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Novel urinary tubular injury markers reveal an evidence of underlying kidney injury in children with reduced left ventricular systolic function: a pilot study

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Abstract

Background—Evolving data suggest tubular injury markers (TIM) to be diagnostic and prognostic biomarkers of kidney injury in adults with chronic cardiac dysfunction. Such data are not well delineated in asymptomatic children with cardiomyopathy. This study sought to evaluate kidney involvement in children with left ventricular (LV) systolic dysfunction.

Methods—We conducted a cross-sectional case-control study in 61 asymptomatic children (aged 1.7–21.9 years) with dilated cardiomyopathy (DCM) and LV ejection fraction (LVEF) < 55 %. Routine conventional kidney function markers and the following urinary TIM were measured: KIM-1, IL-18, neutrophil gelatinase-associated lipocalin (NGAL), and L-FABP. Characteristics and TIM data of cases were compared with those of 61 age- and gender-matched healthy controls.

Results—Children with DCM had higher TIM concentrations compared with controls for IL-18 (28.2 pg/mg, IQR [15.9–42.5] vs 19.0 [12.6–28.6], $p < 0.001$), NGAL (13.2 ng/mg [6.5–44.3] vs 8.3 [3.1–17.5], $p = 0.01$), and KIM-1 (386 pg/mg [248–597] vs 307 [182–432], $p = 0.02$). All conventional kidney function markers were within normal limits in the DCM cohort. A combined model using cut-off values of KIM-1 ≥ 235 , IL-18 ≥ 17.5 , and (BNP) > 15 pg/ml resulted in distinction between patients with mildly depressed LV (55 > LVEF ≥ 45) and those with LVEF < 45 %. The sensitivity of this model was 80 % when any of the cut-off values was met and specificity 83 % when all cut-off values were met.

Conclusions—Our data suggest that asymptomatic children with LVEF < 55 % might have subclinical kidney injury that cannot be detected with conventional kidney function markers. TIM in conjunction with other cardiac function markers may be utilized to distinguish asymptomatic

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children with DCM and moderate or worse LV dysfunction (LFEV < 45 %) from those with mild LV dysfunction (55 > LVEF 45 %).

Keywords

Cardiomyopathy; Cardiorenal syndrome; Chronic kidney disease; Tubular injury markers; Children

Introduction

Cardiorenal syndrome (CRS) describes a variety of acute and chronic conditions in which the primary failing organ can be either the heart or the kidney [1, 2]. Type I CRS describes acute kidney injury (AKI) as a consequence of acute decompensating cardiac function (ADHF), whereas type 2 CRS describes chronic kidney disease (CKD) as a consequence of chronic heart failure. Although several epidemiological studies have evaluated the prevalence and outcome of type 2 CRS in adults [3, 4], drawing any conclusions based on the results is difficult because of the overlap between patients with ADHF (i.e., type 1 CRS) and chronic congestive heart failure (CHF) type 2. Moreover, adults with CHF are likely to have other comorbidities that might contribute to the changes in kidney function, which makes finding the optimal population to study type 2 CRS challenging. Unfortunately, epidemiological data of type 2 CRS have been limited to one registry-based study [5] in children with dilated cardiomyopathy (DCM).

Serum creatinine (SCr) has been the most frequently implemented clinical marker of kidney function in CRS; however, precise evaluation of kidney function is limited by the delayed elevation of creatinine after kidney injury and the significant functional loss before this elevation occurs. In response to these limitations, intensive basic and translational research has targeted the discovery of novel urinary tubular injury markers (TIMs) to uncover kidney dysfunction before SCr elevation in the AKI setting. Data support the use of TIMs as feasible diagnostic and predictive indices for kidney involvement in patients with concurrent co-morbidities such as CHF [6–8]. To date, the role of these TIMs in children with cardiomyopathy has not been explored.

In this report, we conducted a pilot case–control study to evaluate the difference in TIM concentrations between healthy children and those with DCM, assess the correlation between left ventricular (LV) systolic function and TIM, and evaluate the diagnostic utility of TIMs in distinguishing impaired LV systolic function.

