SUPPLEMENTARY APPENDIX

Hemoglobin concentration, total hemoglobin mass and plasma volume in patients: implications for anemia

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Supplementary Information

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Participant recruitment and testing sites

Patients were recruited from those managed at University College Hospital (University College London Hospitals NHS Foundation Trust, UCLH), Southampton General Hospital (University Hospital Southampton NHS Foundation Trust) and the Royal Free Hospital (Royal Free London NHS Foundation Trust, RFH). Supplementary Figure 1 shows a Consolidated Standards of Reporting Trials (CONSORT)- style flow diagram indicating included and excluded patients and specific sub-groups. HV, patients with IBD and those awaiting surgery were recruited from and tested at UCLH only. Patients with CLD were recruited from and tested at all three sites, and those with CHF at UCLH and Southampton. Healthy volunteers were members of staff at UCLH or University College London (UCL) recruited by word of mouth or email advertisement. Patients were identified and recruited at outpatient clinics or when attending other routine clinical appointments.

Eligibility criteria

Inclusion criteria for all participants were, age >18 years, and ability give informed consent and to comply with basic breath-holding instructions. Healthy volunteers were additionally free from known chronic disease or use of regular medications. In CHF (clinically diagnosed and under treatment, confirmed- as clinically indicated- by the presence of a circulating concentration of B-type natriuretic peptide (BNP) >200 pg ml^{-1}), additional inclusion criteria were echocardiographic or cardiac magnetic resonance imaging (cMRI) evidence of left ventricular (LV) systolic impairment as defined by a left ventricular ejection fraction of less than 50%; New York Heart Association (NYHA) class II-IV; and not fully stabilised, i.e. still symptomatic with shortness of breath and/or peripheral oedema and/or clinical signs of bibasal crepitations or peripheral oedema.

Patients with CLD all had a diagnosis of alcoholic liver disease, primary sclerosing cholangitis, hepatitis C or cryptogenic cirrhosis. A confirmed diagnosis of IBD (either Crohn's disease or ulcerative colitis) was required for inclusion into this group. For surgical patients, inclusion was based on a patient being scheduled to undergo elective major surgery, regardless of specialty.

Excluded were prisoners, or those with a baseline %COHb greater than 5% (as can occur in smokers), presence of haemoglobinopathy.

Blood sampling

At UCLH and RFH, [Hb] was determined from a fingertip capillary blood sample (Hb 201 Microvette, Hemocue AB, Angelholm, Sweden), analysed immediately (HemoCue® Hb 201+, Hemocue AB, Angelholm, Sweden) when [Hb] could not be obtained from venous blood at the time of testing or was not available on the same day as tHb-mass measurement from hospital electronic records. At UCLH and RFH, fingertip capillary samples (200 µl) were collected before and 6- and 8-min after the start of CO rebreathing (Na-heparinized 200 µl RAPIDLyte Multicap Capillary tubes, Siemens Healthcare Diagnostics Inc, Deerfield, USA), with samples analysed within 15 minutes for percent carboxyhaemoglobin (%COHb) using a blood gas analyser (Hemoximeter; Cobas b 221 POC system, Roche Diagnostics Ltd, Switzerland). At Southampton, [Hb] was measured in venous blood. An intravenous cannula was inserted prior to tHb-mass testing at Southampton, allowing venous blood samples (200 µl) to be collected before and at 6- and 8-mins after CO rebreathing via a Na-heparinized blood gas syringe (RAPIDLyte, Siemens Healthcare Diagnostics Inc, Deerfield, USA). %COHb was determined at Southampton using the RAPIDPoint 500 Blood Gas System (Siemens Healthcare Diagnostics Inc, Deerfield, USA). All blood samples for the determination of [Hb],

haematocrit (Hct) and %COHb were collected under the same conditions, from the same anatomical site, and with patients in a seated position. Blood samples were analysed within 10-15 minutes of one another due to %COHb being analysed after the oCOR test using a blood gas machine (which takes 15 minutes). Blood sampling from venous, arterial and capillary blood yields an identical Δ%COHb and therefore identical tHb-mass values. [1]

