Haematocrit is invalid for estimating red cell volume: a prospective study in male volunteers

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Abstract: BACKGROUND: Although haematocrit and haemoglobin value are concentrations, they are commonly used to guide clinical decisions involving red cell and plasma volumes. A study challenging this convention systematically co-determined and compared these measures. MATERIALS AND METHODS: Using a non-radioactive double-tracer technique to assess blood volume components, measurements were taken once in 46 healthy male endurance athletes. The best predictors of blood composition were derived from the first 36 athletes by automated stepwise forward selection of non-invasive metric parameters (age, weight, height, body surface area and body mass index) and the resulting formulae validated in the remaining ten volunteers. Haematocrit, haemoglobin concentration, red cell volume and plasma volume were measured again 4 weeks later in eight randomly selected volunteers. RESULTS: Red cell volume (2,282±283 mL) did not correlate with either haematocrit (0.42±0.02) or haemoglobin concentration (14.2±0.8, P>0.05, resp.), but was predictable from body surface area (red cell volume [mL]=1,547 × body surface area [m2]-723; r=.88, P<0.01). A similar accuracy was unobtainable using any potential predictor for plasma or blood volume, haematocrit or haemoglobin concentration. Red cell volume showed high intra-individual stability when measured again after 4 weeks, whereas plasma volume oscillated in both directions by up to 22%. DISCUSSION: Only red cell volume shows sufficiently stable intra- and interindividual values to be an accurate, objective indicator of normality in blood composition. The measurement technique is feasible in the outpatient setting and this parameter provides effective, robust, and readily available diagnostic information that might be useful in numerous clinical situations. Its clinical significance does, however, remain to be demonstrated.

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Haematocrit is invalid for estimating red cell volume: a prospective study in male volunteers

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Background. Although haematocrit and haemoglobin value are concentrations, they are commonly used to guide clinical decisions involving red cell and plasma volumes. A study challenging this convention systematically co-determined and compared these measures.

Materials and methods. Using a non-radioactive double-tracer technique to assess blood volume components, measurements were taken once in 46 healthy male endurance athletes. The best predictors of blood composition were derived from the first 36 athletes by automated stepwise forward selection of non-invasive metric parameters (age, weight, height, body surface area and body mass index) and the resulting formulae validated in the remaining ten volunteers. Haematocrit, haemoglobin concentration, red cell volume and plasma volume were measured again 4 weeks later in eight randomly selected volunteers.

Results. Red cell volume (2,282±283 mL) did not correlate with either haematocrit (0.42±0.02) or haemoglobin concentration (14.2±0.8, $P>0.05$, resp.), but was predictable from body surface area (red cell volume [mL]=1,547 x body surface area [m^2]–723; $r=.88$, $P<0.01$). A similar accuracy was unobtainable using any potential predictor for plasma or blood volume, haematocrit or haemoglobin concentration. Red cell volume showed high intra-individual stability when measured again after 4 weeks, whereas plasma volume oscillated in both directions by up to 22%.

Discussion. Only red cell volume shows sufficiently stable intra- and interindividual values to be an accurate, objective indicator of normality in blood composition. The measurement technique is feasible in the outpatient setting and this parameter provides effective, robust, and readily available diagnostic information that might be useful in numerous clinical situations. Its clinical significance does, however, remain to be demonstrated.

Keywords: haematocrit, haemoglobin, red cell volume, plasma volume, endothelial surface layer.

Introduction

The gold standard method of direct measurement of red cell volume (RCV) and/or plasma volume (PV) using radioactive tracers is too elaborate[1], expensive and invasive for incorporation into clinical routine. This, however, leaves physicians unhappy about having to make significant decisions involving infusion and transfusion therapy without robust information on their actual target. Explicit attempts to achieve at least an indirect estimate of pre-operative blood volume or RCV underline the strong desire for more accurate knowledge of quantitative blood composition[2]. Despite mounting evidence against using haemoglobin concentration (Hb) and haematocrit (Hct) to screen for hypovolaemia or determine the requirement for packed red cell transfusion[3], physicians on wards and in peri-operative and intensive care settings still mainly resort to such practice[4]. These largely interchangeable twin surrogates have become enshrined in clinical

guidelines. Thus in pregnancy or postpartum, iron is often administered once the Hb falls below a certain threshold, sometimes even without the additional measurement of ferritin, while the World Health Organization has made an upper threshold of Hb a major diagnostic criterion in myeloproliferative neoplasms[5]. In other contexts, as the short half-life of erythropoiesis-stimulating agents makes the direct detection of blood doping difficult after more than 2-3 days[6,7], the World Anti-Doping Agency includes Hb and Hct among other indirect parameters of erythropoiesis in its screening procedures, with no attempt at blood component quantification[8]. The tacit, shared assumption underlying each of these protocols, namely that Hb and Hct are sufficiently reliable indicators of RCV for routine use, must be questioned.

