Effect of absolute blood volume measurement guided fluid management on the incidence of intradialytic hypotension associated events: a randomised controlled trial

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GRAPHICAL ABSTRACT

ABSTRACT

Background. Ultrafiltration to target weight during haemodialysis is complicated by intradialytic hypotension associated adverse events (IHAAE) in 10-30% of dialysis treatments. IHAAE are caused by critical reductions in absolute blood volume (ABV), due to the interaction of ultrafiltration, refill and compensatory mechanisms. Non-randomised studies suggested that ABV-guided treatment, using an
indicator dilution technique employing the blood volume monitor on the dialysis machine, could reduce the incidence of IHAAE.

**Methods.** We performed an open label randomised controlled trial. Patients were randomly assigned to adjustment of target weight guided by ABV measurements or standard care. Primary outcome was change in the incidence of IHAAE from baseline, defined as the percentage of treatment episodes in a four-week period where the patient had a systolic blood pressure <90 mmHg or symptoms of impending hypotension. ABV measurements were compared to anthropomorphometric estimation and the gold standard using isotope dilution.

**Results.** Fifty-six patients were randomised, of whom 29 were allocated to ABV-guided treatment and 27 to standard care. Overall baseline incidence of IHAAE was 26.0%. ABV-guided treatment significantly reduced the incidence of IHAAE compared to standard care, with a mean change from baseline of -9.6% (95% CI: -17.3; -1.8%) versus +2.4% (-2.3; +7.2%). The adjusted difference between the groups was 10.5% (1.3; 19.8%, p = 0.026). ABV measurement had moderate agreement with other methods to estimate blood volume. The sensitivity for the previously suggested threshold of a post-dialysis normalised blood volume of 65 ml/kg was observed to be 74% in this study.

**Conclusions.** ABV-guided volume management significantly reduced IHAAE compared to standard care. The clinical relevance of the previously suggested threshold of 65 ml/kg cannot be firmly concluded on the basis of our results. If confirmed in a larger trial, this intervention could potentially change dialysis practice and impact patient care in a clinically meaningful way.

**KEY LEARNING POINTS**

**What was known:**
- Fluid management remains a challenge in haemodialysis treatment, often resulting in intradialytic hypotension-associated adverse events (IHAAE).
- Clinical assessment, bio-impedance spectroscopy and online relative blood volume measurements are used to establish target weight, although these tools fall short to prevent IHAAE.
- Measurement of absolute blood volume (ABV) is proposed as a promising tool to improve fluid management in haemodialysis patients.

**This study adds:**
- This is the first trial to assess the effect of ABV measurement guided fluid management on the incidence of IHAAE compared to standard care.
- Adjustment of target weight guided by ABV measurement led to a significant reduction of IHAAE.
• ABV measurement provides reproducible results and shows moderate agreement with other methods to calculate absolute blood volume, including the gold standard method.

Potential impact:
• ABV measurement could be a helpful tool to prevent IHAAE in haemodialysis patients, in order to improve quality of life and reduce morbidity and mortality.

Keywords: fluid management, haemodialysis, intradialytic hypotension, intradialytic morbid events

INTRODUCTION
The determination of target weight in haemodialysis patients is one of the important clinical dilemmas in nephrology. Target weight is usually established by gradually modulating post-dialysis body weight while observing the patient for the development of hypotension associated adverse events (IHAAE) such as dizziness, cramps or loss of consciousness. Underestimation of target weight results in dialysis-induced hypotension due to the failure of cardiovascular compensatory mechanisms once the total blood volume falls beyond a certain threshold. The prevalence of intradialytic hypotension associated adverse events ranges from 10 to 30% [1-3]. IHAAE are associated with an impaired quality of life and increased cardiovascular morbidity and mortality, caused by end-organ hypoperfusion [3-5].