Materials and methods

Study design and population

We conducted an observational cross-sectional case–control study of children followed at the Cardiomyopathy and Heart Failure clinic at Cincinnati Children’s Hospital Medical Center (CCHMC) between November 2011 and December 2013. Patients with a known history of DCM were screened to match the following inclusion criteria: age between 6 months and 21 years and evidence of LV persistent systolic dysfunction, defined as LV ejection fraction (LVEF) less than 55 % by an echocardiogram performed within 7 days of

the enrollment date. Exclusion criteria included patients with: clinical evidence of ADHF, mixed DCM with obstructive or restrictive features, arrhythmogenic right ventricular dysplasia, aortic stenosis, uncorrected complex congenital heart disease, a history of CKD, and known primary renal or urological disorders.

Measurements

Following provision of informed consent by the patient's caregiver, the predefined clinical variables were extracted from the medical records. A urine sample was collected on the same day as the clinic visit. Each urine sample was divided into two separate aliquots. The first sample was processed within 4 h of collection by the central laboratory of the CCHMC to obtain the following measures: urinalysis, urinary protein (uPr), urinary microalbumin (uMAIb), and urinary creatinine (uCr) as per the standard measures and methods of the central laboratory of the CCHMC. The second sample was kept in a 4 °C refrigerator for a maximum of 24 h until processing. During processing, specimens were centrifuged at 3,000 rpm at 4 °C for 15 min. The supernatant was divided into up to nine 1-mL cryovials, depending on the collected urine volume, and stored at -80 °C. The stored urine samples were analyzed in the Division of Nephrology Research Laboratory to measure the concentration of the following biomarkers: kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), interleukin 18 (IL-18), and liver-fatty acid binding protein (L-FABP). The biomarker concentrations were indexed to uCr to adjust for a sample dilution.

Urinary KIM-1 ELISA was constructed using commercially available reagents (Duoset DY1750; R & D Systems, Minneapolis, MN, USA). Urinary NGAL was measured using a commercially available assay (NGAL ELISA Kit 036; Bioporto, Grusbakken, Denmark). Urinary IL-18 and L-FABP were measured using commercially available ELISA kits (Medical & Biological Laboratories, Nagoya, Japan and CMIC, Tokyo, Japan respectively) as per the manufacturers' instructions.

Data regarding serum concentrations of creatinine (SCr), cystatin-C, and B-type natriuretic peptide (BNP) were extracted from the medical records when available within 30 days before or after the day of the clinic visit. SCr and cystatin-C were used to calculate the estimated glomerular filtration rate (eGFR) using modified Schwartz [9] and Larsson [10] formulas respectively.

Echocardiographic measurements were obtained using standard imaging techniques on a Vivid 7 GE ultrasound imaging system (Milwaukee, WI, USA) or a Philips iE33 × MATRIX ultrasound system (Andover, MA, USA). Images were obtained in standard views according to the American Society of Echocardiography's guidelines [11, 12]. Inter- and intra-observer variability for the measurements in the CCHMC's echocardiographic laboratory were less than or equal to 5 %. All the echocardiograms and LFEV measurements were reviewed and validated by JLJ, who was blinded to individual patient kidney function or TIM results to decrease measurement-related errors.

Study data were collected and managed using REDCap [13]. REDCap is a secure web-based application designed to support data capture for research studies.

Controls

Eligible and consenting patients were matched to healthy children from the Cincinnati Genomic Control Cohort (CGCC). This cohort consists of more than 350 healthy children between 3 and 18 years of age. Recruits were obtained through a plan developed to ensure appropriate racial and ethnic community-based representation of the seven counties of northern Kentucky and Ohio that comprise greater Cincinnati [14]. Potential subjects recruited from the community were scheduled for an office visit where candidates filled in a medical questionnaire, received a physical examination, and where samples of blood, urine, and hair were collected. Children with the presence of known genetic diseases or chronic medical conditions were excluded from the cohort. More specifically for the subset used for our study, children with a history of urological or kidney disease or injury were excluded from the cohort. Urine samples were stored and processed as explained above. Patients and controls were matched 1:1 based on age and gender, as the concentrations of TIMs correlate with these two variables [14]. Patients younger than 3 years or older than 18 years of age were matched to the youngest or oldest controls possible respectively.

