Measurement of total circulating blood volume following subarachnoid haemorrhage: methodological aspects

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SUMMARY The total circulating blood volume (TCBV) and total body/venous haematocrit ratio (Htb/Hv) was determined by simultaneous measurement of the red cell volume (RCV) and the plasma volume (PV) in 10 subarachnoid haemorrhage (SAH) patients, 10 supine bedresting control patients and 20 ambulant out-patients. The mean Htb/Hv of the SAH patients, 0.866, was found to be significantly lower than that of the supine controls, 0.908, and the ambulant patients, 0.909, ($p < 0.01$). Using the ratio 0.866 the total circulating blood volume of the SAH patients was calculated from either their RCV or their PV and compared with their measured TCBV. Expressed as a percentage of measured TCBV the mean errors of these single volume determinations were 2.84% and 1.76% respectively. The significance of these changes in the Htb/Hv ratio of SAH patients is discussed in relation to the circulatory disturbances they suffer.

Patients with subarachnoid haemorrhage (SAH) are particularly vulnerable to the insidious development of hypovolaemia,¹⁻³ which may exacerbate the effects of cerebral vasospasm and cerebral ischaemia. Correction of hypovolaemia in these circumstances may help reverse ischaemic neurological deficits.⁴ Thus the ability to measure total circulating blood volume (TCBV) is of importance to both the clinical management of these patients and to further research aimed at understanding the pathophysiology of hypovolaemia accompanying intracranial disease. The present study was planned to examine factors which might influence the accuracy and practicability of blood volume measurement in SAH patients with particular regard to changes in the total body/venous haematocrit ratio (Htb/Hv), and the validity of single volume measurements of TCBV.

Physiological considerations

Although there is no direct method of determining the TCBV of a patient it is generally accepted that the sum of the simultaneously measured red cell volume (RCV) and plasma volume (PV) accurately reflects

TCBV.^{5,6} In practice simultaneous measurement of RCV and PV is both technically demanding and time-consuming and may not be appropriate to the management of critically ill patients. For these reasons many workers choose to measure one or other of the two blood compartments, RCV or PV, and derive the TCBV using the haematocrit:

$$\text{Haematocrit} = \frac{\text{RCV}}{\text{RCV} + \text{PV}} \quad \text{where } \text{RCV} + \text{PV} = \text{TCBV}$$

$$\text{thus TCBV} = \frac{\text{RCV}}{\text{Haematocrit}} \quad \text{or} \quad \text{TCBV} = \frac{\text{PV}}{1 - \text{Haematocrit}}$$

The haematocrit shown above relates the total RCV to the total PV and is usually referred to as the total body haematocrit (Htb). It is in fact a theoretical value only obtained when both RCV and PV are measured. In reality the haematocrit of blood varies throughout the body and when the haematocrit of venous blood (Hv), which is usually sampled in clinical practice, is measured it is invariably found to be higher than the Htb. If TCBV is calculated using the uncorrected Hv significant errors occur.⁷ These changes in haematocrit are largely due to the rheological properties of whole blood. As blood passes through a vessel a gradient of flow velocities is estab-

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lished from a stationary layer of plasma at the endothelial surface to a fast moving layer of blood in the centre. Red cells tend to flow in the centre leaving plasma rich blood peripherally, creating a "haematocrit gradient" to match the flow gradient.⁸ With decreasing vessel size the ratio of surface area to volume increases and flow rates decrease. The relative proportion of slow moving blood of low haematocrit which is close to the endothelial surface increases. At the extremes of the microcirculation single red cells surrounded by plasma negotiate capillaries. As a consequence the haematocrit in the capillary bed is lower than that in larger capacitance vessels. This phenomenon was first observed *in vitro* by Fahraeus in 1929⁹ and subsequently confirmed experimentally.^{10 11}