Currently used methods to establish target weight including clinical assessment, bio-impedance spectroscopy and online relative blood volume (RBV) measurements all have their own limitations [6, 7]. RBV measurements are based on real-time non-invasive techniques during haemodialysis and provide the ratio of current blood volume to the blood volume at the start of haemodialysis, using measurements of blood constituents such as haematocrit. These measurements reflect changes in blood volume during dialysis without providing any information about the initial hydration status or the initial absolute blood volume (ABV). Additionally, several conditions such as ultrafiltration, exercise, postural change, recruitment of the unstressed venous blood volume and fluid infusions during dialysis influence these measurements, resulting in an incorrect estimation of RBV [6]. It is therefore hardly surprising that RBV measurement has not fulfilled its promise as a useful tool in the prediction and prevention of IHAAE [8].

A tool that could measure ABV might be able to predict IHAAE more accurately, since the occurrence of IHAAE depends on the decline of ABV beyond the critical threshold, where cardiovascular and neurohumoral compensatory mechanisms can no longer maintain blood pressure. Such a tool would have to be “point of care”, which disqualifies the gold standard method of radioisotope blood volume measurements [9]. The RBV monitoring tool on haemodialysis machines was recently used to calculate ABV, using the principle of indicator dilution by infusion of a known volume of isotonic ultrapure...
dialysate [10, 11]. These studies identified a critical threshold for ABV of 65 ml/kg target weight that predicted the occurrence of IHAAE [12]. In a pre-post intervention study among 45 haemodialysis patients, they found a reduction in the incidence of IHAAE from 12% to 0.9% after adjustment of target weight in patients with an ABV below that critical threshold [13].

We conducted a randomised controlled trial in haemodialysis patients to examine the efficacy of ABV-guided volume management in reducing the incidence of IHAAE compared to standard care.

MATERIALS AND METHODS

Trial design

An open label randomised controlled trial was conducted. Because of patient engagement in haemodialysis treatment, it was not possible to blind them. The treating physician was not blinded to the adjustment in target weight but was unaware of ABV measurement and IHAAE questionnaire outcomes. The study protocol was approved by an independent ethics committee. This study was registered at ClinicalTrials.gov (Identifier: NCT05872984). All patients provided written informed consent. Patients were recruited between July 2020 and January 2021. Study follow-up was completed in May 2021.

Patients

Adult patients on maintenance haemodialysis for at least three months on a three times weekly haemodialysis scheme were eligible. Patients with severe volume overload, heart failure or liver failure, with a contraindication to bolus fluid infusion at start of dialysis were excluded from participation. Additional exclusion criteria were residual diuresis >500 ml/24h, central venous access, single needle treatment, clinically relevant fistula dysfunction with spKt/V <1.2, and an inability to provide informed consent.

Trial procedure and intervention

After inclusion, patients entered a 4-week observational phase to assess their baseline incidence of IHAAE, using a predefined questionnaire (Supplement 1). ABV was measured twice in all patients during the mid-week haemodialysis sessions in the fifth and sixth week. Both the patients and their treating physicians were not informed on the results of these measurements. Subsequently patients were randomised in a 1:1 ratio to adjustment of target weight guided by ABV measurements (intervention group) or standard care (control group), using permuted block randomisation with randomly varying block sizes of 4, 6 and 8. In patients in the intervention group with a normalised absolute blood volume (Vₚₙ) <65 ml/kg at the end of dialysis, target weight was increased once by 0.5 kg. ABV measurement was repeated after 2 weeks and incidence of IHAAE was
assessed for another four weeks (Figure 1). Adjustment of target weight in the control group was at the discretion of the treating physician in accordance with clinical practice. Additionally, eight patients were randomly selected to measure total blood volume using radioactive labelled albumin. This is based on the principle of isotope dilution and is considered the gold standard. After administration of a bolus of 50 mg/kg $^{125}$I-labelled albumin, total blood volume was calculated from the known administered dosage and the radioactivity concentration measured in plasma. This measurement was taken before the dialysis session where the ABV measurement was performed prior to starting ultrafiltration.

