Clinical update: perioperative fluid management

In a recent randomised trial in 1000 patients, the ARDS Clinical Trials Network concluded that a balanced fluid regimen (conservative management) over the first 7 days was beneficial in patients with acute lung injury.[1] Despite the primary outcome of 60-day mortality not being significantly different compared with a cumulative fluid excess of 7 L, conservative management shortened the duration of intensive care (14·6 *vs* 16·8 days) and improved lung function, leading to a shorter period of mechanical ventilation (13·4 *vs* 15·9 days).

The study draws attention to the debate on perioperative fluid management. Although fluid restriction is accepted in thoracic surgery,[2] the situation differs in the general surgical population. Many believe that liberal fluid use is appropriate perioperatively to prevent anaesthesia-induced hypotension and, therefore, to protect organ function, especially in the kidneys.[3] The background is an assumed large intravascular volume-deficit after preoperative fasting, an increased evaporative fluid loss, and an inevitable shift towards the third space (ie, an ill-defined compartment thought to reflect otherwise unexplainable perioperative fluid losses) during major surgery.[4]

The belief is that vasoconstriction enables the awake and fasted adult to maintain adequate blood pressure despite hypovolaemia. This mechanism is thought to fail during induction of anaesthesia, because of decreased sympathetic tone. The resulting decrease in blood pressure, as well as use of vasoconstrictors, is blamed as the trigger for perioperative acute renal failure.[3] Thus, preoperative volume loading is considered indispensable and fluid boluses are part of most recommendations for perioperative treatment.[3,5] Other widely used guides to fluid therapy are cardiac filling pressures and even peripheral venous pressure, qualitatively assessed by infusion flow rate, as well as end-tidal carbon dioxide pressure and central venous oxygen saturation.

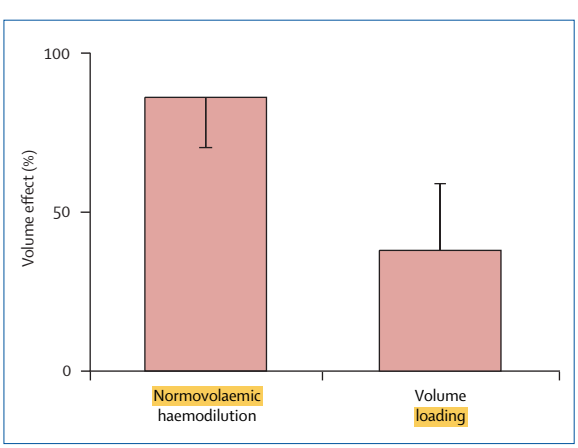

Figure: **Context-sensitivity of volume effects of iso-oncotic colloids in normovolaemic individuals**
Experiment used 5% human albumin. Normovolaemic haemodilution=removal of mean 1150 (SD 196) mL blood and simultaneous replacement by 1333 (204) mL colloid (n=15).[8] Volume loading=infusion of 1379 (128) mL colloid (n=10).[9] Bar=SD, difference p<0·05.

However, fluid preloading and liberal intraoperative fluid substitution are not evidence-based procedures. Preoperative deficits and insensible losses are highly overestimated[6] and prophylactic fluid boluses have no major effect on the incidence or severity of anaesthesia-related hypotension.[7] Furthermore, volume effects are context-sensitive: a simultaneous infusion of iso-oncotic colloids during acute bleeding (ie, when carefully maintaining intravascular normovolaemia) led to volume effects of over about 90%.[8] By contrast, about two-thirds of an additional bolus in a normovolaemic patient leaves the vasculature towards the interstitial space within minutes (figure).[9]

A liberal fluid regimen has not previously been shown to decrease the incidence of acute renal failure. Nor is there evidence that kidney function deteriorates postoperatively in normovolaemia when urinary output was moderately reduced perioperatively. Rather, protection of fluid compartments is a physiological reaction to surgical stress.[10] In contrast with general opinion, cardiac filling pressures are poor predictors of volume state,[11,12] and a changing peripheral venous pressure can have many causes, most of them trivial. A decreasing end-tidal carbon dioxide pressure can, among other causes, indicate low cardiac output, which is, however, not necessarily related to hypovolaemia. As a commonly used alternative for the more invasively obtainable pulmonary arterial oxygen saturation, detection of central venous oxygen saturation can indicate a change in tissue oxygen supply. However, global volume is an indirect measure, one that only partly affects direct measures such as cardiac output or haemoglobin concentration. Consequently, these traditional variables, despite being widely used because of lack of an alternative in practice, are not suitable to justify any fluid regimen.

There are two main, partly competing, efforts to update traditional perioperative infusion: fluid substitution with a fixed regimen and estimations of actual fluid losses, and fluid optimisation with secondary circulatory variables. The idea behind optimisation is to obtain supranormal values of tissue-oxygen delivery.[13] With this concept transferred to the general surgical patient, a maximum stroke volume, achieved by oesophageal doppler-monitored fluid boluses, was considered to represent the optimum fluid load. Indeed, compared with fluid substitution at the discretion of the anaesthetist, this goal-directed approach led to a significantly reduced hospital stay (7 *vs* 9 days) and fewer intermediate and major postoperative complications (2% *vs* 15%) after elective colorectal surgery.[14] Additionally, patients treated intraoperatively with colloid boluses (7 mL/kg at first, then 3 mL/kg), to maintain a predefined minimum aortic flow and to optimise stroke volume individually, tolerated diet significantly earlier (2 *vs* 4 days after intervention).[14] The beneficial effect of doppler-guided fluid-optimisation seems to rise with age and frailty, and obviously it is advantageous to avoid crystalloids in such procedures.[15] Also, several indirect variables were tested as predictors of fluid responsiveness. Less invasive measurements, such as variation in the systolic arterial or pulse pressure (on the principle that intrathoracic pressure changes cyclically during mechanical ventilation, decreasing venous backflow and consequently stroke volume, preferentially in hypovolaemic patients) strongly distinguish between responders and non-responders to fluid challenge. Therefore, they are increasingly replacing the unreliable static cardiac-filling pressures.[16]

Nevertheless, circulatory optimisation, achieved by optimising these easily applicable surrogate variables, has not yet been reliably translated into improved outcomes and, for practical reasons, oesophageal doppler cannot be routine. Interestingly, protocol-based fluid restriction reduces the incidence of perioperative complications, cardiopulmonary events and disturbances of bowel motility, while improving wound and anastomotic healing.[5,17–19]

Whereas many studies of major non-abdominal surgery are underpowered, the findings for major abdominal surgery are promising. In 2002, Lobo and co-workers investigated 20 adults after elective colonic resection.[19] Intraoperatively, fluid use was similar in all patients, but postoperatively they were randomised to a restrictive (≤2 L a day) or a standard (≥3 L a day) protocol. The standard protocol caused a significant weight gain of 3 kg, a later return of bowel function, and a longer hospital stay. In a larger trial in 80 patients undergoing colorectal surgery, MacKay and colleagues did not confirm these findings, despite their protocols for postoperative fluid management seeming, at first sight, to be similar to Lobo's (table).[20] But patients in MacKay's randomised groups were intraoperatively managed with relative fluid restriction (basal rate 10 mL/kg per h) compared with that in Lobo's study (about 18 mL/kg per h). The resulting different postoperative starting points are reflected by the respective values of perioperative weight change and total fluid intake on the day of operation. A sufficient postoperative fluid balance was not possible, because oral fluid intake was only "encouraged", but not reported by MacKay. Nevertheless, with no patient receiving more than 3 L of intravenous fluid a day, even perioperatively, MacKay's standard group was actually treated too restrictively to cause measurable harm.

Rather, MacKay's findings underline the importance of a rational concept for perioperative treatment. In a multicentre study in a homogeneous group of 141 patients undergoing major colorectal surgery, perioperative intravenous fluid restriction (mean 2740 *vs* 5388 mL) significantly reduced the incidence of

	Lobo et al[19]		MacKay et al[20]	
	Standard	Restrictive	Standard	Restrictive
Daily intravenous intake (L)				
Day of operation	5·6*‡	3·0*‡	2·8†	2·0†
1st postoperative day	3·0*‡	1·6*‡	2·6†	2·0†
2nd postoperative day	2·7*‡	1·3*‡	2·5†	0·0†
3rd postoperative day	2·7*‡	1·0*‡	0·5†	0·0†
Daily oral intake (L)*‡				
1st postoperative day	0·1	0·2	Not reported	Not reported
2nd postoperative day	0·2	0·6	Not reported	Not reported
3rd postoperative day	0·4	0·8	Not reported	Not reported
Weight change (kg)*‡§				
1st postoperative day	2·7	0·7	1·1	−0·5
2nd postoperative day	3·3	0·0	1·2	−0·4
3rd postoperative day	2·7	−0·7	1·1	−0·7
Endpoints (days)†				
Time to first flatus	4·0	3·0	2·9	2·9
Postoperative hospital stay	9·0	6·0	7·2	7·2

Values are *mean or †median. ‡Values are estimated from graphs. §Cumulative values *vs* preoperative state.

