


# Serum creatinine and cystatin C-based estimates of glomerular filtration rate are misleading in acute heart failure

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## Abstract

**Aims** We aimed to test whether the endogenous filtration markers serum creatinine or cystatin C and equation-based estimates of glomerular filtration rate (GFR) based on these markers appropriately reflect changes of measured GFR in patients with acute heart failure.

**Methods** In this prospective cohort study of 50 hospitalized acute heart failure patients undergoing decongestive therapy, we applied an intravenous visible fluorescent injectate (VFI), consisting of a low molecular weight component to measure GFR and a high molecular weight component to correct for measured plasma volume. Thirty-eight patients had two sequential GFR measurements 48 h apart. The co-primary endpoints of the study were safety of VFI and plasma stability of the high molecular weight component. A key secondary endpoint was to compare changes in measured GFR (mGFR) to changes of serum creatinine, cystatin C and estimated GFR.

**Results** VFI-based GFR measurements were safe and consistent with plasma stability of the high molecular weight component and glomerular filtration of the low molecular weight component. Filtration marker-based point estimates of GFR, when compared with mGFR, provided only moderate correlation (Pearson's  $r$ , range 0.80–0.88, depending on equation used), precision ( $r^2$ , range 0.65–0.78) and accuracy (56%–74% of estimates scored within 30% of mGFR). Correlations of 48-h changes GFR estimates and changes of mGFR were significant ( $P < 0.05$ ) but weak (Pearson's  $r$ , range 0.35–0.39). Observed decreases of eGFR by more than 15% had a low sensitivity (range 38%–46%, depending on equation used) in detecting true worsening mGFR, defined by a  $>15\%$  decrease in mGFR.

**Conclusions** In patients hospitalized for acute heart failure, serum creatinine- and cystatin C-based predictions performed poorly in detecting actual changes of GFR. These data challenge current clinical strategies to evaluate dynamics of kidney function in acute heart failure.

**Keywords** Acute heart failure; Worsening kidney function; Acute kidney injury; CKD-EPI formula; Measured GFR; Visible fluorescent injectate

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## Introduction

The kidney's glomerular filtration rate (GFR) critically contributes to heart failure pathophysiology<sup>1,2</sup> and is generally closely monitored in patients undergoing decongestive therapy for acute heart failure (AHF), usually by measuring serial serum creatinine (SCr) concentrations or less frequently by measuring serum cystatin C (CysC) concentrations. Increases in SCr and CysC in AHF patients are usually considered an ominous sign of 'worsening renal function' (WRF). Definitions of WRF based on these markers have been labelled as risk factors or primary endpoints in heart failure studies.<sup>3–7</sup> A growing body of evidence, however, indicates that changes of SCr or CysC suggesting worsening GFR are neither closely associated with structural tubular injury nor with adverse outcomes in patients with AHF undergoing decongestive therapy, especially when accompanied by evidence of efficient decongestion, diuresis and hemoconcentration.<sup>8–12</sup>

It has been demonstrated in stable chronic heart failure populations that formula-based point estimates of GFR (eGFR) have a relatively poor precision and accuracy in predicting measured GFR (mGFR).<sup>13–15</sup> This may be further aggravated in the setting of AHF, where extracellular fluid expansion and volume shifts under intensified decongestive therapy may lead to (1) dynamic alterations of GFR that undermine the steady-state assumptions of GFR-estimating equations or (2) dilution or concentration of SCr and CysC independent from GFR alterations.

Established equations frequently applied in clinical practice to determine eGFR based on SCr and/or CysC, age, gender and ethnicity were developed in individuals with stable steady-state equilibrium of kidney function. They include the *Chronic Kidney Disease Epidemiology Collaboration* (CKD-EPI) equations<sup>16</sup> and the *Modification of Diet in Renal Disease Study* (MDRD) equation<sup>17</sup> (see *Table S1* for details). In addition, a kinetic eGFR equation (keGFR) has been proposed when SCr concentrations are not in steady-state equilibrium.<sup>18</sup> Whether SCr, CysC or filtration marker-based equations accurately reflect GFR and its dynamic changes in patients with AHF is unknown, because studies of point or serial measurements of actual GFR in AHF patients have not been conducted.

Nevertheless, clinicians will usually consider SCr or CysC as surrogates of GFR in AHF patients and may apply eGFR equations, even under non-steady-state conditions. The term 'worsened renal function' is not uniformly defined in the heart failure field and usually refers to a percentage decrease of estimated GFR based on SCr and/or CysC<sup>19</sup> or to an absolute or relative increase of SCr as utilized in the KDIGO definition of acute kidney injury (AKI).<sup>20</sup> For instance, the recent ROSE-AHF trial defined WRF as a 20% decrease of eGFR based on the CKD-EPI equation, which utilizes SCr and CysC.<sup>8</sup>

Misjudgements of GFR trajectories in the context of AHF may affect therapeutic decision making. For instance, a diagnosis of creatinine-based worsening GFR may prompt

premature discontinuation of aggressive diuresis or even contribute to volume administration or the initiation of kidney replacement therapy. In addition, pharmacological heart failure therapy with renin–angiotensin system blockers may be withheld, if worsening GFR is assumed to be present. In contrast, a failure to diagnose true worsening of GFR in these subjects may lead to prolonged structural kidney injury, resulting in chronic kidney disease (CKD) progression.<sup>21–23</sup>

Data regarding the changes of actual GFR during heart failure therapy are currently lacking. To address this knowledge gap, we conducted a clinical study in patients undergoing decongestive therapy for AHF and utilized a recently developed fluorescent tracer-based method<sup>24</sup> to rapidly measure GFR at two consecutive time points during heart failure therapy. We assessed safety and functionality of the fluorescent tracer-based method and compared mGFR to SCr- and CysC-based estimates of GFR and different definitions of worsening GFR.

## Methods

### Population

This prospective multicentric Phase 2b study was performed at the Nephrology and Cardiology Departments of Charité – Universitätsmedizin Berlin and at the Cardiology Department of Kerckhoff Klinik, Bad Nauheim, between January and July 2019. Fifty patients undergoing either intravenous or oral decongestive diuretic therapy for AHF were enrolled following written informed consent. Eligible participants had to have a diagnosis of AHF and were aged  $\geq 18$  years with evidence of AHF based on presence of  $\geq 1$  symptom (dyspnoea, orthopnoea or oedema) and  $\geq 1$  sign (rales, peripheral oedema, ascites or pulmonary vascular congestion on chest radiography).<sup>25</sup> Exclusion criteria included pregnancy, acute onset of myocardial infarction, unstable angina pectoris, new onset of atrial fibrillation, requirement of intravenous vasodilators or inotropic agents, acute or chronic need for renal replacement therapy, significant non-cardiac diseases, severe infections and internal bleeding. Non-sterile participants agreed to use effective methods of contraception.

