Title

Are non-invasive estimations of plasma volume an accurate measure of congestion in patients with chronic heart failure?

Short title

Estimating plasma volume in patients with chronic heart failure

Authors

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Abstract (250 words)

Aim: We report associations between different formulae for estimating plasma volume status (PVS) and clinical and ultrasound markers of congestion in patients with chronic heart failure (CHF) enrolled in the Hull Lifelab registry.

Methods and Results: Cohort 1 comprised patients with data on signs and symptoms at initial evaluation (n=3505). Cohort 2 included patients with ultrasound assessment of congestion (lung B-line count, inferior vena cava (IVC) diameter, jugular vein distensibility (JVD) ratio) (N=341). Two formulae for PVS were used: (a) Hakim (HPVS) and (b) Duarte (DPVS). Results were compared with clinical and ultrasound markers of congestion. Outcomes assessed were mortality and the composite of heart failure (HF) hospitalisation and all-cause mortality. In cohort 1, HPVS was associated with mortality (hazard ratio (HR) per unitary increase = 1.02 (1.01 – 1.03); P<0.001). In cohort 2, HPVS was associated with B-line count (HR) = 1.05 (95% confidence interval (CI) 1.01 – 1.08); P=0.02) and DPVS with the composite outcome (HR = 1.26 (1.01 – 1.58); P=0.04). HPVS and DPVS were strongly related to haemoglobin concentration and HPVS to weight. After multivariable analysis, there were no strong or consistent associations between PVS and measures of congestion, severity of symptoms, or outcome. By contrast, log[NTproBNP] was strongly associated with all three.

Conclusions: Amongst patients with CHF, HPVS and DPVS are not strongly or consistently associated with clinical or ultrasound evidence of congestion, nor clinical outcomes after multivariable adjustment. They appear only to be surrogates of the variables from which they are calculated with no intrinsic clinical utility.

Introduction

Plasma volume expansion, driven by neuro-hormonal activation, is the first step in decompensation from a euvolaemic state to overt clinical congestion in patients with chronic heart failure (CHF) [1]. Worsening congestion is associated with a worse prognosis [2], but monitoring congestion is difficult: clinical examination is unreliable [3,4], and ultrasound techniques may be useful but require specialist equipment and expertise [5]. Non-invasive methods to assess congestion remotely in patients with CHF might be helpful, particularly in the wake of the COVID-19 pandemic [6].

While direct measurement of plasma volume is invasive and costly, there are two formulae which can estimate plasma volume status (PVS) from routinely collected variables: the Hakim PVS formula (HPVS) using sex, weight and haemoglobin [7]; and the Duarte PVS formula (DPVS) formula using haemoglobin and haematocrit [8]. Higher PVS (greater congestion) by either formula is associated with a greater risk of adverse outcome in many selected and unselected cohorts of patients with acute [9-11],,, or chronic heart failure [7,12,13].[7,,]

Estimations of PVS should correlate with markers of congestion but there are conflicting data on the correlations between estimations of PVS and signs and symptoms of congestion[7,9-15],, and limited data on associations with ultrasound markers of congestion [9,16]. Accordingly, we investigated the associations between HPVS and DPVS and clinical and

ultrasound markers of congestion in two cohorts of patients recruited to the Hull LifeLab database.

Methods

Patient population

Between September 2000 and October 2016, all clinical, demographic, biochemical and echocardiographic data on patients referred from primary or secondary care to a specialist heart failure clinic serving a local population of about 550,000 people was recorded on a secure database (The Hull LifeLab) – cohort 1.

We also investigated a cohort of patients attending a routine follow up visit to the LifeLab clinic enrolled between April 2016 and March 2017 who underwent comprehensive ultrasound assessment of congestion: inferior vena cava (IVC) diameter, jugular vein distensibility (JVD) ratio, and lung B-line count – cohort 2. This population has been described elsewhere [5], and consists of patients seen at either at baseline or a routine follow up appointment during the recruitment period (supplementary figure 1). All analyses were performed separately in each cohort.

All subjects gave their written informed consent for their data to be used. The study conforms to the principles outlined in the Declaration of Helsinki and was approved by relevant ethical bodies. All patients with an NTproBNP measurement, documentation of left ventricular systolic function, recorded symptoms and signs, haematocrit, haemoglobin, height and weight were used for analysis (N=3505 in cohort 1; N=341 in cohort 2).

Heart failure was defined as the presence of signs or symptoms consistent with the diagnosis (a mandatory requirement for referral to the clinic) and **either** left ventricular systolic dysfunction (LVSD) moderate or worse (left ventricular ejection fraction (LVEF) <40%) (defined as heart failure with reduced ejection fraction: HeFREF); **or** LVSD between mild and mild-to-moderate (inclusive) (LVEF 40-49%) *and* raised N-terminal pro B-type natriuretic peptide (NTproBNP) plasma concentrations (>125 ng/L) (defined as heart failure with a mid-range ejection fraction: HeFMREF); **or** no or trivial LVSD (LVEF ≥50%) *and* raised NTproBNP concentrations (heart failure with a normal ejection fraction: HeFNEF) [17]. The severity of LVSD was based on the British Society of Echocardiography guidelines at the time of the clinic visit (supplementary table 1). The World Health Organisation definition of anaemia was used (<13 g/dL for men; <12 g/dL for women) and obesity was defined as a body mass index (BMI) of ≥30 kg/m^2.