Table: **Semiquantitative comparison of two studies on postoperative fluid handling**

major and minor complications.[17] Despite limited fluid application and a perioperative decrease in urine output, acute renal failure did not occur in any restrictively treated patient. Others found similar results, including a decreased hospital stay under fluid restriction, in a more heterogeneous group of 152 patients scheduled for mixed-abdominal surgery.[5] In 2006, a systematic review of 80 randomised trials recommended to avoid fluid overload in major surgical procedures.[18]

But what, exactly, is overload? Despite the beneficial effects of restrictive fluid management in major abdominal surgery, extrapolations to the perioperative treatment for an individual remain difficult. There are large differences in the definitions of liberal or standard and restrictive (table), which reflect the lack of standardisation and make any pooling of data impossible. In most previous investigations with a strict protocol, a locally used regimen was simply entitled the liberal protocol, and the investigators compared this with their own restrictive idea. But, when comparing these restrictive regimens to measured values of preoperative blood volume after overnight fasting and insensible perspiration, fluid restriction in those studies was only "less liberal". Preoperative fasting does not normally cause intravascular hypovolaemia,[8,9] and the measured basal evaporative water loss is only about 0·5 mL/kg per h, increasing to a maximum of 1 mL/kg per h during major surgery.[6] In addition, a measurable weight gain even in restricted study groups[5,17] indicates that there is still potential for improvement.

Adequate substitution of fluid needs before, during, and after major abdominal surgery can improve outcome. The same is true for doppler-guided achievement of supranormal predefined goals for cardiac output. Future studies should compare these two approaches, erroneously called fluid restriction and optimisation, respectively, to make a decision about which is the better choice. However, we should also be able to provide a rational fluid regimen to the many patients in whom extended monitoring is not possible for logistical or financial reasons. To achieve this end, more well-powered trials are needed, comparing the current standard to a fluid regimen that is based on scientific data about perioperative losses.

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We declare that we have no conflict of interest.

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Changes in Blood Volume and Hematocrit during Acute Preoperative Volume Loading with 5% Albumin or 6% Hetastarch Solutions in Patients before Radical Hysterectomy

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Background: **The impact of acute preoperative volume loading with colloids on blood volume has not been investigated sufficiently.**

Methods: **Before surgery, in 20 patients undergoing major gynecologic procedures, volume loading was performed during anesthesia by infusing approximately 20 ml/kg of colloid at a rate of 90 ml/min (group I: 5% albumin solution; group II: 6% hetastarch solution; n = 10 each). Plasma volume (indocyanine green dilution technique), erythrocyte volume (labeling erythrocytes with fluorescein), hematocrit, total protein, and hetastarch plasma concentrations (group II) were measured before and 30 min after the end of infusion.**

Results: **More than 1,350 ml of colloid (approximately 50% of the baseline plasma volume) were infused within 15 min. Thirty minutes after the infusion had been completed, blood volume was only 524 ± 328 ml (group I) and 603 ± 314 ml (group II) higher than before volume loading. The large vessel hematocrit (measured by centrifugation) dropped more than the whole body hematocrit, which was derived from double-label measurements of blood volume.**

Conclusions: **The double-label measurements of blood volume performed showed that 30 min after the infusion of approximately 20 ml/kg of 5% albumin or 6% hetastarch solution (within 15 min), only mean 38 ± 21% and 43 ± 26%, respectively, of the volume applied remained in the intravascular space. Different, *i.e.*, earlier or later, measuring points, different infusion volumes, infusion rates, plasma substitutes, or possibly different tracers for plasma volume measurement might lead to different results concerning the kinetics of fluid or colloid extravasation.**

VOLUME loading (VL) with colloids is common clinical practice in the perioperative period, *e.g.*, before and during spinal or epidural anesthesia, before declamping the aorta during vascular surgery, or during preoperative acute hypervolemic hemodilution. However, the exact impact of VL with colloids on blood volume (BV), plasma volume (PV), and large vessel hematocrit (Hct_{lv}) have not been investigated sufficiently. Double-label measurements of BV can give information about fate, distribution, or redistribution of infused colloids. In this clinical study, to our knowledge, for the first time both compartments of BV were measured before and 30 min after an exactly defined VL with 5% albumin or 6% hetastarch solutions.

Materials and Methods

The study was approved by the ethics committee at our institution, and all patients gave written informed consent. Twenty patients with a preoperative diagnosis of carcinoma of the cervix who were scheduled for radical hysterectomy were studied. All patients were American Society of Anesthesiologists physical status I–II without cardiovascular or pulmonary dysfunctions.

After arrival in the operating room, monitors were applied, and lumbar epidural catheters were placed in all patients. However, epidural anesthesia was not started until the 30 min post-VL measurements. After placement of the epidural catheter, general anesthesia was induced with fentanyl, thiopental, and *cis*-atracurium, and, after tracheal intubation, was maintained with 0.4–1.5 vol% isoflurane in a 50% oxygen–nitrous oxide mixture. Mechanical ventilation was performed to maintain arterial oxygen partial pressure at 200–250 mmHg and arterial carbon dioxide partial pressure at approximately 40 mmHg. Radial artery and central venous catheters were inserted. Cooling of the patients was prevented by means of a warming blanket. Before VL, no intravenous infusions were applied (except for very small amounts, which were necessary to inject the intravenous drugs). Perioperative monitoring included electrocardiogram, direct arterial blood pressure, central venous pressure, pulse oxymetry, repeated determinations of hemoglobin concentration (at least every 30 min; cyanhemoglobin method), and arterial blood gases. After a time interval of at least 20 min after induction of anesthesia, baseline measurements of PV (with indocyanine green [PV_{ICG}]), erythrocyte volume (EV), Hct_{lv}, and serum total protein concentration were performed during periods of stable anesthesia and hemodynamics.

Determination of Plasma Volume with Indocyanine Green

Immediately before each dye injection, a calibration curve was constructed by measuring two times 10 ml of

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the patient's blood having two known indocyanine green (ICG) concentrations (1.25 and 2.5 μg/ml of whole blood, respectively; ICG-Paesel, Frankfurt a. M., Germany). The light absorption of the blood was measured at 800 and 900 nm in a spectrophotometer that was developed by one of the authors (H. B.). After calibration, 0.25 mg/kg of ICG was injected into the central venous catheter as a bolus dose over 5 s (zero time = time of injection). For measuring ICG concentration, blood was continuously withdrawn (between the second and the fifth minute after injection) from the arterial catheter through a cuvette by means of a calibrated pump. The cuvette was attached to the spectrophotometer. For calculation of PV_{ICG}, see below (Calculations).

Determination of Erythrocyte Volume

The method for measuring EV, using autologous erythrocytes stained with sodium fluorescein (SoF; Fluorescein-Lösung 10%, Alcon Pharma, Freiburg, Germany) and flow cytometry was developed in our laboratory and published in detail previously.[1,2] In brief, after arrival in the operating room, 40 ml of patient's blood was taken for labeling erythrocytes with SoF. In the laboratory, the blood was centrifuged, and thereafter, the erythrocyte suspension was incubated with 48 mg of SoF for 5 min. To prevent an excess of unbound fluorescein, the cells were washed twice using a solution containing calcium (Calcium Braun 10%, Braun Melsungen, Germany) and were resuspended to the volume of the initial blood sample (40 ml) using Ringer's lactate. For repeated measurements before and after VL, the cells were then divided in two aliquots for the two measurements.

Immediately before injection of ICG (see PV_{ICG} measurements in Determination of Plasma Volume with Indocyanine Green), labeled erythrocytes were injected into the central venous catheter (20 ml per measurement). Four, 6, and 8 min after the injection of fluorescein-labeled erythrocytes, samples were drawn from the arterial catheter, stored on ice, and analyzed in the laboratory by flow cytometry (FACScan, Becton Dickinson, Heidelberg, Germany; using an argon laser at 488 nm). For calculations of EV, see below (Calculations).

Determination of Large Vessel Hematocrit and Total Protein Plasma Concentration

Large vessel hematocrit and total protein concentration were measured in arterial blood samples that were drawn approximately 1 min before the injection of ICG. Hct_{lv} was measured in triplicate without correction for plasma trapping by centrifugation of the blood samples (12,000 rpm for 4 min). Total protein was determined using the Biuret method (variation coefficient < 2%).

Volume Loading Procedure

After baseline measurements (of PV_{ICG}, EV, Hct_{lv}, and total protein concentration), VL was performed by infusing approximately 20 ml/kg of two different colloid solutions within 15 min at a rate of approximately 90 ml/min. Two groups of patients (n = 10 each) were investigated sequentially over a time span of 1.5 yr. In group I, 5% albumin solution (Centeon Pharma GmbH, Marburg, Germany) was used, and in group II, 6% hetastarch solution (molecular weight: 200,000 ± 25,000; degree of substitution: 0.5; Fresenius AG, Bad Homburg, Germany) was used for VL.

After completion of the infusion and a steady state interval of 30 min without any further infusions, all aforementioned measurements (PV_{ICG}, EV, Hct_{lv}, and total protein concentration) were taken again in identical sequence. At the measuring point after VL in group II, hetastarch plasma concentration was also measured according to the protocol presented below. Strictly after these measurements, epidural analgesia was started and 10 min after surgery began.