Data were handled in respect of patient anonymity and confidentiality. The study was approved by the regional ethics board Berlin and conducted in accordance with Declaration of Helsinki guidelines (EduraCT Number 2018-002638-18, <https://clinicaltrials.gov/ct2/show/NCT03808948>).

### Measurement of GFR

mGFR was determined by measuring the plasma clearance of an intravenously injected fluorescently labelled dextran, which is freely filtered by the glomerulus and not reabsorbed into the blood. The visible fluorescent injectate (VFI) (FAST

BioMedical, Indianapolis, IN, USA), consists of 12 mg of a 150-kDa carboxymethylated dextran, conjugated to a rhodamine dye (FD003), and 35 mg of a 5-kDa carboxymethylated dextran, conjugated to fluorescein (FD001).

A volume of 3.0 mL was infused intravenously. Blood samples were collected right before and 15, 30, 60 and 180 min after injection. Blood plasma was run on a validated BioAnalytical HPLC assay at Covance Laboratories in Salt Lake City, Utah. Plasma volume (PV) was determined using the average FD003 concentrations of the early 15 and the 60 min time point. The low molecular weight dextran is freely filtered, consistent with inulin.<sup>26</sup> mGFR determined by VFI technology has been shown to be nearly identical to mGFR determined by a 6-h iohexol protocol (considered a gold standard in the field) in normal subjects and subjects with CKD.<sup>24</sup> The concentration of the small dextran GFR marker at time zero was back-calculated from the measured PV by dividing the known dose into the measured PV. The time points were then fitted using a two-compartment model, and the resulting area under the curve was calculated. The use of the time point 0 determination helped to better resolve the shape of the clearance curve. mGFR was calculated and adjusted to body surface area as described previously. The FAST patented software technology only reports an mGFR value for assays where the four-point clearance curve yields a valid two-exponent data fit, where both rates are positive and conform to the expected fast and slow decay profiles.

## Study design

GFR was measured in the morning after enrollment (Fay 1) and  $48 \pm 5$  h after the first measurement (Day 3, whenever feasible). Body weight and height were determined, and venous blood was drawn to determine SCr (Jaffé, IDMS standardized) and CysC (immunoturbidimetry) before injection of the fluorescent tracer. mGFR results were not available to treating physicians. Subjects were followed for 30 days after the last VFI injection and assessed for adverse and serious adverse events.

The co-primary endpoints of the study were the assessment of safety of VFI (the safety endpoint was predefined as an absence of compound-related treatment-emergent severe adverse events) and the confirmation of plasma stability of the FD003 high molecular weight marker over the 15-, 30-, and 60-min blood draws (defined as differences  $< 10\%$  of mean plasma concentrations of FD003 between time points 15 and 30 min and between time points 30 and 60 min, respectively). No formal hypothesis test was used to calculate the sample size. The sample size was set to 50 patients, which was deemed adequate, given the primary endpoints were consistent with those of a Phase 2 study. The comparison of GFRs determined by VFI mGFR technology with estimated

GFR using established equations was a predefined secondary endpoint. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

## Equations estimating GFR and definitions of worsening GFR

Five equations were used as SCr- and CysC- based estimations of GFR: simplified MDRD (sMDRD),<sup>17</sup> CKD-EPI<sub>SCr</sub>,<sup>27</sup> CKD-EPI<sub>CysC</sub>,<sup>16</sup> CKD-EPI<sub>SCr-CysC</sub>,<sup>16</sup> and kinetic eGFR.<sup>18</sup> Table S1 shows these equations in detail. True worsening mGFR was diagnosed when changes in mGFR from Day 1 to Day 3 indicated an mGFR decrease  $> 15\%$ . KDIGO criteria for AKI are based on changes of SCr (e.g. Stage 1 is defined by an increase of SCr by  $< 0.3$  mg/dL or an increase of SCr by 1.5-fold).<sup>28</sup> To assess whether a change of mGFR (as measured by serial VFI measurements) was equivalent to AKI, we used mGFR and back-calculated hypothetical idealized SCr values when applying a reverse CKD-EPI<sub>SCr</sub> equation (see Supporting Information). This allowed us to make an mGFR-based 'true AKI' diagnosis, which was then compared with the actual SCr-based AKI definition.

## Statistical analysis

Descriptive analyses were done by reporting the quantiles of the empirical distributions of the respective variables, for example, mean and standard deviation for continuous data, median and interquartile range for skewed distributed data and frequencies with percentages for categorical variables. Student's *t*-test was used to compare continuous variables. For known skewed data, the Mann–Whitney *U* test was used. The Wilcoxon test was used to compare dependent samples for continuous variables. Chi-square tests were used to compare independent categorical variables.

Correlations between mGFR and eGFR using different GFR estimation equations and correlations between percentage changes in mGFR and eGFR were provided using Pearson's correlation coefficients. The predictive performance of the equations was evaluated by precision, accuracy and bias. Precision was evaluated by the amount of expected variation in the estimates using linear regression and reporting  $r^2$ . Accuracy was assessed by comparing the results of eGFR with those of mGFR and counting the number of subjects with predicted eGFR values within the 15% ( $P_{15}$ ) and 30% ( $P_{30}$ ) range of mGFR. Bias was defined as mean difference of the estimated and measured value. Further, 95% confidence intervals (CI) and standard deviation were calculated where appropriate. Agreements between measured and estimated GFR were assessed with Bland–Altman plots. Predefined limits of agreement were set to  $\pm 6$  mL per min per  $1.73$  m<sup>2</sup> based on clinical

considerations in AHF patients, who often exhibit pre-existing CKD. In CKD Stage G3A, G3B or G4, differences of eGFR and mGFR that are more than 6 mL/min would place >40% of patients into different GFR stages, which might have clinically meaningful implications.

The statistical analysis was performed with the use of SPSS (Chicago) Version 23, GraphPad Prism Version 8 and R Core Team (2018, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). All reported *P*-values are two tailed. Due to the exploratory character of the study, all reported results and *P*-values have to be considered as non-confirmatory.