Echocardiographic measurements in cohort 2

Echocardiography was performed after clinical examination using a Vivid 7 system (GE healthcare, UK) operating at 1.7-3.4 mHz. Ultrasound assessment of congestion was performed after echocardiography. LVEF was measured using Simpson's biplane method, right ventricular function was measured using tricuspid annular plane systolic excursion, left atrial volume was measured in the apical four chamber view and indexed to body surface area (LAVI), systolic trans-tricuspid gradient was estimated using the Bernoulli equation.

The IVC diameter was measured 2 cm from the RA in the sub-costal view and collapse on sniff was measured as $\geq$ or <50%. Jugular vein diameter was measured in M-mode or 2D echocardiography at rest and during Valsalva (forced expiration against a closed glottis) with the patient supine at a 45 degree angle using a linear high frequency (10 mHz) probe as described elsewhere [5].[5] Lung B-lines were recorded in a 28-zone scan across the anterior chest (2^{nd} to 5^{th} intercostal space on the right hemithorax, and 2^{nd} to 4^{th} intercostal space on the left hemithorax, in the parasternal, mid-clavicular and mid axillary lines) with the patient in a near-supine position using a standard echo probe. Each window was recorded for a maximum of 5 seconds and the total number of B-lines counted. All images were reviewed offline by an experienced operator (PP) [5].

Plasma volume equations and congestion definitions

HPVS

The HPVS formula calculates the difference between actual plasma volume (calculated from a patient's weight, sex and haematocrit) [18] and ideal plasma volume (estimated euvolaemic state based on sex and weight) [19] expressed as a percentage of the ideal plasma volume.

Actual plasma volume (mL) = (1 – haematocrit) × (A + (B × weight in kilograms))

A = 1530 for men and A = 864 for women; B = 41 for men and B = 47.9 for women

Ideal plasma volume = C x weight in kilograms

C = 39 for men and C = 40 for women

HPVS = ((Actual plasma volume – ideal plasma volume) / ideal plasma volume) × 100

Patients who have a calculated *actual* plasma volume greater than the calculated *ideal* plasma volume will have a positive HPVS (>0%) and were classed as congested.

DPVS

The DPVS formula estimates plasma volume based on a patient's haematocrit and haemoglobin [8,20]. Those with greater congestion will have higher DPVS.

DPVS = [100 – haematocrit (percentage) / haemoglobin (g/dL)]

Proposed cut-offs to define congestion by DPVS range from 5.3 dL/g, to 5.5 dL/g [20]. To identify trends in the data and explore possible cut offs, we split the patients into quartiles of increasing DPVS where Q4 (highest value of DPVS) was the most congested. Compared to patients in Q1-3, those in Q4 in both cohorts had a much higher NTproBNP, were much more likely to have New York Heart Association (NYHA) class III or IV symptoms and clinical congestion score ≥3, and had a far higher mortality. The lowest value of DPVS in Q4 was 5.2 dL/g in cohort 1 and 5.3 dL/g in cohort 2, therefore we used 5.2 dL/g as the cut point to define "congestion" by DPVS.

Clinical congestion

We used a score we have previously described to categorise patients as 'severely congested' on the basis of lung auscultation (normal, basal crackles or mid-zone or diffuse crackles), jugular vein pulse (JVP) assessment (not raised, raised 1-4cm or raised to earlobes), peripheral oedema (none, ankles or knees or above) and liver examination (non-palpable or palpable) [5]. One point is scored for each degree of severity and patients with a score of ≥3 were classed as congested.

Ultrasound congestion

The presence of congestion by ultrasound was defined by IVC diameter >2cm, JVD ratio <4 or total lung B-line count >14 [5].

Statistical Analysis

Patients in each cohort were split into groups based on the presence or absence of congestion by HPVS, DPVS, and clinical congestion score. For cohort 2 only, patients were additionally split based on ultrasound measures.

Categorical data are presented as percentages, continuous data are presented as median and interquartile range (IQR).

Independent samples t-tests were used to compare continuous variables, chi-squared tests were used to compare categorical variables and Fisher's exact test was used to compare dichotomous categorical variables between the groups. Kruskal-Wallis and Mann-Witney-U tests were used to compare non-normally distributed continuous and categorical variables, respectively, across the groups.

The relationship between estimations of PVS as continuous variables and other variables was assessed by Pearson correlation coefficients. The relationship between the presence of congestion by the different definitions was assessed by uni- and multi- variable binary logistic regression. We used variance inflation factor (VIF) to assess the severity of

collinearity between different estimations of PVS and the variables from which each measure was calculated. A VIF of 1 indicates no collinearity, 1-5 moderate collinearity, and >5 extreme collinearity.

Outcomes assessed were all-cause mortality and all-cause mortality or heart failure hospitalisation. Associations between estimations of PVS, clinical and ultrasound congestion, and outcome were assessed using a Cox proportional hazard regression model which included a small number of clinically relevant variables to avoid statistical over-fitting that were chosen *a priori*. These variables were age, sex, symptom severity (NYHA class III or IV versus I or II), log[NTproBNP], haemoglobin, urea, albumin, and the prescription of loop diuretic (versus no loop diuretic). Proportionality of hazards was checked by residual plotting. Each measure of congestion was entered separately into the multivariable models.

Statistical analysis was carried out using SPSS 26 software. The two-tailed level of statistical significance was set at $P < 0.05$.