Calculation of tHb-mass

tHb-mass was calculated using a specifically design excel spreadsheet (Microsoft Excel 2011 for Apple Macintosh) using the formula:

$$\text{tHb-mass (g)} = K \times \text{MCO(ml)} \times 100 \times (\Delta\%\text{COHb} \times 1.39)^{-1}$$

K = barometric pressure x 760^{-1} x [1(0.003661 x temperature)]

$\text{MCO} = \text{CO}_{adm} - (\text{CO}_{system + lung\ (after\ disconnection)} + \text{CO}_{exhaled\ (after\ disconnection)}$

CO_{adm} = CO volume administered into the system

$\text{CO}_{system + lung\ (after\ disconnection)}$ = CO concentration in spirometer x (spirometer volume + remaining volume in the lung after disconnection)

$\text{CO}_{exhaled\ (after\ disconnection)}$ = end-tidal CO concentration x alveolar ventilation x time

Δ%COHb = difference between baseline %COHb and %COHb post CO administration (average of 6- and 8-min %COHb values)

1.39 = Hüfners number (constant) (ml CO x g Hb^{-1})

where:

lung residual volume, 1500 ml in men, 1200 ml in women; alveolar ventilation, 5000 ml min^{-1}, CO concentration is in parts per million (ppm)

Blood volume (BV), plasma volume (PV) and red cell volume (RCV) were calculated

from mean corpuscular haemoglobin concentration (MCHC), [Hb] and tHb-mass, as below:

BV (ml) = tHb-mass (g)/[Hb] ($g \cdot dl^{-1}$) • 100
RCV (ml) = tHb-mass (g)/MCHC • 100
PV (ml) = BV – RCV

When Hct and [Hb] values were obtained from capillary blood at UCLH and the RFH these values were corrected to venous conditions using the following formulas [2, 3]:

[Hb] ($g \cdot dl^{-1}$) = [Hbcapillary] • 0.8787 + 1.24
Hct (%) = [Hctcapillary] • 0.8425 + 5.23

Estimation of normal adult values for PV were calculated using the formulas from the 1995 Expert Panel on Radionuclides of the International Council for Standardisation in Haematology. [4]

Equation 1. Estimation of normal male plasma volume

$$\text{Mean normal PV (ml)} = 1578 \times S$$

Equation 2. Estimation of normal female adult plasma volume

$$\text{Mean normal PV (ml)} = 1395 \times S$$

Where S = body surface area (m^2) calculated using the formula of Du Bois and Du Bois. [5]

PV values were adjusted for age, sex, weight, and height using a published formula to calculate normal volumes as derived from >100,000 measurements of height and weight from Metropolitan Life tables. [6] Normal PV was classified as measured volumes within ± 8% of the expected normal volume on an individual level. Mild to moderate volume

expansion was considered >8% to ≤25% positive deviation from expected norms, and severe as >25% above the expected normal volume. [6]

Supplementary references

1. Garvican LA, Burge CM, Cox AJ, Clark SA, Martin DT, Gore CJ. Carbon monoxide uptake kinetics of arterial, venous and capillary blood during CO rebreathing. Exp Physiol. 2010;95(12):1156-1166.

