biomarkers of established renal dysfunction. However, according to our findings, neither urine cystatin-C nor NGAL levels at day 3 may serve to identify those hospitalised ADHF patients at risk of developing kidney injury within the following days.

Cystatin-C and protein-X values significantly correlated in ADHF-RD+ patients at admission ($r= 0.453$) as they did in the ADHF-DKI group ($r=0.523$). In contrast, there was no statistical relationship between both variables when this correlation was analysed within the ADHF-RD- group ($p=0.5986$). Combination of protein-X and cystatin-C values at day 3 discriminated ADHF-DKI patients by C-statistics analysis ($p<0.05$).

ADHF patients have been followed up over one year after hospital discharge to determine the potential association between the identified biomarkers and the incidence of clinical events. Forty-two percent of the ADHF patients had cardiac and/or renal major adverse clinical events during the follow-up period. Fifty-six percent were patients of the ADHF-RD+ group, with renal dysfunction already at inclusion in the study. Our findings suggest that ADHF-RD+ patients with higher protein-X urine levels at admission and during hospital stay tend to require further hospitalisations throughout the following year. Up to now no consistent association has been found between protein-X levels and incidence of major adverse event in patients ADHF patients with normal renal function at study inclusion. However, we cannot rule out these results being partially affected by the sample size.

Although this study is based on a small group of patients the results suggest urinary protein-X as a potential early biomarker of renal dysfunction in hospitalised ADHF-patients and supports the combined value of protein-X and cystatin-C to gain better discrimination of ADHF patients with incident kidney injury during hospitalisation.

3. Clinical and social impact

As said above, cardiorenal syndrome is a very common pathological process that complicates the treatment and worsens patient outcome in acute decompensated heart failure. Creatinine is nowadays the gold standard used for identification of kidney dysfunction. However, creatinine changes are only evident when renal damage is already present.

For this reason, we designed EDRIAHF, a multidisciplinary project aimed to identify and prove the value of proteins that might serve as biomarkers of kidney insufficiency in patients hospitalised for ADHF before creatinine and urea levels rise. In this respect, the project's findings evidence that a high level of protein-X at 72h hospitalisation associates with a loss of renal function within the next days in ADHF patients. Although this finding still needs to be proved in larger groups of ADHF patients, it might provide a new tool in early diagnosis of renal dysfunction in patients hospitalised due to acute decompensated heart failure. This would facilitate clinicians' decision-making and allow a better prognostic stratification of patients hospitalised for ADHF. Moreover, identification of a new biomarker for early detection of renal injury would directly benefit patients. Indeed, worsening of renal function in ADHF patients implies longer hospital stay, worse prognosis and more frequent hospital readmissions due to complications from heart or kidney failure. Such a new biomarker would help to detect kidney failure before damage is already produced. Therefore, ADHF patients would have better prognosis, shorter hospital stays and fewer hospital readmissions.

4. Dissemination and congresses

Until now, the results of the study have been presented in international and national scientific forums including the Congress of the European Society of Cardiology (ESC), Sociedad Española de Cardiología, and Eurothrombosis (organised by the Working Group on Thrombosis of the ESC) among others (see list below). Two manuscripts are currently in preparation and a third manuscript with the results of a still ongoing part of the study is foreseen.

List of presentations in Congresses

European Society of Cardiology Congress (Paris, August 2018). Rapid fire presentation: *Differential urine proteomic signature in early phase of renal insufficiency in patients with acute heart failure.* Diaz-Riera, E; López, L; García-Arguinzonis, M; Badimon, L; García-Moll, X; Padró, T.

Eurothrombosis (Barcelona, October 2018). Poster: *Antithrombin III is found in high levels in urine of patients with acute heart failure and kidney dysfunction.* Diaz-Riera, E; García-Arguinzonis, M; López, L; García-Moll, X; Badimon, L; Padró, T.

Congreso de la Sociedad Española de Cardiología (Seville, October 2018). Poster: *Disfunción renal precoz en pacientes con insuficiencia cardíaca aguda: Análisis proteómico en orina*. Diaz-Riera, E; García-Arguinzonis, M; López, L; García-Moll, X; Badimon, L; Padró, T.

Clinical Proteomics (Barcelona, November 2018). Poster: *Differential urine proteomics signature for early diagnostic of renal insufficiency in patients with acute heart failure*. Diaz-Riera, E; García-Arguinzonis, M; López, L; García-Moll, X; Badimon, L; Padró, T.

EMLTD Congress on Thrombosis (Athens, June 2019). Poster: *Antithrombin III turnover is exacerbated in patients with heart failure and renal dysfunction*. Diaz-Riera, E; García-Arguinzonis, M; López, L; García-Moll, X; Badimon, L; Padró, T.

Congreso de la Sociedad Española de Cardiología (Barcelona, October 2019). Pòster: *Biomarcadores tempranos de disfunción renal en pacientes con insuficiencia cardíaca descompensada: Estudio proteómico*. Diaz-Riera, E; García-Arguinzonis, M; López, L; García-Moll, X; Badimon, L; Padró, T.