**Measurement of ABV**

Measurement of ABV was performed during the mid-week dialysis session [13]. At the start of dialysis, before starting ultrafiltration, a 240ml bolus ultrapure dialysate was infused into the extracorporeal circuit of the dialysis machine with an infusion rate of 200ml/min. RBV measurements before and after this infusion are performed automatically by the RBV module of the Fresenius 5008 dialyser. Within one minute after infusion of the bolus the increase in RBV appeared in the display of the dialysis machine. The dialysis treatment was initiated after these measurements. ABV at start and end of dialysis were calculated from these RBV values, using the formulas described by others [10, 11].

ABV at start of dialysis (initial ABV) is calculated with formula 1, where $V_{bolus}$ is the volume of the infused bolus (ml), $RBV_{before bolus}$ and $RBV_{after bolus}$ are the RBV measured immediately before and after bolus infusion (%).

1. Initial ABV ($L$) = \[
\frac{V_{bolus}}{RBV_{after bolus} - RBV_{before bolus}} \times 0.1
\]

ABV at the end of dialysis (final ABV) is calculated with formula 2, where final RBV (%) is the measured RBV at the end of dialysis.

2. Final ABV($L$) = \[
Initial ABV \times \text{final RBV}\]

Normalized absolute blood volume ($V_s$) was calculated by dividing the ABV by the patient target weight (ml/kg).

**Outcomes**

The prespecified primary outcome was change in incidence of IHAAE from baseline to post intervention. These change scores were compared between the intervention and control group. IHAAE
was defined as the occurrence of a systolic blood pressure <90 mmHg or a sudden decrease in systolic blood pressure of \( \geq 20 \text{ mmHg} \) during dialysis regardless of symptoms, or symptoms related to impending hypotension including dizziness, light-headedness, sweating, nausea, vomiting, cramps, vision disturbances, altered motor, verbal or cognitive functions or unconsciousness. Blood pressure was measured according to protocol immediately before and after both initiation and termination of dialysis, during dialysis at hourly intervals and additionally in case of symptoms. We assessed post-dialysis weight as a non-prespecified explanatory analysis. The secondary outcomes of interest were baseline incidence of IHAAE in the entire study population, reproducibility of ABV measurement, agreement of ABV measurement with other methods to determine blood volume (anthropomorphometric estimation using Nadler’s formula and the isotope dilution technique), and sensitivity and specificity of the proposed threshold of \( V_s \) of 65 ml/kg. These secondary outcomes were collected before randomisation. Data on serious adverse events were collected prospectively.

**Statistical analysis**

Previously published data showed >90% reduction of IHAAE after adjusting target weight based on ABV measurement [13]. It was estimated that a sample size of 120 patients would provide 80% power to detect a statistically significant difference between the two groups in the primary outcome under the assumptions of a 23% incidence of IHAAE with a standard deviation of 30.5%, a 75% decline in IHAAE, and a 10% loss to follow-up.

Analyses were performed according to intention-to-treat and included data from all patients who had undergone randomisation. Baseline characteristics are presented as numbers, percentages and means with standard deviations. Incidence of IHAAE at baseline and after intervention was calculated by dividing the number of dialysis treatments with one or more symptoms attributable to IHAAE by the total number of dialysis treatments with a completed questionnaire in the relevant 4-week interval. Some questionnaires were missing due to logistical reasons, which were assumed to be missing completely at random. The primary endpoint was analysed with the use of ANCOVA, with the following pre-defined covariables: baseline IHAAE, pre-dialysis blood pressure and the use of anti-hypertensive agents. We used Bland-Altman analysis to evaluate the agreement within subsequent ABV measurements, and agreement between ABV and other methods to calculate ABV. Statistical analyses were performed with SPSS statistics 25 (SPSS Inc., Chicago, IL, USA) and R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria).
Dialysis prescription

All patients were dialyzed using the Fresenius 5008 HD machine (Fresenius Medical Care), using a Fresenius FX800 Cordiax filter. A standardised dialysis prescription was used with a blood flow of 300 ml/min, dialysate flow of 500 ml/min and post-dilution hemodiafiltration using the Autosub-plus modality and the blood temperature module of the Fresenius machine. Ultrafiltration needs were based on pre-dialysis weight compared to the prescribed target weight maximized at 3200 ml/4 hr.