## Results

### Patient characteristics and outcomes

We enrolled 50 hospitalized patients with AHF undergoing diuretic decongestive therapy, which included at least an intravenous loop diuretic in 40 patients (80%) and at least an oral loop diuretic in 10 patients (20%). Baseline characteristics are shown in *Table 1*.

mGFR was determined using VFI technology 2 days (median; IQR 1–3 days) after hospital admission (Day 1 of the study). A second GFR measurement was performed

**Table 1** Baseline characteristics of the cohort at enrollment (Day 1)

	All patients	Patients with two GFR measurements 48 h apart
	50	38
<b>Demographic data</b>		
Age (years)	72.5 ± 13.7	71.9 ± 14.4
Male	40(80)	29(76.3)
Caucasian	48(96)	36(94.7)
<b>Clinical data at enrolment</b>		
Weight (kg)	89.2 ± 22.2	89.8 ± 23.2
Height (cm)	172.8 ± 10.6	172.1 ± 11.7
BMI (kg/m <sup>2</sup> )	29.7 ± 6	30.1 ± 5.8
Systolic blood pressure (mmHg)	126 ± 23.6	126.3 ± 24.7
Diastolic blood pressure (mmHg)	71.6 ± 14.1	71.7 ± 15.1
Mean arterial pressure (mmHg)	89.8 ± 14.2	89.9 ± 14.9
Heart rate (bpm)	73.5 ± 15.8	76 ± 13.6
NYHA Class II	7 (14)	4(10.5)
NYHA Classes III–IV	43 (86)	34(89)
LVEF (%)	47.9 ± 15.5 <sup>b</sup>	46 ± 14.8 <sup>c</sup>
HFpEF	29 (58) <sup>b</sup>	23(60.5) <sup>c</sup>
HFmrEF	9(18) <sup>b</sup>	5(13.2) <sup>c</sup>
HFrEF	10(20) <sup>b</sup>	9(23.7) <sup>c</sup>
<b>Co-morbidities</b>		
Diabetes	21(42)	17(44.7)
Coronary artery disease	31(62)	25(65.8)
Hypertension	46(92)	35(92.1)
Peripheral arterial occlusive disease	10(20)	7(18.4)
Baseline eGFR (CKD-EPI <sub>SCr</sub> ) (mL per min per 1.73 m <sup>2</sup> )	49.8 ± 21.2	50.1 ± 21.2
CKD-Stage 1–2	21(42)	16(42)
CKD-Stage ≥3	29(58)	22(58)
<b>Medication at enrolment</b>		
RAASI	36 (72)	28(73.7)
Loop diuretic	50 (100)	38(100)
Thiazide diuretic	8 (16)	6(15.8)
Aldosterone antagonist	19(38)	15(39.5)
β-Blocker	37 (74)	29(76.3)
<b>Laboratory values at enrolment</b>		
SCr (mg/dL)	1.8(1.3–2.1)	1.8(1.2–2.2)
CysC (mg/L)	2.2(1.6–2.7)	2.2(1.7–2.5)
NT-proBNP (ng/L)	3737(1822–7427) <sup>a</sup>	3647(1830–8076.5) <sup>b</sup>
Haematocrit, (%)	34.3(30.2–40) <sup>c</sup>	34.3(30–39.7) <sup>c</sup>

Data are presented as mean ± standard deviation, median (interquartile range) or number (percentage), as appropriate. No statistically significant differences between patients with two GFR measurements, and patients with one GFR measurement only were noted except for heart rate (*P*, Student's *t*-test, Mann–Whitney *U* test, chi-square test, as appropriate). Data are rounded to one decimal place; missing data are indicated by superscript letters.

BMI, body mass index; CKD, chronic kidney disease; Cys C, cystatin C; GFR<sub>Baseline</sub>, eGFR CKD-EPI<sub>SCr</sub> based on the clinically adjudicated out-patient baseline creatinine; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; RAASI, renin–angiotensin–aldosterone system inhibitor; SCr, serum creatinine, CysC, Cystatin C.

<sup>a</sup>*n* = 3.

<sup>b</sup>*n* = 2.

<sup>c</sup>*n* = 1.

48 h later in 38/50 (76%) patients (Day 3 of the study). VFI consists of two components, a high molecular weight component (FD003) and a low molecular weight component (FD001). Following VFI injection, FD003 is retained in the intravascular space, whereas FD001 is freely filtered by the glomerulus. Blood draws were obtained 15, 30, 60 and 180 min after VFI injection to determine plasma concentrations of FD001 and FD003. PV was calculated from the plasma concentration of FD003 based on the indicator dilution principle. The starting concentration of FD001 was calculated based on the PV, and measured FD001 kinetics over the following 180 min were fitted to calculate mGFR.

The first co-primary endpoint of this study was safety. Twenty-one (42%) patients had treatment-emergent severe adverse events (including one death and four rehospitalizations) in this AHF population, but all were considered typical in AHF setting and unrelated to the VFI compound. This indicated that the predefined primary safety endpoint of the study was met. There were 37 patients (74%) with a total of 177 treatment-emergent adverse events (TEAEs). One hundred sixty-four TEAEs were considered not related or unlikely related to VFI. Thirteen TEAEs of 10 patients (20%) were considered to be possibly, probably or surely related to study drug (Table S2). These included diarrhoea (one moderate, three mild), hypersensitivity or pruritus (two moderate, one mild), vertigo (one mild) and laboratory abnormalities (five mild). Two patients were discontinued due to urticaria and pruritus after VFI administration. These symptoms resolved quickly with administration of antihistamine therapy.

The second co-primary endpoint of this study was stability of FD003 within the intravascular compartment, which is considered to be a critical prerequisite for calculating PV. The concentration of FD003 was stable throughout the sampling period. Differences of mean plasma concentrations of FD003 between time points 15 and 30 min ( $-2.36 \pm 7.94\%$  on Day 1 and  $-3.12 \pm 11.29\%$  on Day 3) and between time points 30 and 60 min ( $-0.8 \pm 5.6\%$  on Day 1 and  $-0.79 \pm 8.67\%$  on Day 3) were below the pre-specified 10% margin. A small subset of patients showed a  $>10\%$  decline

in the concentration of FD003, but these declines were restricted to individual time points per patient and did not show a systematic decline over time in any individual. The observed plasma stability indicates that FD003 follows the indicator dilution principle in acute decompensated heart failure patients. Kinetics of FD001 were compatible with elimination kinetics by glomerular filtration in all 50 patients.

### Static relationships of creatinine- and cystatin C-based GFR estimates and mGFR

We first compared eGFR calculated on Day 1 according to five established equations (sMDRD, CKD-EPI<sub>SCr</sub>, CKD-EPI<sub>CysC</sub>, CKD-EPI<sub>SCr-CysC</sub> and keGFR) to mGFR determined on the same day (Tables 2 and S3). Mean eGFRs according to sMDRD, CKD-EPI<sub>SCr</sub> and keGFR were significantly higher when compared with mean mGFR, whereas mean eGFR according to CKD-EPI<sub>CysC</sub> on Day 1 was significantly lower than mGFR. CKD-EPI<sub>SCr-CysC</sub> did not differ significantly from mGFR. Similar results were observed on day 3 (Table S3 and Figures S1 and S2). None of the equations met predefined limits of agreement of  $\pm 6$  mL per min per  $1.73 \text{ m}^2$ . Bland–Altman plots indicated that all equations tended to underestimate mGFR in the low GFR range ( $<40$  mL per min per  $1.73 \text{ m}^2$ ) and overestimate GFR in the high GFR range ( $>40$  mL per min per  $1.73 \text{ m}^2$ ) (Figure S3).

