



Discordance between estimated and measured changes in plasma volume among patients with acute heart failure

Jutta S. Swolinsky¹ , Enkhtuvshin Tuvshinbat¹, David M. Leistner^{2,3,4}, Frank Edelmann^{3,4,5}, Fabian Knebel⁶, Niklas P. Neger¹, Caroline Lemke¹, Robert Roehle^{3,7,8}, Michael Haase^{9,10}, Maria Rosa Costanzo¹¹, Geraldine Rauch⁷, Veselin Mitrovic¹², Edis Gasanin¹², Daniel Meier¹³, Peter A. McCullough¹⁴, Kai-Uwe Eckardt¹, Bruce A. Molitoris^{13,15} and Kai M. Schmidt-Ott^{1,3,16*} 

¹Department of Nephrology and Medical Intensive Care, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Hindenburgdamm 30, Berlin, 12203, Germany; ²Department of Internal Medicine and Cardiology, Campus Benjamin Franklin, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; ³Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Germany; ⁴DZHK (German Centre for Cardiovascular Research), partner site Berlin, Berlin, Germany; ⁵Department of Internal Medicine and Cardiology, Campus Virchow Klinikum, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; ⁶Department of Cardiology and Angiology, Campus Mitte, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; ⁷Institute of Biometry and Clinical Epidemiology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; ⁸Clinical Study Center, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany; ⁹Medical Faculty, Otto von-Guericke-University Magdeburg, Magdeburg, Germany; ¹⁰Diaverum Renal Care Center, Potsdam, Germany; ¹¹Advocate Heart Institute, Naperville, IL, USA; ¹²Kerckhoff Klinik, Bad Nauheim, Germany; ¹³FAST BioMedical, Indianapolis, IN, USA; ¹⁴Cardiorenal Society of America, Phoenix, AZ, USA; ¹⁵Indiana University, Indianapolis, IN, USA; and ¹⁶Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

Abstract

Aims In acute heart failure (AHF), changes of venous haemoglobin (Hb) concentrations, haematocrit (Hct), and estimated plasma volume (ePV) have been proposed as surrogates of decongestion. These estimates are based on the theoretical assumptions that changes of Hb concentrations and Hct are driven by the intravascular volume status and that the intravascular Hb pool remains stable. The objective of this study was to assess the relationship of changes of measured plasma volume (mPV) with changes of Hb, Hct, and ePV in AHF.

Methods and results We studied 36 AHF patients, who received two sequential assessments of mPV, measured red cell volume (mRCV) and measured total blood volume (mTBV) (48 h apart), during the course of diuretic therapy using a novel visible fluorescent injectate (VFI) technique based on the indicator dilution principle. Changes of ePV were calculated based on the Kaplan–Hakim or Strauss formula. AHF patients receiving diuretics (median intravenous furosemide equivalent 160 mg/48 h) displayed a wide range of changes of mPV (−25.4% to +37.0%). Changes in mPV were not significantly correlated with changes of Hb concentration [Pearson's r (r) = −0.241, P = 0.157], Hct (r = −0.307, P = 0.069), ePV_{Kaplan–Hakim} (r = 0.228, P = 0.182), or ePV_{Strauss} (r = 0.237, P = 0.163). In contrast to theoretical assumptions, changes of mTBV were poorly correlated with changes of Hb concentrations and some patients displayed unanticipated variability of mRCV, suggesting an unstable intravascular red cell pool.

Conclusions Changes of Hb or Hct were not reflective of directly measured changes of intravascular volume status in AHF patients. Basing clinical assessment of decongestion on changes of Hb or Hct may misguide clinical decision-making on an individual patient level.

Keywords Acute heart failure; Estimated plasma volume (ePV); Measured plasma volume (mPV); Strauss' formula; Haematocrit

Received: 22 July 2021; Revised: 4 October 2021; Accepted: 11 November 2021

*Correspondence to: Kai M. Schmidt-Ott, Department of Nephrology and Medical Intensive Care, Charité - Universitätsmedizin Berlin, Hindenburgdamm 30, 12203 Berlin, Germany. Tel: +49 30 450 414 671; Fax: +49 30 450 514 971. Email: kai.schmidt-ott@charite.de

Introduction

More than 90% of hospitalizations in heart failure (HF) patients are due to signs and symptoms of fluid overload, and congestion remains the primary challenge in acute HF (AHF).¹ Monitoring congestion is important in all stages of HF management: in the outpatient setting to prevent decompensation, in the emergency department to identify AHF, and during the course of decongestive treatment to guide therapy and to identify patients nearing discharge from an AHF hospitalization.

The integrative assessment of congestion and volume status is based on clinical congestion scores, biomarkers, and imaging tools.² Clinical assessment, containing, for example, change in body weight, oedema grade, and dyspnoea grade, is typically performed repeatedly within routine care, but evaluation is error prone or examiner dependent and requires clinical expertise. Clinical scores are time consuming and limited in detecting mild congestion.

It has been suggested that changes in the readily available parameters haemoglobin (Hb) concentration and haematocrit (Hct) may provide surrogates of effective decongestion and, hence, may be helpful in evaluating the effects of therapies in AHF patients.^{2,3} This suggestion is based on several large retrospective post hoc studies that have indicated an association between haemoconcentration (defined by increasing Hb concentrations or Hcts) and beneficial clinical outcomes in patients hospitalized for AHF.^{4–8}

Formula-based estimates of absolute plasma volume (PV) using the Kaplan–Hakim formula⁹ are based on Hct, sex, and body weight. Several studies have indicated a moderate to good correlation of measured plasma volume (mPV) and estimated plasma volume (ePV) by Kaplan–Hakim.^{10,11} An estimated percentage change in plasma volume (% Δ ePV) between two time points can be obtained by sequentially applying the Kaplan–Hakim formula or by using the Strauss formula, which considers changes of Hct and Hb concentrations (Supporting Information, *Figure S1*).¹² Similar to the outcome studies above, an increasing ePV based on the Strauss formula has been associated with unfavourable outcomes in AHF patients,^{13,14} while a decreasing Strauss-based ePV at hospital discharge was associated with decreased AHF-related readmissions and mortality.¹⁵