The purpose of the present study was to challenge the conventional approach by assessing the ability of Hct and Hb to predict individual RCV and PV in a highly standardised population of comparably trained endurance athletes. We set out to test for interindividual constancy, i.e. predictability of RCV, PV, blood volume (BV), Hct, and Hb from external metric characteristics under steady-state conditions. In cases in which we found a significant correlation between the predicted and actual value in a first (training) subset of our population, we sought to validate the underlying automatically generated formula prospectively against the actual values measured in a second (validation) subset. Our other objectives were to test the intra-individual stability of quantitative blood composition, assess the feasibility of a well established, less invasive and non-radioactive alternative to the gold standard method of BV assessment, and discuss the impact of its potential introduction into routine use.

Materials and methods

The study protocol was approved by the Zurich cantonal institutional review board and all volunteers gave written informed consent to participation in the study. Inclusion criteria were male gender, age 18 to 45 years, and regular amateur participation in competitive endurance disciplines such as cycling, swimming, and running for at least 3 years. The aim of setting these criteria was to limit intra-individual scatter related to gender, sickness and physical constitution within our sample population. Exclusion criteria were iron deficiency (ferritin <15 μg/L), anaemia (Hb <11.7 g/dL), blood transfusion in the previous 4 months, and ongoing prescription medication.

Each subject was studied once, between 08:00 and 10:00, except for eight randomly selected subjects who were studied again 4 weeks later (see below). On their scheduled study morning subjects were asked to eat their normal breakfast, omitting caffeinated drinks (clear fluids were allowed), and come to our laboratory without having exercised beforehand. On arrival, they reclined on an examination couch while a 17-gauge cannula was placed in an antecubital vein. Measurements were taken within 15 to 30 min (a minimum of 15 min in the supine position being observed to allow stable equilibration of body fluids).

Determination of blood components

RCV and PV were co-determined using a non-radioactive, double-label technique based on measuring the compartmental concentrations of intravenously injected tracers. Distribution spaces were calculated from these concentrations and the amount of tracer injected.

Measurement of plasma volume with indocyanine green

A bolus of indocyanine green (ICG, Fa. Paesel, Frankfurt a. M., Germany), which binds rapidly to plasma proteins and is eliminated exclusively by the liver, was injected into a peripheral vein. Given the dye's short half-life of about 3 min, the theoretical plasma concentration at the injection time has to be extrapolated from several timed samples taken after complete mixture with the plasma. From this concentration and the amount of dye injected the initial distribution space of ICG, i.e. the PV, can be calculated. The technical details and theoretical background of this method have been described extensively elsewhere[9,10].

In brief, two 10 mL venous blood samples were withdrawn immediately before injecting an intravenous bolus of 0.25 mg/kg ICG over 5 sec at t_0. Starting from 2 min post-t_0, ten 2.5 mL venous blood samples were taken precisely every 20 sec into lightly heparinised tubes and centrifuged immediately to obtain the plasma.

A two-point system calibration was performed by spiking the two pre-injection samples with known amounts of ICG. This gave two known plasma concentrations (1.25 and 2.5 μg/mL) whose

specific extinctions, due exclusively to the ICG in the plasma sample, were then derived by subtracting extinction at 900 nm (zero ICG extinction) from that at 805 nm (maximal ICG extinction). Thanks to the known linear dependency of plasma concentration on specific extinction, the resulting equation allowed concentrations to be derived from any extinction value. We also measured the specific extinctions of the ten venous plasma samples taken from each volunteer's circulation. ICG concentrations at t_0 were derived from monoexponential extrapolation of the light absorption curve resulting from the respective measuring time points back to t_0, assuming a one-compartment model. The calibration led directly to the theoretical plasma concentration at injection time (CP_0). The PV could then be calculated as follows:

$$PV[mL]=D[mg] \times CP_0^{-1}[mg \times mL^{-1}]$$

D being the amount of dye injected.

