Relative Blood Volume Changes Underestimate Total Blood Volume Changes during Hemodialysis

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Background: Measurements of relative blood volume changes (ΔRBV) during hemodialysis (HD) are based on hemoconcentration and assume uniform mixing of erythrocytes and plasma throughout the circulation. However, whole-body hematocrit (Ht) is lower than systemic Ht. During HD, a change in the ratio between whole-body to systemic Ht (F cell ratio) is likely to occur as a result of a net shift of low Ht blood from the microcirculation to the macrocirculation. Hence, ΔRBV may differ significantly from total blood volume changes (ΔTBV). Therefore, this study compared ΔRBV and ΔTBV during HD.

Design, participants, and measurements: Plasma and erythrocyte volumes were measured using 125I- and 123I-radiiodinated albumin and 51Cr-labeled erythrocytes, respectively. After validation of the standardized method in two patients on a nondialysis day, seven patients completed the protocol during HD. 125I-albumin and 51Cr-labeled erythrocytes were administered 20 min before the start of HD. 123I-albumin was administered at 160 min into the HD session to quantify and correct for 125I-albumin leakage. ΔRBV was measured continuously throughout HD. The F cell ratio was derived from whole-body and systemic Ht.

Results: Total ultrafiltration volume was 2450 ± 770 ml. TBV declined from 5905 ± 824 to 4877 ± 722 ml during HD. Thus, TBV declined 17.3 ± 4.4%, whereas the RBV decline was only 8.2 ± 3.7% (P = 0.001). The F cell ratio increased from 0.896 ± 0.036 to 0.993 ± 0.049 during HD (P = 0.002).

Conclusions: ΔRBV significantly underestimates ΔTBV during HD. The rise in F cell ratio strongly suggests that during HD, blood translocates from the microcirculation to the macrocirculation, probably as a cardiovascular compensatory mechanism in response to hypovolemia.


Technological advances have allowed the development of continuous, noninvasive measurement of relative blood volume changes (ΔRBV) during hemodialysis (HD). Continuous registration of ARBV during HD with ultrafiltration (UF) is advocated as a tool to maintain an adequate volume of the intravascular compartment to avoid HD hypotension (1–3). However, the use of ΔRBV measurements in clinical practice depends on its accuracy in reflecting the change in whole-body blood volume or total blood volume (ΔTBV). Noninvasive measurements of ΔRBV are based on hemoconcentration of blood constituents that remain in the circulation during HD with UF. Mostly erythrocytes are used for these measurements. ΔRBV devices that are based on hemoconcentration of afferent blood can adequately represent ΔTBV only in case of uniform mixing of plasma and erythrocytes throughout the different vascular beds of the circulation (4–6). However, this assumption is not valid: The whole-body hematocrit (Ht) is lower than the Ht of arterial or venous blood (7,8). The difference is due to a dynamic reduction in microvascular Ht in capillaries and venules (<200 μm), known as the Fahraeus effect (9). The difference between arterial or venous Ht and whole-body Ht is expressed as the F cell ratio (the ratio of whole-body Ht to arterial or venous Ht) and approximates 0.91 in nondialysis individuals (10,11). The lack of uniform mixing of erythrocytes throughout the circulation would not induce a divergence between ΔRBV and ΔTBV calculation if the difference in Ht between the different vascular beds would remain constant during HD— in other words, if the F cell ratio would not change (12). However, a change in F cell ratio may occur during HD with UF (13,14). As a consequence, the observed ΔRBV may differ significantly from the ΔTBV (13).

To our knowledge, no studies to date that have compared absolute ΔTBV, which is the sum of erythrocyte and plasma volume, using methods that are generally accepted as the gold standard: Radiiodinated albumin (125I) and 51Cr-labeled erythrocytes (15,16), during HD with UF with its relative counterpart: ΔRBV measurements as measured by hemoconcentration of afferent blood. In this study we compared hemoconcentration-based ΔRBV and ΔTBV measured by repetitive measurement of
absolute plasma and erythrocyte volumes during HD with UF in eight patients. In addition, we calculated the F cell ratio because a change in this factor may explain a possible discrepancy between ∆RBV and ∆TBV measurements.