Determination of Hetastarch Plasma Concentration

Measurement of hetastarch in plasma was conducted by a modified method described by Förster *et al.*[3] Plasma (0.5 ml) was transferred into a screw-topped tube containing 0.25 ml of 35% potassium hydroxide and placed into a boiling water bath for 45 min. After cooling and addition of 7.5 ml of ethanol (100%), the suspension was refrigerated at 4°C for 12 h. The samples were then centrifuged at 3,500 rpm at 0°C for 60 min. The supernatant was separated, and the remaining fluid was mixed with 2.5 ml of 2 M hydrochloric acid and then again placed in a boiling water bath for 120 min. After a second cooling procedure, the suspension was transferred into a 10-ml tube. Sodium hydroxide (2.5 ml) was added, and the suspension was filled with water up to the 10-ml mark of the tube. The same procedure was conducted with a hetastarch standard dilution sample (1.2 g/dl hetastarch in water). A total of 0.5 ml of the hydrolyzed sample or the hydrolyzed standard sample was transferred into a cuvette, 2 ml of gluco-quant suspension (hexokinase-glucose-6-phosphate-dehydrogenase; Boehringer, Mannheim, Germany) was added, and then the first light absorption (E1) was measured at 340 nm with a spectrophotometer (Cary 100 Bio, Varian, Melbourne, Australia). Afterward, 0.04 ml of gluco-quant was added, and after 10 min, the second light absorption (E2) was also determined at 340 nm. The difference (dE) between both values (E2 − E1) is proportional to the hetastarch concentration. The hetastarch concentration in the plasma sample (c) was derived by:

$$c = c\ \text{standard} \times dE\ \text{sample}/dE\ \text{standard} \qquad (1)$$

where c standard = 1.2 g/dl.

Mean difference and SD of 200 *in vitro* measurements with different known hetastarch concentrations in plasma samples were −0.016 g/dl and ± 0.106 g/dl,

respectively, in comparison with the predicted (known) hetastarch concentrations.

Calculations

Measured BV was derived by BV = PV_{ICG} + EV, and whole body hematocrit (Hct_{wb}) was calculated as Hct_{wb} = EV/BV.

Calculations Concerning the Determination of Plasma Volume. Indocyanine green concentration at injection time was derived by monoexponential extrapolation of the light absorption curve between minutes 2–5 back to zero time (using Excel for Windows, Microsoft, Redmond, Washington). If this value is put into the calibration curve, CBo, the theoretical whole blood concentration of the dye at injection time, is obtained. Theoretical plasma concentration of the dye at injection time (CPo) was calculated as:

$$CP_o = CB_o/(1 - Hct_{lv}). \quad (2)$$

Measurements of PV_{ICG} were calculated as:

$$PV_{ICG} = D/CPo, \quad (3)$$

where D is the amount of dye injected.

Calculations Concerning the Determination of Erythrocyte Volume. Erythrocyte volume was calculated according to:

$$EV\ (ml) = (Ei \times Vi \times Hct_{lv})/(Ep \times FEf) \quad (4)$$

where Ei = number of erythrocytes injected per milliliter of tagged cell suspension, Vi = volume of injected cell suspension in milliliters, Hct_{lv} = large vessel hematocrit of the subject's arterial blood (measured in triplicate), Ep = number of erythrocytes per milliliter in the patient's arterial blood (measured in triplicate), and FEf = fraction of fluorescent erythrocytes determined by flow cytometry.

The fraction of fluorescent erythrocytes determined by flow cytometry was taken as the mean value from determinations of samples drawn at 4, 6, and 8 min after injection, counting in triplicate the fluorescent erythrocytes in 50,000 cells by means of the flow cytometer. Ei and Ep were obtained using a cell counter (530 nm; Coulter Electronics, Miami, FL).

Statistical Analysis

As all measured and calculated data were distributed normally (assessed by Kolmogorov-Smirnov tests) and are presented as mean values with SDs. For demographic data, Student *t* tests for unpaired data were performed. A two-way analysis of variance for repeated measures was performed comparing intragroup and intergroup differences of measured and calculated variables. *Post hoc* testing was conducted using the Student-Newman-Keuls method for multiple comparisons. $P < 0.05$ was considered significant.

Table 1. Patient Characteristics

	Group I (n = 10)	Group II (n = 10)
Age	39 ± 11	44 ± 12
Height (cm)	165 ± 5	168 ± 5
Weight (kg)	64 ± 13	65 ± 12
Colloid infused (ml)	1,379 ± 128 (Albumin)	1,417 ± 209 (Hetastarch)

Values are mean ± SD.

$P < 0.05$ difference between groups.

Results

Demographic data of the patients in the two groups (table 1) did not show any significant intergroup difference. There was also no significant difference in the volumes of colloid infused for VL between the groups. Measured and calculated variables (before and after VL) in the two groups are shown in table 2. In group I, 30 min after infusion of 1,379 ± 128 ml (22.1 ± 3.6 ml/kg) of 5% albumin solution, PV_{ICG} was only 507 ± 350 ml higher than before VL. As was to be expected, EV remained almost constant. Hct_{lv} (measured by centrifugation) decreased more (−6.5 ± 1.4%) than Hct_{wb} (−2.8 ± 2.7%; determined by double-label measurements of BV; see Calculations). Therefore, the ratio of Hct_{wb} and Hct_{lv} (Hct_{wb}/Hct_{lv}) increased significantly from 0.83 to 0.93. Thirty minutes after VL with 5% albumin solution, there was no significant change in total protein concentration in relation to the value before VL.

Patients in group II (see also tables 1 and 2) had a colloid infusion (6% hetastarch solution) of almost the same amount as patients in group I (22.1 ± 3.0 ml/kg). In comparison with group I, no significant differences were found in PV_{ICG}, EV, Hct_{lv}, Hct_{wb}, and the ratio of Hct_{wb}/Hct_{lv} before and after VL. Thirty minutes after the infusion of 1,417 ± 209 ml of 6% hetastarch solution, PV_{ICG} was only 597 ± 296 ml higher than before VL ($P > 0.05$ in relation to group I). Also in group II, Hct_{lv} decreased more than Hct_{wb}, resulting in a significant increase in the ratio of Hct_{wb}/Hct_{lv} from 0.84 to 0.95. VL with 6% hetastarch resulted in a significant total protein concentration decrease from 64 to 44 g/l. Mean hetastarch concentration in group II patients was 18 ± 1 g/l 30 min after completion of the infusion.

Discussion

The most surprising results of the study were the small differences in BV and PV_{ICG} before *versus* 30 min after the end of VL with the colloids used (table 2). Before our study, we expected (and we assume most anesthesiologists do) that 30 min after infusion, the nearly isooncotic colloids used would remain in blood to perhaps 80–90%. A volume effect around 90% of 5% albumin solution[4,5]

Table 2. Measured and Calculated Variables before and after Volume Loading (VL) with 5% Albumin (Group I) or 6% Hetastarch Solution (Group II)

	Group I (n = 10)		Group II (n = 10)	
	Before VL	After VL (Albumin)	Before VL	After VL (Hetastarch)
PV_{ICG} (ml)	3,014 ± 585	3,521 ± 673*	2,984 ± 685	3,581 ± 706*
EV (ml)	1,175 ± 223	1,192 ± 226	1,231 ± 171	1,238 ± 155
BV (ml)	4,189 ± 769	4,713 ± 868*	4,215 ± 728	4,818 ± 721*
Hct_{lv} (%)	34.0 ± 2.9	27.5 ± 1.9*	35.3 ± 4.5	27.3 ± 3.7*
Hct_{wb} (%)	28.2 ± 2.8	25.4 ± 2.2*	29.7 ± 5.1	26.1 ± 4.6*
Hct_{wb}/Hct_{lv}	0.829 ± 0.050	0.925 ± 0.073*	0.840 ± 0.078	0.953 ± 0.067*
Total protein concentration (g/l)	60 ± 4	61 ± 3	64 ± 5	44 ± 5*†
Hetastarch plasma concentration (g/l)	—	—	—	18 ± 1

Values are mean ± SD; for calculations see also Methods.

* $P < 0.05$ intragroup difference with respect to value before VL. † $P < 0.05$ difference between groups.

PV_{ICG} = plasma volume; EV = erythrocyte volume; BV = blood volume (PV_{ICG} + RCV); Hct_{lv} = large vessel hematocrit; Hct_{wb} = whole body hemocrit; Hct_{wb}/Hct_{lv} = ratio between whole body hemocrit and large vessel hemocrit.

and 105% of 6% hetastarch solution (molecular weight: 200,000; degree of substitution: 0.62)[4] was already demonstrated during preoperative normovolemic hemodilution by means of the same PV measuring method as used in the current study. Figure 1 demonstrates, however, that in the current investigation only 38 ± 21% and 43 ± 26%, respectively, of the infused 5% albumin or 6% hetastarch solutions could be found in the intravascular space 30 min after VL. The large SDs and the respective minima and maxima (fig. 1) demonstrate a large interindividual variability. As there was only a weak correlation between BV before VL and the increase in BV 30 min after VL ($r = 0.29$; $P = 0.21$), from our data, we have no evidence that patients with low baseline BV values had a larger increase in BV than patients with a high baseline BV. After VL, Hct_{lv} (measured by centrifugation of arterial blood samples) decreased more than Hct_{wb} (derived by double-label measurements of BV; table 2), resulting in a significant increase in the ratio of Hct_{wb}/Hct_{lv}. Therefore, one might assume that Hct_{lv} decreased disproportionally in relation to the increases in BV and PV_{ICG}. For assessing the possible physiological and clinical importance of our findings, first, one basic question should be asked: Are the small increases observed in BV and PV_{ICG} simply a reflection of any measurement error or artifact? To answer this question, the three basic measuring methods (Hct_{lv}, EV, and PV_{ICG}) used in the current study are evaluated.