Red cell volume measurement with sodium fluorescein

RCV was derived by injecting a known amount of autologous red cells labelled with sodium fluorescein (Fa. Alcon, Freiburg, Germany), a non-radioactive marker, and determining their fraction in venous blood samples after recirculation[11,12]. A 30 mL sample of peripheral venous whole blood was taken from each volunteer to label the red blood cells. The blood was centrifuged and the red cell suspension incubated with 48 mg sodium fluorescein for 5 min. To remove unbound fluorescein, the cells were washed twice in calcium gluconate solution, and then resuspended to the volume of the initial blood sample (30 mL) using Ringer's lactate solution. The labelled red cells were re-infused via the peripheral cannula. Three samples were drawn from the peripheral cannula between 5 and 10 min after injection, stored on ice, and analysed by flow cytometry (FACScan, Becton Dickinson, Heidelberg, Germany) using an argon laser at 488 nm.

RCV was then calculated as follows:

$$RCV[mL]=(E_i \times V_i \times Hct_{LV})/(E_p \times F_{Ef})$$

where E_i=number of red cells injected per mL of labelled cell suspension, V_i=volume of injected cell suspension [mL], Hct_{LV}=subject's arterial blood Hct (measured in triplicate), E_p=number of red cells per mL in the subject's arterial blood (measured in triplicate), and F_{Ef}=fluorescent erythrocyte fraction determined by flow cytometry.

The fluorescent erythrocyte fraction determined by flow cytometry was the mean of all three samples, each counted in triplicate as the number of fluorescent erythrocytes in 50,000 cells. E_i and E_p were obtained using a cell counter (530 nm; Coulter Electronics, Miami, FL, USA).

Determination of haematocrit

Hct was determined in venous blood samples (collected into ethylene diamine tetra-acetic acid-treated tubes, Vacutainer™, Becton Dickinson, Plymouth, UK) taken about 1 min before injecting ICG. The Hct was measured in triplicate by centrifugation (15,000 *g* for 4 min), without correction for plasma trapping. To differentiate this Hct from the calculated theoretical whole-body Hct (Hct_{WB}; see below), we refer to this measured value hereinafter as the large-vessel Hct (Hct_{LV}).

Further calculations

Body surface area (BSA) was derived from body height (H) and body weight (W) using the formula of Du Bois and Du Bois[13]:

$$BSA[m^2]=0.007184 \times H[cm]^{0.725} \times W[kg]^{0.425}.$$

Body mass index (BMI) was calculated using the standard formula:

$$BMI[kg/m^2]=W[kg]/H[m]^2.$$

BV was derived by adding measured values of PV and RCV:

$$BV[mL]=PV[mL]+RCV[mL].$$

Mean Hct_{WB} was calculated from the RCV and BV:

$$Hct_{WB}[fraction]=RCV[ml] \times BV[ml]^{-1}.$$

Normally, Hct_{WB} is slightly lower than Hct_{LV} because of the considerable non-circulating fraction of PV sequestered in the endothelial surface layer (ESL)[9], representing an exclusion zone for blood cells. The F-cell ratio quantifying this difference between the two haematocrit values was calculated as:

$$F\text{-}cell=Hct_{WB}[fraction] \times Hct_{LV}[fraction]^{-1}.$$

The red cell distribution space was interpreted as the circulating fraction of PV (PV_{circ}). Using the red cells as tracer, it was calculated as[14]:

$$PV_{circ}[ml]=(1–Hct_{LV}\ [fraction])\ x\ RCV[mL]/ Hct_{LV}[fraction].$$

The total volume of the ESL, the non-circulating fraction of PV, was calculated as:

$$ESL[mL]=PV[mL]–PV_{circ}[mL].$$

Normal RCV and PV values in male non-athletes were derived from BSA as recommended by the Expert Panel on Radionuclides of the International Council for Standardisation in Haematology[1]:

$$RCV_{normal}[mL]=(1{,}486\ x\ BSA[m^2]–825);$$
$$PV_{normal}[mL]=1{,}578\ x\ BSA[m^2].$$

Selection and testing of potential predictors of "normal" red cell, plasma and blood volumes in endurance athletes in order to screen for "abnormal" values