The relationship between the total body haematocrit and the venous haematocrit remains remarkably constant in normal subjects and both anaemic and polycythaemic patients with haematocrits ranging from 9% to 82%.¹²⁻¹⁴ It is usually expressed as a ratio, Htb/Hv, with a commonly agreed value of 0.91. This ratio can be used to estimate the total body haematocrit from the venous haematocrit: $Htb = 0.91Hv$. TCBV may then be calculated as follows:

$$TCBV = \frac{RCV}{0.91Hv} \text{ or } TCBV = \frac{PV}{1-0.91Hv}$$

The use of this method to determine blood volume assumes that no significant change has occurred in the Htb/Hv ratio. Such an assumption may be justified in clinical situations where extensive experience of blood volume measurement has already been obtained. But in circumstances where the ratio is likely to be disturbed by circulatory changes its absolute value should be established before calculating any of the blood volumes from RCV or PV alone.

Modern techniques for the measurement of RCV and PV are based on an isotope dilution technique. Their accuracy depends on complete and homogeneous mixing of the isotopically-labelled marker substance in the space to be measured, the stability of the marker and its accurate measurement following dilution. Ideally the marker should remain entirely within the space or should leave it at a predictable and constant rate. Autogenous ⁵¹Cr labelled red blood cells (RBCs) fulfil these requirements and are most commonly used for determining RCV. PV is conveniently measured using radio-iodinated human serum albumin. However albumin is continuously lost from the circulation to the lymphatic and reticulo-endothelial systems^{15 16} with the result that the uncorrected albumin space is greater than the true PV. This problem may be overcome by counting the activity of a number of dilutional samples at set intervals and extrapolating

back to time-zero, giving a measure of perfect or instantaneous mixing of the labelled albumin within the plasma.

Materials and methods

Ten consecutive and unselected patients (ages 37-55 years, mean 47) in whom a diagnosis of SAH had been confirmed by lumbar puncture and/or CT scanning were studied on the day following their admission to the Wessex Neurological Centre. The studies took place between the second and tenth day after SAH, reflecting the pattern of referral to the unit. The conscious level of the patients was recorded using the Glasgow Coma Score, GCS.¹⁷ Five patients with a GCS of 13 or more were receiving free diet and fluids before their admission, the remaining five, with scores of 12 or less had received supplementary intravenous fluids, 1500-2000 ml per day. Ten control subjects matched for age, sex, and weight were selected from amongst patients resting in bed whilst awaiting myelographic examination of non-malignant cervical and lumbar spine disease. The patients or their close relatives and the controls gave informed consent to the study and local ethical committee approval was received. During the course of the study data were collected from 20 unselected and unmatched ambulant out-patients referred to the Department of Nuclear Medicine for measurement of RCB and PV to confirm or refute the diagnosis of absolute polycythaemia in the presence of an elevated packed cell volume.

The RCV and PV were determined in each case at 9.00 am following the recommendations of the International Committee for Standardisation in Haematology.⁷ In brief, the spun red cells from 8 ml of fresh blood were incubated for 20 minutes with 750 KBq ⁵¹Cr (Amersham code No CJS 1P) and then washed in normal saline. The red cells together with 300 KBq ¹²⁵I human serum albumin (¹²⁵I-HSA Amersham code No IM 17P) were then used to prepare standard solutions, before weighing, injection into the patient, and then reweighing. Fifteen and 30 minutes after injection heparinised blood samples were withdrawn from the patient via separate venepunctures. After separation and lysis the ¹²⁵I and ⁵¹Cr were counted separately in the plasma and whole blood. The haematocrit of the venous blood was determined using a microhaematocrit method. The RCV and PV were then calculated from the concentrations of the standards, the venous haematocrit and the mean counts of the standards, blood and plasma corrected to time zero. TCBV was calculated as the sum of RCB and PV and total body haematocrit as $RCV/TCBV$.