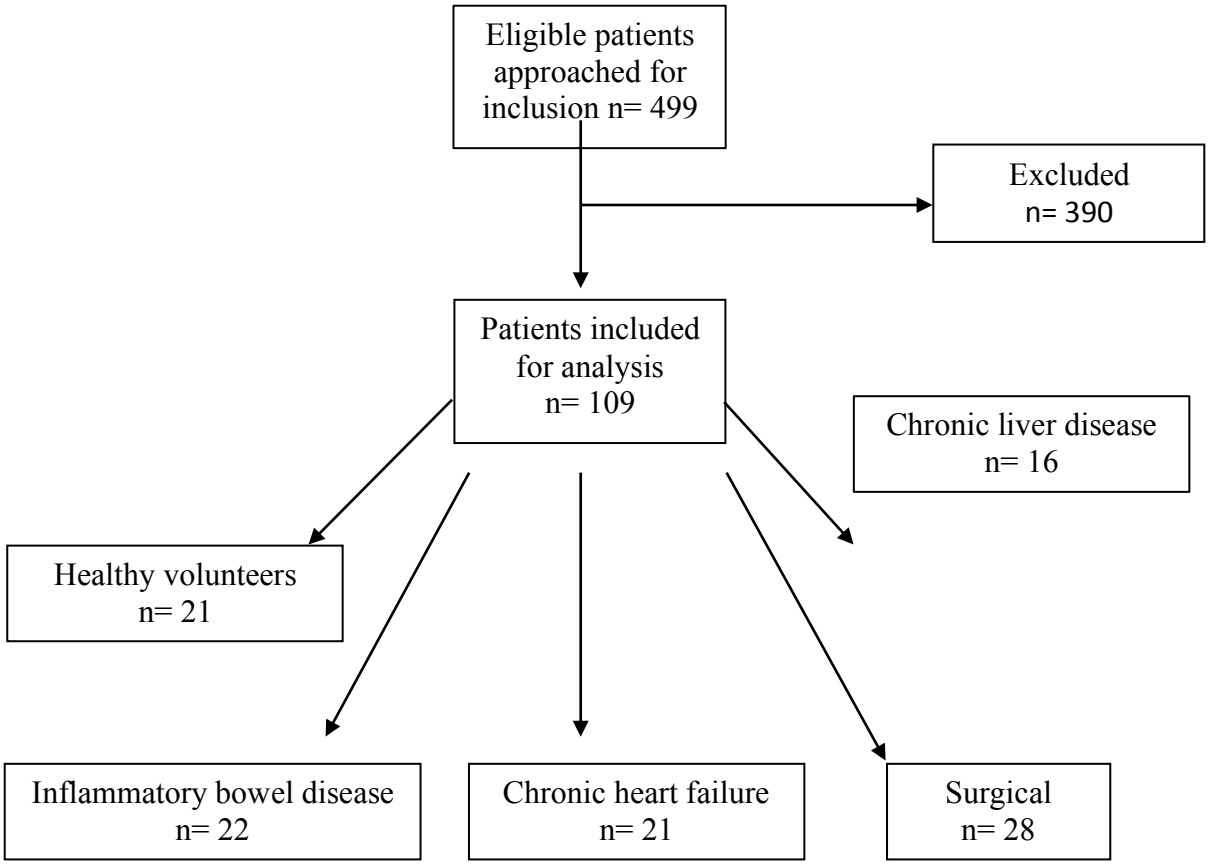
2. Chaplin H, Jr., Mollison PL, Vetter H. The body/venous hematocrit ratio: its constancy over a wide hematocrit range. J Clin Invest. 1953;32(12):1309-1316.

3. Schumacher YO, Pottgiesser T, Ahlgrim C, Ruthardt S, Dickhuth HH, Roecker K. Haemoglobin mass in cyclists during stage racing. Int J Sports Med. 2008;29(5):372-378.

4. Pearson TC, Guthrie DL, Simpson J, et al. Interpretation of measured red cell mass and plasma volume in adults: Expert Panel on Radionuclides of the International Council for Standardization in Haematology. Br J Haematology. 1995;89(4):748-756.

5. Du Bois D, Du Bois E. Clinical calorimetry: Tenth paper to estimate the approximate surface area if height and weight are known. Arch Intern Med. 1916;17:863-871.

6. Feldschuh J, Enson Y. Prediction of the normal blood volume. Relation of blood volume to body habitus. Circulation. 1977;56(4 Pt 1):605-612.



Supplementary Figure 1. Consolidated Standards of Reporting Trials (CONSORT)- style flow diagram indicating included and excluded patients and specific sub-groups. Excluded patients are those who declined participation, failed to respond to correspondence via letter and telephone contact or could not be contacted despite multiple attempts by the research team.

Table-S1. Surgical specialty for planned surgical procedure (n= 28).

Surgical specialty	**Frequency N (%)**
Upper gastrointestinal	8 (28.5%)
Lower gastrointestinal	8 (28.5%)
Orthopaedic	7 (25%)
Other	3 (10.7%)
Urology	2 (7.1%)

Table-S2. Characteristics and aetiology of inflammatory bowel disease patients.