The overall performance of formula-based estimates of mGFR was evaluated by analysing correlation (Pearson's  $r$ ), precision ( $r^2$ ), accuracy ( $P_{15}$ ,  $P_{30}$ ) and bias (Tables 2 and S4). Filtration marker-based estimates of GFR at any given time, when compared with mGFR, provided only moderate correlation (Pearson's  $r$ , range 0.80–0.88, depending on equation used), precision ( $r^2$ , range 0.65–0.78) and accuracy (56%–74% of estimates scored within 30% of mGFR).

To analyse determinants of systematic error, we evaluated the predictive performance of the equations by subgroups. Age significantly affected bias of CKD-EPI<sub>CysC</sub> and CKD-EPI<sub>SCr-CysC</sub>. Endogenous marker concentrations strongly affected bias of all equations with lower SCr and CysC, leading to

**Table 2** Performance of SCr- and CysC-based equations when compared with measured GFR in AHF patients at enrollment (Day 1)

	Mean $\pm$ SD	Bias mean $\pm$ SD	Pearson's $r$	Precision $r^2$	Accuracy	
					$P_{15}$	$P_{30}$
mGFR (mL per min per $1.73 \text{ m}^2$ )	35 $\pm$ 11.7					
sMDRD (mL per min per $1.73 \text{ m}^2$ )	40.4 $\pm$ 17.1*	5.4 $\pm$ 10.2	0.81	0.66	40%	70%
CKD-EPI <sub>SCr</sub> (mL per min per $1.73 \text{ m}^2$ )	40.4 $\pm$ 18.5*	5.4 $\pm$ 11.3	0.81	0.66	40%	66%
CKD-EPI <sub>CysC</sub> (mL per min per $1.73 \text{ m}^2$ )	31 $\pm$ 16.6*	-4.0 $\pm$ 9.9	0.81	0.66	40%	56%
CKD-EPI <sub>SCr-CysC</sub> (mL per min per $1.73 \text{ m}^2$ )	34.6 $\pm$ 17	-0.4 $\pm$ 9.6	0.84	0.70	30%	74%
keGFR (mL per min per $1.73 \text{ m}^2$ )	41.4 $\pm$ 18*	6.6 $\pm$ 11.7	0.80	0.65	26%	66%

Accuracy  $P_{15}$  and  $P_{30}$  refers to per cent of GFR estimates that are within 15% and 30% of measured GFR, respectively. GFR data and bias are presented as mL/min/ $1.73 \text{ m}^2$ .

SD = standard deviation.

\* $P < 0.01$  vs. mGFR.

marked overestimation of mGFR by sMDRD, CKD-EPI<sub>SCr</sub> and keGFR, whereas higher SCr and CysC concentrations were associated with underestimation of GFR by CKD-EPI<sub>CysC</sub> and CKD-EPI<sub>SCr-CysC</sub> (Table 3).

### Comparison of relative changes of serum creatinine and serum cystatin C and corresponding changes of mGFR

The repeat measurement design of our study enabled us to assess relative changes of mGFR between Day 1 and day 3 and compare them to changes of SCr, CysC and GFR estimates. We analysed the 48-h changes of mGFR and the corresponding changes of eGFR in individual study patients. The data indicated that the change of eGFR provided profoundly inadequate estimates of the true mGFR trajectory in a subset of AHF patients (Figure 1). Correlation coefficients of 48-h changes of GFR estimating formulas and corresponding changes of mGFR indicated weak, but significant correlations (Pearson's  $r$ , range 0.35–0.39, depending on equation used) (Figure S4). A statistically significant but weak negative correlation was observed between 48-h changes in CysC and corresponding 48-h changes of mGFR ( $r = -0.37$ ,  $P = 0.022$ ), whereas SCr changes did not correlate significantly with 48-h changes of mGFR ( $r = -0.31$ ,  $P = 0.057$ ) (Figure S5). Observed decreases of eGFR by more than 15% had a low sensitivity (range 38%–46%, depending on equation used) in detecting true worsening mGFR, defined by a >15% decrease in mGFR (Figure 2 and Table 4). Similarly, KDIGO creatinine criteria for AKI had a poor sensitivity in detecting corresponding decreases of mGFR (Figure 2 and Table 4). Changes of SCr, CysC and eGFR from Day 1 to Day 3 frequently provided false-negative signals in patients with declines of mGFR and occasionally triggered false-positive signals (Figure 2). Accordingly, trajectories of SCr, CysC and eGFR from Day 1 to Day 3 in individuals with a true worsening mGFR > 15% frequently failed to reveal corresponding increases in filtration markers and decreases of eGFR (Figure 3). Conversely, patients with stable or increasing mGFR occasionally exhibited erroneous increases of filtration markers providing false-positive signals. Hence, definitions of 'worsening kidney function' based on the serial application of eGFR equations or based on KDIGO AKI criteria had weak test characteristics, in particular a poor sensitivity, in detecting worsening mGFR in AHF patients.

## Discussion

### Key findings and novelty

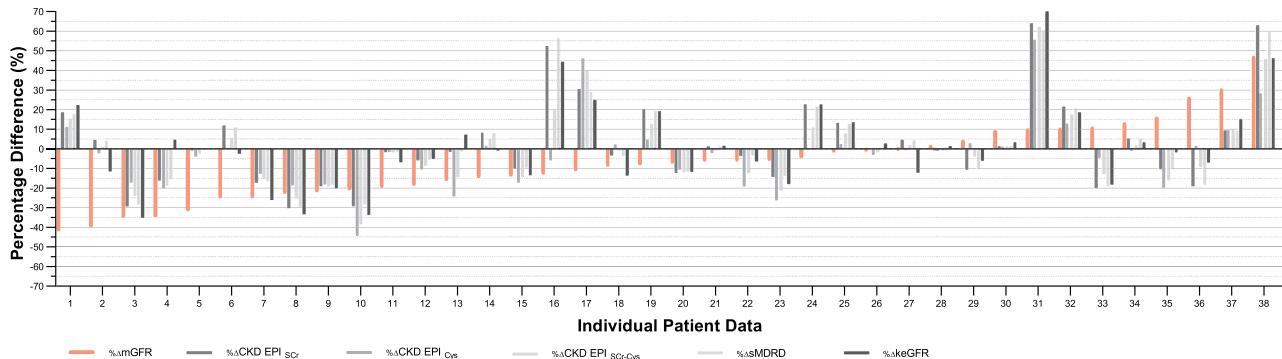
To our knowledge, this is the first study comparing estimates of GFR with mGFR in an AHF population. Repeated GFR