Analytical and statistical methods

Comparative statistics were performed between cases and controls. Categorical data were analyzed using Chi-squared tests. Skewed distributions were described with medians and interquartile ranges (IQR) and compared using the Wilcoxon rank-sum test. Normal distributions were described with means and standard deviations (SD) and analyzed using two-tailed Student's *t* tests. Univariate linear regression models were created to evaluate the correlation between the LVEF as a continuous dependent variable and the different conventional kidney dysfunction markers and TIM as predictors. Normality of data distribution was tested using the Kolmogorov–Smirnov test. Skew-distributed variables were normalized using the logarithmic distribution in the regression models. Receiver operating characteristic (ROC) analysis was performed to quantify individual and combined diagnostic utility of TIM to discriminate between cases (LVEF < 55 %) and controls. Controls were presumed to have an LVEF = 55 %. A similar analytical approach was employed to evaluate the diagnostic utility of TIM and BNP to distinguish cases with moderate or greater systolic LV dysfunction (LVEF < 45 %) from those with mild dysfunction (55 > LVEF ≥ 45 %). TIM and BNP cut-off values resulting in 80 % sensitivity were derived. Alpha was set at 0.05 for significance. The analyses and graphics were conducted using SAS version 9.3 (SAS Institute, Cary, NC, USA).

Results

Characteristics of cases and controls

Between November 2011 and December 2013, a total of 821 clinic visits were screened, from which 119 patients met the inclusion criteria. Participation consent and urine samples were obtained from 61 of these patients, who were included in the analysis. For patients with multiple visits, samples were collected on the first visit only.

The majority of cases involved primary (combined familial and idiopathic) DCM (46 %). In general, patients had mildly to moderately depressed LV systolic function with mean LVEF

42.3 (12.1) %. Twenty-nine patients (47.5 %) had LFEV between 54 and 50 %, 5 (8.2 %) had LVEF between 49 and 45 %, 8 (13.1 %) had LVEF between 44 and 40 %, and 19 (31.2 %) had LFEV <40 %.

The characteristics of the cases and controls are depicted in Tables 1 and 2. No significant differences in age, gender, ethnicity, weight, height, body mass index (BMI) or heart rate were observed between the cases and controls. However, controls were more likely to have elevated systolic and diastolic blood pressure at the time of evaluation. In the case cohort, conventional kidney function markers, including blood urea nitrogen (BUN), cystatin-C-based eGFR, creatinine-based eGFR, urinary protein/Cr, and microalbumin/Cr ratios were within normal limits. Cases had higher urinary concentrations of KIM-1, NGAL, and IL-18 than controls (Table 3).

The predictive characteristics of urinary tubular injury markers to distinguish cases with LVEF <55 % from healthy controls

Table 4 depicts the diagnostic characteristics and cut-off values consistent with a sensitivity of 80 % of each novel TIM to predict an LVEF of <55 %. In this model, all healthy controls were assumed to have an LVEF of 55 %. The predictive value of IL-18 was the best among the four TIMs, with an area under the curve (AUC) of the receiver operator curve (ROC) equal to 0.74, followed by NGAL, with an AUC of 0.64. Except for the statistically significant positive likelihood ratio (+) LR of 1.4 (95 % CI: 1.07–1.82) and negative likelihood ratio (–) LR of 0.46 (95 % CI: 0.27–0.81) for IL-18, none of the TIMs showed good diagnostic utility. Combining IL-18 with one or more of the other TIMs did not improve the AUC of IL-18 in predicting LVEF <55 %. Stepwise incorporation of NGAL, KIM-1, and L-FABP with IL-18 demonstrated incremental improvements in the specificity for the prediction of EF <55 % (from 0.43 to 0.71 [95 % CI: 0.59–0.80]).

Conventional kidney function markers do not correlate with LVEF

Table 5 depicts the results of univariate linear regression models between LVEF and all kidney function and injury markers in our cohort. None of the conventional kidney function markers correlated with LVEF. Among TIMs, only KIM-1 correlated significantly and inversely with LVEF ($r = -0.30$, $p = 0.01$; Fig. 1). Furthermore, no significant correlation was found between KIM-1 and BNP.