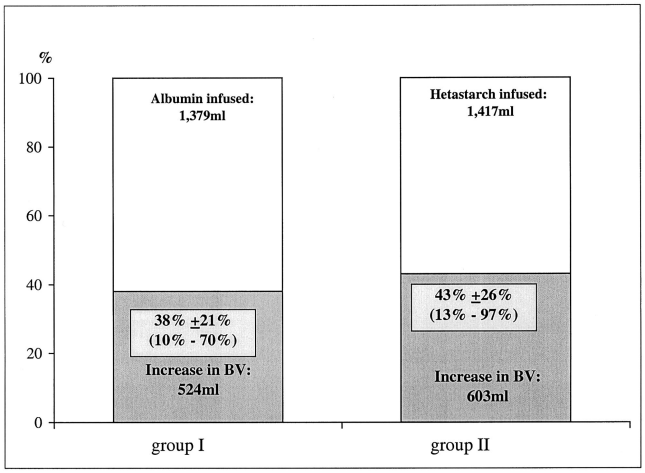

Fig. 1. Increase in blood volume (BV) 30 min after volume loading in relation to the amount of colloid infused (mean value, SD, and range).

Measurement of Large Vessel Hematocrit

As previously mentioned, Hct_{lv} measurements were taken in triplicate by centrifugation of arterial blood samples at 15,000*g* for 4 min without correction for plasma trapping. Two decimals were used, and the variation coefficient of repeated measurements was less than 2%. We did not correct for plasma trapping because previous work from our laboratory demonstrated that when the aforementioned centrifugation procedure was used, the effect of plasma trapping was minimal (< 1%).[6] The amount of trapped plasma depends, *e.g.*, on the centrifugal force and the length of centrifugation.[7,8] This amount is approximately 3%[8–10] or even lower, as a more recent investigation showed.[11] Assuming plasma trapping of 3%, we can simulate the respective changes in our results.

If the Hct_{lv} values before and after VL are reduced by 3%, this will result in a reduction of EV in both groups before and after VL by exactly 3% (approximately 37 ml; see Calculations). In both groups, PV_{ICG} will increase by a mean of 48 ml (1.6%) before VL and 40 ml (1.1%) after VL. As expected, a small decrease in EV combined with a small increase in PV_{ICG} will result in only minimal changes in BV in relation to the values shown in table 2 (in both groups approximately +12 ml before and +4 ml after VL, respectively). In addition, the relation of Hct_{wb}/Hct_{lv} before and after VL in both groups will be altered only minimally by a correction of Hct_{lv} for plasma trapping as Hct_{wb} will decrease to almost the same extent (approx-

imately 3%) as Hct_{lv} decreases (3%). In conclusion, correcting the Hct_{lv} values for plasma trapping of an unknown amount would not result in any fundamental change of our results.

Erythrocyte Volume Measurement with Sodium Fluorescein

A close correlation between EV measurements using radiochromium and those using SoF-labeled erythrocytes was previously demonstrated by Lauermann *et al.*[12] In our laboratory, mean difference and variation coefficient for EV double measurements with SoF in healthy volunteers were 0.6 and 3.1%, respectively.[2] The results of an *in vivo* validation of our EV measuring method were published recently. After preoperative acute normovolemic hemodilution in 16 patients, the amount of erythrocytes removed (399 ± 81 ml; calculated from volume and hematocrit of the whole blood in the hemodilution bags) could be determined precisely by means of EV measurements before and after acute normovolemic hemodilution. Mean difference between the amount of erythrocytes removed and the difference between EV before and after acute normovolemic hemodilution was 6 ± 50 ml (± 4.2% with respect to the EV before hemodilution measured with the SoF technique; n = 16).[1] After the labeling procedure (see Determination of Erythrocyte Volume), measuring samples of the injected cell suspension by flow cytometry showed that 100% of the injected erythrocytes were labeled with SoF. Therefore, we conclude that the EV measurement used in this study offered very precise data.

Plasma Volume Measurement with Indocyanine Green

This method is referred to as the "whole blood method" for PV_{ICG} determination, methodologic aspects of which were published previously.[13] Within 10 min, it gives reproducible results immediately in the operating room (mean difference and variation coefficient between double measurements: 0.3 and 6.2%, respectively).[14] In the current investigation, PV_{ICG} measurements were taken almost simultaneously with EV measurements. However, any interference of the two tracers is very unlikely because of the different light absorption and fluorescence characteristics of ICG and SoF. In blood, ICG has its peak of light absorption at 805 nm and has no light absorption below 600 nm,[15] whereas SoF has an absorption maximum between 485 and 500 nm and does not absorb any light above 600 nm.[16] Maximum fluorescence emission for ICG and SoF appears at 835 and 520 nm, respectively, without any overlapping.[15,16]

Critical View on the Blood Volume Measuring Method with Respect to Circulating and Noncirculating Compartments in the Intravascular Space

The precision of EV and PV_{ICG} measurements used in this study have proven to be high. However, one might ask for the real distribution spaces of the tracers used within the time of our measurements (4–8 min after the injection of SoF-labeled erythrocytes and 2–5 min after the injection of ICG, respectively). To answer this question we must examine the distribution and the flow characteristics of erythrocytes and plasma in the vascular bed, including microcirculatory networks. Classic methods estimating microvascular hematocrit used the anatomic width of a microvessel and the number and mean corpuscular volume of erythrocytes on static pictures of microvessels.[17,18] Respective calculations led to the assumption that the hematocrit in microvessels is substantially lower than in large vessels.[18] This refers to the fact that in microvessels, erythrocytes typically travel near the central line of the blood stream and have a higher velocity than mean blood flow, a hypothesis brought forward by Fahraeus as early as 1928.[19] One important premise of the "classic model" is that the entire (total) PV is always circulating in macrovascular and microvascular beds.[18] An interpretation of our data in line with this classic model would mean that (1) our measured EV represents the circulating EV, (2) PV_{ICG} should represent the total and circulating PV, and (3) the small increase in PV_{ICG} in both groups 30 min after the completion of VL leads to the conclusion of a considerable extravasation of the administered fluids out of the intravascular space.

The results of several other recent studies using direct intravitalmicroscopy or other indirect estimations, however, questioned this classic model and the extent of Fahraeus' hypothesis.[20–22] These investigations have shown that there is a difference between the anatomic width of a microvessel and the width of the space available for circulating erythrocytes. An exclusion zone for erythrocytes adjacent the endothelial surface could be demonstrated. This zone was termed "plasma layer" or "endothelial surface layer" (ESL) and has a thickness of approximately 0.4–1.5 μm.[18,23–25] It contains fluid (without erythrocytes), is immobile or moves very slowly, and is in a dynamic equilibrium with the flowing plasma.[25] This new model implies that PV can be separated into two compartments: a circulating PV (PV_{circ}) and a resting, noncirculating PV in the ESL. In line with the new model, erythrocytes are circulating in the PV_{circ}, and the hematocrit in the central, circulating column of microvessels does not substantially differ from Hct_{lv}.[20–22] Moreover, the volume of ESL probably does not remain constant in case of replacing or diluting plasma with artificial fluids. Evidence was found that in such cases, a substantial part of the absorbed layer of plasma components (ESL) can be dissolved into the flow-

ing blood, thereby widening the circulating column and decreasing the thickness of the ESL.[25,26]

How can our data be interpreted in line with the new model? It is obvious that our measured EV should exclude the ESL and that it only concerns the circulating compartment of the intravascular space. However, because PV can be separated into a circulating compartment and a noncirculating compartment (the ESL), two different distribution spaces for ICG are conceivable. These are the PV_{circ}—if ICG does not enter the ESL within the time of our measurements—or total PV, which is the sum of PV_{circ} and noncirculating PV, if ICG also enters the plasma of the ESL and equilibrates with it. Vink and Duling[27] were able to demonstrate that small anionic tracers (0.4–40 kd) entered the ESL with a half-time of 11–60 min, whereas small neutral tracers such as rhodamine entered the ESL within one transit time and equilibrated with the ESL within 1 min.[27] ICG is an anionic tracer (775 Da), but it combines with plasma proteins (mainly α lipoproteins) within a few seconds.[28,29] This dye was repeatedly used for infrared fluorescence videomicroscopy of skin capillaries.[30–33] These investigations showed that on the arteriolar or venular side of finger capillaries in normal subjects, the width of the circulating erythrocyte column averaged 68% in comparison with the width of the microvessels determined with ICG within 5 min after injection. As no leakage of the dye across the endothelial cells could be observed, the respective difference in diameters was attributed to the plasmatic zone between the erythrocyte column and the capillary wall (ESL). ICG did fill the total diameter of the capillaries (including the ESL) within 1 min.[30–33]

Because of the hypothesis (according to the new model) that the hematocrit in the circulating compartment in microvessels is not substantially different from Hct_{lv}, we should be able to calculate PV_{circ} with the aid of our measured EV and Hct_{lv} values and compare it with PV_{ICG}.