We recruited a total of 46 volunteers who we randomly divided into a training set (n=36) and a validation set (n=10). From the training set we randomly selected a further subgroup (n=8) for repeated study 4 weeks later. Our study was performed outside the competition period, the athletes were training extensively and continuously to maintain their condition. The eight randomly selected volunteers were strongly discouraged to change their training behaviour during the observation period. We used program R 2.7.1 from the R environment (http://www.r-project.org) to implement an automated stepwise forward selection procedure from the training set of potential metric predictors for RCV, PV and BV (age, weight, height, BSA and BMI). These parameters were readily available, non-invasive and, most importantly, not directly dependent on the blood compartments. The algorithm started with an empty model and evaluated one predictor at a time. The best predictor, giving the lowest *P* value, was added and the algorithm restarted with this new model, with the remaining predictors being added one at a time. Predictors were discarded if they lost statistical significance after another variable was added. The predictive performance of the resulting best formulae was assessed using the validation set.

Statistical analysis

Spearman's rank correlation was used to study associations between two variables. A level of statistical significance of less than 0.05 was used in all tests. Data are given as the mean ±SD. All measured and calculated values were normally distributed (according to the Kolmogorov-Smirnov test). The Pearson's product-moment correlation coefficient was used to test for associations between continuous variables. Student's *t*-test was used to compare groups, whereas paired Student's t tests were used for the intra-individual follow-up at 4 weeks, being insensitive to possible deviations from normal distribution. All analyses were performed using SPSS 18 (SPSS, Chicago, IL, USA).

Results

Table I shows the demographic data. Table II presents the measured and calculated results for the training and validation subsets. Figures 1, 2 and 3 refer to the training set only. Hb values ranged widely in our population, but RCV and PV correlated significantly with BSA (Figure 1). Outliers were particularly prominent in the PV data. Although RCV did not correlate with either Hct or Hb, a slight negative relationship was observed between PV and Hb

Table I - The volunteers' characteristics (potential metric predictors).

	Training set (n=36)		Validation set (n=10)	
Age, years	33±7	(20-45)	30±7	(21-45)
Height, cm	180±5	(168-93)	177±7	(168-187)
Weight, kg	75±9	(60-100)	71±6	(64-83)
Body surface area, m^2	1.94±0.13	(1.68-2.31)	1.87±0.11	(1.73-2.08)
Body mass index, kg/m^2	23.1±1.9	(19.8-28.7)	22.5±0.8	(21.0-23.6)

Values are mean±SD (min-max).
Differences between the two subsets were not statistically significant (P>0.05).

Table II - Measured and calculated variables.

	Training set (n=36)		Validation set (n=10)	
RCV, mL	2,282±283	(1,834-2,853)	2,093±208	(1,803-2421)
RCV, mL/m² BSA	1,173±107	(996-1,390)	1,117±59	(1,044-1212)
RCV, mL/kg BW	30±3	(23-26)	30±1	(28-32)
PV, mL	3,739±502	(2,632-5,352)	3,528±440	(2,978-4,138)
PV, mL/m² BSA	1,922±209	(1,500-2,500)	1,891±259	(1,566-2,191)
PV, mL/kg BW	50±6	(41-62)	50±7	(41-59)
BV, mL	6,022±712	(4,486-8,205)	5,622±511	(4,781-6,475)
BV, mL/m² BSA	3,095±269	(2,556-3,832)	3,008±263	(2,667-3,358)
BV, mL/kg BW	80±8	(71-95)	80±8	(70-89)
Hb, g/dL	14.2±0.8	(12.9-15.8)	14.4±0.9	(12.3-15.4)
Hct_{LV}	0.42±0.02	(0.38-0.46)	0.42±0.03	(0.35-0.47)
Hct_{WB}	0.38±0.03	(0.33-0.44)	0.37±0.04	(0.33-0.43)
Hct_{WB}/Hct_{LV}	0.91±0.05	(0.78-1.00)	0.89±0.07	(0.80-1.00)
ESL, mL	517±334	(0-1,349)	604±420	(8-1,128)

Values are mean±SD (min-max).
BSA indicates body surface area; BV, blood volume (RCV + PV); BW, body weight; ESL, endothelial surface layer; Hb, haemoglobin concentration in peripheral blood; Hct_{LV}, large-vessel haematocrit; Hct_{WB}, whole-body haematocrit (RCV/BV); PV, plasma volume; RCV, red cell volume.
Differences between the two subsets were not statistically significant (P>0.05).