Results

In the baseline cohort 3505 had complete data on echocardiography, NTproBNP, and symptoms and signs. 1201 (34%) had HeFREF, 784 (22%) had HeFMREF and 1520 (43%) had HeFNEF. In the ultrasound congestion cohort 341 had complete data; 124 (36%) had HeFREF, 68 (20%) had HeFMREF, and 149 (44%) had HeFNEF. The prevalence of

congestion by each definition in each cohort is shown in figure 1. Patient characteristics by different definitions of congestion are shown in tables 1 and 2 and supplementary table 2.

Table 1 - Clinical characteristics by congestion status in cohort 1

Variable	Clinical Congestion Score			H-PVS			D-PVS		
	No congestion <3 N =3056	Congested ≥3 N = 449	P	No congestion ≤0% N =2926	Congested >0% N = 579	P	No congestion <5.2 dL/g N =2675	Congested ≥5.2 dL/g N = 830	P
Demographics & Symptoms									
Age - years	73 (66 - 80)	78 (71 - 83)	<0.001	73 (66 – 79)	79 (73 – 84)	<0.001	73 (65 – 80)	77 (71 – 82)	<0.001
Sex (males) – N (%)	1857 (61)	283 (63)	0.38	1787 (61)	353 (61)	0.96	1726 (64)	414 (50)	<0.001
Weight – kg	79 (67 – 91)	77 (66 - 92)	0.75	82 (71 – 94)	62 (54 – 70)	<0.001	80 (68 – 93)	73 (63 – 86)	<0.001
Anaemia – N (%)	959 (31)	225 (50)	<0.001	699 (24)	485 (84)	<0.001	368 (14)	816 (98)	<0.001
BMI >30 kg/m^2 – N (%)	1105 (36)	171 (38)	0.43	1237 (42)	39 (7)	<0.001	1023 (38)	253 (30)	<0.001
NYHA III/IV – N (%)	727 (24)	285 (64)	<0.001	796 (27)	216 (37)	<0.001	693 (26)	319 (38)	<0.001
HeFREF – N (%)	1017 (33)	184 (41)	0.003	989 (34)	212 (37)	0.30	947 (35)	254 (31)	0.04
HeFMREF – N (%)	685 (22)	99 (22)		652 (22)	132 (23)		588 (22)	196 (24)	
HeFNEF – N (%)	1354 (44)	166 (37)		1285 (44)	235 (41)		1140 (43)	380 (46)	
Clinical congestion									
Peripheral oedema† – N (%)	570 (19)	370 (82)	-	763 (26)	177 (31)	0.03	627 (23)	313 (38)	<0.001
Lung crackles – N (%)	278 (9)	252 (56)	-	398 (14)	132 (23)	<0.001	357 (13)	173 (21)	<0.001
Raised JVP – N (%)	104 (3)	407 (91)	-	386 (13)	125 (22)	<0.001	331 (12)	180 (22)	<0.001
Clinical congestion score ≥3	-	-	-	337 (12)	112 (19)	<0.001	284 (11)	165 (20)	<0.001
Blood results									
NTproBNP – ng/L	905 (364 – 2110)	2957	<0.001	890 (361 – 2073)	2355	<0.001	885 (348-2072)	1780 (731-4124)	<0.001

		(1182 – 6270)			(958 – 5424)				
Haemoglobin – g/dL	13.4 (12.2 – 14.5)	12.6 (11.5 – 13.9)	<0.001	13.6 (12.6 – 14.7)	11.3 (10.4 – 12.2)	<0.001	13.8 (13.0-14.8)	11.2 (10.5-11.7)	<0.001
Medications									
Loop diuretic – N (%)	1855 (61)	363 (81)	<0.001	1791 (61)	427 (74)	<0.001	1604 (60)	614 (74)	<0.001
Plasma Volume Estimations									
H-PVS - %	-10 (-16 - -4)	-7 (-14 – 0)	<0.001	-12 (-17 - -7)	5 (2 – 9)	-	-13 (-18 - -8)	0 (-4 – 6)	<0.001
D-PVS – dL/g	4. 6 (±1.0)	5.0 (±1.3)	<0.001	4.3 (3.8 – 4.8)	5.8 (5.2 – 6.5)	<0.001	4.2 (3.7 – 4.6)	5.8 (5.4 – 6.4)	-
H-PVS >0% – N (%)	467 (15)	112 (25)	<0.001	-	-	-	153 (6)	426 (51)	<0.001
D-PVS ≥5.2 dL/g – N (%)	665 (22)	165 (37)	<0.001	404 (14)	426 (74)	<0.001	-	-	-

Legend

†Affecting ankles or above. Abbreviations used: H-PVS – plasma volume status as calculated from the Hakim formula ; D-PVS – plasma volume status as calculated by the Duarte formula; BMI – body mass index; NYHA – New York Heart Association; JVP – jugular venous pulse; NTproBNP – N-terminal pro B-type natriuretic peptide; N – number.