Nevertheless, it is unclear whether changes of Hb concentration, Hct, and ePV based on the Kaplan–Hakim or Strauss formula have clinical benefit in guiding therapy on an individual patient level, because prospective clinical studies are missing. In addition, changes of Hb concentration and Hct have never been tested against direct mPV in AHF patients. Definitions of haemoconcentration and Strauss' formula assume that changes in Hb concentrations over time are inversely proportional to changes of intravascular total blood volume (TBV). This may be true if the total intravascular Hb mass is stable and evenly distributed in different vascular

beds. An additional theoretic assumption that has not been validated is that changes in Hct over time reflect the changes in the ratio of red cell volume (RCV) to TBV.¹⁶ However, each of these assumptions may be violated in AHF patients, who frequently experience blood losses or receive blood transfusions during hospitalization, events that will alter RCV, Hct, and Hb concentrations independently of changes in PV. In addition, redistribution of red blood cells between the central and the splanchnic circulations may occur in AHF, which may induce dynamic changes of peripheral venous Hb concentrations and Hct independent of changes in TBV.¹⁷

In this study, we determined changes of mPV in AHF patients during 48 h of decongestive therapy based on the indicator dilution principle using a novel intravenously injected fluorescence dye labelled dextran. This allowed us to compare Hb concentration changes, Hct changes, and ePV changes with directly measured changes of PV. In addition, we were able to investigate whether theoretical assumptions underlying Hb-based and Hct-based definitions of 'congestion' and 'decongestion' and Strauss' formula-based PV estimates may be violated in AHF patients. These data represent the first time these associations have been tested.

Methods

Study cohort

The data presented are results of a pre-specified secondary analysis from a prospective phase 2b study that enrolled hospitalized patients who were treated for AHF using decongestive therapy (study identifier, EmPaKt-CHF; EudraCT number, 2018-002638-18, <https://clinicaltrials.gov/ct2/show/NCT03808948>), which was performed between January and July 2019 at the nephrology and two cardiology departments of Charité – Universitätsmedizin Berlin and at the cardiology department of Kerckhoff Klinik Bad Nauheim.¹⁸ Eligible participants were aged ≥ 18 years and had a diagnosis of AHF and were undergoing either intravenous or oral diuretic treatment. Evidence of AHF was based on presence of ≥ 1 symptom (dyspnoea, orthopnoea, or oedema) and ≥ 1 sign (rales, peripheral oedema, ascites, or pulmonary vascular congestion on chest radiography).¹⁹ 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 kidney replacement therapy, significant non-cardiac diseases (e.g. malignancies and neurodegenerative diseases), severe infections, and internal bleeding. Nonsterile participants agreed to use effective methods of contraception.

All 50 study subjects received a quantitative measure of PV at enrolment. A subset of 36 patients with a second PV

measurement 48 h after enrolment was analysed in this study, and changes in mPV were compared with changes of formula-based estimates of PV. Treating physicians were blinded to the directly mPV values.

Participants gave written informed consent. Data were handled in respect of patient anonymity and confidentiality. The study protocol was approved by the regional ethics board and conducted in accordance with the Declaration of Helsinki guidelines. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Study-related parameters

Plasma volume was measured after enrolment (Day 1) and 48 ± 5 h after the first measurement (Day 3). mPV was determined using visible fluorescent injectate (VFI) (FAST Biomedical, Indianapolis) as previously described.²⁰ VFI consists of 12 mg of a 150 kDa carboxymethylated dextran, conjugated to a rhodamine dye (FD003) that allows repeat PV measurements for at least 6 h after injection, and 35 mg of a 5 kDa carboxymethylated dextran, conjugated to fluorescein (FD001), that is freely filtered by the kidney. The VFI technique can measure both PV and glomerular filtration rate (GFR) in a clinically actionable fashion.^{20,21} The large PV fluorescent marker has a half-life of nearly 100 h in the vascular system, and the small GFR marker's clearance is determined by the subject's GFR.

A volume of 3.0 mL was injected intravenously over 30 s. Blood samples of 3 mL 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. PV was determined using the average FD003 concentrations of the early 15 min and the 60 min time point using the indicator dilution principle after distribution in the vascular system. Functionality and safety of the VFI technique, including stable plasma concentrations of the PV marker FD003 over the 15 and 60 min time points, are reported elsewhere.¹⁸

Venous blood (3 mL) was drawn to determine Hb concentration and Hct before injection of the fluorescent tracer to calculate ePV according to the Strauss formula.¹² Hct was determined by multiplying the red cell count by the mean cell volume (Labor Berlin, Germany). Hb concentration was measured photometrically (Labor Berlin). Measured red cell volume (mRCV) was calculated from mPV and total body haematocrit (TBHct²²):

$$\text{mRCV} = \frac{\text{mPV}}{(1 - \text{TBHct})} - \text{mPV}$$

Measured total blood volume (mTBV) was calculated from mPV and TBHct²²:

$$\text{mTBV} = \frac{\text{mPV}}{(1 - \text{TBHct})}$$

Estimated PV was calculated using the Kaplan–Hakim formula⁹:

$$\text{ePV}_{\text{Kaplan-Hakim}} = (1 - \text{haematocrit}) \times [a + (b \times \text{weight in kg})]$$

$a = 1530$ in males and 864 in females; $b = 41$ in males and 47.9 in females.

Figure 1 Study flow chart. PV, plasma volume; VFI, visible fluorescent injectate.

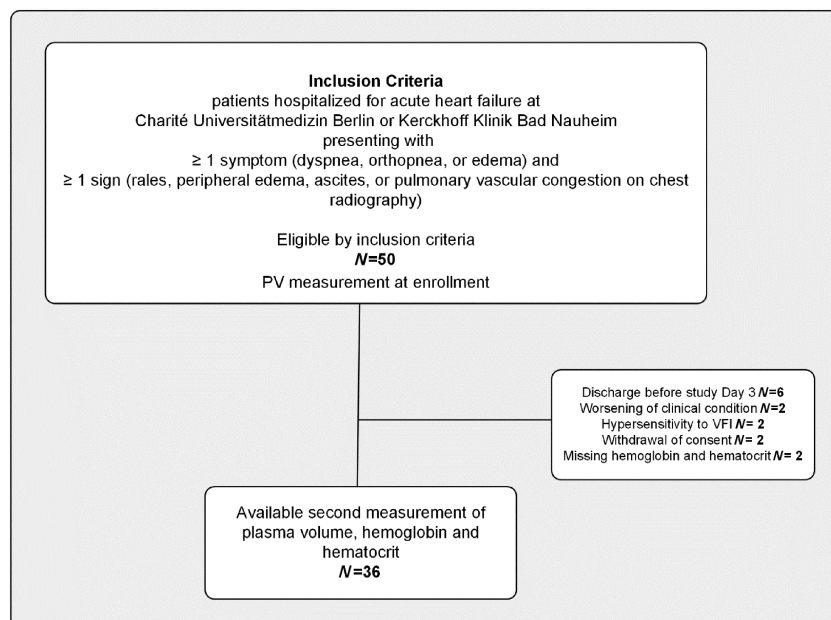


Table 1 Baseline characteristics of the cohort at enrolment (Day 1)