Variable	**IBD (n= 22)**
Gender	
Male (%)	50%
Age (yr)	50 (33-62)
Height (cm)	170.2 ± 7.3
Weight (kg)	77.7 ± 18.1
Aetiology of IBD	
Crohn's disease	4 (18%)
Small bowel CD	2 (9%)
Terminal ileal CD	1 (4.5%
Ulcerative colitis	9 (41%)
Ulcerative proctitis	3 (14%)
Pan UC	2 (9%)
Collagenous colitis	1 (4.5%)

IBD, Inflammatory bowel disease; CD, Crohn's disease; UC, Ulcerative colitis

Table-S3. Characteristics, medications and aetiology of liver disease patients.

Variable	**LD (n= 16)**
Gender	
Male (%)	75%
Age (yr)	51 (48-55)
Height (cm)	174.8 ± 8.9
Weight (kg)	85.3 ± 18.5
Primary aetiology of liver disease	
Hepatitis	3 (19%)
ALD	8 (50%)
Cryptogenic cirrhosis	2 (12%)
Sclerosing cholangitis	2 (12%)
Other	1 (6%)
Hypertension	1 (6%)
Medication	
Beta blocker	3 (19%)
ACE inhibitor	0 (0%)
Statin	0 (0%)
Diuretic	5 (31%)
Diuretic times per day	
None	11 (69 %)
Once	5 (31 %)
Bi daily	0

ALD, decompensated alcoholic liver disease; ACE inhibitor, angiotensin-converting-enzyme inhibitor.

Table-S4. Characteristics, medications and aetiology of chronic heart failure patients.

Variable	**CHF (n= 22)**
Gender	
Male (%)	82%
Age (yr)	68 (61-75)
Height (cm)	173.5 ± 8.1
Weight (kg)	80.4 ± 15.9
Aetiology of heart failure	
Ischaemic heart disease	7 (32%)
Cardiomyopathy	10 (45%)
Ischaemic	5 (22%)
Non-ischaemic	5 (22%)
Other	5 (22%)
Hypertension	7 (32%)
NHYA class	
I	2 (9%)
II	14 (64%)
III	4 (18%)
IV	2 (9%)
LVEF (%)	33.2 ± 11.4
Medication	
Beta blocker	19 (86%)
ACE inhibitor	20 (91%)
Statin	16 (73%)
Diuretic	17 (77%)
Diuretic dose (mg)	25 ± 29
Diuretic times per day	
None	5 (22%)
Once	14 (64%)
Bi daily	3 (14 %)

NYHA, New York Heart Association Class; LVEF (%), left ventricular ejection fraction; ACE inhibitor, angiotensin-converting-enzyme inhibitor

Table-S5. Relationship between total haemoglobin mass and haemoglobin concentration in sub-groups based on plasma volume status.

	Plasma volume status		
Sub group	**Normal**	**Mild to moderate**	**Severe**
IBD	(n= 9)	(n= 7)	(n= 5)
	r= 0.86, p= 0.003	r= 0.94, p< 0.002	r= 0.78, p= 0.118
Surgical	(n= 8)	(n= 14)	(n= 5)
	r= 0.94, p= 0.001	n= 0.78, p= 0.001	r= 0.80, p= 0.104
CHF	(n= 4)	(n= 6)	(n= 11)
	r= 0.95, p= 0.046	r= 0.86, p< 0.029	r= 0.59, p= 0.055
HV	(n=2)	(n= 8)	(n= 11)
		r= 0.91, p< 0.002	r= 0.89, p< 0.0001

r, Pearson's correlation coefficient; PV values were adjusted for age, sex, weight, and height using a published formula to calculate normal volumes as derived from >100,000 measurements of height and weight from Metropolitan Life tables. [6] Normal PV was classified as measured volumes within ± 8% of the expected normal volume on an individual level. Mild to moderate volume expansion was considered >8% to <25% deviation from expected norms, and severe as >25% of the expected normal volume. [6] Insufficient numbers to perform sub-group analysis in chronic liver disease group. IBD, inflammatory bowel disease; CHF, chronic heart failure; HV, healthy volunteers.