Table 2 – Clinical & echocardiographic characteristics by congestion status in cohort 2

Variable	Clinical Congestion Score			H-PVS			D-PVS		
	No congestion <3 N =296	Congested ≥3 N = 45	P	No congestion ≤0% N =276	Congested >0% N = 65	P	No congestion <5.2 dL/g N =241	Congested ≥5.2 dL/g N = 100	P
Demographics & Symptoms									
Age - years	74 (67 – 82)	80 (74 – 85)	<0.001	73 (66 – 80)	82 (76 – 85)	<0.001	73 (66 – 81)	78 (73 – 84)	<0.001
Sex (males) – N (%)	195 (66)	34 (76)	0.23	184 (67)	44 (68)	1.00	159 (66)	69 (69)	0.62
Weight - kg	84 (71 – 98)	80 (75 – 97)	0.76	88 (76 – 102)	66 (55 – 75)	<0.001	86 (74 – 99)	77 (69 – 93)	0.04
Anaemia – N (%)	103 (35)	27 (60)	0.002	71 (26)	59 (91)	<0.001	50 (21)	80 (80)	<0.001
BMI >30kg/m^2 – N (%)	135 (46)	19 (42)	0.75	150 (54)	3 (5)	<0.001	123 (51)	30 (30)	<0.001
NYHA III or IV – N (%)	52 (18)	33 (73)	<0.001	62 (23)	23 (35)	0.04	51 (21)	33 (33)	0.03
HeFREF – N (%)	107 (36)	17 (38)	0.97	100 (36)	23 (35)	0.77	87 (36)	37 (37)	0.48
HeFMREF – N (%)	59 (20)	9 (20)		53 (19)	15 (23)		52 (22)	16 (16)	
HeFNEF – N (%)	131 (44)	19 (42)		123 (45)	27 (42)		102 (42)	47 (47)	
Clinical congestion									
Peripheral oedema† – N (%)	72 (24)	45 (100)	-	95 (34)	22 (34)	1.00	69 (29)	47 (47)	0.002
Lung crackles – N (%)	12 (4)	28 (62)	-	27 (10)	13 (20)	0.03	22 (9)	18 (18)	0.03
Raised JVP – N (%)	29 (10)	41 (91)	-	48 (17)	22 (34)	0.01	42 (17)	28 (28)	0.04
Clinical congestion score ≥3	-	-	-	32 (12)	13 (20)	0.10	23 (10)	22 (22)	0.004
Blood results									
NTproBNP – ng/L	1079 (397 – 2222)	3273 (1860 – 6840)	<0.001	1117 (432 – 2304)	2282 (909 – 3809)	<0.001	1144 (419 – 2323)	1808 (781 – 3627)	0.002
Haemoglobin – g/dL	13.3 (12.1 – 14.4	12.2 (11 – 13.5)	0.003	13.5 (12.5 – 14.5)	11.3 (10.6 – 12.2)	<0.001	13.6 (12.8 – 14.6)	11.8 (108 – 12.3)	<0.001
Medications									
Loop diuretic – N (%)	220 (74)	38 (84)	0.19	206 (75)	52 (80)	0.42	174 (72)	83 (83)	0.04

Estimations of Plasma Volume Status									
HPVS - %	-9 (-15 - -3)	-7 (-12 - -1)	0.11	-11 (-16 - -7)	5 (2 – 9)	-	-12 (-17 - -6)	-2 (-8 – 6)	<0.001
DPVS – dL/g	4.5 (4.0 – 5.2)	5.2 (4.3 – 5.5)	0.03	4.5 (4.0 – 5.1)	5.4 (4.9 – 6.2)	<0.001	4.3 (3.9 – 4.7)	5.8 (5.5 – 6.3)	-
HPVS >0% - N (%)	52 (18)	13 (29)	0.10	-	-	-	22 (9)	43 (43)	<0.001
DPVS ≥5.2 dL/g – N (%)	78 (26)	22 (50)	0.004	57 (21)	43 (66)	<0.001	-	-	-
Echocardiographic characteristics									
LVEDV - mL	142 (109 – 195)	140 (100 – 220)	0.79	148 (107 – 200)	130 (108 – 169)	0.14	146 (105 – 199)	137 (110 – 184)	0.87
LVESV - mL	76 (49 – 124)	69 (46 – 153)	0.36	76 (49 – 132)	69 (46 – 98)	0.36	78 (45 – 130)	70 (49 – 119)	0.71
LVEF - %	46 (35 – 55)	48 (28 – 57)	0.27	47 (35 – 56)	47 (35 – 53)	0.75	45 (35 – 56)	49 (33 – 55)	0.63
LA diameter	4.3 (3.8 – 4.8)	4.8 (4.4 – 5.3)	<0.001	4.4 (3.8 – 4.9)	4.3 (3.6 – 4.9)	0.15	4.3 (3.8 – 4.8)	4.6 (3.8 – 5.0)	0.04
LAVI – ml/m²	42 (33 - 56)	55 (41 – 77)	<0.001	43 (34 – 56)	50 (35 – 70)	0.01	42 (33 – 56)	48 (36 – 69)	0.001
E – m/s	0.9 (0.6 – 1.1)	1.1 (0.9 – 1.3)	<0.001	0.9 (0.7 – 1.1)	0.9 (0.6 – 1.1)	0.58	0.9 (0.6 – 1.1)	1.0 (0.7 – 1.2)	0.46
Septal E/e'	13.3 (10.4 – 18.0)	19.2 (12.8 – 27.6)	<0.001	14.2 (10.6 – 20.0)	13.3 (11.2 – 19.6)	0.66	14.4 (10.8 – 19.5)	13.1 (10.6 – 20.1)	0.78
Lateral E/e'	10.2 (7.5 – 13.9)	13.0 (9.1 – 17.5)	<0.001	10.5 (7.7 – 14.2)	10.9 (7.6 – 14.4)	0.28	10.6 (7.6 – 14.6)	10.2 (7.8 – 14.0)	0.63
TAPSE – cm	2.0 (1.5 – 2.3)	1.4 (1.2 – 1.8)	<0.001	1.9 (1.5 – 2.3)	1.9 (1.4 – 2.2)	0.08	1.9 (1.5 – 2,3(	1,9 (1.4 – 2.2)	0.21
TR gradient – mmHg	27 (21 – 36)	38 (31 – 47)	<0.001	29 (21 – 38)	31 (25 – 41)	0.21	28 (21 – 36)	30 (25 – 40)	0.21
MR - *none / trivial*	149 (50)	13 (29)	0.01	141 (51)	20 (31)	0.002	120 (50)	41 (41)	0.20
mild	135 (45)	28 (62)		126 (46)	37 (57)		110 (46)	53 (53)	
moderate / severe	13 (5)	4 (9)		9 (3)	8 (12)		11 (4)	6 (6)	
TR - *none / trivial*	175 (59)	6 (13)	<0.001	160 (58)	20 (31)	<0.001	141 (58)	39 (39)	0.01
mild	114 (38)	34 (76)		109 (39)	39 (60)		94 (39)	53 (53)	
moderate / severe	7 (3)	5 (11)		7 (3)	6 (9)		6 (3)	7 (7)	
Congestion by ultrasound									
IVC diameter – cm	1.9 (1.6 – 2.2)	2.6 (2.4 – 3.1)	<0.001	2.0 (1.6 – 2.3)	2.0 (1.7 – 2.6)	0.24	1.9 (1.6 – 2.3)	2.0 (1.7 – 2.6)	0.003
IVC >2cm – N (%)	104 (36)	40 (89)	<0.001	112 (42)	31 (48)	0.40	97 (41)	6 (47)	0.40
Visible intrahepatic veins – N (%)	230 (79)	42 (93)	0.02	215 (79)	56 (86)	0.23	190 (80)	81 (82)	0.76