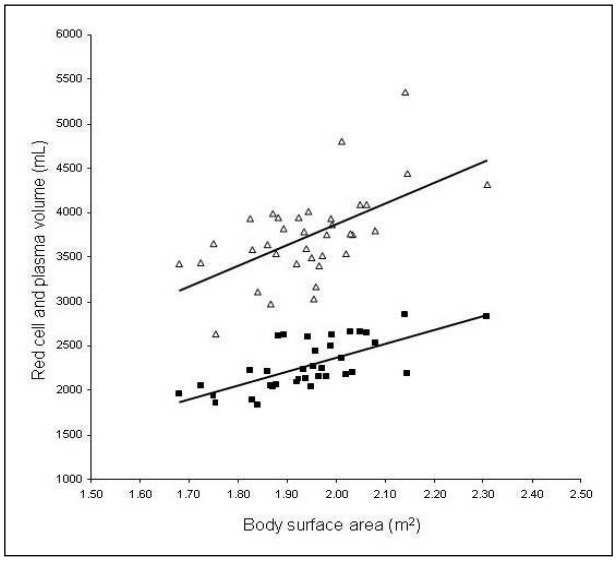

Figure 1 - Correlation of red cell volume (black squares, r=0.69, P<0.001) and plasma volume (white triangles, r=0.58, P<0.001) with body surface area using Pearson's product-moment correlation coefficient (training set, n=36).

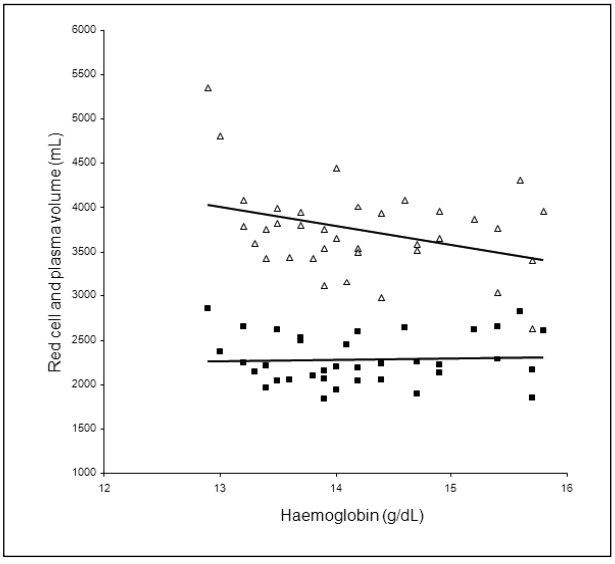

Figure 2 - Correlation of red cell volume (black squares, P>0.05) and plasma volume (white triangles, r=0.35, P<0.05) with haemoglobin concentration using Pearson's product-moment correlation coefficient (training set, n=36).

(Figure 2). The results confirmed the expected dependence of Hct_{LV} on Hb (Figure 3). In the 36 athletes in the training set RCV was only slightly, if significantly, increased over the normal adult range[1] (2,282±283 *vs.* 2,063±186 mL, P<0.001), whereas PV and BV were both considerably increased[1] (3,739±502 *vs.* 3,067±198 mL and 6,022±712 *vs.* 5,130±384 mL, both P<0.001).

The mean overall study time per volunteer (n=46) from couch to cannula removal was 49±2 min.

Stepwise forward selection of potential external metric predictors (Table I) of measured Hb and Hct

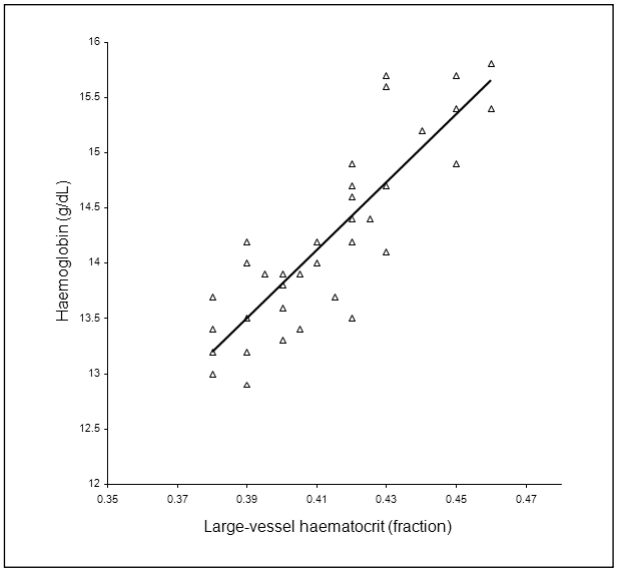

Figure 3 - Correlation of large-vessel haematocrit with haemoglobin concentration using Pearson's product-moment correlation coefficient (r=0.85, P<0.001) (training set, n=36).