Materials and Methods

Patients
The standardized method for determining plasma and erythrocyte volumes (radioiodinated 125I-albumin and 51Cr, respectively) was evaluated for its stability for repeated measurements in two HD patients on a nondialysis day in a prestudy test. Subsequently, eight patients underwent measurements of absolute plasma and erythrocyte volumes during a single HD session according to the same protocol.

To be eligible, patients had to fulfill the following criteria: (1) Non-hypotension-prone male HD patients who were treated with HD for at least 6 mo and had stable values for hemoglobin (>7 mmol/L); (2) unaltered erythropoietin dosage for at least 2 mo; (3) an arteriovenous fistula without recirculation as established by Transonic flow measurements (Transonic Systems, Ithaca, NY); and (4) no residual renal function (diuresis <100 ml/24 h). Exclusion criteria were (1) the absence of informed consent, (2) Recent hemorrhage, and (3) diabetes. The last exclusion criterion was applied because participating patients were not allowed to eat or drink during the study.

Study Protocol

Determination of Erythrocyte and Plasma Volume. Plasma and erythrocyte volume measurements are routinely used to assess erythrocyte and plasma volume in the diagnosis of polycythemias. The standard technique for measuring plasma volume involves the use of 51Cr-labeled albumin (15,16). For the measurement of the erythrocyte volume, 51Cr-labeled erythrocytes are commonly used (15,16). The principle is based on the so-called “isotope dilution technique.” Volumes are calculated from the known dosage administered and the radioactivity concentration measured in whole blood or plasma (15). A single dose of 51Cr-labeled erythrocytes will suffice for the duration of this study because the radioactivity half-life is 28 d.

Because transcapillary albumin leakage occurs in both healthy control subjects and HD patients (17), it is not possible to measure reliably the plasma volume over a longer period of time when using only one radioiodinated marker. We therefore determined plasma volume using two radioiodinated albumin injections. We chose 125I-albumin and 123I-albumin because these isotopes differ in γ-ray energy (27 to 32 and 159 keV, respectively) and, thus, allow separate detection in the γ counter. The administration of the second radioiodinated isotope, 180 min after administration of the first isotope, allows quantification of and correction for the transcapillary escape rate of albumin (TER Alb). The quantification of TER Alb was based on the first 125I plasma volume measurement and the 123I plasma volume determination after 180 min of HD because at this time point, the plasma volume as assessed simultaneously by 125I and 123I should yield identical results. In this study, we assumed that TER Alb was constant during HD in the individual participant. All values for plasma volume displayed in this study are corrected for TER Alb.

Radioiodination 

51Cr labeling of erythrocytes and 125I-albumin labeling were performed under aseptic conditions in the radiopharmacy unit of the Nuclear Medicine Department under supervision of a hospital pharmacist. 51Cr-sodium chromate and 125I-sodium iodide (125I-Na) were obtained from GE Health (Eindhoven, Netherlands). 125I-human serum albumin (125I-HSA) was obtained from Merck Frosst Canada (Kirkland, QU, Canada). An extensive description of the used methods is described in detail elsewhere (15,16). In short, the 51Cr-sodium chromate solution was added to the patients’ erythrocytes. The mixture was incubated for 45 to 60 min at a temperature of 37°C. Thereafter, 25 mg of ascorbic acid was added to the labeled erythrocyte suspension. A known amount of the well-mixed labeled erythrocyte suspension was injected together with a known amount of 123I-HSA to the patient.