**Table 3** Determinants of systematic error

	Bias sMDRD			Bias CKD EPI <sub>SCr</sub>			Bias CKD EPI <sub>CysC</sub>			Bias CKD EPI <sub>SCr-CysC</sub>			Bias keGFR		
	n	Mean ± SD	P	Mean ± SD	P	Mean ± SD	P	Mean ± SD	P	Mean ± SD	P	Mean ± SD	P		
Age (years)	≤77.5	5.9 ± 10.5	0.743	7.5 ± 12.2	0.181	-0.5 ± 11.1	0.01	2.4 ± 10.7	0.034	8.0 ± 12	0.307	8.0 ± 12	0.307		
	>77.5	4.9 ± 10.2		3.2 ± 10.2		-7.56 ± 7.1		-3.3 ± 7.6		4.7 ± 10.3		4.7 ± 10.3			
BMI (kg/m <sup>2</sup> )	≤29.7	6.2 ± 11.0	0.607	6.1 ± 12.8	0.647	-3.6 ± 11.0	0.729	0.1 ± 11.0	-0.68	7.1 ± 12.8	0.67	7.1 ± 12.8	0.67		
	>29.7	4.7 ± 9.5		4.6 ± 9.8		-4.5 ± 8.8		-1.0 ± 8.3		5.7 ± 9.4		5.7 ± 9.4			
SCr (mg/dl)	≤1.75	11.4 ± 9.6	<0.0001	12.3 ± 10.6	<0.0001	-0.9 ± 10.8	0.023	4.44 ± 9.5	<0.0002	12.8 ± 10.3	<0.0001	12.8 ± 10.3	<0.0001		
	>1.75	-0.6 ± 6.9		-1.5 ± 7.0		-7.2 ± 7.8		-5.3 ± 7.0		0.01 ± 7.9		0.01 ± 7.9			
CysC (mg/dl)	≤2.22	11.5 ± 9.4	<0.0001	12.5 ± 10.3	<0.0001	0.5 ± 10.5	0.001	5.4 ± 10.5	<0.0001	13.1 ± 9.9	<0.0001	13.1 ± 9.9	<0.0001		
	>2.22	-0.7 ± 7.0		-1.8 ± 7.0		-8.6 ± 6.8		-6.3 ± 6.2		-0.3 ± 7.9		-0.3 ± 7.9			
CKD stage	≤Stage 3a	0.04 ± 8.0	<0.0001	-1.0 ± 8.0	<0.0001	-7.5 ± 7.3	0.02	-5.2 ± 7.2	0.001	0.02 ± 8.3	<0.0001	0.02 ± 8.3	<0.0001		
	>Stage 3a	10.0 ± 10.0		10.8 ± 11.0		-1.1 ± 10.8		3.7 ± 9.6		11.9 ± 10.5		11.9 ± 10.5			
LVEF <sup>a</sup>	≤50%	4.5 ± 11.8	0.652	4.8 ± 13.4	0.871	-3.6 ± 11.2	0.528	-0.40 ± 11.5	0.772	5.7 ± 13.2	0.784	5.7 ± 13.2	0.784		
	>50%	5.9 ± 8.6		5.4 ± 8.8		-5.4 ± 8.0		-1.2 ± 7.2		6.6 ± 8.7		6.6 ± 8.7			

Mean bias (± standard deviation) on Day 1 stratified by demographic and clinical parameters. Cut-offs were set at median or near median (when based on an established clinical cut-off). P shows P-value of between subgroup comparisons of bias (Student's t-test). Bias is presented as mL per min per 1.73 m<sup>2</sup>.  
<sup>a</sup>Missing data: n = 2.

**Figure 1** Individual patient data of 48 hour changes in mGFR and corresponding changes in eGFR based on CKD-EPI<sub>SCr</sub>, CKD-EPI<sub>Cys</sub>, CKD-EPI<sub>SCr-Cys</sub>, sMDRD and keGFR. Patients are sorted by direction and intensity of the change in mGFR during the 48-h study period. Please note that several patients (e.g. Patients 1, 2, 17, 31, 35 and 36) display a substantial mismatch between the formula-based estimated change of GFR and the true change of measured GFR.



measurements by the VFI technology established safety and functionality of this technique in AHF and enabled us to evaluate changes of mGFR over time and analyse the dynamic performance of filtration markers and filtration marker-derived GFR estimating equations and comparison vs. definitions of ‘worsening renal function’ or ‘AKI’. The key finding of this study is that time courses of SCr, CysC or eGFR (by any formula) provide insufficient information regarding the time course of mGFR and have limited sensitivity in detecting true worsening GFR.