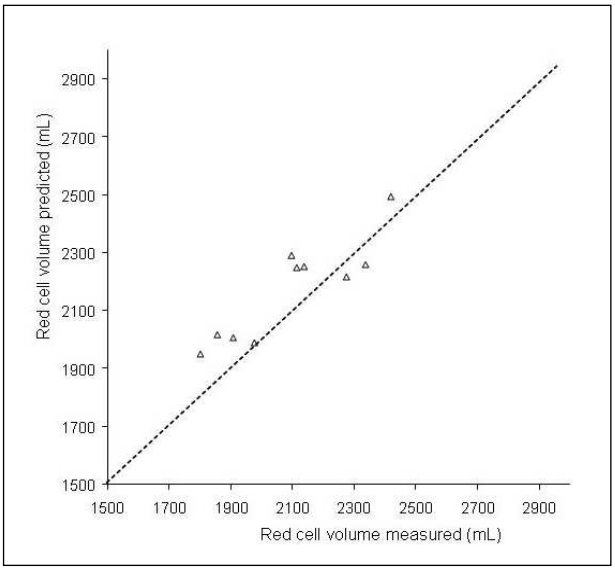

Figure 4 - Correlation between measured red cell volume and that predicted using a formula derived from the training set (n=36) in a new validation set (n=10, r=0.88, P<0.01). Dotted line=line of equality.

showed no correlation in the training set, while RCV, PV and BV values generated the following formulae:

RCV[mL]=1,547 x BSA[m^2]–723 (r=0.68, P<0.01);
PV[mL]=2,312 x BSA[m^2]–754 (r=0.56, P<0.01);

and

BV[mL]=3,859xBSA[m^2]–1477 (r=0.67, P<0.01).

Applying these calculations to the measurements made in the validation set (n=10) in order to assess their generalisability and predictive performance showed that only the predicted RCV value correlated significantly with the measured value (r=0.88, P<0.01 [Figure 4]). P values for PV and BV were 0.99 and 0.28 (not shown).

Follow-up of eight patients randomly selected from the training set showed no significant overall changes after 4 weeks in mean RCV, PV, Hct_{LV}, or total ESL volume (2,229±300 mL *vs.* 2,240±311 mL; 3,763±852 mL *vs.* 3,749±642 mL; 0.42±0.03 *vs.* 0.42±0.01; and 609±320 mL *vs.* 634±431 mL; all P>0.05, Figures 5-7). Intra-individual RCV proved highly stable, with a mean difference of 53±47 mL (maximum: 119 mL, equivalent to only 5%), whereas PV changed unpredictably in both directions by up to 1,054 mL (22%; mean 404±332 mL; Figure 5). This was related to antidromic individual changes in Hct of up to 0.03 (8%; the mean intraindividual difference was 0.02±0.01; Figure 6). Total ESL volume fluctuated intra-individually by up to 769 mL (82%; the mean intra-individual difference was 280±236 mL; Figure 7), for no detectable reason.

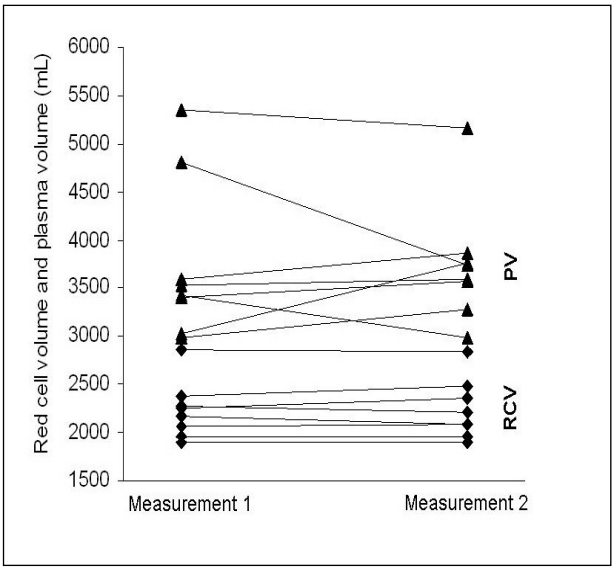

Figure 5 - Intra-individual changes in red cell volume (RCV, diamonds) and plasma volume (PV, triangles) measured on two occasions 4 weeks apart in eight patients randomly selected from the training set.