	All patients
	36
Demographic data	
Age (years)	72.6 ± 13.1
Male	27 (75)
Caucasian	35 (97.2)
Clinical data at enrolment	
Weight (kg)	89.9 ± 23.7
BMI (kg/m ²)	30.1 ± 6
BSA (m ²) ^a	2.0 ± 0.31
Systolic blood pressure (mmHg)	126.7 ± 24.8
Diastolic blood pressure (mmHg)	72.1 ± 15.4
Heart rate (b.p.m.)	75.2 ± 13.5
NYHA Class II	4 (11.1)
NYHA Class III–IV	32 (88.9)
LVEF (%) ^b	46.9 ± 14.2
HFpEF	22 (61.1)
HFmrEF	5 (13.9)
HFrEF	8 (22.2)
AHF type	
Acute decompensation of chronic heart failure	35 (97.2)
De novo AHF	1 (2.8)
Clinical presentation	
Acute decompensated heart failure	23 (63.9)
Acute pulmonary oedema	11 (30.6)
Isolated right ventricular failure	2 (5.6)
Factors triggering AHF	
Ischaemia	3 (8.3)
Valvular disease	6 (16.6)
Worsening of pulmonary disease	3 (8.3)
Infection	2 (5.6)
Non-adherence of salt/fluid intake or medication	7 (19.4)
Uncontrolled arrhythmia	3 (8.3)
Uncontrolled hypertension	2 (5.6)
Anaemia	1 (2.8)
Renal impairment	4 (11.1)
Unknown	4 (11.1)
Comorbidities	
Diabetes	17 (47.2)
Coronary artery disease	25 (69.4)
Hypertension	33 (91.7)
CKD Stage 1–2	15 (41.7)
CKD Stage ≥3	21 (58.3)
Medication before hospitalization	
Oral loop diuretic	29 (80.6)
RAASI	27 (75)
Thiazide diuretic	4 (11.1)
Aldosterone antagonist	14 (38.9)
Beta-blocker	28 (77.8)
Medication at enrolment	
Intravenous loop diuretic	30 (83)
Oral loop diuretic only	6 (17)
RAASI	27 (75)
Thiazide diuretic	5 (13.9)
Aldosterone antagonist	15 (39.5)
Beta-blocker	29 (76.3)
Intravenous furosemide equivalent dose at enrolment day (mg) ^c	80 (60–135)
Laboratory values at enrolment	
eGFR CKD EPI _{Scr} (mL/min/1.73 m ²)	39.3 ± 17.4
NT-proBNP (ng/L) ^b	3895 (1822–82 93)
Hct (%)	34.9 ± 6.9
Hb (g/dL)	11.6 ± 2.3
Volume measures and estimates	
mTBV (L)	5.1 ± 1.3
mRCV (L)	1.6 ± 0.5
mPV (L)	3.5 ± 0.9

AHF, acute heart failure; BMI, body mass index; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; Hct, haematocrit; 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; mPV, measured plasma volume; mRCV, measured red cell volume; mTBV, measured total blood volume; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; RAASI, renin-angiotensin-aldosterone system inhibitors.

Data are presented as median and interquartile range or number and percentage, as appropriate. Data are rounded to one decimal place except NT-proBNP (next integer).

^aBSA = body surface area according to Dubois *et al.*³⁰

^bMissing data: *n* = 1.

^cFurosemide equivalent dose: 20 mg furosemide i.v. ≅ 40 mg furosemide p.o. ≅ 20 mg torasemide p.o.

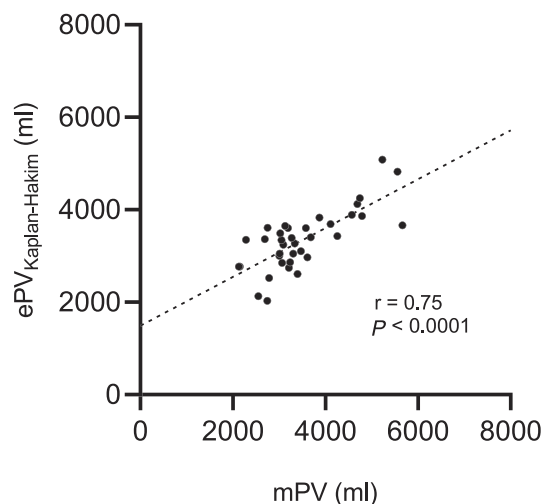
Estimated 48 h change of PV was calculated using percentage change of the Kaplan–Hakim formula-derived estimates (%ΔePV_{Kaplan–Hakim}):

$$\% \Delta ePV_{\text{Kaplan-Hakim}} = \frac{ePV_{\text{Kaplan-Hakim}}(\text{Day 3}) - ePV_{\text{Kaplan-Hakim}}(\text{Day 1})}{ePV_{\text{Kaplan-Hakim}}(\text{Day 1})} \times 100$$

Estimated 48 h change of PV was also calculated using the Strauss formula (%ΔePV_{Strauss})¹² where Day 1 designates enrolment day and Day 3 the day of second PV measurement 48 h later:

$$\% \Delta ePV_{\text{Strauss}} = 100 \times \left[\left(\frac{\text{Hb}(\text{Day 1})}{\text{Hb}(\text{Day 3})} \right) \times \left(\frac{1 - \text{Hct}(\text{Day 3})}{1 - \text{Hct}(\text{Day 1})} \right) \right] - 100$$

Figure 2 Correlation of mPV and the corresponding ePV based on the Kaplan–Hakim formula. Pearson’s correlation coefficient (*r*) and the associated *P*-value are shown. ePV, estimated plasma volume; mPV, measured plasma volume.