Hepatic vein diameter – cm	0.6 (0.4 – 0.9)	1.1 (0.8 – 1.3)	<0.001	0.7 (0.5 – 0.9)	0.7 (0.5 – 1.0)	0.49	0.7 (0.5 – 0.9)	0.8 (0.5 – 1.1)	0.01
JVD ratio	5.6 (3.7 – 7.4)	2.2 (1.4 – 3.0)	<0.001	5.5 (3.2 – 7.2)	4.6 (2.8 – 6.7)	0.10	5.7 (3.6 – 7.7)	4.6 (2.6 – 6.1)	<0.001
JVD ratio <4	78 (28)	32 (82)	<0.001	83 (32)	27 (43)	0.10	68 (30)	42 (46)	0.01
B-line count	6 (2 – 13)	26 (15 - 37	<0.001	6 (2 – 15)	12 (4 – 27)	0.01	6 (2 – 16)	9 (3 – 22)	0.04
B-line count >14	69 (23)	37 (82)	<0.001	74 (27)	32 (49)	0.001	67 (28)	39 (39)	0.05

Legend

†Affecting ankles and above. Abbreviations used: H-PVS – plasma volume status as calculated from the Hakim ; D-PVS – plasma volume status as calculated by the Duarte formula; BMI – body mass index; NYHA – New York Heart Association; JVP – jugular venous pulse; NTproBNP – N-terminal pro B-type natriuretic peptide;LVEDV – left ventricular end diastolic volume; LVESV – left ventricular end systolic volume; LVEF – left ventricular ejection fraction; LA – left atrium; LAVI – left atrial volume index; TAPSE – tricuspid annular plane systolic excursion; TR – tricuspid regurgitation; MR – mitral regurgitation; IVC – inferior vena cava; JVD – jugular venous distensibility; N – number.

In both cohorts, patients with congestion by any definition were older, had more severe symptoms, had higher NTproBNP but lower haemoglobin, and were more likely to be taking a loop diuretic than those without congestion. Almost all patients classed as congested by either DPVS or HPVS were anaemic compared to around half of patients with a clinical congestion score ≥3 or congestion on ultrasound.

Patients classed as congested by clinical congestion score were more likely to be classed as congested by HPVS (cohort 1 only), DPVS (both cohorts), and any of the ultrasound definitions of congestion.

During a median follow up of 1492 days (interquartile range 512 – 1825) in cohort 1, 1216 patients died and a further 873 patients were hospitalised with HF. During a median follow up of 600 days (363 – 749) in cohort 2, 71 patients died and a further 56 were hospitalised with HF.

HPVS

HPVS and DPVS were strongly positively correlated with each other. HPVS had strong inverse correlations with weight and haemoglobin, as expected, and weak positive correlations with log[NTproBNP], LAVI, TR gradient, and B-line count (figure 2). The VIF for HPVS and haemoglobin was 2.6 and 2.3 in cohorts 1 and 2, respectively, and the VIF for HPVS and weight was 1.6 and 1.9, respectively (figure 2). Consequently, almost all patients

with congestion by HPVS were anaemic and almost no patient with congestion by HPVS was obese (tables 1 and 2, figure 3A).

Greater congestion measured by HPVS as either a continuous or categorical variable was associated with clinical measures of congestion, and New York Heart Association (NYHA) class III or IV symptoms on univariable logistic regression in both cohorts. However, after adjustment for haemoglobin, only the association with lung B-line count >14 remained statistically significant while the association with symptoms changed direction – those with greater congestion were *less* likely to have severe symptoms (table 3).

HPVS as a continuous or categorical variable was associated with both outcomes in both cohorts on univariable analysis. However, only increasing HPVS as a continuous variable was associated with all-cause mortality in cohort 1 after multivariable adjustment (hazard ratio (HR) = 1.02 (95% confidence interval (CI) =1.01 – 1.03); χ^2=10;P<0.001) (supplementary table 3). Restricting the multivariable model to just age, sex and haemoglobin did not change the associations between HPVS and either outcome in either cohort.