HSA (Cealb 20%; Sanquin, Amsterdam, Netherlands) was labeled in the radiopharmacy department with 125I-Na using a standard iodogen (Pierce Biotechnology, Rockford, IL) method. The mixture was purified over a PD-10 column (Sephadex G-25M; Amersham Biosciences AB, Uppsala, Sweden) and overnight dialysis (Slide-A-Lyzer; Pierce Biotechnology). Radiochemical purity was >98%.

Protocol. The study protocol was identical during the prestudy test and the HD study except for the start of HD (T = 0) during the latter study. HD commenced 20 min after the first injection with the radiopharmaceuticals. This time interval was chosen to allow adequate distribution of the radioiodinated isotopes (15,16). For avoidance of the widely known influence of postural changes on blood volume (18) and F cell ratio (19,20), patients were placed in a supine position 20 min before blood sampling for erythrocyte labeling with 51Cr and remained in this position until the study protocol was completed (from 100 min before the start of HD until the stop of HD). After completion of the erythrocyte labeling with 51Cr, the patients’ own blood supplemented with 125I-albumin was administered. The interval between blood sampling for the labeling of the erythrocytes and the re-administration of the isotopes was 60 min. During the prestudy test and the HD study, 123I was given 180 min after the administration of 125I (160 min into the HD session). Blood was sampled four times at 10-min intervals immediately after both injections that contained the isotope(s). After four blood samples, the interval between two samplings was increased to 20 min. In total, blood was sampled 17 times. At each sampling, a total of 5.5 ml of blood was withdrawn. Each sampling included erythrocyte and plasma volume calculation as well as determination of whole-body and systemic Ht. Because the average Ht in our patient group was 0.40 ± 0.05 at the start of HD, the sampling of a total of 93.5 ml of blood equals an erythrocyte and plasma volume of approximately 37.4 and 56.1 ml, respectively. In the calculations of erythrocyte and plasma volume, we made no corrections for this sampling of blood because this plasma volume is very small in comparison with the expected change in plasma volume and because this erythrocyte volume is negligible compared with the patients’ erythrocyte volume. A standard dialysis needle was used for blood sampling. A vein on the nondialysis access side was cannulated for the purpose of administering the radiopharmaceuticals.

Patients were asked to refrain from coffee, tea, alcohol, and tobacco use from the night before the study until completion of the study. With the exception of a glass of water (125 ml) at room temperature after 20 and 200 min into the HD session, patients were not allowed to eat or drink throughout the study to avoid any influence of food intake on blood volume (21). BP and heart rate were measured simultaneously during each time blood was sampled.

The study was approved by the Medical Ethics Committee of the University Medical Center Groningen. Written informed consent was obtained from all participating patients. The study was performed in accordance with the principles of the Declaration of Helsinki and guidelines for Good Clinical Practice.

Dialysis Settings. All eight patients underwent dialysis for 4 h with bicarbonate dialysis in a single session. A low-flux polysulfone hollow-fiber dialyzer (F8; Fresenius Medical Care, Bad Homburg, Germany) was used. Blood flow rates and dialysate flow rates were set at 250 and 300 ml/min, respectively. Dialysate composition was as follows: Sodium 139 mmol/L, potassium 1.0 mmol/L, calcium 1.5
mmol/L, magnesium 0.5 mmol/L, chloride 108 mmol/L, bicarbonate 34 mmol/L, acetate 3.0 mmol/L, and glucose 1.0 g/L. Dialysate temperature was set at 36.0°C. HD was performed with the Integra HD apparatus (Gambro-Hospal, Lyon, France). \( \Delta RBV \) were measured with Hemoscan (Gambro-Hospal). In addition, we assessed \( \Delta RBV \) from changes in Ht (\( \Delta RBV-Ht \)) in afferent blood because this method is considered to be the reference method for determining \( \Delta RBV \) during HD (5,22).