The predictive characteristics of urinary tubular injury markers to distinguish cases with remarkable LV depression (LVEF <45 %) from those with mild depression (55 > LVEF 45 %)

Table 6 shows the diagnostic characteristics and cut-off values consistent with sensitivity of 80 % for each novel TIM and BNP to predict LVEF <45 % in our case cohort. The AUC of BNP was 0.70 with a specificity of 0.48 (95 % CI: 0.28–0.69) at the specified cut-off value. Among TIMs, KIM-1 and IL-18 had an AUC-ROC of 0.61 and 0.59 respectively, whereas the AUCs of NGAL and L-FABP did not exceed 0.50. When used as solo tests, the specificity of BNP, KIM-1, and IL-18 was 0.48, 0.29, and 0.22 respectively. However, combining these three markers in a single predictive model improved the specificity to 0.83 (95 % CI: 0.71–0.92) with a negative predictive value of 0.79 (95 % CI: 0.67–0.88).

Discussion

The coexistence of compromised kidney function and heart disease in children and adults is associated with a poor prognosis and prolonged hospitalization [17–19]. The deleterious impact of kidney dysfunction may exceed the impact of the primary cardiac lesion on morbidity. Blinder et al. reported that the mortality risk of postoperative stage II AKI exceeds the risk of death from single ventricle physiology [19]. Most current knowledge regarding the epidemiology and outcome of kidney dysfunction in the presence of existing cardiac comorbidity in children is extracted from the literature evaluating post-operative AKI in neonates and infants undergoing surgical intervention for congenital heart disease (i.e., CRS type 1) [19, 20]. To date, the association between kidney dysfunction and asymptomatic LV systolic dysfunction in children with cardiomyopathies has not been evaluated systemically.

In this pilot study, we demonstrated that children with a history of DCM and persistent evidence of at least mildly depressed LFEV have higher urinary concentrations of NGAL, IL-18, and KIM than age- and gender-matched, randomly selected, healthy controls. Moreover, conventional markers suggest that children with DCM might have normal kidney function and no evidence of kidney damage. These two findings suggest underlying kidney injury in these patients that cannot be detected by the currently available kidney dysfunction/injury markers, a fact proposing the potential diagnostic value of the novel markers in addition to the traditional kidney function markers. Not merely revealing evidence of injury, elevated urinary TIM concentrations can represent prognostic markers for incident heart failure risk, as seen in adults [21]. Moreover, IL-18 showed reasonable discrimination for LVEF of <55 % (AUC of 0.74). To our knowledge, this is the first report of a urinary biomarker being predictive of reduced LV systolic function in children with DCM. Moreover, this represents an opportunity to recognize evidence of kidney injury in the absence of change in conventional markers of kidney function and may offer an opportunity to detect future decline at an earlier stage in the clinical course. Despite the differences noted in TIM urinary concentrations between cases and controls, it should be noted that our data suggest an overlap between the two groups, with a limitation of TIMs to precisely distinguish cases from controls.

Our findings are important for a number of reasons. First, higher urinary KIM-1 concentrations are independently associated with incident heart failure after adjusting for age and other morbidities, such as diabetes, hypertension, coronary heart disease, and albuminuria, even in the healthy elderly population without heart failure [21]. In the same report, elevated IL-18 concentration showed the same association; however, this association was not significant after adjusting for albuminuria. In our cohort, microalbuminuria did not correlate with LVEF. Second, some studies have reported that high urinary KIM-1 and/or IL-18 concentrations are associated with the development of incident CKD and/or rapid progression of kidney disease independent of eGFR and proteinuria in adults and in human immunodeficiency virus-infected women [22, 23].

Similarly, no significant correlation was found between LVEF and any of the conventional kidney function markers, indicating no evidence of overt kidney dysfunction in this cohort