### Functionality and safety of VFI technique

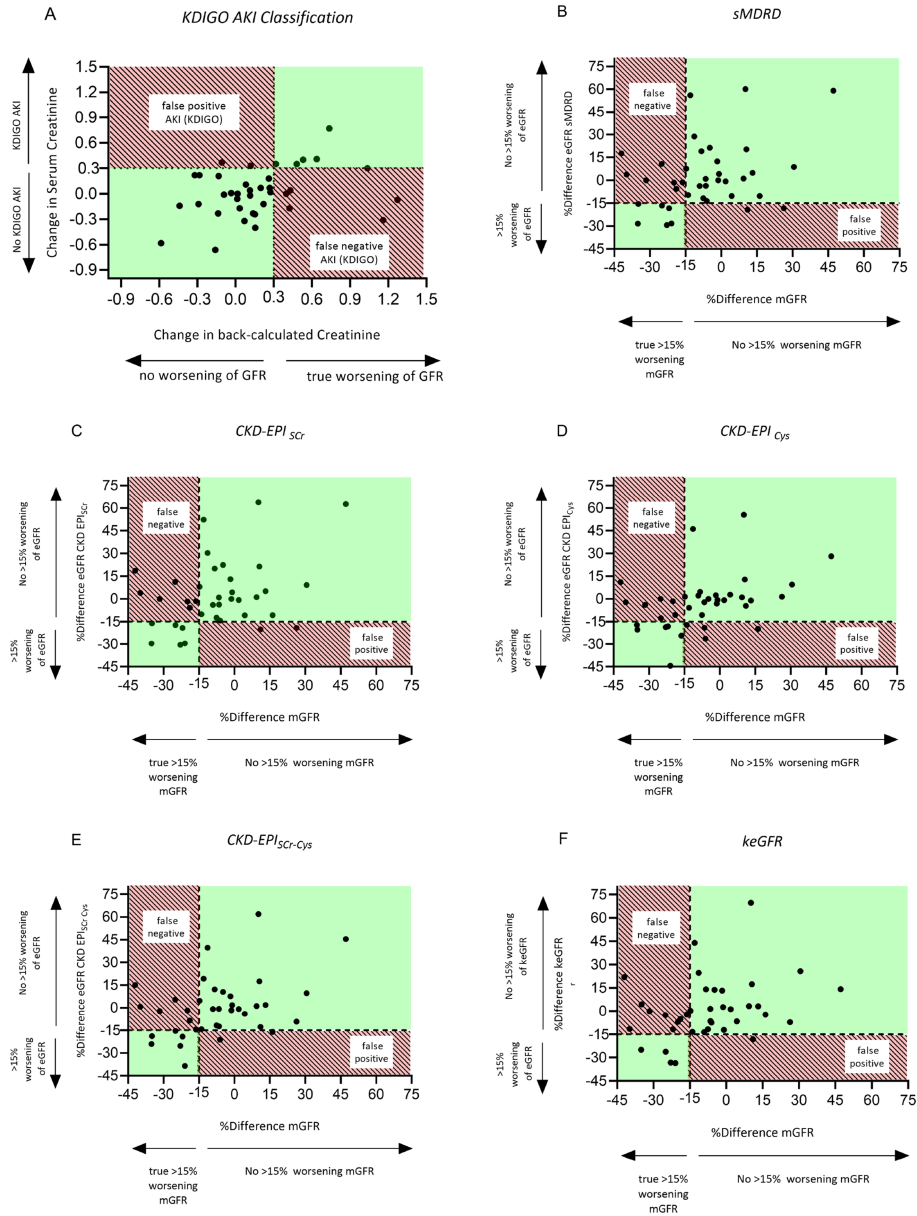
Previous techniques for GFR measurements, such as inulin, iothalamate or iohexol clearance, are time consuming (>5 h), making them less suitable in the setting of worsening GFR.<sup>29</sup> In contrast, the VFI-based technique used in this study allows determination of the clearance rate constant based on a low molecular labelled dextran and PV based on a high molecular weight dextran, allowing accurate GFR calculation within a relatively short time window of 180 min and facilitating repeat GFR measurements.<sup>24</sup> In the current study, plasma kinetics of the low molecular labelled dextran were compatible with glomerular filtration, whereas the high molecular weight dextran was stably retained in the plasma compartment. The VFI technique for GFR measurement has been validated against iohexol GFR measurements,<sup>24</sup> a widely accepted standard technique for GFR assessment.<sup>30</sup> Using the VFI technique as comparator in healthy subjects and CKD patients, the CKD-EPI<sub>SCr</sub>-derived eGFR had a high accuracy ( $P_{30}$  90.6%) and precision ( $r^2$  0.92) and an acceptably small systemic error of 4.4 mL per min per  $1.73 \text{ m}^2$  in predicting mGFR.<sup>24</sup> The first co-primary endpoint of this study was safety, and for this purpose, adverse events were

analysed. Though no severe adverse events were considered related to the VFI compound, a subset of patients exhibited mild or moderate events suggestive of allergic reactions, including two cases of transient pruritus and urticaria, which resolved within 30–60 min after antihistamine treatment. A previously published study using VFI reported no serious adverse events.<sup>24,31</sup> Placebo-controlled data on the VFI compound are currently limited to one small Phase 1 study, in which VFI or placebo was administered in 32 healthy subjects. TEAEs were recorded in 15/24 patients in the VFI group (63%) and in 7/8 patients in the placebo group (88%). None of these adverse events was severe, and the high percentage of events in the placebo group reflects the mild nature of the adverse events.

### Performance of static GFR estimates based on filtration markers in AHF patients

The KDIGO Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease 2012<sup>32</sup> recommends to report GFR estimates that have a comparable or better accuracy ( $P_{30}$ ) than CKD-EPI<sub>SCr</sub> (2009),<sup>27</sup> CKD-EPI<sub>CysC</sub> (2012) and CKD-EPI<sub>SCr-CysC</sub> (2012) ( $P_{30}$  87.2%, 85.9% and 91.5%, respectively<sup>16</sup>). The National Kidney Foundation (NKF) recommends an accuracy performance of at least 75% to be considered in clinical decisions and advises against the use of GFR equations that do not meet this margin as tools in clinical decision making.<sup>33,34</sup> In our AHF cohort, we observed accuracies ( $P_{30}$ ) between 56% and 74%, depending on the equation. Notably, the most frequently applied CKD-EPI<sub>SCr</sub> and sMDRD equations, which rely solely on SCr as a filtration marker, provided  $P_{30}$  values of only 63%–66% and 66%–70%, respectively, which are markedly below recommended thresholds. Among all equations, the CKD-EPI<sub>SCr-CysC</sub> provided the highest

**Figure 2** Changes in mGFR and corresponding changes in SCr and GFR estimates. (A) The difference in  $SCr_{Day1} - SCr_{Day3}$  (in mg/dL), which is used for the KDIGO AKI definition (increase  $\geq 0.3$  mg/dL within 48 h indicates Stage 1 AKI), is plotted against ‘the change of back-calculated creatinine’, that is, the idealized hypothetical SCr values that would have corresponded to  $mGFR_{Day1}$  and  $mGFR_{Day3}$  based on reverse application of the  $CKD-EPI_{SCr}$  equation (for details, see Methods section). (B–F) The percent differences of  $eGFR_{Day3} - eGFR_{Day1}$  (B)  $CKD-EPI_{SCr}$  (C)  $CKD-EPI_{CysC}$  (D)  $CKD-EPI_{SCr-CysC}$  (E)  $sMDRD$  and (F)  $keGFR$  are plotted against the per cent differences in  $mGFR_{Day3} - mGFR_{Day1}$ . Green area represents agreement of AKI diagnosis or GFR loss based on eGFR and mGFR, as appropriate. Red-striped area represents mismatch of AKI diagnosis or GFR loss based on eGFR and mGFR, as appropriate.



(but still suboptimal) accuracy ( $P_{30}$ ) of 71%–74%. Whereas no previous study had evaluated mGFR in AHF patients, several previous studies have assessed mGFR in chronic heart failure patients.<sup>13–15</sup> Published accuracies ( $P_{30}$ ) for different eGFR equations in these patients range from 58% to 89%.<sup>13–15</sup> Together, these data suggest that filtration markers have limited performance in heart failure patients in general, but become even less valid in AHF.