The PV_{circ} can be calculated as:

$$PV_{circ} = (EV/Hct_{lv}) - EV \quad (5)$$

If the distribution space for ICG represents total PV, the difference between PV_{ICG} and PV_{circ} would give an estimate of the volume of ESL:

$$\text{Estimated volume of ESL} = PV_{ICG} - PV_{circ}. \quad (6)$$

Because of these assumptions, figures 2A and 2B would demonstrate the relation between PV_{ICG}, PV_{circ}, and the estimated volume of ESL before and after VL for both groups. At first glance, it can be seen that in both groups the proportions seem to be reasonable, as PV_{ICG} is always higher than PV_{circ}. It was already demonstrated that 30 min after VL in groups I and II, PV_{ICG} was only 507 ± 350 ml and 597 ± 296 ml higher than before VL, respectively (see also table 2). PV_{circ}, however, increased

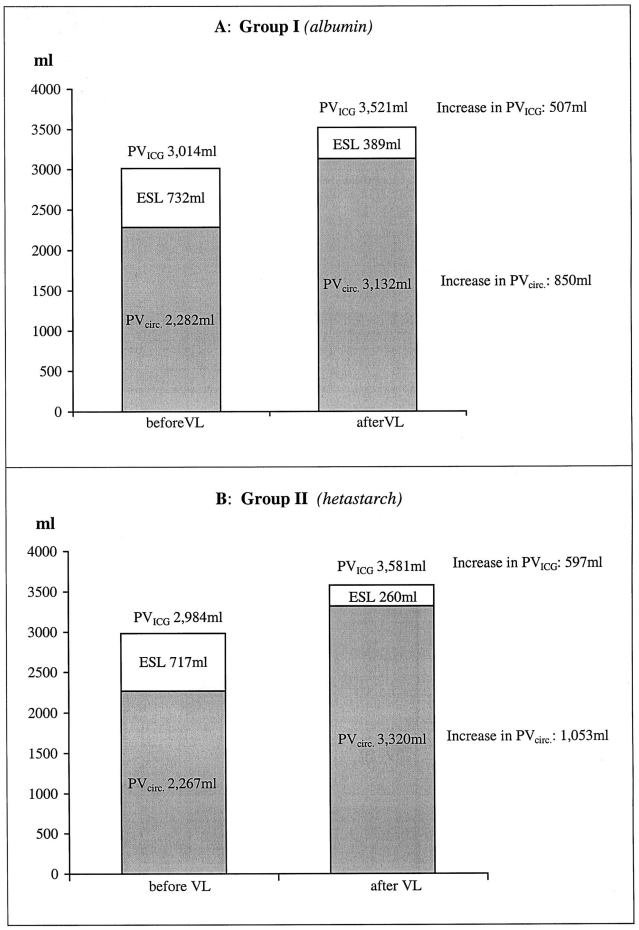

Fig. 2. (*A*) Relation of plasma volume measured with indocyanine green (PV_{ICG}), circulating plasma volume (PV_{circ}), and estimated volume of the endithelial surface layer (ESL) before and 30 min after volume loading (VL). Group I (albumin). (*B*) Relation of PV_{ICG}, PV_{circ}, and ESL before and 30 min after VL. Group II (hetastarch).

more, namely, 850 ± 134 ml in group I and 1,053 ± 199 ml in group II. This implies a decrease in the volume of ESL from approximately 700 to 400 ml (group I) and from approximately 700 to 300 ml (group II) 30 min after the completion of VL.

An interpretation in line with the new model could mean that there was only a small increase in total PV (PV_{ICG}) in relation to the amount of VL, that PV_{circ} increased more than PV_{ICG}, and that the larger increase in PV_{circ} in relation to the increase in PV_{ICG} was caused by a considerable decrease ("washout") in the volume of ESL.

Up to now, the distribution space of ICG in the time of our measurements (2–5 min after injection) is not completely known. Consequently, we cannot finally decide which interpretation, the one according to the classic model or the other one according to the new model, is the right one. The aforementioned intravital videomicroscopic data, however, make us assume that in our experiment, the distribution space of ICG (PV_{ICG}) was more likely the total intravascular PV than the PV_{circ} according to the new model. Regardless of whether

Table 3. Balance of the Intravascular Protein and Hetastarch According to the Classic Model and the New Model

	Classic Model (Calculations Based on PV_{ICG})		New Model (Calculations Based on PV_{circ})	
	Group I (n = 10)	Group II (n = 10)	Group I (n = 10)	Group II (n = 10)
Albumin infusion (g)	69	—	69	—
Hetastarch infusion (g)	—	85	—	85
PV before VL (PV_{ICG} or PV_{circ}) (ml)	3,014 (PV_{ICG})	2,984	2,282 (PV_{circ})	2,267
Total protein concentration before VL (g/l)	60	64	60	64
IVP before VL (g)	181	191	137	145
PV after VL (PV_{ICG} or PV_{circ}) (ml)	3,521 (PV_{ICG})	3,581	3,132 (PV_{circ})	3,320
Total protein concentration after VL	61	44	61	44
IVP after VL (g)	215	158	191	146
Deficit in IVP after VL (g)	35	33	15	−1
Hetastarch plasma concentration after VL	—	18	—	18
Intravascular hetastarch after VL (g)	—	64	—	60
Deficit in hetastarch after VL (g)	—	21	—	25

Values are mean.

PV = plasma volume; VL = volume loading; IVP = intravascular protein.

PV_{ICG} represents the sum of circulating and noncirculating PV (new model) or just the circulating (and entire) PV (classic model), the small increase in PV_{ICG} 30 min after the completion of VL in any case leads to the conclusion of considerable extravasation of fluid out of the intravascular space. In other words, in line with both interpretations, the difference between the measured (small) increase in PV_{ICG} 30 min after VL and the amount of volume infused should represent the total loss of fluid out of the intravascular space 30 min after the completion of VL (fig. 1). As long as the exact volume of ESL is unknown, as tracers that allow an accurate distinction between circulating and noncirculating plasma are not available, the generated data may be a step forward in the ongoing debate about the impact and amount of ESL.

Considerations About the Fate of the Colloids Infused (Albumin or Hetastarch)

As may be taken from table 2, total protein concentration remained constant after VL with 5% albumin solution, whereas it significantly decreased after the infusion of 6% hetastarch solution. An estimate of the total amount of intravascular protein (IVP) before and 30 min after VL should result from the product of the respective total protein plasma concentration and PV:

$$IVP_{\text{before VL}} = \text{total protein concentration}_{\text{before VL}} \times PV_{\text{before VL}} \quad (7)$$

$$IVP_{\text{after VL}} = \text{total protein concentration}_{\text{after VL}} \times PV_{\text{after VL}} \quad (8)$$

The deficit in IVP after VL can be estimated by the difference between the increase in IVP and the amount of albumin infused:

$$\text{Deficit in } IVP_{\text{after VL}} = \text{albumin infusion} - (IVP_{\text{after VL}} - IVP_{\text{before VL}}) \quad (9)$$

In group II, the amount of hetastarch remaining in the intravascular space 30 min after VL can be calculated as:

$$\text{Intravascular hetastarch}_{\text{after VL}} = \text{hetastarch plasma concentration}_{\text{after VL}} \times PV_{\text{after VL}} \quad (10)$$

The deficit in intravascular hetastarch after VL can be estimated by the difference between the amount of hetastarch infused and the amount of intravascular hetastarch after VL:

$$\text{Deficit in intravascular hetastarch} = \text{hetastarch infusion} - \text{intravascular hetastarch}_{\text{after VL}} \quad (11)$$

At this point, the question of which PV is to be taken for such estimates arises. Because total protein as well as hetastarch plasma concentrations were determined from arterial blood samples, these should be concentrations with respect to the circulating intravascular compartment. According to the classic model, PV_{ICG} should be used for PV, whereas according to the new model, PV_{circ} (based on EV and Hct_{lv} measurements) should be the right variable for such estimates. Table 3 shows the respective estimates according to the classic and new models.

Following the classic model, in group I, IVP increased 30 min after VL with 69 g of albumin from a mean of 181 g to only 215 g, so that 35 g of the protein infused (approximately 50%) did not remain in the intravascular space. In group II, there was a deficit in hetastarch of 21 g (64 – 85 g; table 3), which was accompanied by a surprising mean decrease (deficit) in IVP of 33 g.

With respect to PV_{circ} according to the new model, however, there was a deficit in (circulating) IVP of only 15 g in group I and no decrease or deficit in (circulating) IVP in group II. In group II, the deficits in intravascular

hetastarch calculated by means of PV_{ICG} or PV_{circ} were not substantially different (table 3; classic model: 21 g *vs.* new model: 25 g). Unfortunately, it is unknown which and how many plasma proteins may constitute the ESL.[25] As a result, considerations about a "washout" of not only fluid but also of protein into the circulating compartment as a result of the decrease in the ESL after VL can only be speculative.

In conclusion, in line with both interpretations of our data, one according to the classic model and the other according to the new model, considerable deficits or losses of protein and hetastarch 30 min after VL could be observed. However, by means of the measuring methods used in this investigation, the fate of the colloids infused could not be cleared up completely.

In summary, in a special clinical setting, in 20 patients during anesthesia and before surgery, we measured BV by means of a double-label technique as the sum of PV_{ICG} and EV before and 30 min after VL with two different, nearly isooncotic colloid solutions (20 ml/kg at a rate of 90 ml/min). Thirty minutes after the infusion of more than 1,350 ml of 5% albumin (group I) or 6% hetastarch solutions (group II), an increase in BV of only 524 ± 328 ml (group I) and of only 603 ± 314 ml (group II) could be measured. Different, *i.e.*, earlier or later, measuring points, different infusion volumes, infusion rates, plasma substitutes, and possibly different tracers for PV measurement may lead to different results concerning the kinetics of fluid or protein extravasations. Further investigations in this field are urgently needed.