with the use of typical surveillance techniques. However, the fact that KIM-1 was elevated correlated inversely with LVEF suggests tubular damage that worsens with decreased LVEF. Although originally identified as an AKI marker, there is some evidence that KIM-1 may predict chronic tubular damage, with subsequent progression of CKD. KIM-1 tubular expression was significantly more pronounced in biopsies obtained from patients with different proteinuric, inflammatory, and fibrotic renal diseases than controlled biopsies with no pathological evidence of kidney disease. Moreover, a significant association was found between KIM-1 and interstitial fibrosis, indicating the promising role of KIM-1 as a marker for chronic renal etiologies [24]. Damman et al. demonstrated that elevated KIM-1 was the strongest independent predictor of worsening kidney function in adults with congestive heart failure in a multivariate Cox regression after adjusting for age, BMI, heart failure class, LVEF, and other comorbidities [25]. In their report, KIM-1 was superior to both NGAL and N-acetyl-beta-D-glucosaminidase in predicting worsening kidney function. Although extensively studied in acute settings, the impact and outcome of chronically elevated urinary injury markers are not well studied in the pediatric population. To our knowledge, our study is the first to demonstrate the association of KIM-1 with LVEF in pediatric patients. Such an association has been reported in adults with congestive heart failure [26, 27]. However, these studies included cohorts with more advanced degrees of LV systolic dysfunction and/or those admitted with ADHF. The populations in these studies had numerous comorbidities, such as chronic hypertension, atherosclerosis, and diabetes that likely contribute to kidney damage and dysfunction and confound the effect of LV dysfunction on kidney function. We suggest that our population might represent a “pure” population for studying CRS type II, as most of our population is free of the comorbidities seen in adults. This offers fertile ground for ongoing cardiorenal study and longitudinal evaluation, not only to assess for changes in TIM, but also the impact of these levels on the long-term risk of CKD. In addition, the majority of our patients do not have underlying CKD, which may confound results.

This study suggests that further investigation of KIM-1 and IL-18 might potentially be employed as a diagnostic utility for moderately or greater reduced LV systolic function (LVEF <45 %). When used alone, KIM-1 and IL-18 showed modest diagnostic characteristic values of LVEF <45 %, with AUCs of 0.61 and 0.59 respectively. We sought to evaluate a diagnostic model that has both a cardiac marker, i.e., BNP, and kidney injury markers, i.e., KIM-1 and IL-18. For specific cut-off values, any of the three markers can predict LVEF <45 % with good sensitivity, but with poor specificity. Combining the three markers in a single model resulted in a high specificity of 83 %. Given this result, the combined “TIM–BNP” model may be useful as a diagnostic tool for deteriorating LV systolic function, saving further expensive and invasive diagnostic testing. The improved prognostic utility of a cardiac marker, i.e., BNP combined with TIM, was introduced by Maisel et al., showing that the urinary NGAL level at discharge improved the mortality prediction of BNP by 29.8 % [8]. To our knowledge, our results are the first to reveal the potential role of using BNP, KIM-1, and IL-18 to identify such a possibility in pediatric populations.

The current definitions of many renal disorders such as AKI and CKD use kidney function markers such as SCr, proteinuria, and urine output for identification. On the other hand, there is a growing belief that early injury markers such as KIM-1 and NGAL can substitute

for the traditional dysfunction markers so that these disorders can be better recognized. CRS is not an exception; the current clinical practice of identifying CRS type II requires evidence of chronic kidney dysfunction. Accordingly, while associated with worse patient outcomes, patients with LV dysfunction and high injury markers and normal kidney function markers are not counted to have CRS. Integration of novel biomarkers into the diagnostic criteria used for CRS may be justified.

Our study has important limitations. First, our data were subject to the inherent limitations associated with a relatively small single-center study. Second, we used LVEF from echocardiography to evaluate LV function; other measures such as MRI or cardiac catheterization would provide more accurate measures and fewer errors estimating LVEF. However, the originality of our hypothesis does not justify such an expensive and somewhat aggressive approach. Lastly, sub-classification analysis between different LV etiologies could not be conducted given the relatively small number of patients in each category. With these limitations, our study documents evidence of kidney injury in mildly to moderately depressed LV systolic function in children, and is to our knowledge the first to use combined serum and urinary biomarkers to predict depressed LVEF in children.

Translational perspectives

There is evidence suggesting that children with decreased left ventricular systolic function might have subclinical kidney injury that can be revealed using novel tubular injury markers. Further translational research should be focused on testing the diagnostic characteristics of different tubular injury markers to utilize kidney dysfunction in children and adults with different types of cardiomyopathies.