Calculations

The following calculations were used in this study:

Total blood volume = erythrocyte volume + plasma volume
Whole-body Ht = erythrocyte volume/(erythrocyte volume + plasma volume)

\[ F \text{ cell ratio} = \frac{\text{whole-body } Ht}{\text{venous } Ht} \]

\[ \Delta TVB \text{ in } \% = \frac{[(TBV_0 - TBV_t)/TBV_0] \times 100}{[Ht_0/Ht_t] - 1} \times 100 \]

in which \( TBV_0 \) and \( Ht_0 \) and \( TBV_t \) and \( Ht_t \) represent TBV and Ht at the start of HD and at a certain moment during HD, respectively.

Statistical Analyses

All data were analyzed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA). Data are presented as mean ± SD unless stated otherwise. Comparisons were made with a paired t test or an unpaired t test when appropriate. In addition, Bland-Altman analysis was used for the evaluation of differences between \( \Delta RBV \) and \( \Delta TBV \). From these results, the Pearson correlation coefficient \( r \) was derived. \( P < 0.05 \) was considered significant.

Table 1

<table>
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<tr>
<th>Patient</th>
<th>EV (ml)</th>
<th>PV (ml)</th>
<th>TBV (ml)</th>
<th>UF</th>
<th>TER(_{ab}) (%)/h</th>
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<td>120 min</td>
<td>180 min</td>
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\( ^{a} \text{0 min and 240 min denote the start and end of the hemodialysis session, respectively. EV, erythrocyte volume; PV, plasma volume; TBV, total blood volume; TER}_{ab}\text{, transcapillary escape rate of albumin; UF, ultrafiltration.} \)
Table 2. ΔTBV and ΔRBV at hourly intervals in the individual patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>ΔTBV (%)</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
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<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
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<td>-13.7</td>
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<td>-3.2</td>
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<td>-5.5</td>
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<tr>
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<td>-8.1</td>
<td>-13.1</td>
<td>-14.0</td>
<td></td>
<td>-6.4</td>
<td>-8.9</td>
<td>-10.5</td>
<td>-12.2</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-4.3</td>
<td>-10.0</td>
<td>-13.3</td>
<td>-17.3</td>
<td></td>
<td>-3.6</td>
<td>-5.4</td>
<td>-7.0</td>
<td>-8.2</td>
<td></td>
</tr>
</tbody>
</table>

ΔRBV, change in relative blood volume; ΔTBV, change in total blood volume.

Discussion

This study shows directly that ΔRBV as assessed by hemoconcentration of afferent blood significantly underesti-
would not change (19,23). This study, however, clearly demonstrates that the F cell ratio increases markedly during HD. We hypothesize that this reflects translocation of blood with a lower Ht from the microcirculation to the macrocirculation, thereby inducing a dilution of the central blood compartment. Therefore, the hemoconcentration of blood that reaches the ΔRBV monitor is not representative of the hemoconcentration of the TBV. As a result, ΔRBV monitors underestimate the real ΔTBV during HD.

Mitra et al. (13) indirectly established that the F cell ratio might rise during HD. These authors used Indocyanine green to determine plasma volume. Erythrocyte volume was subsequently calculated from plasma volume and venous Ht. However, the use of calculated erythrocyte volumes necessitates the input of an estimated average F cell ratio because an individual F cell ratio cannot be derived from plasma volume alone. This may induce an error because population values for the F cell ratio have been reported as 0.864 (24) and 0.91 (25). On an individual level, F cell ratios have been reported to display wide scattering from 0.76 to 1.15 (25). Therefore, the input of a mean F cell ratio for different patients may introduce a substantial error in calculating erythrocyte volumes in patients who have an F cell ratio that differs from the mean value (15,25). In our study, the mean pre-HD F cell ratio differed from the mean population values mentioned. In addition, our patients displayed a wide interindividual variability in F cell ratio (from 0.84 to 0.95 before HD). Nevertheless, our study does support the hypothesis from Mitra et al. In their study, they could not explain the apparent rise in erythrocyte volume other than by an increase in F cell ratio during HD with UF (13). Our direct F cell measurements underline this hypothesis. It is interesting that the difference between ΔRBV and ΔTBV that we found was of the same order of magnitude as in the study of Mitra et al. (8.2 ± 3.7 and 7.7 ± 10.6%, respectively).