Results

The details of the patients and controls, including their ages, sex, weight, peripheral venous haematocrit and Htb/Hv are shown in tables 1, 2 and 3. In addition, tables 1, 2 and 3 include the individual blood volumes of the patients and controls. A tendency to a reduction in the circulating blood volumes of the SAH patients when compared with the supine controls is evident but this does not reach statistical

Table 1 *SAH patients. Venous haematocrits, blood volumes and Htb/Hv*

Name	Age (yr)	Sex	Wt kg	Days after SAH	GCS	Hb g/dl	Hv %	PV litres	RCV litres	TCBV litres	Htb/Hv
1	38	F	56	9	11	11.8	36	2.27	1.05	3.32	0.86
2	54	F	53	5	14	14.6	43	1.93	1.00	2.93	0.82
3	55	F	63	3	5	15.3	49	2.07	1.54	3.61	0.86
4	55	F	58	10	9	14.7	45	1.91	1.26	3.17	0.87
5	42	M	85	3	12	15	44	2.48	1.59	4.07	0.89
6	53	F	73	9	13	15.7	43	2.38	1.47	3.85	0.88
7	46	M	70	8	14	15.4	46	2.83	1.95	4.78	0.87
8	43	M	85	4	14	15.4	47	3.33	2.44	5.77	0.89
9	37	M	70	2	10	13.5	43	2.93	1.70	4.65	0.84
10	48	F	83	5	13	15.1	48	2.14	1.61	3.75	0.88
Mean	47		70				Mean	2.43	1.56	3.99	0.866
SEM	2.2		3.8				SEM	0.15	0.13	0.28	0.0007

Table 2 *Supine controls. Venous haematocrits, blood volumes and Htb/Hv*

Control	Age (yr)	Sex	Wt kg	Hb g/dl	Hv %	PV litres	RCV litres	TCBV litres	Htb/Hv
1	38	F	67	12.7	35	2.32	1.13	3.45	0.91
2	42	F	52	12.1	33	3.12	1.34	4.46	0.91
3	41	F	76	13.3	40	2.89	1.65	4.54	0.90
4	50	F	58	14.2	40	2.57	1.53	4.1	0.93
5	56	M	74	13.8	45	3.27	2.24	5.51	0.89
6	59	F	61	11.6	40	2.12	1.15	3.27	0.88
7	57	M	67	15.6	48	2.59	2.05	4.64	0.92
8	59	M	92	14.9	40	3.33	1.96	5.29	0.93
9	37	M	70	11.9	45	2.39	1.68	4.07	0.91
10	46	F	72	11	39	2.56	1.42	3.98	0.90
Mean	48		70		Mean	2.72	1.61	4.33	0.908
SEM	2.78		3.5		SEM	0.13	0.12	0.23	0.0005

Table 3 *Ambulant controls. Venous haematocrits, blood volumes and Htb/Hv*

Control	Age (yr)	Sex	Wt kg	Hct %	PV litres	RCV litres	TCBV litres	Htb/Hv
1	31	M	70	55	2.22	2.16	4.38	0.89
2	48	M	92	50	3.26	2.12	5.38	0.86
3	47	M	81	53	2.78	2.79	5.57	0.94
4	41	M	65	66	3.28	5.26	8.54	0.92
5	49	M	79	51	2.50	2.10	4.60	0.99
6	49	F	63	51	2.16	2.03	4.19	0.94
7	48	M	66	44	2.31	1.76	4.07	0.97
8	61	M	82	53	2.37	2.32	4.69	0.92
9	51	M	70	51	2.75	2.3	5.05	0.88
10	76	M	77	48	2.11	1.65	3.76	0.89
11	43	M	80	44	3.26	2.12	5.38	0.89
12	66	M	92	55	3.21	3.34	6.55	0.89
13	70	M	67	55	3.12	2.93	6.05	0.87
14	49	M	87	46	2.50	2.12	4.62	0.86
15	64	F	66	50	2.04	2.02	4.06	0.99
16	67	F	83	60	1.94	2.30	4.24	0.88
17	42	M	79	56	2.46	2.64	5.10	0.92
18	29	M	82	51	2.48	2.15	4.63	0.90
19	62	F	60	52	1.77	1.60	3.37	0.90
20	69	F	63	52	2.40	2.06	4.46	0.88
Mean	53		75		2.546	2.39	4.93	0.909
SEM	2.9		2.2		0.11	0.18	0.25	0.0008