Conclusion

Elevated urinary TIM concentrations in children with DCM suggest evidence for subclinical renal tubular injury that can-not be revealed using conventional kidney function markers. TIMs may discriminate children with depressed LVEF, but more importantly may allow for closer renal surveillance in this population. Our results further support the concept of the CRS in pediatric cardiomyopathy patients. Further research should be employed to evaluate the prognosis of elevated TIM in childhood DCM.

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Compliance with ethical standards The study was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board. Predefined clinical variables were extracted from medical records following provision of informed consent by the patient caregiver.

Abbreviations

ADHF Acute decompensated heart failure

AUC	Area under the curve
BNP	B-type natriuretic peptide
CCHMC	Cincinnati Children's Hospital Medical Center
CGCC	Cincinnati Genomic Control Cohort
CHF	Congestive heart failure
CKD	Chronic kidney disease
CRS	Cardiorenal syndrome
DCM	Dilated cardiomyopathy
eGFR	Estimated glomerular filtration rate
IL-18	Interleukin 18
IQR	Interquartile range
KIM-1	Kidney injury molecule-1
L-FABP	Liver-fatty acid binding protein
LV	Left ventricular
LVEDD	Left ventricular end-diastolic dimension
LVEF	Left ventricular ejection fraction
LVESD	Left ventricular end-systolic dimension
LVFS	Left ventricular fractional shortening
LVNC	Left ventricular non-compaction
NGAL	Neutrophil gelatinase-associated lipocalin
OR	Odds ratio
ROC	Receiver operating characteristic
SCr	Serum creatinine
TIM	Tubular injury markers
uCr	Urinary creatinine
uMAlb	Urinary microalbumin
uPr	Urinary protein

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Fig. 1.
Linear regression correlation between urinary KIM-1/Cr ratio and left ventricular ejection fraction (LVEF)

Table 1

Characteristics of cases and controls

Characteristic variable	Patients <i>n</i> = 61	Controls <i>n</i> = 61	<i>p</i> value
Age in years, mean (SD)	13.8 (6.0)	13.0 (5.0)	0.85
Gender, male, <i>n</i> (%)	34 (55.7)	34 (55.7)	1.00
Ethnicity, <i>n</i> (%)			0.11
Caucasian	45 (73.8)	47 (77.1)	
African–American	8 (13.1)	12 (19.7)	
Hispanic	3 (4.9)	2 (3.3)	
Other	5 (8.2)	0 (0)	
Weigh percentile for age, median (IQR)	68.0 (33.8–90.1)	67.7 (41.2–90.8)	0.96
Height % for age, median (IQR)	70.0 (31.5–90.1)	76.2 (36.6–92.4)	0.67
BMI			
BMI % for age, median (IQR)	65.0 (35.0–92.0)	71.0 (36.1–88.4)	0.77
BMI Z score, median (IQR)	0.56 (−0.3–1.5)	0.56 (−0.4–1.2)	0.67
Vital signs			
Heart rate in beat/min, median (IQR)	78.5 (64–88)	85 (72–95.5)	0.14
SBP			
Absolute value in mmHg			
Absolute value in mm g, mean (SD)	108 (15.0)	117 (12.8)	<0.01 *
Absolute value in mmHg, percentile	53.7 (11.2)	61.4 (15.5)	<0.01 *
DBP			
Absolute value in mmHg			
Mean (SD)	59.9 (9.6)	66.5 (6.9)	<0.01 *
Percentile	41.7 (13.4)	49.8 (11.9)	<0.01 *
Normal blood pressure, <i>n</i> (%)	45 (73.8)	39 (63.9)	0.02 *
SBP or DBP between 90th and 95th %, <i>n</i> (%)	12 (19.7)	8 (13.1)	
SBP or DBP between 95th and 99th % + 5 mmHg, <i>n</i> (%)	2 (3.3)	11 (18.0)	
SBP and DBP more than 99th% + 5 mmHg, <i>n</i> (%)	2 (3.3)	0 (0)	
Missing data, <i>n</i> (%)	0 (0)	3 (4.9)	

BMI body mass index, *DBP* diastolic blood pressure, *DCM* dilated cardiomyopathy, *IQR* interquartile range, *SBP* systolic blood pressure, *SD* standard deviation