RESULTS

The study had to be terminated before reaching target recruitment due to a lagging inclusion induced by the COVID-19 pandemic. A total of 59 patients were included. During the baseline period one patient withdrew consent, one withdrew from dialysis and one did not meet the inclusion criteria (Figure 2). Fifty-six patients underwent randomisation. Clinical and demographic characteristics were similar in the two treatment groups (Table 1). Sixty-one percent were male, mean age was 68 years and mean dialysis vintage was 40 months. Comorbidities were common and the majority of patients used at least one antihypertensive drug. While there were no overall differences in the use of antihypertensive drugs, calcium channel blockers and ACE inhibitor use was higher in the intervention group. Ultrafiltration requirement, pre-dialysis systolic and diastolic blood pressure and pre-dialysis V,s did not differ either. Although two patients died during the course of the study, we collected sufficient follow-up data to include them for analysis. Causes of death were unrelated to the study; sepsis and out of hospital cardiac arrest.

Primary outcome

Twenty-nine patients were allocated to the intervention group of which 18 had a post-dialysis V,s <65 ml/kg requiring an adjustment of their target weight with 0.5 kg. After this adjustment six additional patients achieved V,s ≥65 ml/kg. With respect to the baseline incidence, ABV-guided treatment reduced the incidence of IHAAE with 9.6% (95% CI: -17.3; -1.8%), compared to an increase of 2.4% (-2.3; +7.2%) in the control group. After adjusting for the baseline incidence of IHAAE, the use of antihypertensive drugs, pre-dialysis systolic and diastolic blood pressure, the difference between the changes from baseline in the two groups was 10.5% (1.3; 19.8%, p = 0.026), see Table 2. In a supplementary analysis based on the incidence of IHAAE during the last two weeks of the intervention period only, under the assumption that it might take some time for the cardiovascular system and neurohumoral mechanisms to adjust to a new target weight, the results were similarly in favour of the intervention group. Mean change in incidence from baseline was -12.7% (95% CI: -21.5; -3.8%) in the intervention group versus +4.9% (-2.8; 12.6%) in the control group, with an adjusted difference of 16.3% (5.1; 27.6%, p = 0.005), see Table 2. The mean change in post-dialysis weight between pre- and
post-intervention did not differ significantly between the intervention and control group \( (p = 0.0664) \),
and similarly, there was no significant difference in the mean intra-dialytic weight reduction, see
Appendix 1.

**Secondary outcomes**
The mean baseline incidence for IHAAE was 26.0% in the total study population (*Table 3*). As shown in
*Table 4*, 35 patients experienced light-headedness at least once and 26 suffered from systolic
hypotension at least once. Hypotensive events were often accompanied by light-headedness, dizziness and cramps.
The mean difference between two subsequent ABV measurements did not significantly differ from zero \((1.13 \text{ ml/kg}, 95\%\text{CI: } -4.61; 2.35)\), indicating no systematic bias. However, the limits of agreement encompassed quite a wide range \((-26.15\) to \(23.89 \text{ ml/kg})\) indicating variability in repeated measurements (*Table 5*).
The total blood volume measurement using the isotope dilution technique was performed in eight patients. One obviously erroneous measurement was excluded from the analysis. Anthropomorphometric estimation was calculated in 56 patients. Comparison of the ABV measurement with the anthropomorphometric estimation revealed moderate agreement, characterized by a mean difference of 0.3 litres \((95\%\text{CI: } -0.63; 0.04)\) with substantial variability \((\text{limits of agreement } -2.56; 1.97)\). In our small sample, ABV measurement overestimated the blood volume with 1.17 litres \((95\%\text{CI: } -1.71; -0.63)\) on average compared to isotope dilution measurement (*Table 5, Figure 3*).