### Performance of filtration markers in dynamically assessing WRF in AHF patients

Changes of SCr or CysC over time are used to diagnose ‘worsening renal function’ or ‘AKI’ according to widely accepted criteria in and outside the heart failure literature.<sup>3,19,28,35,36</sup> Though it is presumed that increases of SCr or CysC indicate



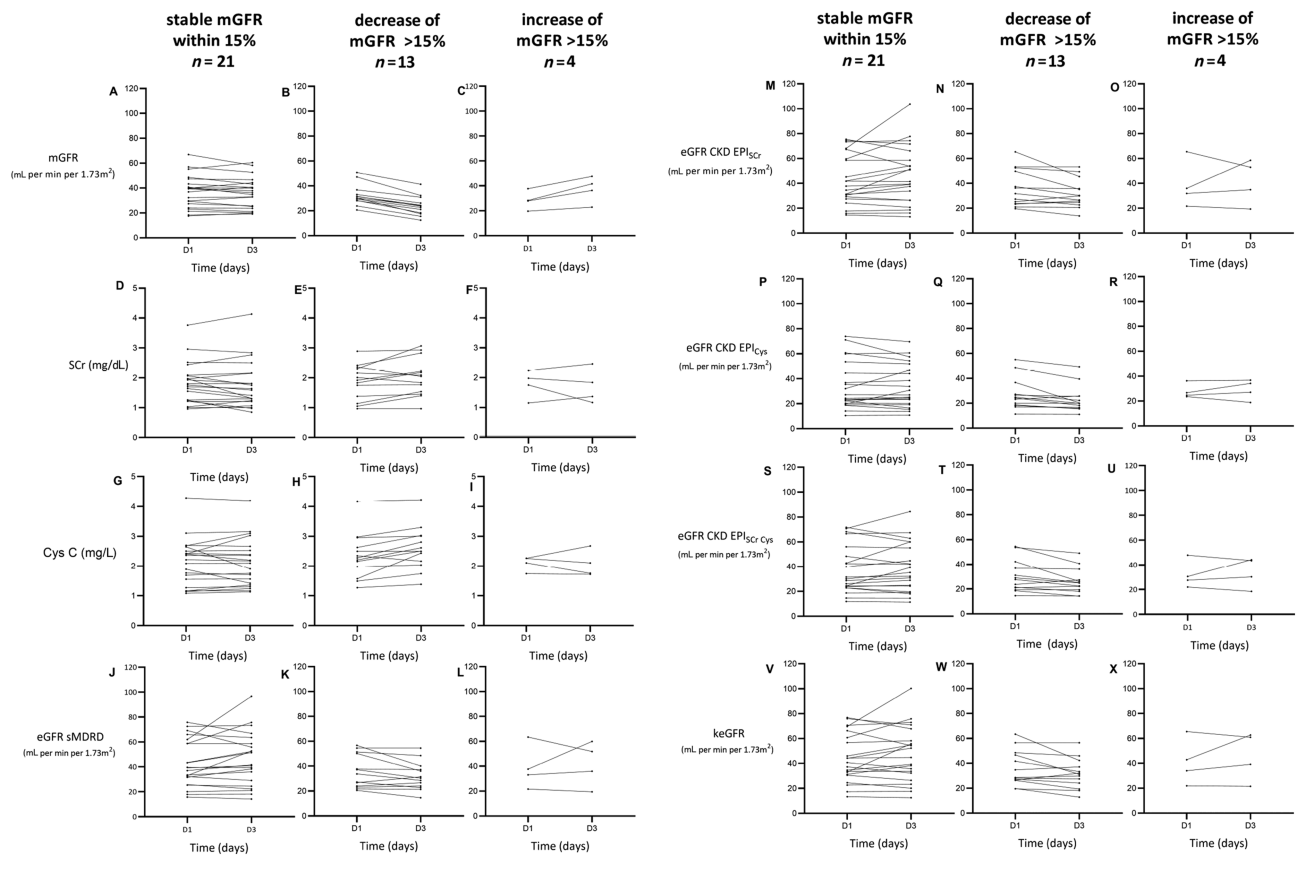
**Table 4** Test characteristics of KDIGO AKI creatinine criteria and of eGFR-based definitions of ‘worsening renal function’ in predicting true GFR loss

	Sensitivity	Specificity	NPV	PPV	+LR	–LR
KDIGO AKI creatinine criteria predicting true corresponding worsening of mGFR	55% (23–83%)	93% (76–99%)	83% (65–94%)	75% (35–97%)	7.4 (1.7–31)	0.5 (0.3–0.9)
>15% eGFR sMDRD loss predicting >15% mGFR loss	46% (19–75%)	92% (74–99%)	77% (58–90%)	75% (35–97%)	5.8 (1.3–24.7)	0.6 (0.3–1)
>15% eGFR CKD-EPI <sub>Scr</sub> loss predicting >15% mGFR loss	46% (19–75%)	92% (74–99%)	77% (58–90%)	75% (35–97%)	5.8 (1.3–24.7)	0.6 (0.3–1)
>15% eGFR CKD-EPI <sub>CysC</sub> loss predicting >15% mGFR loss	46% (19–75%)	84% (64–96%)	75% (55–89%)	60% (26% – 88)	2.9 (0.9–8.4)	0.6 (0.4–1.1)
>15% eGFR CKD-EPI <sub>Scr-CysC</sub> loss predicting >15% mGFR loss	46% (19–75%)	92% (74–99%)	77% (58% – 90%)	75% (35% – 97%)	5.8 (1.3–24.7)	0.6 (0.3–1)
>15% keGFR loss predicting >15% mGFR loss	38% (14–68%)	92% (74–99%)	74% (55–88%)	71% (29–96%)	4.8 (1.1–21.5)	0.7 (0.4 – 1)

Data are presented as estimates (95% CI) and rounded to the nearest integer.

+LR, positive likelihood ratio; –LR, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

**Figure 3** Time courses of SCr (D–F), CysC (G–I) and different GFR estimates: sMDRD (J–L), CKD-EPI<sub>Scr</sub> (M–O), CKD-EPI<sub>Cys</sub> (P–R), CKD-EPI<sub>ScrCys</sub> (S–U), keGFR (V–X) in patients with stable mGFR (difference of  $mGFR_{Day3} - mGFR_{Day1} > -15\%$  and  $< +15\%$ ;  $n = 21$  (A)), true decrease of (difference of  $mGFR_{Day3} - mGFR_{Day1} \leq -15\%$ ;  $n = 13$  (B)) and true increase of mGFR (difference of  $mGFR_{Day3} - mGFR_{Day1} \geq +15\%$ ;  $n = 4$  (C)) within 48 h.



decreasing GFR, no studies with serial mGFR assessments are available to date.