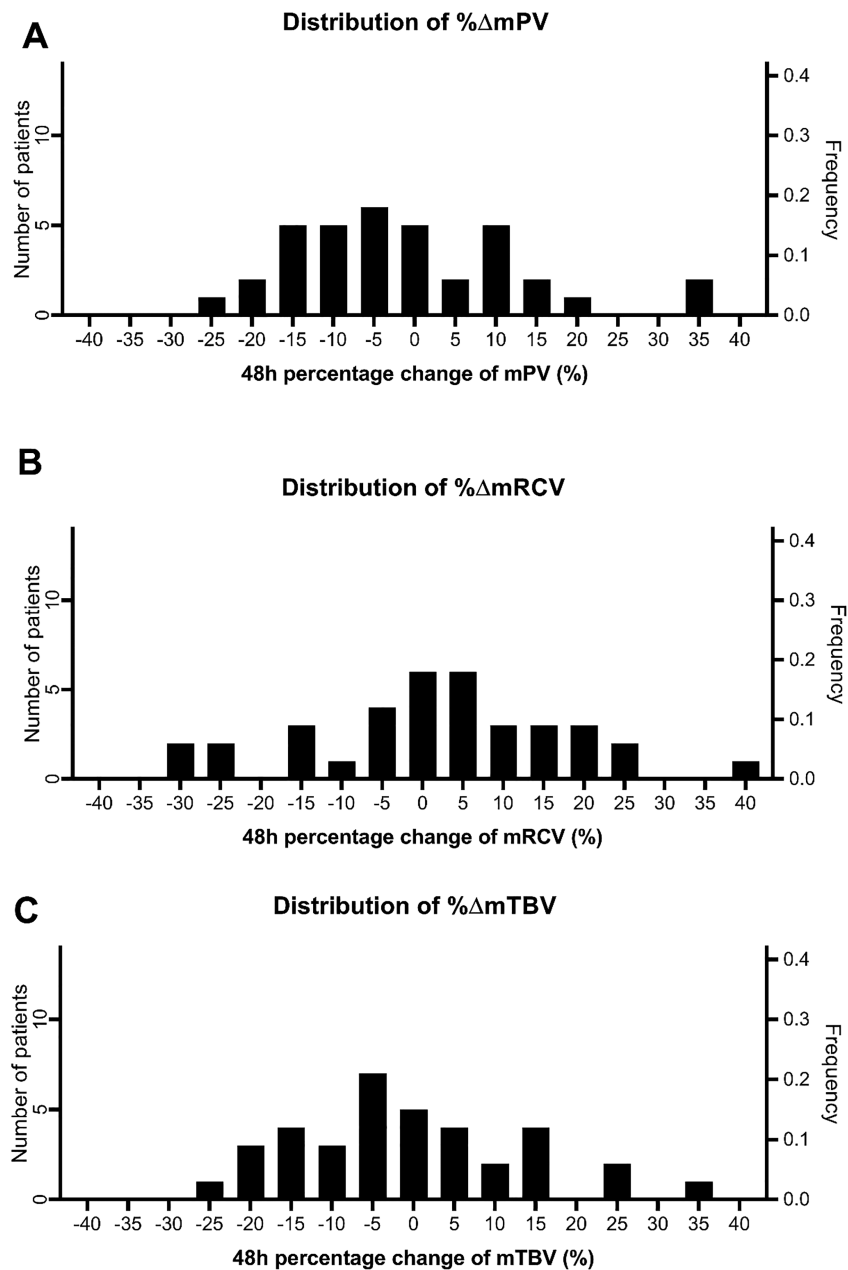


Statistical analyses

Descriptive analyses were performed 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 (IQR) for skewed distributed data, and frequencies with percentages for categorical variables. The Wilcoxon test was used to compare dependent samples for continuous variables.

Correlations between percentage changes in mPV and ePV were determined using Pearson's correlation coefficients. The Bland–Altman plots were used to analyse the agreement of percentage changes in mPV and percentage changes in ePV. Statistical analyses were performed using SPSS (Chicago) Version 25 and GraphPad Prism Version 8. 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.

Figure 3 Frequency distribution of percentage changes of (A) mPV, (B) mRVC, and (C) mTBV within 48 h of diuretic treatment. mPV, measured plasma volume; mRVC, measured red cell volume; mTBV, measured total blood volume.



Results

Figure 1 represents a flow chart of the inclusion of 36 AHF patients who had two sequential PV measurements during the course of decongestive therapy. The first measurement was performed 2 days (median; IQR 1–3 days) after hospital admission (Day 1), and the second measurement was performed 48 ± 5 h later (Day 3). The administered intravenous furosemide equivalent dose was 80 mg (median; IQR 60–135 mg) on Day 1 and 70 mg (median; IQR 32.5–120 mg) on Day 2. Cumulative median 48 h dose was 160 mg/48 h (IQR 102.5 to 240 mg/48 h).

The demographics and clinical characteristics for the study cohort of 36 patients are shown in Table 1. Most of the patients were male (75%) and Caucasian (97.2%). The mean age was $72.6 (\pm 13.1)$ years. The majority of patients had heart failure with a preserved ejection fraction (HFpEF, 61.1%) and pre-existing chronic kidney disease (CKD) Stage 3 or higher (58.3%).

First, we evaluated the correlation and agreement of a Kaplan–Hakim formula-based estimate of absolute PV ($ePV_{\text{Kaplan-Hakim}}$) with mPV on enrolment. While we found a significant positive correlation between $ePV_{\text{Kaplan-Hakim}}$ and mPV ($r = 0.75, P < 0.0001$; Figure 2), the Bland–Altman plots revealed relatively wide limits of agreement between $ePV_{\text{Kaplan-Hakim}}$ and mPV (-1345 to $+1022$ mL) and a bias of -161 mL, indicating that $ePV_{\text{Kaplan-Hakim}}$ slightly underestimated mPV (Supporting Information, Figure S2).

The cohort demonstrated a wide spectrum of changes of mPV, mRCV, and mTBV during 48 h of diuretic therapy (Figure 3). Changes of mPV ranged from -25.4% to $+37.0\%$, changes of mRCV ranged from -30.9% to $+38.1\%$, and changes of mTBV ranged from -25.3% to $+34.0\%$. Most patients displayed decreases of mPV and mTBV during the 48 h of decongestive therapy. In contrast, fewer patients displayed decreases of mRCV and the distribution of changes of mRCV was wide, suggesting an unanticipated variability of mRCV in some patients.

We next investigated average changes in mPV, mRCV, mTBV, Hb concentration, Hct, and body weight during 48 h of decongestive therapy on a population scale (Table 2). While body weight decreased significantly from Day 1 to Day 3 (89.9 ± 23.7 kg vs. 88.8 ± 22.7 kg, $P = 0.001$), none of the other parameters displayed significant population-wide differences during the time course of decongestive therapy. This was consistent with the wide ranges of changes described above. In agreement with a previous study,²³ we observed no apparent relationship between the amount of change in PV and the change in body weight (Supporting Information, Figure S3).