DPVS

DPVS had and extremely strong inverse correlation with haemoglobin (figure 2), and weak positive correlations with log[NTproBNP], LAVI, TR gradient, IVC diameter and B-line count, and weak negative correlations with weight and JVD ratio. The VIF for DPVS and haemoglobin was 14.4 and 1.6 in cohorts 1 and 2 respectively suggesting extreme collinearity

in cohort 1 (figure 2). Consequently, almost all patients with congestion by DPVS were anaemic (tables 1 and 2, figure 3). By comparison, only 50% of patients with congestion by clinical examination or ultrasound were anaemic (tables 1 and 2, supplementary table 2).

After adjustment for haemoglobin, DPVS was not associated with clinical or ultrasound measurements of congestion, nor symptoms (table 3).

DPVS as a continuous or categorical variable was associated with both outcomes in both cohorts on univariable analysis. However, only increasing DPVS as a continuous variable was associated with all-cause mortality or HF hospitalisation in cohort 2 after multivariable adjustment (HR = 1.26 (95% CI =1.01 – 1.58); χ^2=4; P=0.04) (supplementary table 3). Restricting the multivariable model to just age, sex and haemoglobin did not change the associations between HPVS and either outcome in either cohort.

By contrast logNTproBNP was strongly associated with clinical and ultrasound measures of congestion, NYHA class III or IV symptoms, and both endpoints in both cohorts after multivariable adjustment (supplementary table 3).

Table 3 – Binary logistic regression for congestion variables associated with different objective measures of congestion

Variable	Univariable			Adjusted for Haemoglobin – g/dL		
	OR	χ^2	P	OR	χ^2	P
	Clinical congestion score ≥ 3 in cohort 1 N=3505					
HPVS - %	1.03 (1.02 – 1.04)	30	<0.001	0.99 (0.97 – 1.01)	2	0.22
HPVS >0% (vs. ≤0%)	1.84 (1.46 – 2.33)	26	<0.001	1.10 (0.83 – 1.46)	0	0.51
DPVS – dL/g	1.39 (1.28 – 1.52)	58	<0.001	0.94 (0.68 – 1.28)	0	0.68
DPVS ≥5.2 dL/g	2.09 (1.69 – 2.58)	47	<0.001	1.16 (0.83 – 1.62)	1	0.38
Log[NTproBNP]	5.17 (4.22-6.32)	256	<0.001	4.70 (3.82 – 5.77)	215	<0.001
	Clinical congestion score ≥ 3 in cohort 2 N=341					
HPVS - %	1.03 (0.99 – 1.06)	3	0.11	-	-	-
HPVS >0% (vs. ≤0%)	1.91 (0.94 – 3.88)	3	0.08	1.03 (0.44 – 2.44)	0	0.95
DPVS – dL/g	1.42 (1.04 – 1.94)	5	0.03	1.08 (0.72 – 1.63)	0	0.71
DPVS ≥5.2 dL/g	2.67 (1.41 – 5.07)	9	0.003	1.89 (0.88 – 4.07)	3	0.10
Log[NTproBNP]	15.20 (6.26 – 36.91)	36	<0.001	13.69 (5.56 – 33.75)	32	<0.001
	NYHA III/IV in cohort 1 N=3505					
HPVS - %	1.02 (1.01 – 1.03)	20	<0.001	0.98 (0.97 – 0.99)	10	0.002
HPVS >0% (vs. ≤0%)	1.59 (1.32 – 1.92)	24	<0.001	1.06 (0.85 – 1.33)	0	0.59
DPVS – dL/g	1.32 (1.23 – 1.41)	60	<0.001	0.95 (0.73 – 1.23)	0	0.68
DPVS ≥5.2 dL/g	1.79 (1.52 – 2.11)	48	<0.001	1.14 (0.88 – 1.46)	1	0.32
Log[NTproBNP]	2.66 (2.31 – 3.06)	183	<0.001	2.44 (2.11 – 2.83)	144	<0.001
	NYHA III or IV in cohort 2 N=341					
HPVS - %	1.02 (1.00 – 1.05)	3	0.10	0.98 (0.94 – 1.02)	1	0.28
HPVS >0% (vs. ≤0%)	1.89 (1.06 – 3.38)	5	0.03	1.17 (0.58 – 2.36)	0	0.66
DPVS – dL/g	1.30 (1.01 – 1.67)	4	0.04	1.00 (0.72 – 1.39)	0	0.99
DPVS ≥5.2 dL/g	1.84 (1.09 – 3.08)	5	0.02	1.24 (0.67 – 2.30)	0	0.50
Log[NTproBNP]	6.31 (3.46 – 11.51)	36	<0.001	5.70 (3.09 – 10.50)	31	<0.001
	IVC diameter >2cm in cohort 2 N = 335					
HPVS - %	1.00 (0.98 – 1.02)	0	0.93	-	-	-
HPVS >0% (vs. ≤0%)	1.10 (0.63 – 1.93)	0	0.74	-	-	-
DPVS – dL/g	1.10 (0.87 – 1.39)	1	0.44	-	-	-
DPVS ≥5.2 dL/g	1.24 (0.76 – 2.03)	1	0.39	-	-	-
Log[NTproBNP]	7.08 (4.02 – 12.46)	46	<0.001	-	-	-
	JVD ratio <4 in cohort 2 N = 319					
HPVS - %	1.03 (1.00 – 1.05)	4	0.04	0.99 (0.95 – 1.02)	1	0.42
HPVS >0% (vs. ≤0%)	1.61 (0.91 – 2.83)	3	0.10	0.90 (0.45 – 1.77)	0	0.75
DPVS – dL/g	1.42 (1.11 – 1.83)	8	0.01	1.14 (0.83 – 1.56)	0	0.61
DPVS ≥5.2 dL/g	2.02 (1.22 – 3.33)	8	0.01	1.38 (0.77 – 2.49)	1	0.29
Log[NTproBNP]	15.49 (7.58 – 31.65)	57	<0.001	14.15 (6.88 – 29.07)	52	<0.001