In this study of AHF patients, we found that the dynamic changes of SCr, CysC and eGFRs estimated with several equations provided poor estimates of the actual mGFR time

course. In addition, the presumptive decline in GFR underlying KDIGO SCr-based AKI criteria weakly correlated to changes of mGFR. Endogenous filtration markers like SCr or CysC require steady-state conditions to provide proper estimates of GFR.<sup>37</sup> Kinetic eGFR calculated based on SCr

dynamics was previously proposed to provide a more accurate account for acute changes of SCr levels based on mathematical models,<sup>18</sup> but it has not been validated against GFR measurements. Here, we show that keGFR did not substantially outperform other eGFR equations both as a static GFR estimate and as an estimate of GFR changes over time.

These observations indicate that GFR-independent determinants of SCr and CysC influence their blood levels and therefore contribute to the weak performance of eGFR equations in AHF. Sarcopenia, immobilization and cardiac cachexia in heart failure patients may contribute to reduced creatinine production from muscle tissue.<sup>38–40</sup> CysC is considered largely independent of muscle wasting and sarcopenia, but an association between elevated CysC levels and higher fat mass has been described.<sup>41</sup> The lower muscle mass and higher fat percentage in elderly heart failure patients could partially explain the observed averaged underestimation of mGFR by CKD-EPI<sub>CysC</sub> and overestimation by CKD-EPI<sub>SCr</sub>. In addition, the expansion of extracellular space in AHF patients enlarges the volume of distribution of creatinine, thereby potentially lowering SCr and CysC concentration. Conversely, quick contractions of extracellular fluid during decongestive therapy may lead to GFR-independent increases of SCr and CysC levels.

There is evidence that increased CysC levels are associated with risk of cardiovascular diseases<sup>42</sup> and CKD-EPI<sub>CysC</sub> has been shown to have a better predictive value for adverse outcomes in AHF patients than CKD-EPI<sub>SCr</sub> or CKD-EPI<sub>SCr-CysC</sub>.<sup>43</sup> Consistently, in our cohort, we observed a more accurate, but still imperfect, reflection of mGFR changes by CysC.

## Clinical impact

During clinical management in AHF, the detection of eGFR decreases will often trigger important clinical decisions. However, our study shows that SCr has limited value in identifying decreasing or increasing GFR in AHF patients. This may result in inappropriate dose adjustments of medications, including renin–angiotensin–aldosterone (RAAS) inhibitors and diuretics, deferral of procedures involving contrast agents or discontinuation of potentially nephrotoxic drugs. In addition, a failure to detect true worsening GFR in these subjects may lead to prolonged kidney injury, resulting in CKD progression.<sup>44,45</sup> Detecting an increasing GFR based on SCr reductions is often viewed as a sign of therapeutic success, but our data suggest that a decrease of SCr may not always be accompanied by corresponding increases of mGFR. Hence, the limitations of SCr in AHF need to be considered clinically.

To date, the ability to measure GFR in a timely fashion that would influence clinical decision making has not been possible. Therefore, the potential impact of mGFR on clinical decision making is unknown. However, a multitude of studies assessed the prognostic value of rises in SCr or CysC or decreases of eGFR (defined as ‘worsening renal function’)

during decongestive therapy. But neither rise in SCr nor increase in tubular injury biomarkers was found to be associated with post-discharge adverse outcomes.<sup>8,46</sup> Our data suggest that ‘worsening renal function’ in these studies may not necessarily have been a reflection of true worsening of mGFR. Hence, the prognostic impact of true GFR loss in AHF should be evaluated in order to assess the relationship between actual functional loss and adverse outcomes. It is likely that knowing the dynamics of mGFR during therapy for AHF patients will result in more complete, effective and safe clinical decisions.

## Study limitations

Our study has limitations. First, our cohort of patients with AHF was predominantly male and almost exclusively Caucasian. Because patients in intensive care units receiving i.v. vasodilators and inotropics were excluded, the results of this study cannot be generalized to these subsets of heart failure patients. Second, the sample size of the study was relatively small (50 patients) and repeat GFR assessments (Day 3) were only available in a sub-cohort (38 patients). Third, though all patients enrolled into the study were undergoing decongestive therapy for AHF, the timing of mGFR assessments varied relative to hospital admission. Fourth, intensity and duration of diuretic therapy were not standardized. Hence, the serial GFR measurements within a 48-h window provided a snapshot of GFR dynamics but did not reflect the GFR trend of the whole decongestive treatment process. Finally, the cohort was rather heterogeneous with regard to baseline characteristics and included both HFrEF and HFpEF patients with ischaemic and non-ischaemic causes. The different degree of neurohumoral activity, patterns of cardiac remodelling, and differential response to drug therapies suggest that HFpEF and HFrEF are two distinct disease processes.<sup>47,48</sup> Our study was not powered to investigate the influence of HF type on mGFR trajectories.

## Conclusion

In this prospective study of patients with AHF undergoing decongestive therapy, we found that frequently used clinical estimates of GFR based on the filtration markers SCr and CysC failed to meet previously recommended margins of accuracy and precision. Most importantly, changes of filtration markers and their derived GFR estimates frequently failed to detect true worsening of mGFR. Our findings highlight the limitations of filtration markers to evaluate dynamics of kidney function in patients with AHF and suggest that caution should be applied when using these markers to guide clinical decision making. Measuring GFR directly using minimally invasive techniques, such as the VFI technique utilized here, could provide a practical alternative to ensure accurate monitoring of GFR.

## Conflict of interest

JSS: none, NPN: none, DML: none, FE: none, FK: none, ET: none, CL: none, RR: none, MH: consultant to FAST BioMedical, RMC: consultant to FAST BioMedical, GR: none, VM: none, EG: none, DM: Employee of FAST BioMedical, PAM, consultant To FAST BioMedical, KUE: none, BAM: Founder, partial owner and Medical Director of FAST BioMedical, KMSO: received grant support, paid to Charité Berlin, from FAST BioMedical.

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## Permission note

All material is original to this submission.

## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Equations for GFR estimation.

**Table S2.** Adverse Events Considered Possibly, Probably, or Surely Related to the VFI Compound.

**Table S3.** mGFR, endogenous filtration markers and estimates of GFR.

**Table S4.** Performance of Scr- and CysC-based equations when compared with measured GFR in AHF patients at day 3.

**Figure S1.** Scatterplots of distribution and cumulative proportions of eGFR and mGFR at enrollment (day 1).

**Figure S2.** Scatterplots of distribution and cumulative proportions of eGFR and mGFR on day 3.

**Figure S3.** Agreement between estimates of GFR versus measured GFR at enrollment (day 1).

**Figure S4.** Correlation of percentage 48 h changes of eGFR with corresponding percentage 48 h changes of mGFR.

**Figure S5.** Correlation of percentage 48 h changes of Scr (A) and of CysC (B) with corresponding 48 h changes of mGFR.

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