*
p < 0.05

Table 2

Clinical and cardioechographic characteristics of patients

Characteristics	Data
Underlying cardiac disease, <i>n</i> (%)	
Idiopathic DCM	18 (29.5)
Familial or genetic DCM	10 (16.4)
DCM secondary to cardiotoxic agent	10 (16.4)
DCM with complex congenital heart disease	12 (19.7)
LVNC	11 (18.1)
Time since diagnosis in years, median (IQR)	1.9 (0.7–3.4)
BNP level in pg/ml, median (IQR)	20 (12–62)
Kidney function markers	
Serum blood urea nitrogen in mg/dl, mean (SD)	13.1 (4.6)
Serum creatinine in mg/dl, mean (SD)	0.63 (0.27)
Cystatin C-based eGFR in ml/min/1.73 m ² , mean (SD)	113.5 (28.5)
Creatinine-based eGFR in ml/min/1.73 m ² , mean (SD)	111.4 (31.2)
Patients with eGFR < 60 ml/min/1.73 m ² , <i>n</i> (%)	0 (0)
Urinary protein/creatinine ratio	
Ratio in mg/mg, median (IQR)	0.12 (0.07–0.2)
Number of patients with ratio <0.2 mg/mg ^b , <i>n</i> (%)	9 (14.7)
Urinary albumin/creatinine	
Ratio in mg/g, median (IQR)	7.8 (5.5–20.5)
Number of patients with ratio > 30 mg/g ^a , <i>n</i> (%)	4 (6.5)
Echocardiographic measurements	
Left ventricular ejection fraction ^c , mean (SD)	42.3 (12.1)
Left ventricular fractional shortening ^d , mean (SD)	28.2 (7.0)
Left ventricular end-diastolic dimension Z-score, mean (SD)	1.9 (0.8)
Left ventricular mass/height ^{2.7} , median (IQR)	36.6 (30.0–46.3)

BNP B-type natriuretic peptide, DCM dilated cardiomyopathy, eGFR estimated glomerular filtration rate, IQR interquartile range, LVNC, left ventricular non-compaction, SD standard deviation

^aNormal value of albumin/creatinine ratio <30 mg/g [15]

^bNormal value of protein/creatinine ratio <0.2 [16]

^cNormal value range: 55–70 %

^dNormal value range: 28–44 %

Table 3

Differences in urinary tubular injury marker (TIM) concentrations between patients and controls

Urinary tubular biomarkers, median (IQR)	Cases (<i>n</i> = 61)	Controls (<i>n</i> = 61)	<i>p</i> value
Urinary KIM-1/creatinine ratio in pg/mg	386.0 (248.7–597.6)	307.3 (182.8–432.5)	0.02 [*]
Urinary NGAL/creatinine ratio in ng/mg	13.2 (6.5–44.3)	8.3 (3.1–17.5)	0.01 [*]
Urinary IL-18/creatinine ratio in pg/mg	28.2 (15.9–41.5)	19.0 (12.6–28.6)	<0.001 [*]
Urinary L-FABP/creatinine ratio in ng/mg	4.1 (1.8–11.1)	2.6 (1.7–3.4)	0.06

IL-18 interleukin 18, *KIM-1* kidney injury molecule-1, *NGAL* neutrophil gelatinase-associated lipocalin, *L-FABP* liver-fatty acid binding protein

^{*}
p < 0.05

Table 4

The predictive characteristics of urinary tubular injury markers to distinguish cases with a left ventricular ejection fraction (LVEF) of < 55 % from controls

Predictive marker	Area under the curve	Best cut-off value for sensitivity close to 80 %	Sensitivity (95 % CI)	Specificity (95 % CI)	Positive predictive value (95 % CI)	Negative predictive value (95 % CI)
KIM-1	0.61	218	0.80 (0.67–0.89)	0.32 (0.20–0.45)	0.54 (0.44–0.65)	0.61 (0.42–0.78)
NGAL	0.64	5.13	0.80 (0.68–0.89)	0.33 (0.22–0.47)	0.55 (0.44–0.66)	0.63 (0.43–0.78)
IL-18	0.74	17.5	0.80 (0.68–0.89)	0.43 (0.30–0.57)	0.61 (0.50–0.72)	0.66 (0.48–0.80)
L-FABP	0.60	1.31	0.80 (0.68–0.89)	0.15 (0.08–0.27)	0.49 (0.39–0.59)	0.43 (0.23–0.66)
IL-18 + NGAL (combined)	0.75	As above	0.67 (0.53–0.78)	0.54 (0.42–0.65)	0.51 (0.40–0.63)	0.69 (0.56–0.80)
IL-18 + NGAL + KIM-1 (combined)	0.75	As above	0.56 (0.42–0.69)	0.63 (0.51–0.73)	0.52 (0.39–0.65)	0.66 (0.54–0.77)
All TIMs combined	0.75	As above	0.52 (0.40–0.66)	0.71 (0.59–0.80)	0.57 (0.56–0.77)	0.67 (0.56–0.77)