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Changes in Intravascular Volume during Acute Normovolemic Hemodilution and Intraoperative Retransfusion in Patients with Radical Hysterectomy

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Background: **Changes in blood volume during acute normovolemic hemodilution (ANH) and their consequences for the perioperative period have not been investigated sufficiently.**

Methods: **In 15 patients undergoing radical hysterectomy, preoperative ANH to a hematocrit of 24% was performed using 5% albumin solution. Intraoperatively, saline 0.9% solution was used for volume substitution, and intraoperative retransfusion was started at a hematocrit of 20%. Plasma volume (indocyanine green dilution technique), hematocrit, and plasma protein concentration were measured before and after ANH, before retransfusion, and postoperatively. Red cell volume (labeling erythrocytes with fluorescein) was determined before and after ANH and postoperatively.**

Results: **Mean normal plasma volumes (1,514 ± 143 ml/m^2) and reduced red cell volumes (707 ± 79 ml/m^2) were measured preoperatively. Blood (1,150 ± 196 ml) was removed and replaced with 1,333 ± 204 ml of colloid. Blood volume before and after ANH was equal and amounted to 3,740 ml. Intraoperatively, plasma volume did not increase until retransfusion despite infusing 3,389 ± 1,021 ml of crystalloid (corrected for urine output) to compensate for an estimated surgical blood loss of 727 ± 726 ml. Postoperatively, after retransfusion of all autologous blood, blood volume was 255 ± 424 ml higher than preoperatively before ANH. Despite mean calculated blood loss of 1,256 ± 892 ml, only one patient received allogeneic blood.**

Conclusions: **During ANH, normovolemia was exactly maintained. After surgical blood loss of 1,256 ± 892 ml, crystalloid and colloid supplies of 5,752 ± 1,462 ml and 1,667 ± 548 ml, respectively, and complete intraoperative retransfusions of autologous blood in every patient, mean blood volume was 250 ml higher than preoperatively before ANH. (Key words: Autologous blood; colloid infusion; erythrocyte volume; plasma volume; surgical blood loss.)**

ALTHOUGH blood and blood products are safer than ever before, transfusion of allogeneic blood and blood components still involves some risks.[1,2] In an effort to avoid such risks, acute normovolemic hemodilution (ANH) has become popular.[3] ANH is defined as "the removal of blood from a patient immediately before operation, either before or shortly after induction of anesthesia, and simultaneous replacement with an appropriate volume of crystalloid or colloid fluids, alone or in combination, such as to maintain the circulating volume."[4] Effectiveness of ANH, however, is controversial.[5–8] Several mathematical considerations dealing with the effectiveness of ANH are based on the condition of accurately maintaining normovolemia in the perioperative period.[9–14] However, we are unaware of any information documenting the exact impact of ANH and intraoperative retransfusion on blood volume. Maintaining perioperative normovolemia involves the exact replacement of surgical blood loss with appropriate intravenous infusions volume per volume. This may be difficult, because estimating surgical blood loss is difficult.[15,16] In addition, mathematical efforts to simulate ANH are based on estimated "normal" and not on measured red cell, plasma, and blood volumes; consequently, their results are of limited validity. In this clinical study, both compartments of blood volume were measured in the course of ANH and at the end of surgery.

Materials and Methods

The study was approved by the ethics committee at our institution and all patients gave written informed consent. Fifteen patients with the preoperative diagnosis of carcinoma of the cervix scheduled for radical hyster-

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ectomy were studied. All patients were American Society of Anesthesiologists physical status 1 or 2 and had no cardiovascular or pulmonary dysfunction.

Before induction of general anesthesia, all patients received a lumbar epidural catheter; however, epidural anesthesia was not started until completion of ANH. General anesthesia was induced with fentanyl, thiopental, and *cis*-atracurium and, after tracheal intubation, maintained with isoflurane 0.4–1.5 vol% in a 50% oxygen–nitrous oxide balance. Mechanical ventilation was performed to maintain alveolar oxygen partial pressure at 200–250 mmHg and alveolar carbon dioxide partial pressure at approximately 40 mmHg. Intraoperative monitoring included electrocardiography, direct arterial blood pressure, central venous pressure, pulse oximetry, repetitive determinations of hemoglobin concentration and hematocrit (at least every 30 min), and arterial blood gases. Additional fentanyl and *cis*-atracurium were given intraoperatively as appropriate.

Experimental Procedure

All measurements were performed during periods of stable anesthesia and hemodynamics. Before ANH, no intravenous infusions were applied. In a time interval of 30 min, duplicate baseline measurements of plasma volume (PV), hematocrit, and plasma protein concentration (Prot) were carried out. Red cell volume (RCV) was simultaneously measured with the first of the duplicate measurements of PV (measuring procedures of the different measuring methods discussed later).

After baseline measurements, blood was removed at a rate of about 60 ml/min and simultaneously replaced with 5% albumin solution at almost the same rate. First, approximately 500 ml/m^2 of blood were removed. We aimed at infusing 15% more colloid than the amount of blood removed. The hemodilution bags were weighed on a precision scale so that the volume of blood withdrawn could be evaluated immediately. For fine tuning, frequent determinations of hematocrit were carried out at the end of ANH to reach a target hematocrit of 24%. The hemodilution procedure took about 20 min.

After completion of ANH and a steady state interval of 30 min without any further infusions but before beginning surgery, simultaneous single measurements of PV, hematocrit, Prot, and RCV were taken. After these measurements, 15 ml bupivacaine 0.5% and 0.1 mg fentanyl were injected into the epidural catheter; 10 min later, surgery began with skin incision. At 1.5-h intervals, 5 ml of bupivacaine 0.5% were given intraoperatively to maintain the blocked level at approximately the sixth thoracic dermatome.

The study protocol comprised exact infusion, retransfusion, and transfusion strategies for the intraoperative period. From the beginning of surgery to the point of retransfusion, 0.9% saline solution was infused continuously. The amount of intraoperative crystalloid infusion was continuously adapted to the current estimated surgical blood loss and the current urine production using the following formula: Target amount of crystalloid infusion = estimated blood loss $\times$ 5 + urine output.

An experienced anesthetist estimated blood loss by visual assessment of swabs, gowns, and the suction system. If hemodynamic variables tended to reflect hypovolemia (*i.e.,* decrease in central venous pressure by $>$ 7 mmHg in relation to preoperative post-ANH measurements; four cases), 250–1,000 ml of 5% albumin solution were administered in addition.

The intraoperative transfusion trigger for beginning retransfusion was established at a hematocrit of 20%. Immediately before intraoperative retransfusion of autologous blood, simultaneous single measurements of PV, hematocrit, and Prot were taken in every patient. When this hematocrit value was reached, fraction of inspired oxygen was switched to 1.0 to increase the physically dissolved oxygen. In most cases, the retransfusion procedure took place late during the surgery, when major blood loss had ceased. In eight cases, the transfusion trigger was not reached; in these cases, all autologous blood was retransfused during closure of the abdominal wall. Initially, we planned to transfuse allogeneic blood after retransfusion of ANH blood if hematocrit decreased to less than 20%, but this did not occur in any patient.

In a time interval of 30 min, postoperative duplicate measurements of PV, hematocrit, and Prot were taken immediately after closure of the abdominal wall while the patient remained under stable anesthesia without obvious blood loss. Postoperative RCV was simultaneously measured with the first of the duplicate measurements of PV.

Determination of Plasma Volume

Immediately before each dye injection, a calibration curve was constructed by twice measuring 10 ml of a patient's blood having two known indocyanine green concentrations (1.25 and 2.5 μg/ml of whole blood, respectively; indocyanine green, Paesel, Frankfurt, Germany). Optical density of blood (corrected for blank) was read at 800 and 900 nm in a densitometer developed by one of the authors (H. B.). At the same time, blood

samples were taken to determine hematocrit (centrifugation of blood samples without correction for plasma trapping; variation coefficient < 2%) and Prot (Biuret method; variation coefficient < 2%). Afterward, in a dose of 0.25 mg/kg of body weight, ICG was injected as a bolus over 5 s into the central venous catheter (T_o = time of injection). From the second to the fifth minute after injection, blood was continuously withdrawn by means of a calibrated pump from the arterial catheter through a cuvette, which was attached to the densitometer in a closed system. Density of blood at injection time was derived by monoexponential extrapolation of the density curve between minutes 2 and 5 back to T_o. If this value is put into the calibration curve CB_o, the theoretical whole-blood concentration of the dye at injection time, which is the initial distribution volume, can be obtained.

Theoretical plasma concentration of the dye at injection time (CP_o) was calculated as $CP_o = CB_o/(1 - \text{hematocrit})$. PV was calculated as $PV = D/CP_o$, where D is the injected amount of dye. This method is referred to as the whole-blood method for PV determination, methodological aspects of which were published previously.[17] This method provides reproducible results within 10 min in the operating room (mean difference and variation coefficient between double measurements: 0.3% and 6.2%, respectively).[18]

Determination of Red Cell Volume

The method of using autologous erythrocytes stained with sodium fluorescein in flow cytometry determinations was developed in our laboratory and published in detail previously.[15,19] In brief, 20 ml of the patient's blood were labeled with 50 mg of sodium fluorescein and injected into a peripheral vein. After injection, samples were drawn from the arterial catheter at 4, 6, and 8 min, stored on ice, and analyzed by flow cytometry (Becton Dickinson, Heidelberg, Germany). RCV was calculated according to the following formula:

$$\text{RCV (ml)} = (RC_i \times V_i \times \text{hematocrit}_p)/(RC_p \times F_{RCf})$$

where RC_i = number of injected erythrocytes per milliliter of tagged cell suspension, V_i = volume of injected cell suspension in milliliters, hematocrit_p = hematocrit of the subject's venous blood, RC_p = number of erythrocytes per milliliter in the patient's venous blood, and F_{RCf} = fraction of fluorescent erythrocytes determined by flow cytometry.