We next compared changes in mPV with changes in Hb concentrations, in Hct, and in ePV (based on the Kaplan–Hakim and Strauss formula) during the 48 h study period. Correlation analyses indicated that changes in mPV displayed

Table 2 Absolute and percentage changes in plasma volume, total blood volume, red cell volume, and the Kaplan–Hakim and Strauss estimates in acute heart failure patients undergoing decongestive therapy

	mPV (L)	mTBV (L)	mRCV (L)	$ePV_{\text{Kaplan-Hakim}}$ (L)	% $\Delta ePV_{\text{Strauss}}$	Hct (%)	Hb (g/dL)	Body weight (kg)
Enrolment value	3.5 ± 0.9 (2.1 to 5.7)	5.1 ± 1.3 (3.0 to 8.2)	1.6 ± 0.5 (0.8 to 2.9)	3.3 ± 0.6 (2.0 to 5.1)	-	34.94 ± 6.9 (23.0 to 50.0)	11.6 ± 2.3 (7.1 to 16.4)	89.9 ± 23.7 (49.7 to 167.2)
48 h value	3.4 ± 0.9 (2.1 to 5.8)	5.0 ± 1.3 (2.9 to 8.6)	1.6 ± 0.5 (0.8 to 3.1)	3.3 ± 0.7 (1.9 to 5.0)	-	35.2 ± 6.3 (26.2 to 53.0)	11.6 ± 2.1 (8.6 to 17.7)	$88.8 \pm 22.7^*$ (48.3 to 158)
Absolute change	-0.06 ± 0.5 (-0.8 to +1.2)	-0.06 ± 0.7 (-1.2 to +1.5)	0.01 ± 0.3 (-0.7 to +0.6)	-0.03 ± 0.2 (-0.4 to +0.5)	-	0.23 ± 3.56 (-10.8 to +9.2)	0.06 ± 0.89 (-2.1 to +2.6)	-1.04 ± 1.95 (-9.2 to +1.6)
Percentage change	$-1.1 \pm 14.3\%$ (-25.4% to +37.0%)	$-0.6 \pm 13.6\%$ (-25.3% to +34.0%)	$+1.6 \pm 16\%$ (-30.9% to +38.1%)	-0.9 ± 5.7 (-13.3% to +18.77%)	$-0.13 \pm 13.9\%$ (-33.1% to +41.5%)	$1.5 \pm 10.34\%$ (-25.3% to +28.8%)	$0.9 \pm 9.0\%$ (-16.0% to +36.6%)	$-1.04 \pm 1.7\%$ (-5.5% to +1.39%)

ePV, estimated plasma volume; Hb, haemoglobin; Hct, haematocrit; mPV, measured plasma volume; mRCV, measured red cell volume; mTBV, measured total blood volume. Data are presented as mean \pm standard deviation (range). Data are rounded to one decimal place except absolute volume changes (two decimal places). * $P = 0.001$ significant change from enrolment value.

no significant correlation with changes in Hb concentrations ($r = -0.241$, $P = 0.157$), changes in Hct ($r = -0.307$, $P = 0.069$), changes in ePV_{Strauss} ($r = 0.237$, $P = 0.163$), or changes in $ePV_{\text{Kaplan-Hakim}}$ ($r = 0.228$, $P = 0.182$) (Figure 4).

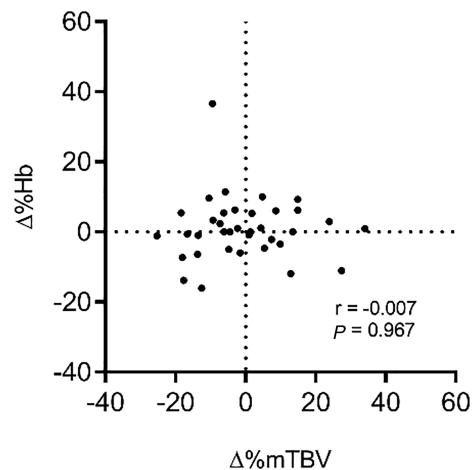
The Bland-Altman plots of $\Delta\%ePV$ and $\Delta\%mPV$ showed wide limits of agreement (from -27.4% to $+27.9\%$ for the Kaplan-Hakim and from -32.8% to $+35.4\%$ for the Strauss formula; Supporting Information, Figure S4), confirming the poor agreement between formula-derived estimated changes and actual measured changes in PV. The accuracies of the Kaplan-Hakim and Strauss formula in identifying a percentage change of ePV that was within ± 10 percentage points of the mPV change were only 50%, respectively.

We next aimed to obtain insights into the causes for these limited associations of ePV estimates (Strauss' formula) with actual changes of PV. We performed correlation analyses and found that the percentage changes of Hb concentrations and percentage changes of measured TBV did not correlate ($r = -0.007$, $P = 0.967$; Figure 5). This indicated that a key assumption underlying Strauss' formula, namely, the negative correlation of Hb concentration changes and TBV changes, was not fulfilled in AHF patients.

We found that 19 patients had a relatively stable RCV (change of RCV less than $\pm 10\%$). In these patients, the Strauss formula performed well, providing a significant and positive correlation of ePV with changes of mPV ($r = 0.779$, $P < 0.0001$; Figure 6A). In contrast, 17 patients showed unstable RCV (change of RCV more than $\pm 10\%$). In these patients, ePV displayed no correlation with actual changes of mPV ($r = 0.089$, $P = 0.734$; Figure 6B).

Strauss' formula is also based on the assumption that PV and RCV undergo changes of the same directionality; that is, an increase of PV would be accompanied by an increase of RCV and vice versa. We therefore examined the associations of mPV and mRCV in our cohort. While percentage changes of mPV and percentage changes of mRCV displayed a positive correlation on a population level ($r = 0.62$, $P < 0.0001$), individual patients displayed substantial deviations from expected changes of mRCV (Figure 7A and 7B). In search of potential explanation of these changes, we found

Figure 5 The assumption of an inverse relationship of Hb changes and TBV changes is violated in acute heart failure patients. Correlation of 48 h percentage change ($\Delta\%$) of TBV and percentage change of Hb concentration. Pearson's correlation coefficient (r) and the associated P -value are shown. Hb, haemoglobin; mTBV, measured total blood volume.



that three patients with substantial decreases in RCV had clinical evidence of bleeding between the two PV measurements whereas one patient with a substantial increase in RCV had received red blood cell transfusions between the two PV measurements. Nevertheless, in the majority of cases of instable RCV, no clinical explanation was apparent from the available data.

Discussion

This study demonstrates that, in AHF patients undergoing decongestive therapy, (i) changes of Hb concentration or Hct correlate poorly with changes of mPV and (ii) the Kaplan-Hakim and Strauss formula-based estimates of changes in PV perform poorly in identifying actual changes of PV. Although the Kaplan-Hakim formula provides an acceptable

Figure 4 Correlation of 48 h percentage change ($\Delta\%$) of mPV and the corresponding $\Delta\%$ of Hb concentrations (A), $\Delta\%$ of Hct (B), and $\Delta\%$ of ePV based on the Kaplan-Hakim (C) and Strauss formula (D). Pearson's correlation coefficient (r) and the associated P -value are shown. ePV, estimated plasma volume; Hb, haemoglobin; Hct, haematocrit; mPV, measured plasma volume.