Table 4 Total body/venous haematocrit ratio

	SAH patients	Supine controls	Ambulant controls
Mean Htb/Hv	0.866	0.908	0.909
Range	0.82-0.89	0.88-0.93	0.86-0.99
SEM	0.0007	0.0005	0.0008
P	-----	<0.01	-----
	-----	<0.01	-----

significance when analysed using Student *t* test for unpaired data (see discussion). Although there was no significant difference in the PV of the ambulant group their RCV and consequently TCBV were higher due to the presence of polycythaemia in a number of subjects.

Table 4 summarises the Htb/Hv data. The variance of the data was analysed and Duncan's multiple range test applied to test for statistically significant differences. The Htb/Hv ratio of the SAH patients was significantly different from both the supine and ambulant controls, $p < 0.01$.

The TCBV of the patients was calculated from either their PV or RCV and Hv corrected by the ratio 0.866 established above, and these results compared with the measured TCBV (table 5). The differences

were expressed as a percentage error of the measured TCBV. Both methods gave acceptable results in terms of laboratory error and clinical application. In particular the use of PV alone yielded a mean error of 1.76%. If a ratio of 0.91 had been used with either RCV or PV the mean errors would have been 4.24% and 4.1% respectively.

Finally, assuming a linear fall in counts from the 125I-HSA between the 15 minute and 30 minute samples the individual slopes (counts/time) of the SAH patients and supine controls were compared using Student's *t* test for unpaired data. No significant difference was detected (see table 6).

Discussion

The mean Htb/Hv ratios of the supine controls and ambulant patients in this study were 0.908 and 0.909 respectively, agreeing closely with the values obtained by previous workers. A supine control group was chosen for comparison with the bed-resting SAH patients because changes in posture may affect plasma proteins and the haematocrit.¹⁸ The addition of an ambulant patient group allowed us to confirm the constancy of the Htb/Hv ratio measured by our technique over a larger range of haematocrit values and to compare this with other studies.

Table 5 TCBV (litres) SAH patients. Measured and derived values. % Errors

Measured	RCV/(0.866 Hv)	% Error	PV/(1-0.866 Hv)	% Error
3.32	3.37	1.50	3.29	0.9
2.93	2.69	8.19	3.07	4.9
3.61	3.63	0.55	3.60	0.27
3.17	3.23	1.89	3.13	1.1
4.07	4.17	2.45	4.01	1.47
3.85	3.95	2.59	3.79	1.56
4.78	4.90	2.51	4.70	1.67
5.77	5.99	3.81	5.62	2.25
4.65	4.57	1.72	4.70	1.07
3.75	3.87	3.2	3.66	2.40
	Mean % Error	2.84		1.76
	SEM	0.66		0.44

Table 6 125I-HSA clearance. Patients and controls

Patients	Counts (15 min)	Counts (30 min)	Slope	Controls	Counts (15 min)	Counts (30 min)	Slope
1	5419	5417	0.2	1	1906	1891	0.9
2	4445	4402	2.86	2	2669	2597	4.73
3	4061	3912	9.93	3	2189	2183	1
4	5580	5244	22.4	4	5844	5684	10.67
5	3946	3907	2.6	5	4634	4598	2.4
6	4649	4549	6.67	6	5020	4802	14.53
7	4579	4455	12.4	7	4276	4268	0.53
8	3475	3320	10.33	8	3572	3524	3.27
9	4538	4354	12.27	9	4332	4256	5.07
10	4060	3904	8	10	5458	5173	19
		Mean	8.78			Mean	6.2
		SEM	2.02			SEM	2.02