The fraction of fluorescent erythrocytes determined by flow cytometry was taken as the mean value from determinations of samples drawn at 4, 6, and 8 min after injection and counting in triplicate the number of fluorescent erythrocytes out of 50,000 cells using the flow cytometer. RC_i and RC_p were obtained using a cell counter (Coulter Electronics, Miami, FL); hematocrit_p was determined by centrifugation (12,000 revolutions/min; 4 min) without correction for plasma trapping. Mean difference and variation coefficient for RCV double measurements with sodium fluorescein in our laboratory were 0.6% and 3.1%, respectively.[19]

Calculations

Body surface area was calculated according to Gehan and George.[20]

The amount of intravascular protein (IVP) was calculated as follows:

$$\text{IVP} = \text{PV} \times \text{Prot} \quad (1)$$

For preoperative and postoperative PV and Prot, the calculated mean of the duplicate determinations was taken.

Whole body hematocrit (WBH) was derived by simultaneous measurement of PV and RCV and calculated as follows:

$$\text{WBH} = \text{RCV}/(\text{RCV} + \text{PV}) \quad (2)$$

F-cell ratio was calculated as follows:

$$\text{WBH/Hct} \quad (3)$$

where Hct is the large vessel hematocrit, which is measured by means of centrifugation of the blood samples.

$$\text{Blood content of ANH bags} = (\text{weight of full ANH bags} - 110\ \text{g})/1.05\ \text{g/ml} \quad (4)$$

with 110 g being the sum of CPDA1 fluid content (70 g) and weight of empty ANH bags (40 g). Specific gravity of blood was taken as being 1.05.[21]

Surgical loss of erythrocytes was calculated according to the following formula:

$$\text{Loss of erythrocytes} = \text{RCV}_{\text{start}} - \text{RCV}_{\text{end}} \quad (5)$$

In this and the following equations, "start" represents preoperative values before ANH, and "end" represents postoperative values. Calculated loss of erythrocytes was completely caused by surgery, because in every patient, all ANH blood was retransfused before the postoperative RCV measurements were taken.

Surgical blood loss was calculated as follows:

$$\text{Blood loss} = \text{loss of erythrocytes}/\text{Hct}_m^{22} \quad (6)$$

where Hct_m is the mean of all hematocrit values measured during surgery (from the beginning of skin incision until closure of the abdominal wall) in a frequency of at least 1 determination in 30 min.

Saved Erythrocytes due to Acute Normovolemic Hemodilution

For theoretical reflections on effectiveness of ANH performed in the current study, a theoretical postoperative hematocrit (Hct_{end_t}) without ANH could be calculated using the following equation:

$$\text{Hct}_{end_t} = \text{Hct}_{start}/e^{(\text{surgical blood loss}/\text{BV}_{start})23} \quad (7)$$

For this and the following equations, mean values of our patients' measured blood volume (BV) and hematocrit before ANH and calculated surgical blood loss (equation 6) were used.

Theoretical mean intraoperative hematocrit (Hct_{m_t}) without ANH could be calculated as follows:

$$\text{Surgical blood loss} = \text{BV}_{start} \times (\text{Hct}_{start} - \text{Hct}_{end_t})/\text{Hct}_{m_t}^{11}$$

$$\text{Hct}_{m_t} = \text{BV}_{start}/\text{surgical blood loss} \times (\text{Hct}_{start} - \text{Hct}_{end_t}) \quad (8)$$

Equation 8 is a linear equation which gives a very close approximation of exponential equation 7 in a hematocrit range of Hct_{end}/Hct_{start} = 1.0–0.5. The mean value of Hct_{end}/Hct_{start} measured in this study was 0.7.

Consequently, theoretical loss of erythrocytes without ANH can be calculated as follows:

$$\text{Surgical loss of erythrocytes}_t = \text{surgical blood loss} \times \text{Hct}_{mt} \quad (9)$$

Again, the mean value of our patients' calculated surgical blood loss was inserted into the equation.

The respective difference in the mean measured loss of erythrocytes provides the theoretical saving of erythrocytes due to ANH:

$$\text{Saved erythrocytes} = \text{measured loss of erythrocytes} - \text{theoretical loss of erythrocytes} \quad (10)$$

Statistical Analysis

As all measured and calculated data described earlier were normally distributed as tested by Kolmogorov–Smirnov tests; they are presented as means ± SD. The amount of blood removed and of colloid supplied during ANH, as well as estimated and calculated blood loss, were compared using paired Student *t* tests. One-way analysis of variance for repeated measures was performed comparing intragroup differences. *Post hoc* testing was conducted using the Student–Newman–Keuls method for multiple comparisons. A value of $P < 0.05$ was considered significant.

Results

In all patients (N = 15), surgical procedures involved pelvic lymphadenectomy and radical hysterectomy. Mean operation time was 3.8 ± 1.1 h. Mean age was 44 ± 14 yr; mean height was 163 ± 7 cm; mean weight was 62 ± 11 kg; and mean body surface area was 1.69 ± 0.16 m^2. Preoperative (before and after ANH), intraoperative (before retransfusion), and postoperative variables are shown in table 1. During ANH, PV increased significantly by 390 ± 236 ml, and RCV decreased significantly by 394 ± 76 ml. BV (PV + RCV) was unchanged. The f-cell ratios (equation 3) did not change during hemodilution.

Before retransfusion, PV did not change with respect to the value after ANH, although more than 4 l of crystalloid were infused to compensate for an estimated blood loss of about 700 ml at that point. As mentioned earlier, at the time of retransfusion, PV, but not RCV, was measured. Therefore, exact values for BV and RCV at that time cannot be given. Calculating BV and RCV before retransfusion (using a mean f-cell ratio of 0.89) would indicate a decrease in BV and RCV of about 250 and 160 ml, respectively, compared with measured values after ANH.

After termination of surgery and retransfusion of all ANH blood in all patients, there was a significantly higher postoperative PV of 524 ± 321 ml with respect to the state before ANH. RCV decreased by 266 ± 210 ml, and consequently, there was an increase in BV of 255 ± 426 ml. Surgical blood loss (equation 6) was underestimated by more than 400 ml. No patient intraoperatively received allogeneic blood; only one patient received two units of allogeneic erythrocytes in the recovery room.

Theoretical postoperative hematocrit (Hct_{end_t}) without ANH (equation 7) amounted to 25.7% in comparison

Table 1. Preoperative, Intraoperative, and Postoperative Variables (N = 15)

	Before ANH	After ANH	Before Retransfusion	Postoperative
PV				
ml	2,550 ± 247	2,941 ± 274*	2,846 ± 464	3,074 ± 376*
ml/m^2	1,514 ± 143	1,748 ± 171*	1,695 ± 305	1,831 ± 266*
RCV				
ml	1,193 ± 157	799 ± 114*	~636 ± 147‡	927 ± 216*
ml/m^2	707 ± 79	472 ± 47*	379	552 ± 129*
BV				
ml	3,745 ± 313	3,740 ± 347	~3,482 ± 561‡	4,000 ± 517*
ml/m^2	2,222 ± 162	2,220 ± 188	2,074	2,383 ± 360*
HCT (%)	35.9 ± 3.5	23.7 ± 2.3*	20.5 ± 3.4‡	25.1 ± 3.8*
f-cell	0.89 ± 0.05	0.90 ± 0.05		0.91 ± 0.05
Plasma protein concentration (g/liter)	62 ± 5	57 ± 4*	45 ± 5‡	47 ± 4*
IVP (g)	157 ± 18	169 ± 21*	127 ± 23‡	144 ± 20*
CVP (mmHg)	8 ± 4	11 ± 4	13 ± 4	14 ± 5*
MAP (mmHg)	69 ± 11	67 ± 14	69 ± 14	70 ± 11
HR (beats/min)	68 ± 12	73 ± 10	75 ± 13	77 ± 13*
Blood removed (ml)		1,150 ± 196		
Colloid infused (ml)		1,333 ± 204†	1,483 ± 291	1,667 ± 548
Infusion of saline solution (ml)		0	4,183 ± 1,408	5,752 ± 1,462
Estimated blood loss (ml)			727 ± 726	853 ± 790
Calculated blood loss (ml)				1,256 ± 892§
Urine production (ml)			794 ± 550	1,543 ± 793

Values are mean ± SD.

ANH = acute normovolemic hemodilution; PV = plasma volume; RCV = red cell volume; BV = blood volume; HCT = hematocrit; IVP = intravascular protein; CVP = central venous pressure; MAP = mean arterial pressure; HR = heart rate; ~ = before retransfusion, BV and RCV were calculated by an f-cell ratio of 0.89.

* $P < 0.05$ difference with respect to value before ANH.

† $P < 0.05$ difference between blood removed and colloid supply.