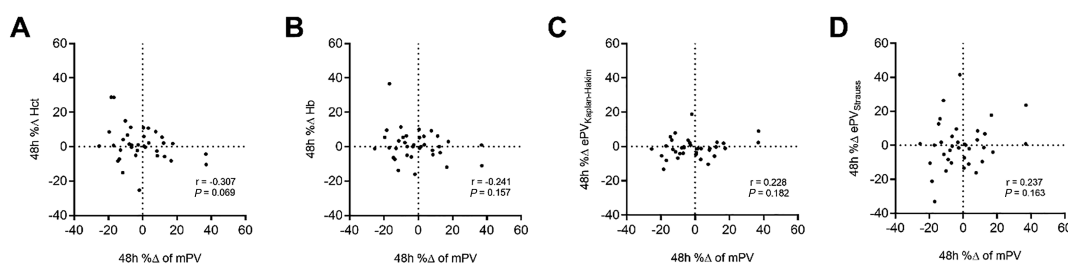


Figure 6 Impact of RCV stability on Strauss’ performance. (A) Correlation of percentage change of ePV_{Strauss} and mPV in patients with mRCV change within ±10%, $n = 19$, $r = 0.779$, $P < 0.0001$. (B) Correlation of percentage change of ePV_{Strauss} and mPV in patients with mRCV change $> +10\%$ and $< -10\%$, $n = 17$, $r = 0.089$, $P = 0.734$. Pearson’s correlation coefficient (r) and the associated P -value are shown. ePV, estimated plasma volume; mPV, measured plasma volume; mRCV, measured red cell volume.

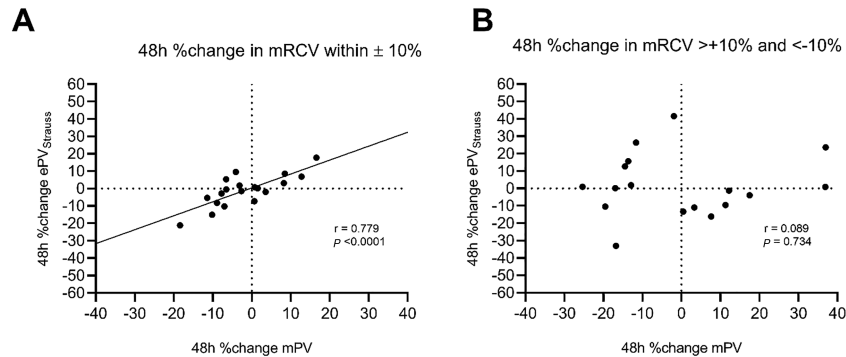
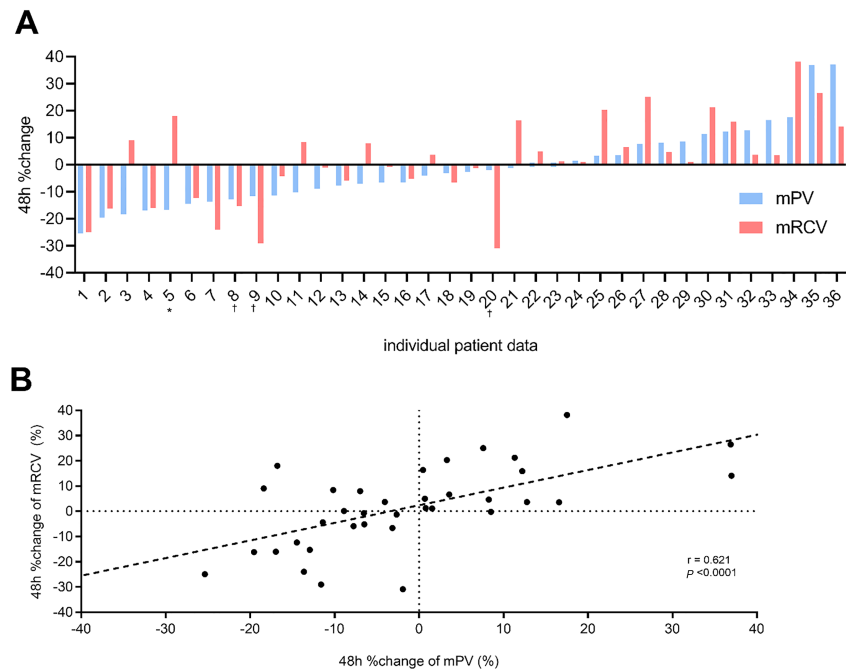


Figure 7 Relationships of PV and RCV in patients with acute heart failure. (A) Percentage changes of mPV and mRCV are shown in individual patients. Patients with decreasing PV are presented on the left, and patients with increasing PV on the right. *Patient received blood transfusion. †Patients with clinical evidence of bleeding event. (B) Correlation of percentage changes of PV and percentage changes of RCV. Pearson’s correlation coefficient (r) and the associated P -value are shown. Note the marked discordance of changes in PV and changes in RCV in some patients. mPV, measured plasma volume; mRCV, measured red cell volume.



population-based estimation of mPV, its ability to estimate individual 48 h changes in PV is poor. Utilization of Hb concentration, Hct, and Strauss’ formula in AHF patients is based on assumptions of a stable intravascular Hb pool and of concordant changes of PV and RCV. Under these assumptions, changes in Hb concentration would be inversely proportional to changes in TBV. However, in our study, no correlation was found between changes in Hb concentration and changes in

TBV. Additionally, changes of RCV were frequently discordant from changes of PV. This indicates that key assumptions underlying Strauss’ equation are frequently violated in AHF patients undergoing decongestive therapy. This may be related to several factors, including blood transfusions, blood losses, or redistribution of red blood cells to and from remote vascular beds (e.g. splanchnic circulation) during the course of therapy of these hospitalized patients.