For the Htb/Hv ratio to depart significantly from the "normal range" profound circulatory disturbances must occur. Animal studies suggest that approximately 20% of the TCBV lies in the capillary bed.¹⁹ Thus in a simple two compartment model of the circulation consisting of a capillary bed with a haematocrit (Hc) and large capacitance vessels whose haematocrit equals the venous haematocrit (Hv) the total body haematocrit is represented by:

$$\text{Hbt} = 0.2 \text{Hc} + 0.8 \text{Hv}$$

Taking the Htb/Hv to be 0.9, the capillary haematocrit can be calculated to be 50% of the venous haematocrit, a figure which compares well with observed values.⁸

Using the same formula a reduction in the Htb/Hv ratio to 0.85 (a fall similar in magnitude to that observed in the SAH patients), if due to changes in the capillary circulation alone would require either a 50% increase in the capacity of the capillary bed or a 50% reduction in the capillary haematocrit. In this context it is interesting to note that patients with severe chronic anaemia in whom adaptive changes in the capillary circulation would be expected, often have Hb/Hv ratios below 0.85,²⁰ but it is difficult to conceive of similar changes occurring during acute illness. In contrast to this a 5% reduction in TCBV if confined solely to the venous circulation could alter the Htb/Hv from 0.9 to 0.85 with no change in venous or capillary haematocrit. Falling Htb/Hv ratios have been observed in the first 48 hours following severe burns when significant hypovolaemia occurs.²¹

Hypovolaemia occurs commonly following a SAH.³ In this series there was an 8% reduction in mean TCBV of the patients compared with the controls. Although this reduction did not reach statistical significance it could, as argued above, easily account for the observed changes in Htb/Hv ratios. A reduction in plasma volume occurs in the supine position.^{22,23} In choosing supine controls to examine changes in the Htb/Hv we accepted that their TCBV would be lower than predicted normal values which other workers have used to examine the incidence of hypovolaemia. The pre-existence of a degree of hypovolaemia in the controls could account for the failure to achieve statistical significance. However when the blood volume of the SAH patients was compared with their predicted values for age, sex and weight a 16.3% reduction in TCBV had occurred ($p < 0.05$ using a paired Student's *t* test for the measured and predicted TCBV).

The aetiology of hypovolaemia following SAH remains obscure. It may relate to dehydration, increased catabolism and decreased erythropoiesis. However it is tempting to speculate that the profound sympathetic disturbances which accompany a

SAH^{24,25} are responsible for increased vasomotor activity tending to vasoconstrict the large venous capacitance vessels more than the capillary bed.²⁶ Thus a combination of altered vasomotor tone and hypovolaemia would both act principally to reduce the venous compartment of the circulation with a consequent fall in the Htb/Hv ratio.

There is much evidence to suggest that the sympathetic system has an important role in regulating trans-capillary exchange of fluid and macromolecules.²⁷ This control probably occurs at the level of the functional pre-capillary sphincter. By closing down the pre-capillary sphincters the high levels of circulating catecholamines accompanying a SAH might be expected to reduce the rate of albumin clearance. In this study there was no significant difference between the albumin clearances of the control and patient groups.

Estimating TCBV from either RCV or PV alone resulted in mean % errors of 2.84% and 1.76% respectively. Three basic sources of error exist when measuring blood volume in this way: firstly that the Htb/Hv of a group of patients departs significantly from the accepted norm and this is not taken into account; secondly, that the Htb/Hv of a patient differs significantly from the group; and, finally, that a technical error in measurement of either RCV or PV occurs. The first error may be dealt with by individual workers establishing a Htb/Hv for a population under study. The second cannot be resolved using RCV or PV alone. With regard to the last, because PV almost invariably exceeds RCV in SAH patients, similar errors in measurement will affect the value of the TCBV least when using PV. With these considerations in mind we feel the use of PV measurement to estimate TCBV in SAH patients to be justified.

In conclusion, (1) a significant reduction in the Htb/Hv ratio follows a SAH; (2) this may be explained in terms of the circulatory changes which accompany a SAH; (3) using a Htb/Hv (in this series 0.866) determined for a group of patients, TCBV may be determined from PV alone with a degree of accuracy acceptable for clinical management and investigation.

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