	Lung B-line count in cohort 2 N = 341					
HPVS - %	1.03 (1.00 – 1.05)	4	0.04	1.05 (1.01 – 1.08)	6	0.02
HPVS >0% (vs. ≤0%)	2.65 (1.52 – 4.61)	12	0.001	2.12 (1.09 – 4.12)	5	0.03
DPVS – dL/g	1.34 (1.06 – 1.70)	6	0.02	1.13 (0.83 – 1.53)	1	0.44
DPVS ≥5.2 dL/g	1.66 (1.02 – 2.71)	4	0.04	1.19 (0.67 – 2.12)	0	0.56
Log[NTproBNP]	14.30 (7.16 – 28.58)	57	<0.001	13.71 (6.78 – 27.71)	53	<0.001

Legend

Abbreviations used: H-PVS – plasma volume status as calculated from the Hakim formula ; D-PVS – plasma volume status as calculated by the Duarte formula; NTproBNP – N-terminal pro-B-type natriuretic peptide; NYHA – New York Heart Association; IVC – inferior vena cava; JVD – jugular vein distensibility

Discussion

Our data suggest that neither the Hakim nor Duarte formula for estimating plasma volume have any clinical utility in patients with CHF, regardless of phenotype. We present several important findings regarding the use of estimations of PVS in patients with CHF derived firstly from a large cohort of unselected outpatients with CHF and secondly, the largest reported cohort of patients with CHF with simultaneous comprehensive ultrasound assessment of congestion. Some of our findings are novel, and some have been reported previously but not fully explored in a clinical context.

We found very close correlations between estimations of PVS and the variables from which they are calculated – particularly haemoglobin. While this is an obvious statement, it has important implications if we are to use estimations of PVS as surrogate measures of congestion. Theoretically, haemoglobin and estimations of PVS measure two different things. Both HPVS and DPVS are derived from equations using haemoglobin but for them to be useful clinically, the each must yield information above and beyond what merely measuring haemoglobin alone might give.

It is therefore important to adjust for haemoglobin concentrations when assessing the association between estimations of PVS and disease severity or outcome; it is an obvious confounder: we found almost no association between either HPVS or DPVS and clinical or ultrasound measures of congestion after adjustment for haemoglobin. Additionally, when the

multivariable outcome model was restricted to just age, sex and haemoglobin there was little evidence of additional value from HPVS or DPVS.

HPVS

Actual plasma volume as calculated from weight and haemoglobin in the Hakim formula has moderate positive correlations with invasively measured plasma volume [7,21].[7,] HPVS had only moderate collinearity and correlation with the variables from which it was calculated, and so it may be argued that it offers some clinical information beyond knowledge of the patient's haemoglobin and weight. For example, it was associated with congestion defined by a lung B-line count independent of haemoglobin.

However, estimating actual plasma volume is only half of the Hakim formula: the calculation of ideal plasma volume is entirely dependent on the patient's weight [18]: ideal plasma volume will be higher (and HPVS lower) in a heavier patient – even if the extra weight is due to fluid retention. As a result, in those investigations of HPVS in which weight is reported (including the present one), the body weight of patients who are classed as congested by a higher HPVS is 16-27 kg lighter than those without congestion [7,10].

HPVS had no association with clinical congestion, severity of symptoms, or congestion defined by IVC diameter or JVD ratio. Furthermore, the prevalence of congestion defined by HPVS was highly dependent on BMI – almost no patient with obesity was classed as

congested by HPVS in either cohort, a phenomenon that was not seen with clinical or ultrasound measures of congestion.

Changes in weight or haemoglobin in patients with CHF are not always due to changes in plasma volume [22,23], but may nonetheless be associated with changes in prognosis [24,25]. In addition to the present study, there are two publications that report a significant association between HPVS and outcome after adjustment for haemoglobin or the presence of anaemia in multivariable outcome models [7,26].[7] Both found significant associations between increasing HPVS and worse prognosis of a similar order of magnitude to the present study. At best, HPVS is perhaps an imprecise measure of congestion.

DPVS

We found extreme collinearity between DPVS and haemoglobin to such an extent the vast majority of patients with congestion by DPVS were anaemic, suggesting that the calculation of PVS added nothing to a simple full blood count. By contrast, we found that only 40-50% of patients with congestion by clinical or ultrasound measures were anaemic [27]. Haemodilution will contribute to a decline in haemoglobin concentration and increase the proportion of patients classified as anaemic, but iron deficiency and a fall in red cell mass will also make an important contribution [28].