Our findings are of importance when it comes to interpreting changes of Hct, Hb concentrations, and ePV in AHF patients. Previous studies have found that increases of Hct or Hb concentrations and decreases of ePV were associated with better outcomes in AHF patients.^{4,6,7,13,24,25} This was interpreted as evidence of a beneficial effect of aggressive decongestion, but our study shows that caution should be applied when making such inferences, because changes of Hb concentrations, Hct, estimates of PV, and changes of mPV were frequently uncoupled in AHF patients. Therefore, it is possible that increases of Hb concentrations or Hct during AHF therapy may be driven by factors unrelated to changes in PV. For instance, adverse outcomes in patients with increasing ePV (or decreasing levels of Hct or Hb) may be related to the prognostic implications of worsening anaemia rather than hypervolaemia.²⁶

Consistent with our observations, Fudim and Miller reported that the $\% \Delta ePV_{\text{Strauss}}$ from hospital admission to discharge in 40 chronic HF patients displayed a weak association with measured changes of PV assessed with an indicator dilution technique that uses radiolabelled albumin.¹¹ While changes of RCV and TBV were not reported in that study, the authors found that, unexpectedly, changes in venous Hb concentrations did not display a negative correlation with quantitative changes in PV. Similar to our findings, Miller and Mullan reported a wide range of RCV change between admission and discharge in patients hospitalized for symptomatic decompensation of chronic HF.²³ In our study, we found clinical explanations for RCV shifts (bleeding and red blood cell transfusions) in a minority of cases. The remaining observed disproportionate increases of RCV could be explained by recruitment of red blood cells from the spleen or splanchnic circulation.^{27,28} In contrast, the observed decreases in RCV that sometimes exceeded the decreases of PV may be explained by re-sequestration of red cells during stabilization in response to decongestive treatment. Furthermore, changes of RCV may be related to repeated phlebotomy or impaired haematopoiesis due to decreased erythropoietin production or abnormal response to erythropoietin associated with cardiac dysfunction.²⁹ Taken together, these data suggest that subsets of AHF patients display an unstable red blood cell pool, which leads to PV-independent alterations of Hb concentrations and Hct over time that contribute to the weak performance of ePV.

We observed a significant decrease in mean body weight during diuretic therapy. In contrast, TBV and PV were increasing in many patients. These observations are consistent with those of Miller and Mullan²³ and suggest that mobilization of expanded interstitial fluid into the intravascular space contributes to PV expansion and identifies the interstitial space as the major source of fluid loss during decongestion.

Finally, the use of a novel fluorescent marker technique, with a rapid clinically actionable readout, will allow for quan-

titative evaluation of PV changes and adjustment of diuretic therapy based on objective data not subjective evaluation as is presently used. The technique also offers the ability for repeat measurements over at least 6 h allowing the clinician insight into clinical therapeutic decisions within this timeframe.

Strengths and limitations

Our study is the first study to investigate measured changes of PV, RCV, and TBV in AHF patients undergoing decongestive therapy. The study is limited by a relatively small sample size of 36 patients. In this study, we defined broad inclusion criteria to represent patients with different types of HF (including patients with preserved and reduced ejection fractions) and a wide range of kidney function. While this represents a strength of our study in terms of generalizability, future studies will be needed to define specific subsets of AHF patients who may benefit from direct measurement of PV. Due to a short observation period, no long-term outcomes are available for this study.

Conclusions

Using a quantitative rapidly actionable measurement of PV, changes in intravascular mPV were not adequately reflected by changes of Hb concentrations, Hct, or changes in the Kaplan–Hakim or Strauss formula-derived ePV in AHF patients. The mechanisms responsible for these discrepancies in AHF patients include an unstable intravascular red blood cell pool and uncoupling of changes of PV and RCV in a subset of patients. Our data suggest that changes of Hct, changes of Hb concentrations, and changes of ePV are of limited value in guiding decongestive therapy in AHF patients and the prognostic impact of these parameters in AHF patients may be driven by PV-independent factors. Direct measurements of PV, as performed in this study, may provide a more accurate assessment of the response of intravascular volume status to decongestive therapy.

Conflict of interest

J.S.S.: none, E.T.: none, D.M.L.: none, F.E.: none, F.K.: none, N.P.N.: none, C.L.: none, R.R.: none, M.H.: consultant to FAST BioMedical, R.M.C.: consultant to FAST BioMedical, G.R.: none, V.M.: consultant to FAST BioMedical, E.G.: none, D. M.: employee of FAST BioMedical, P.A.M.: consultant to FAST BioMedical, K.U.E.: none, B.A.M.: founder, partial owner, and Medical Director of FAST BioMedical, K.M.S.O.: received grant support, paid to Charité Berlin, from FAST Biomedical.

Funding

The study was funded by a grant from FAST BioMedical, Indianapolis, IN, USA. Open Access funding enabled and organized by Projekt DEAL.

Supporting information

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

Figure S1. Schematic model of volume distribution in intravascular space and its changes (A) underlying Strauss formula (B, C).

Figure S2. Agreement between estimated PV by Kaplan-Hakim formula ($ePV_{\text{Kaplan-Hakim}}$) and absolute measured PV (mPV) upon enrollment (day 1). “Difference” indi-

cates difference between $ePV_{\text{Kaplan-Hakim}}$ and mPV. “Average” shows average of $ePV_{\text{Kaplan-Hakim}}$ and mPV. Solid line shows zero. Dotted lines show bias and bias ± 1.96 standard deviation. Data are presented in milliliters.

Figure S3. Absolute 48 h changes of mPV (milliliters) and bodyweight (grams) are shown in individual patients. Patients with decreasing PV are presented on the left, patients with increasing PV on the right.

Figure S4. Agreement between estimated PV change by Kaplan-Hakim ($\Delta ePV_{\text{Kaplan-Hakim}}$) (A) and Strauss’ formula ($\Delta ePV_{\text{Strauss}}$) (B) and measured PV change (ΔmPV) “Difference” indicates difference between ΔePV and ΔmPV change. “Average” shows average of ΔePV and ΔmPV . Solid line shows zero. Dotted lines show bias and bias ± 1.96 standard deviation. Data are presented in percentages.