‡ $P < 0.05$ difference with respect to the value after ANH.

§ $P < 0.05$ difference between estimated and calculated blood loss.

with postoperative hematocrit with ANH of 27.2% (corrected for postoperative hypervolemia). Theoretical mean intraoperative hematocrit (Hct_{m_t}) without ANH (equation 8) amounted to 30.5%, in comparison with measured Hct_m with ANH of 21.9%. Mean theoretical loss of erythrocytes without ANH (equation 9) was 383 ml, in comparison with mean measured loss of erythrocytes with ANH (equation 5) of 266 ml. Consequently, mean calculated amount of erythrocytes theoretically saved due to ANH (equation 10) was 117 ml.

Discussion

As far as we know, double-label measurements of BV in the course of ANH did not exist before this study. We investigated a small number of patients with homogeneous preoperative PV and RCV values undergoing ANH and a defined surgical procedure with a standardized infusion and transfusion strategy.

The objective of reducing allogeneic transfusions has led to diminishing the transfusion trigger to a hemoglobin concentration of 70 g/l[24] or 60 g/l[25] in recent years. Weiskopf *et al.* demonstrated that ANH to a hemoglobin concentration of 50 g/l does not produce evidence of inadequate tissue oxygenation in conscious, healthy, resting volunteers.[26] Such a low hemoglobin level should also be safe in patients without cardiopulmonary diseases during stable anesthesia, which reduces oxygen consumption,[27–29] and hyperoxic ventilation, which in addition improves tissue oxygenation.[30] Postoperative hemoglobin concentration, however, should be high enough such that increased oxygen consumption due to shivering or increased posttraumatic metabolism can be covered and basic physical activities can be performed by patients.[31–33] This situation could lead to introducing two transfusion triggers: One for the intraoperative period during hemodynamic stability, stable anesthesia, and hyperoxic ventilation, and one for the postoperative period, when there are higher oxygen consumption and normoxic ventilation. By accepting a low intraoperative hematocrit (15–20%) and postoperatively increasing this value before extubation by retransfusion of autologous

blood, ANH could realize this principle. We performed ANH to a low but not minimal hematocrit, because in clinical practice, the amount of surgical bleeding during extensive surgery frequently is not constant, and in case of suddenly extensive bleeding, hematocrit could easily decrease below the lowest acceptable value.

Regardless of whether or not using ANH will lead to a reduction in transfusions of allogeneic erythrocytes, its proper use will lead to the highest possible postoperative RCV after major blood losses which do not necessarily involve allogeneic transfusions. Consequently, ANH may offer an economical way of preserving the patients' preoperative resources of erythrocytes. It was not the original intention of this study to completely answer the question of effectiveness of ANH in avoiding allogeneic transfusions; however, these data can be used to aid the efficiency calculations done by others.

Preoperative State

The possibility of already existing slightly hypovolemic states preoperative, which often cannot be recognized by routine monitoring, represent a serious dilemma with regard to perioperative fluid therapy. Comparing our patients' PV values before ANH (table 1) with normal PV values established by Pearson *et al.*[34] (PV = 1,395 × body surface area; 99% limits ± 25%) revealed that mean preoperative PV was 109% of predicted normal. The same comparison for RCV showed a preoperative deficit of 115 ml/m^2 for our patients' RCV (normal RCV for women: 822 ml/m^2; 99% limits ± 25%).[34] The comparison with normal values established in our laboratory in 10 female volunteers (RCV = 848 ± 54 ml/m^2)[19] also showed that RCV of the investigated patients was significantly below normal. A slightly elevated PV and reduced RCV resulted in a normal preoperative BV (about 99% in comparison with normal values established by Pearson *et al.*).[34] The reasons for this deficit in RCV are unclear. Increased vaginal bleeding from the tumor, iron deficiency, or tumor anemia could be some explanations. A preoperative deficit in RCV of more than 100 ml/m^2 obviously may be of importance for the perioperative transfusion strategy as well as for effectiveness of ANH.

Period of Acute Normovolemic Hemodilution

As presented in table 1, in the present investigation, normovolemia was maintained and BV was not elevated after ANH, despite administering 15% more volume of colloid than blood removed. In a previous investigation in our laboratory, PV before and after preoperative ANH was measured in 12 patients also scheduled for gynecologic surgery using the same measuring method for PV as used in this study.[35] Mean PV before ANH was 3,148 ± 327 ml, and 1,018 ± 75 ml of blood, corresponding to 653 ± 47 ml of plasma, were removed. Supplying 1,088 ± 93 ml of 5% albumin solution was supposed to result in a theoretical PV after ANH of 3,583 ml; however, measured PV after ANH was only 3,414 ± 340 ml. Consequently, the deficit of 169 ml was 16.6% in relation to the volume of blood removed. An increased extravasation rate of albumin during or after ANH with 4% albumin solution was shown by Payen *et al.*[36] by means of radioactively labeled human serum albumin. The authors concluded that for ANH, the volume of colloid infused should be higher than that of blood withdrawn. The causes for an increased extravasation rate of albumin in the course of ANH remain to be elucidated. The fact that replacing blood removed with a 10% surplus of 5% albumin solution will better maintain hemodynamic stability during ANH than replacing in a ratio of 1:1 has been determined previously.[26] According to these findings, we decided to infuse 15% more of colloid than blood removed for ANH in the current investigation.

Using the same method for measuring PV as used in this study but without also measuring RCV, Haller *et al.* postulated a systematic increase in f-cell ratio during ANH.[35] Based on the two-compartment model of the entire vascular bed brought forward by LaForte *et al.* this would mean that there is a change in the relation between macrovascular and microvascular hematocrit, a change in the relation between the macrovascular compartment volume and the whole blood volume, or both in the course of ANH.[37,38] In the current investigation using a double-label technique for BV measurement, we could not find a significant difference between f-cell ratios before and after ANH. The reason for these contradictory findings is unclear. We can only conjecture that it could be a question of the rate of blood removal and simultaneous replacement, which might vary from one clinical study to another. Another explanation, which in our opinion is less probable, is that the postulate being based on single-label measurements, which is now contradicted by the gold standard (double-label measurement of BV), is false.

Period before Retransfusion

Before this investigation, we expected PV to increase until retransfusion of autologous blood, due to the standardized crystalloid infusion regimen used, to compensate for surgical bleeding. It was suggested that losses of up to 30–40% of blood volume usually can be treated

adequately with crystalloids.[25] As shown in table 1, values of BV calculated by means of an f-cell ratio of 0.89 led to the assumption of decreasing BV until retransfusion, accompanied by a major decrease in Prot and IVP (12 g/l and 42 g, respectively). Consequently, the applied infusion regimen using crystalloids was not sufficient to compensate for a mean blood loss of 730 ml (20% of patients' BV) during intraabdominal surgery, especially as the calculated surgical blood loss before retransfusion (equation 6; calculated value: 749 ml; RCV before retransfusion calculated by an f-cell ratio of 0.89) indicated that surgical blood loss at that time was correctly estimated.

Preoperative Versus *Postoperative Volume Balance*

After retransfusion and additional colloid supply, PV increased significantly so that mean postoperative BV was about 250 ml higher than the preoperative measurement before ANH. Postoperative normovolemia after major surgery is not a matter of course. A previous investigation in our laboratory using the same PV measuring method as used in this study showed postoperative hypovolemia in patients after surgery due to ovarian cancer.[39] As a result, the reason for postoperative slight hypervolemia in the current investigation requires clarification. The protein balance shows that loss of 96 g of protein (calculated by protein supply − change in IVP) was replaced to a sufficient extent by supplying 83 g of protein. In contrast, there was a deficit in protein supply of 45 g in relation to protein loss (145 g) in the previous investigation of patients with ovarian cancer (mentioned previously). Thus, a sufficiently balanced protein supply seems to be the reason for postoperative slight hypervolemia in the current investigation.

As mentioned previously, in our view, ANH is part of a concept of economically treating patients' preoperative resources of erythrocytes. A recently published meta-analysis did not conclusively answer questions regarding the effectiveness of ANH.[40] The amount of erythrocytes saved by ANH depends on three factors: (1) the initial hematocrit; (2) hematocrit at the end of ANH; and (3) most importantly, the amount of surgical blood loss, which mostly cannot be predicted.[7] As an indication for ANH, an expected blood loss of 20%[4] to 30%[3] of the patients' estimated BV was proposed. In the current investigation, mean surgical blood loss was 1,256 ml (34% of the patients' measured BV). Theoretical saving of only 117 ml of erythrocytes in combination with the postoperative hematocrit of 27.2% (corrected for the high postoperative BV) means that without ANH, the postoperative hematocrit would not have been substantially different, and an intraoperative transfusion of allogeneic blood probably would not have been necessary in more than one patient. However, performing ANH reduced surgical loss of erythrocytes by 44% (117 ml in relation to the measured loss of erythrocytes of 266 ml) and consequently led to a postoperative RCV that was 11% higher.

Our data showed that ANH with a 15% surplus of 5% albumin solution in relation to blood removed maintained normovolemia. After ANH, an infusion regimen supplying only crystalloid did not seem to be sufficient to compensate for a surgical blood loss of about 20% of patients' BV during intraabdominal surgery. Retransfusing of autologous blood led to a higher postoperative BV with respect to the preoperative value before ANH. By reducing surgical loss of erythrocytes, ANH could promote economical treatment of patients' preoperative resources of erythrocytes.

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