In this study, we used 123I-albumin as a second plasma volume marker to quantify and correct for TER_{alb} from 125I-albumin during HD. In healthy control subjects, TER_{alb} has been demonstrated to range from 4.3 to 7.4%/h (17,26–28). Hildebrandt et al. (17) showed a mean TER_{alb} of 9.6% in HD patients before the start of HD, and Geers et al. (26) found a TER_{alb} of 9.8 ± 2.6% in patients with the nephrotic syndrome. As a mean, we found an intradialytic TER_{alb} of 9.1 ± 1.3%, which is in line with the aforementioned studies.

This study has some important pathophysiologic and clinical implications. It shows directly that the F cell ratio rises during HD. This observation strongly suggests that during HD, translocation of blood from the microcirculation to the macrocirculation takes place, which probably is a cardiovascular compensatory mechanism in response to hypovolemia. The underestimation of the real TBV decline may explain why some patients develop hypotension at a relatively small RBV decline. In addition, the poor predictive value of ΔRBV for the occurrence of dialysis hypotension (reviewed by Dasselaar et al. [12]) may well partly be explained by variability in the divergence between ΔRBV and ΔTBV (and thus a variable rise in F cell ratio) during HD. In this study, the extent of the divergence between ΔRBV and ΔTBV indeed differed considerably between individuals. Some patients displayed a relatively small difference between ΔRBV and ΔTBV (e.g., −12.2 versus −14.0% in patient 7), whereas others displayed a substantial difference (e.g., −8.1 versus −22.8% in patient 3). Notably, the divergence between ΔRBV and ΔTBV was of the same order of magnitude in patients with a relative low and high UF volume. In other words, the difference between ΔRBV and ΔTBV was also prominent in the patients with relatively low UF volumes (e.g., −5.7 versus −14.3% with a UF volume of 1200 ml in patient 2).

Factors that may influence the extent of the intradialytic translocation of blood from the microcirculation to the macrocirculation (and thus the extent of the change in F cell ratio) are ambient and dialysate temperature, changes in position, administration of vasoactive drugs and exercise during HD. All of these factors are known to influence ΔRBV (reviewed in reference [12]). It remains to be studied whether these factors also influence ΔTBV and/or the divergence between ΔRBV and ΔTBV.

In this study, we investigated hemodynamically stable patients. Future studies should also address the issue of underestimation of ΔTBV by ΔRBV and the change in F cell ratio in hypotension-prone HD patients. For the moment, however, it is important for the dialysis staff to realize that the relative change in blood volume, as calculated by ΔRBV monitors, may mark-

### Table 3. Individual hourly F cell ratios

<table>
<thead>
<tr>
<th>Patient</th>
<th>F Cell Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>60 min</td>
</tr>
<tr>
<td>1</td>
<td>0.91</td>
</tr>
<tr>
<td>2</td>
<td>0.84</td>
</tr>
<tr>
<td>3</td>
<td>0.92</td>
</tr>
<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>0.90</td>
</tr>
<tr>
<td>6</td>
<td>0.87</td>
</tr>
<tr>
<td>7</td>
<td>0.88</td>
</tr>
<tr>
<td>Mean</td>
<td>0.896</td>
</tr>
</tbody>
</table>

### Figure 3. The mean course of the F cell ratio (C) during HD of all seven patients. *P < 0.05 versus the start of the HD session.
edly underestimate the real decline in blood volume, especially in the second half of the HD session.

**Conclusion**

$\Delta RBV$ significantly underestimates $\Delta TBV$ during HD. The rise in F cell ratio strongly suggests that blood translocates from the microcirculation to the macrocirculation during HD, probably as a cardiovascular compensatory mechanism in response to intravascular volume depletion.

**Acknowledgments**

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**Disclosures**

None.

**References**