Haemoglobin and haematocrit only have weak correlations with invasively measured plasma volume in patients with CHF [29]. It is, therefore, unsurprising that the correlation between plasma volume as calculated by the Strauss formula (from which DPVS is derived) [8] and invasively measured plasma volume in patients with heart failure is modest at best (r=0.29; P=0.003) [21]. Indeed, we found no associations between DPVS and clinical or ultrasound measures of congestion or symptoms severity after adjustment for haemoglobin.

Furthermore, it is not immediately clear what DPVS is measuring: in the literature, DPVS has been used as a unitless measurement [8], or the units have varied by a factor of 100 from mL/g [16], to dL/g [30]. As it is derived from haemoglobin, the presumption is that it measures decilitres (or millilitres) of plasma volume per gram of haemoglobin. For a patient with a haemoglobin of 12 g/dL and DPVS of 4.6 the estimated plasma volume would either be 55.2 mL or 55.2 dL - neither figure is plausible.

There are about 20 published reports assessing the association between estimates of PVS and outcome in patients with heart failure [20]. However, no publication that reports a significant association between DPVS and outcome included haemoglobin or the presence of anaemia in the outcome model [9-13], although one excluded patients with anaemia [12], and one found no difference in the association between DPVS and outcome in patients with or without anaemia [9].

Increasing DPVS is associated with higher risk of adverse outcome in patients with other conditions that are not associated with plasma volume expansion, such as cancer [31], or sepsis [32], suggesting that DPVS is not a surrogate measure of congestion, but a complicated way to apply measurements of haemoglobin.

Low haemoglobin is not always associated with plasma volume expansion but still represents a physiological state that predisposes to worse outcome, regardless of the underlying disease process. As anaemic patients have a higher adverse event rate, it comes as no surprise that DPVS is also associated with worse outcome in some analyses.

Ultimately, our data suggest that NTproBNP has closer and more robust correlations with clinical or ultrasound measures of congestion, symptoms severity, and outcome than does HPVS or DPVS. If one knows the patient's NTproBNP concentration, it is difficult to see what extra clinical or prognostic information is gained by estimating the PVS using either equation.

"The great tragedy of science - the slaying of a beautiful hypothesis by an ugly fact."

Thomas Henry Huxley, "Darwin's Bulldog"[33]

Study limitations

The limitations of retrospective analyses apply to our study and confounding factors cannot be excluded. Our data is a snapshot of a single time-point and no conclusions can be drawn on the importance of changing PVS over time nor the effect of HF medications on different measures of plasma volume. Short-term changes in haemoglobin might be a useful guide to the impact of HF therapy [34]. However, there are processes other than plasma volume expansion that can cause a fall in haemoglobin. Finally, although some may not accept an NTproBNP > 125 ng/L as diagnostic for HeFNEF or HeFMREF, it is consistent with recent European Society of Cardiology guidelines [17].[17]

It might be argued that our analysis should have used previously published cut-offs for both HPVS (≥-4%) and DPVS (≥5.5 dL/g) to define congestion by those estimations of PVS [20]. However, those cut-offs were specific to the populations being studied and may not apply to ours. We used an HPVS >0%, the value at which actual plasma volume exceeds ideal, and a DPVS of ≥5.2 dL/g, the upper quartile of values, to define congestion.

Conclusion

Neither estimation of PVS has consistent or strong associations with clinical or ultrasound measures of congestion, severity of symptoms, or adverse outcome in patients with CHF; by contrast, NTproBNP was strongly and independently associated with all three. DPVS is strongly correlated with haemoglobin, consequently the presence of anaemia strongly dictates

the presence of congestion by DPVS. HPVS is strongly correlated with body weight, consequently the presence of obesity strongly dictates the presence of congestion by HPVS. Therefore, it is unlikely that either HPVS or DPVS has any clinical utility as a measure of congestion.

Figure legends

Figure 1

Abbreviations used: HPVS – plasma volume status as calculated by the Hakim formula; DPVS – plasma volume status as calculated by the Duarte formula; IVC – inferior vena cava; JVD – jugular venous distensibility.

Figure 2

Scatterplots of HPVS and DPVS and the variables from which they are calculated in cohort 1 (panel A) and cohort 2 (panel B), and a univariable correlations in both cohorts (panel C). Positive correlations between variables are shaded red; negative are shaded blue. Where two coefficients are given in a single cell, the top line is r for cohort 1, bottom line is r for cohort 2. Abbreviations used: HPVS – plasma volume status as calculated from the Hakim formula; DPVS – plasma volume status as calculated by the Duarte formula; NTproBNP – N-terminal pro-B-type natriuretic peptide; BMI – body mass index; LAVI – left atrial volume index; TR – tricuspid regurgitation; IVCD – inferior vena cava diameter; JVD – jugular vein distensibility; VIF – variance inflation factor.

Figure 3

Anaemia was defined as <13 g/dL in men and <12 g/dL in women. Obesity was defined as a BMI >30 kg/m^2. Almost all patients with congestion by HPVS or DPVS were anaemic and almost no patient with congestion by HPVS or DPVS were obese. Abbreviations used: BMI – body mass index; CCS – Clinical Congestion Score; DPVS – plasma volume status as

calculated by the Duarte formula; HPVS – plasma volume status as calculated by the Hakim formula;

Supplementary Figure 1

Of the 3505 patients in cohort 1, 164 patients were also included in cohort 2. We performed the same analyses in both cohorts separately. Abbreviations used: LVSD – left ventricular systolic dysfunction; NTproBNP – N-terminal pro-B-type natriuretic peptide; US – ultrasound.

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The data underlying this article will be shared on reasonable request to the corresponding author.

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