In an attempt to validate the accuracy of the proposed critical threshold of a post-dialysis \( V_s \) of 65 ml/kg the incidence of IHAAE was converted to a binary variable based on the median baseline incidence in the total study population. This resulted in a sensitivity of 74%, a specificity of 50%, a positive predictive value of 59% and a negative predictive value of 67% for the threshold of 65 ml/kg (*Table 6*). A Receiver Operating Characteristics (ROC) analysis was performed to assess the diagnostic accuracy of ABV measurement. To determine the optimal threshold, we employed the Youden’s J statistic, which seeks to maximize the sum of sensitivity and specificity. The threshold corresponding to the highest Youden’s J value was 67.5 ml/kg, with an AUC of 0.624 \((0.473 – 0.776)\). When we retained IHAAE as a continuous variable the mean incidence of IHAAE was \(28.7 \pm 24.6\%\) in patients with \( V_s <65 \text{ ml/kg} \), compared to \(22.5 \pm 25.8\%\) in those with \( V_s \geq65 \text{ ml/kg} \) \((p = 0.216)\).

During our study, four patients were admitted to the hospital in both treatment arms, for reasons unrelated to the intervention. No problems due to fluid overload were observed.
This study is the first randomised trial demonstrating that adjustment of target weight based on non-invasive ABV measurements using the RBV module of the Fresenius 5008 dialysis machine, aiming for a normalised blood volume ($V_s$) at the termination of dialysis of ≥65 ml/kg, leads to an absolute reduction in the incidence of IHAAE compared to standard care of 10.5% ($p = 0.026$). These findings confirm the results of previous non-randomized studies [12, 13].

The mean baseline incidence of IHAAE in this trial was 26.0%, which means that an average patient has one or more symptoms of IHAAE in three out of twelve dialysis sessions over a 4-week period. This finding, which is in agreement with reported incidences in the literature, represents a significant treatment burden for patients, which not only impacts patient satisfaction with treatment and quality of life, but is also associated with hospitalisation, cardiovascular events and mortality [2-4]. IHAAE should be seen as a relevant clinical problem and interventions that target this issue are therefore worthwhile.

Previous studies reported favourable reproducibility of the ABV measurement [10, 11]. Although we found no significant mean difference between the two subsequent measurements, the wide limits of agreement raise concerns about the clinical reliability of the measurement at least in our institution. Further research is warranted to determine whether strategies such as implementation as a part of routine clinical practice, adaptation, or automation of the measurement protocol are required to diminish this variability. The ABV method resulted in a significant overestimation of blood volume measured with the gold standard method of isotope dilution in this small sample of 7 patients. Our result for pre-dialysis blood volume using the isotope dilution technique (4.8 ± 0.7 l) was very similar to post-dialysis blood volume measured by others also in seven patients (4.9 ± 0.7 l) [14]. A larger sample might resolve these issues of precision and accuracy.

It was found that average post-dialysis blood volumes at baseline, resulting from clinical judgment, were quite close to the proposed threshold of 65 ml/kg. However, it is obvious that clinical judgement is imperfect as illustrated by the baseline incidence of IHAAE of 26.0%. This is especially true in situations in which body weight might have changed rapidly for example due to periods of illness, hospitalization or surgery. ABV-guided treatment might very well be superior in these instances.

Dichotomising the incidence of IHAAE based on the median value enabled the calculation of sensitivity and specificity. From this analysis it could be concluded that the threshold of 65 ml/kg was not very good at predicting more than average incidence of IHAAE. A threshold of 67.5 ml/kg might have better predictive value, although this cut-off value still provides only moderate sensitivity. This could be due to small size of the study but could also be due to the fact that the occurrence of IHAAE is determined by the interplay of intravascular volume with other factors including ultrafiltration, refill and
cardiovascular and neurohumoral compensatory mechanism. Based on our study, the clinical relevance of the threshold of 65 ml/kg remains inconclusive. A future study might clarify this issue.

A limitation of our study was the absence of formal blinding. To minimize potential bias, we restricted the information provided to patients, nursing staff and the treating physicians. Treating physicians were not informed about the treatment allocation and ABV measurement. Neither physicians nor nurses were provided information about the subsequent adjustment of target weight based on these measurements. Patients were seen by multiple nurses in the course of a 4-week period, which further obscured the reason for changes in target weight. The researchers were not involved in patient care.