References

- Metra M, Davison B, Bettari L, Sun H, Edwards C, Lazzarini V, Piovanelli B, Carubelli V, Bugatti S, Lombardi C, Cotter G, Dei Cas L. Is worsening renal function an ominous prognostic sign in patients with acute heart failure? The role of congestion and its interaction with renal function. *Circ Heart Fail* 2012; **5**: 54–62.
- Girerd N, Seronde MF, Coiro S, Chouihed T, Bilbault P, Braun F, Kenizou D, Maillier B, Nazeyrollas P, Roul G, Fillieux L, Abraham WT, Januzzi J Jr, Sebbag L, Zannad F, Mebazaa A, Rossignol P, INI-CRCT, Great Network, and the EF-HF Group. Integrative assessment of congestion in heart failure throughout the patient journey. *JACC Heart Fail* 2018; **6**: 273–285.
- Vaduganathan M, Greene SJ, Fonarow GC, Voors AA, Butler J, Gheorghiade M. Hemoconcentration-guided diuresis in heart failure. *Am J Med* 2014; **127**: 1154–1159.
- Testani JM, Chen J, McCauley BD, Kimmel SE, Shannon RP. Potential effects of aggressive decongestion during the treatment of decompensated heart failure on renal function and survival. *Circulation* 2010; **122**: 265–272.
- Davila C, Reyentovich A, Katz SD. Clinical correlates of hemoconcentration during hospitalization for acute decompensated heart failure. *J Card Fail* 2011; **17**: 1018–1022.
- van der Meer P, Postmus D, Ponikowski P, Cleland JG, O’Connor CM, Cotter G, Metra M, Davison BA, Givertz MM, Mansoor GA, Teerlink JR, Massie BM, Hillege HL, Voors AA. The predictive value of short-term changes in hemoglobin concentration in patients presenting with acute decompensated heart failure. *J Am Coll Cardiol* 2013; **61**: 1973–1981.
- Greene SJ, Gheorghiade M, Vaduganathan M, Ambrosy AP, Mentz RJ, Subacius H, Maggioni AP, Nodari S, Konstam MA, Butler J, Filippatos G, on behalf of the EVEREST Trial investigators. Haemoconcentration, renal function, and post-discharge outcomes among patients hospitalized for heart failure with reduced ejection fraction: insights from the EVEREST trial. *Eur J Heart Fail* 2013; **15**: 1401–1411.
- Oh J, Kang SM, Hong N, Youn JC, Han S, Jeon ES, Cho MC, Kim JJ, Yoo BS, Chae SC, Oh BH, Choi DJ, Lee MM, Ryu KH. Hemoconcentration is a good prognostic predictor for clinical outcomes in acute heart failure: data from the Korean Heart Failure (KorHF) registry. *Int J Cardiol* 2013; **168**: 4739–4743.
- Hakim R. *Plasmapheresis*. Philadelphia: Lippincott, Williams and Wilkins; 2001.
- Ling HZ, Flint J, Damgaard M, Bonfils PK, Cheng AS, Aggarwal S, Velmurugan S, Mendonca N, Rashid M, Kang S, Papalia F, Weissert S, Coats CJ, Thomas M, Kuskowski M, Cohn JN, Woldman S, Anand IS, Okonko DO. Calculated plasma volume status and prognosis in chronic heart failure. *Eur J Heart Fail* 2015; **17**: 35–43.
- Fudim M, Miller WL. Calculated estimates of plasma volume in patients with chronic heart failure-comparison with measured volumes. *J Card Fail* 2018; **24**: 553–560.
- Strauss MB, Davis RK, Rosenbaum JD, Rossmel EC. Water diuresis produced during recumbency by the intravenous infusion of isotonic saline solution. *J Clin Invest* 1951; **30**: 862–868.
- Duarte K, Monnez JM, Albuissou E, Pitt B, Zannad F, Rossignol P. Prognostic value of estimated plasma volume in heart failure. *JACC Heart Fail* 2015; **3**: 886–893.
- Kobayashi M, Girerd N, Duarte K, Preud’homme G, Pitt B, Rossignol P. Prognostic impact of plasma volume estimated from hemoglobin and hematocrit in heart failure with preserved ejection fraction. *Clin Res Cardiol* 2020; **109**: 1392–1401.
- Hudson SR, Chan D, Ng LL. Change in plasma volume and prognosis in acute decompensated heart failure: an observational cohort study. *J R Soc Med* 2016; **109**: 337–346.
- Greenleaf JE, Convertino VA, Mangseth GR. Plasma volume during stress in man: osmolality and red cell volume. *J Appl Physiol* 1979; **47**: 1031–1038.
- Fudim M, Hernandez AF, Felker GM. Role of volume redistribution in the congestion of heart failure. *J Am Heart Assoc* 2017; **6**: e006817.
- Swolinsky JS, Nerges NP, Leistner DM, Edelman F, Knebel F, Tuvshinbat E, Lemke C, Roehle R, Haase M, Costanzo MR, Rauch G, Mitrovic V, Gasanin E, Meier D, McCullough PA, Eckardt KU, Molitoris BA, Schmidt-Ott KM. Serum creatinine and cystatin C-based estimates of glomerular filtration rate are misleading in acute heart failure. *ESC Heart Fail* 2021; **8**: 3070–3081.
- Felker GM, Lee KL, Bull DA, Redfield MM, Stevenson LW, Goldsmith SR, LeWinter MM, Deswal A, Rouleau JL, Ofili EO, Anstrom KJ, Hernandez AF,

- McNulty S, Velazquez EJ, Kfoury AG, Chen HH, Givertz MM, Semigran MJ, Bart BA, Mascette AM, Braunwald E, O'Connor CM, NHLBI Heart Failure Clinical Research Network. Diuretic strategies in patients with acute decompensated heart failure. *N Engl J Med* 2011; **364**: 797–805.
20. Molitoris BA, George AG, Murray PT, Meier D, Reilly ES, Barreto E, Sandoval RM, Rizk DV, Shaw AD, Peacock WF. A novel fluorescent clinical method to rapidly quantify plasma volume. *Cardiorenal Med* 2019; **9**: 168–179.
21. Rizk DV, Meier D, Sandoval RM, Chacana T, Reilly ES, Seegmiller JC, DeNoia E, Strickland JS, Muldoon J, Molitoris BA. A novel method for rapid bedside measurement of GFR. *J Am Soc Nephrol* 2018; **29**: 1609–1613.
22. Kirsch KA, Johnson RF, Gorten RJ. The significance of the total-body hematocrit in measurements of blood compartments. *J Nucl Med* 1971; **12**: 17–21.
23. Miller WL, Mullan BP. Understanding the heterogeneity in volume overload and fluid distribution in decompensated heart failure is key to optimal volume management: role for blood volume quantitation. *JACC Heart Fail* 2014; **2**: 298–305.
24. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974; **37**: 247–248.
25. Costill DL, Branam L, Eddy D, Fink W. Alterations in red cell volume following exercise and dehydration. *J Appl Physiol* 1974; **37**: 912–916.
26. Silver MA, Anker SD. Anemia and heart failure: guidance for clinicians and trialists. Expert Analysis. American College of Cardiology. <https://www.acc.org/latest-in-cardiology/articles/2021/07/06/12/18/anemia-and-heart-failure> (6 July 2021).
27. Underwood RJ, Griswold HE Jr, Hurst WW. Effect of acute cardiac tamponade on red cell volume, total plasma protein and plasma volume. *Circ Res* 1957; **5**: 257–260.
28. Rapaport E, Weisbart MH, Levine M. The splanchnic blood volume in congestive heart failure. *Circulation* 1958; **18**: 581–587.
29. Ruirok WP, Qian C, Sillje HH, van Goor H, van Veldhuisen DJ, van Gilst WH, de Boer RA. Heart failure-associated anemia: bone marrow dysfunction and response to erythropoietin. *J Mol Med (Berl)* 2011; **89**: 377–387.
30. Du Bois D, Du Bois EF. A Formula to Estimate the Approximate Surface Area if Height and Weight Be Known. *Archives of Internal Medicine*. 1916; **17**: 863–871.