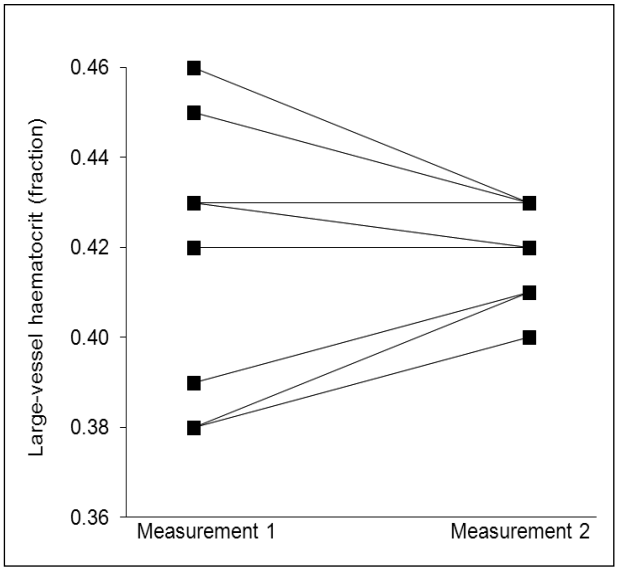

Figure 6 - Intra-individual changes in haematocrit measured on two occasions 4 weeks apart in eight patients randomly selected from the training set.

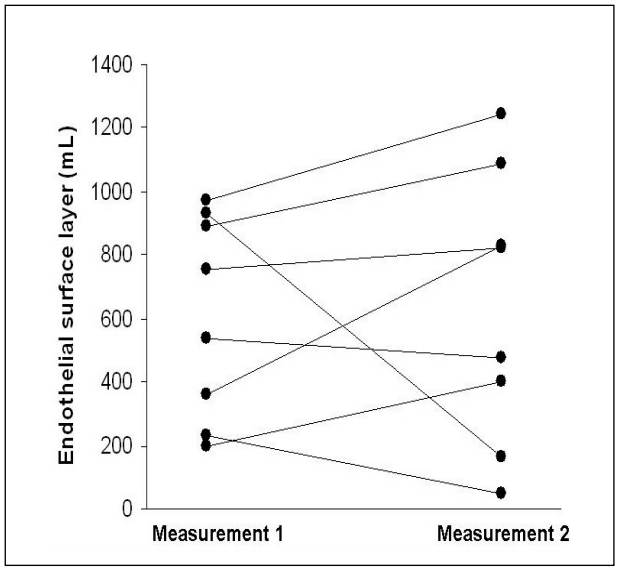

Figure 7 - Intra-individual changes in total endothelial surface layer volume measured on two occasions 4 weeks apart in eight patients randomly selected from the training set.

Discussion

Hct and Hb represent, by definition, the oxygen-carrying capacity of the blood. Therefore, the common therapeutic strategy to transfuse red cells into a given blood volume to increase their concentration appears, at first view, quite reasonable. Unfortunately, it is clearly based on an assumption that is often not fulfilled: a "normal" and constant PV.

Our data contradict the generally assumed correlation between RCV and Hct or Hb in peripheral blood vessels, even for fluid compartments in steady state. The reason is primarily mathematical: the concentration values are inversely related to total distribution space volume. The RCV in the circulation represents total oxygen-binding capacity, while PV is the non-corpuscular fraction of the blood, mainly responsible for cardiac preload and rheology. It follows that Hct is not a purely red cell parameter: it does indeed change with RCV, but it also decreases with an increasing PV and vice versa, as borne out by the slight negative association with PV in our study. Unlike inter- and intra-individually stable RCV, which our data show to be reliably predictable from BSA, PV is a short-term variable, changing intra-individually within seconds due to body position, coughing etc[15]. Standing, for instance, elevates Hct, due to raised hydrostatic pressure in the lower limb venous system force-filtering plasma into tissue, as opposed to the situation when a subject is sitting or lying down[16].