Finally, we feel that patients are unlikely to either under- or overreport signs and symptoms of hypotension on the basis of perceived treatment allocation, since they are well aware of these signs and symptoms and their adverse consequences.

As a second limitation ABV was not calculated at the time of IHAAE. Since the intravascular volume is determined by multiple factors as already stated previously, a post-dialysis Vs of ≥65 ml/kg does not exclude the possibility thatVs declined below this threshold causing IHAAE in the course of a dialysis treatment. However, in a recent study with ABV monitoring every 30 minutes during haemodialysis treatment, the lowest blood volume was usually found at the end of dialysis treatment rather than during an episode of hypotension [15]. Automation of this method using the dialysis machine to enable continuous ABV monitoring might be a worthwhile technological development to influence the incidence of IHAAE. A recent uncontrolled study by the authors that pioneered the ABV method demonstrated the feasibility of this method. IHAAE was not formally assessed in that study [16].

We failed to recruit the required number of patients due to the COVID-19 pandemic. Additionally, mean baseline post-dialysis Vs in our study population based on clinical judgment was already quite close to the proposed threshold of 65 ml/kg, which may have limited the ability to detect the full potential of the intervention. Finally, a once-time increase in total body water of 500 ml resulted in exceeding the threshold of 65 ml/kg in only six out of 18 patients requiring adjustments of target weight. Nevertheless, despite these limitations the intervention still demonstrated a significant effect.

We used a conservative strategy of only increasing target weight once by 0.5 kg to limit the risk of inducing hypervolemia. This strategy achieved the goal of a Vs ≥65 ml/kg in only six out of 18 patients that required adjustment based on the ABV measurement, which resulted in a non-significant change in target weight in the intervention group in a secondary unplanned analysis.

Repeated increments of target weight to achieve a terminal blood volume ≥ 65 ml/kg in all participants would probably have resulted in a significant change in post-dialysis weight. Employing such a strategy in a future trial would warrant measures to avoid hypervolemia. Although it is reasonable to assume that IHAAE is more prevalent in patients with hypovolemia, IHAAE does not preclude euvoolemia, or
even hypervolemia, since its occurrence depends on the interplay of multiple factors as previously stated. In theory the significant reduction in IHAAE achieved by aiming for a post-dialysis blood volume above 65 ml/kg could potentially be due to the induction of hypervolemia. Hypervolemia, which is highly prevalent in HD patients, is associated with adverse cardiovascular outcomes and should be avoided at all cost [17]. Fluid-monitoring using bioimpedance spectroscopy (BIS) should be considered in future research to mitigate the risk of volume overload. We did not employ BIS to monitor patients in this trial. We can therefore not comment on the performance of the ABV method relative to the performance of BIS in the prevention of IHAAE. BIS guided treatment has demonstrated a positive impact on intradialytic hypotension in a few relatively small studies [18-21]. However, BIS, which can provide an estimate of the volume of intracellular and extracellular water, is unable to measure the intravascular blood volume. BIS can therefore not be used to guide a treatment that aims to keep the intravascular volume above a certain threshold. As already stated combining the ABV target of 65 ml/kg to prevent hypovolemia and BIS to prevent hypervolemia might be a more optimal strategy.

It is concluded that ABV-guided adjustment of target weight, in a dialysis population with an incidence of IHAAE that is comparable to the incidence in the literature, led to a significant reduction of IHAAE in comparison to usual care. Despite the inability to confirm the proposed threshold of 65 ml/kg as a diagnostic tool for predicting the absence of IHAAE, the effectiveness of a therapeutic intervention targeting this threshold was successfully demonstrated. A future larger study using repeated increments of target weight to achieve a terminal blood volume ≥ 65 ml/kg in all participants (ideally employing real-time ABV estimates) and BIS to guard against hypervolemia should also address the issues of reproducibility of ABV measurements, the validity of the 65 ml/kg threshold and the difficult issue of blinding. If confirmed in such a trial this intervention would have the potential to change dialysis practice and impact patient care in a clinically meaningful way.