CI confidence interval, *IL-18* interleukin 18, *KIM-1* kidney injury molecule-1, *NGAL* neutrophil gelatinase-associated lipocalin, *L-FABP* liver-fatty acid binding protein, *TIM* tubular injury marker

Table 5

Correlation between left ventricular systolic function represented by the left ventricular ejection fraction (LVEF) as an outcome variable and different kidney function/injury markers as predictor variables using univariate linear regression models

Kidney function marker as a predictor	LVEF as an outcome variable		
	Pearson correlation coefficient (r)	Regression slope (β 1)	p value
Serum blood urea nitrogen	-0.11	-1.01	0.31
Creatinine-based eGFR	0.04	0.29	0.77
Cystatin C-based eGFR	0.21	0.13	0.13
Urinary protein/creatinine ratio	-0.14	-1.93	0.4
Urinary microalbumin/creatinine ratio	-0.17	-2.14	0.22
Urinary KIM-1/creatinine ratio	-0.30	-2.84	0.01 *
Urinary NGAL/creatinine ratio	-0.04	-0.33	0.74
Urinary IL-18/creatinine ratio	-0.14	-1.30	0.08
Urinary L-FABP/creatinine ratio	-0.06	-0.54	0.25

eGFR estimated glomerular filtration rate, *IL-18* interleukin 18, *KIM-1* kidney injury molecule-1, *NGAL* neutrophil gelatinase-associated lipocalin, *L-FABP* liver-fatty acid binding protein

*
 $p < 0.05$

Table 6

The predictive characteristics of urinary tubular injury markers (TIM) to distinguish cases with remarkable left ventricular depression (LVEF < 45 %) from those with mild depression (55 > LVEF 45 %)

Predictive marker	Area under the curve (AUC)	Best cut-off value for sensitivity close to 80 %	Sensitivity (95 % CI)	Specificity (95 % CI)	Positive predictive value (95 % CI)	Negative predictive value (95 % CI)
BNP (pg/ml)	0.70	15	0.80 (0.56–0.94)	0.48 (0.28–0.69)	0.55 (0.35–0.73)	0.75 (0.47–0.92)
KIM-1 (pg/mg)	0.61	235	0.79 (0.56–0.92)	0.29 (0.17–0.42)	0.30 (0.46–0.78)	0.77 (0.55–0.92)
NGAL (ng/mg)	0.50	4.8	0.82 (0.61–0.94)	0.17 (0.08–0.29)	0.28 (0.18–0.39)	0.71 (0.42–0.91)
IL-18 (pg/mg)	0.59	17.5	0.82 (0.61–0.94)	0.22 (0.12–0.35)	0.29 (0.19–0.42)	0.76 (0.50–0.93)
L-FABP/UCr (ng/mg)	0.43	12.1	0.78 (0.56–0.92)	0.15 (0.07–0.27)	0.26 (0.19–0.36)	0.64 (0.35–0.87)
BNP + KIM-1 (combined)	0.68	As per above	0.47 (0.27–0.69)	0.80 (0.67–0.89)	0.48 (0.27–0.69)	0.80 (0.67–0.89)
BNP + KIM-1 + IL-18 combined	0.70	As per above	0.43 (0.23–0.65)	0.83 (0.71–0.92)	0.50 (0.29–0.73)	0.79 (0.67–0.88)

CI confidence interval, *BNP* B-type natriuretic peptide, *IL-18* interleukin 18, *KIM-1* kidney injury molecule-1, *NGAL* neutrophil gelatinase-associated lipocalin, *L-FABP* liver-fatty acid binding protein