A second serious conceptual limitation to the current practice of extrapolating RCV from peripheral blood Hct and Hb is the inter- and intra-individual variability in total ESL volume[9].This recently described red cell exclusion zone on the luminal surface of the endothelial cell layer consists of membrane-bound glycoproteins and proteoglycans that bind plasma constituents by virtue of special charge properties[17]. An intact ESL makes a significant contribution to vascular barrier competence[18] while also mediating other important physiological functions such as anti-inflammation and shear-stress transduction to the endothelial surface[19]. As confirmed by the present study, this non-circulating fraction of PV normally exceeds 0.5 L in adults[14]. Intra-individually it is positively related to the peripheral red cell concentration and, therefore, to Hb and Hct. The smaller the ESL, the more red cells are able to hide within the microcirculation, artificially decreasing the apparent Hb concentration and peripheral venous Hct. The opposite effects occur for a larger ESL. There are no external markers for predicting intra-individual variability in total ESL volume. For this reason it is erroneous to calculate BV from RCV or PV and peripheral Hct, despite the repeated attempts to do so[20], since the error can be as high as 20%. While the demonstrated

intra-individual oscillations in the total volume of this physiologically crucial structure should not have any clinical impact beyond our mathematical calculations it has repeatedly been demonstrated that this might not be the case in critical illness. In particular, the release of inflammatory mediators due to trauma, sepsis and ischaemia/reperfusion, but also of atrial natriuretic peptide during artificial intravenous volume loading caused a significant destruction of the ESL in humans. This led to protein extravasation and tissue oedema, loss of nutritional blood flow, and an increase in platelet and leucocyte adhesion and, in septic shock, was strongly related to overall mortality[21]. ESL protection will be an important issue with great potential in future intensive care medicine. Already today, anaesthetists increasingly try to avoid intravascular hypervolaemia by applying rational, target-oriented fluid management during major surgical interventions[22].

Our data show that in a given subject it is just as inappropriate to assume that the RCV is abnormal on the basis of Hct and Hb values outside the normal range as it is to discount an abnormal RCV on the basis of a normal Hct and Hb: these are randomly influenced variables with no acceptable inter-individual normal value. Nevertheless, as being responsible for an adequate oxygen delivery to the cells, together with cardiac output, a decrease in these concentration measures below the individual "transfusion trigger" requires immediate correction. As it could in fact indicate the normally assumed real decrease in RCV due to acute bleeding, transfusing packed red cells might often be the correct treatment of a decreased Hb value. However, an increase in PV by inappropriate infusion therapy leading to hypervolaemia of the extracellular space could also present like this, with a causal therapy keeping the organism in balance being the use of diuretics in this case. Only direct measurement of the RCV is methodologically safe, free from any contestation and actually helpful.

Considerable efforts have been made to the retrospective generation of predictive formulae for RCV and PV from metric parameters in a given population and, thereby, establishing individual normal values[1]. However, to the best of our knowledge, our study is the first to have also prospectively evaluated predictive performance in a second independent subset. While our training set suggested that PV would prove highly predictable, as previously reported[1], the formula proved invalid in the subsequent validation set. This demonstrated that intra-individual prediction of PV, as of the related red cell concentration, is unreliable, thus reconfirming our previous point.

The observation that endurance athletes might have a slightly higher PV than the normal population is interesting. It illustrates the changes in blood composition behind the well-known pseudo-anaemia consistently diagnosed in this population on the basis of a decrease in peripheral Hct[23]. This was not, however, a central focus of our study, which was primarily methodological.

Our non-radioactive technique not only deepens understanding of a significant practical problem in various clinical fields, but it also provides a possible solution. Previous clinical studies[9,11,24] have already shown that the non-radioactive techniques used here to assess RCV and PV directly provide a reliable, accurate and precise alternative to the gold standard[1], delivering the desired information within less than 1 hour. It has been frequently applied scientifically to patients and volunteers in the past and helped to answer important clinical questions[14,25-28]. Measurements are performed using the same single peripheral cannula required for conventional and clearly inferior screening techniques. As for RCV, comparable efforts have been made in the past to determine Hb mass by carbon monoxide rebreathing studies[29-31]. This scientifically well-established method is indeed convenient and virtually bloodless. However, the information concerning total BV gained from this test lacks precision because the RCV can only be estimated from an Hb mass and total PV cannot reliably be calculated from RCV and Hct, for the reasons set out earlier.

Given its proven feasibility, this currently largely academic procedure for simultaneously measuring RCV and PV could find its way into routine clinical use for selected indications when the physician needs accurate information on the amount of circulating red cells. The clinical significance for patients' outcome and the cost-benefit ratio of this in principle undoubtedly useful additional information do, however, remain to be determined.

The Authors declare no conflicts of interest.

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