CONFLICT OF INTEREST STATEMENT

None declared.

AUTHORS’ CONTRIBUTIONS

M.J. and L.V. were responsible for the study conception, design, conduct, data interpretation, drafting and critical review of manuscript. F.D. contributed to the study design. A.G. and N.G. contributed to the data analysis. All authors read and approved the final manuscript.
This study was an investigator-initiated study, without any external funding.

**DATA AVAILABILITY STATEMENT**

The data underlying this article will be shared on reasonable request to the corresponding author.

**REFERENCES**


**Table 1: Demographic and clinical characteristics of patients at baseline.**

<table>
<thead>
<tr>
<th></th>
<th>ABV-guided treatment (n = 29)</th>
<th>Standard care (n = 27)</th>
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<tr>
<td>Age, years</td>
<td>67.9 ± 11.5</td>
<td>67.5 ±12.1</td>
</tr>
<tr>
<td>Male sex, no (%)</td>
<td>20 (69%)</td>
<td>14 (63%)</td>
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<tr>
<td>Cause of ESKD, no (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerulonephropathy</td>
<td>3 (10%)</td>
<td>3 (11%)</td>
</tr>
<tr>
<td>Tubulointerstitial nephritis</td>
<td>2 (7%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>ADPKD</td>
<td>3 (10%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Renal vascular disease due to hypertension</td>
<td>4 (14%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Renal vascular disease due to other cause or unspecified</td>
<td>4 (14%)</td>
<td>5 (19%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>5 (17%)</td>
<td>7 (26%)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>3 (10%)</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (20%)</td>
<td>7 (26%)</td>
</tr>
<tr>
<td>Comorbidities, no (%)</td>
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<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>12 (41%)</td>
<td>12 (44%)</td>
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<tr>
<td>Clinically evident heart failure</td>
<td>3 (10%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>16 (55%)</td>
<td>14 (52%)</td>
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<tr>
<td>Use of antihypertensive drugs, no (%)</td>
<td>24 (83%)</td>
<td>22 (81%)</td>
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<tr>
<td>Alpha blocker</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
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<tr>
<td>Beta blocker</td>
<td>17 (60%)</td>
<td>19 (70%)</td>
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<tr>
<td>Calcium channel antagonist</td>
<td>7 (24%)</td>
<td>0 (0%)</td>
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<td>ACE inhibitor or ARB</td>
<td>9 (31%)</td>
<td>2 (7%)</td>
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<td>Loop diuretic</td>
<td>8 (27%)</td>
<td>11 (41%)</td>
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<tr>
<td>Aldosterone antagonist</td>
<td>1 (3%)</td>
<td>2 (7%)</td>
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<tr>
<td>Dialysis vintage, months</td>
<td>36.6 ± 28.0</td>
<td>44.1 ± 30.9</td>
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<tr>
<td>Haemodialysis duration, minutes</td>
<td>242.4 ± 16.7</td>
<td>239.5 ± 12.4</td>
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<td>Post-dialysis weight, kg</td>
<td>88.5 ± 26.0</td>
<td>79.3 ± 17.5</td>
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<td>Ultrafiltration, litre</td>
<td>2.4 ± 1.0</td>
<td>2.1 ± 0.7</td>
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<tr>
<td>Pre-dialysis systolic blood pressure, mmHg</td>
<td>138.2 ± 27.5</td>
<td>139.1 ±30.1</td>
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<tr>
<td>Pre-dialysis diastolic blood pressure, mmHg</td>
<td>62.7 ± 13.6</td>
<td>68.6 ± 15.4</td>
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<tr>
<td>Pre-dialysis ABV, litre (n = 54)</td>
<td>6.2 ± 1.6</td>
<td>5.5 ± 1.1</td>
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Table 2: Change from baseline in incidence of IHAAE

<table>
<thead>
<tr>
<th>Change from baseline</th>
<th>Intervention group (N = 29)</th>
<th>Control group (N = 27)</th>
<th>Adjusted mean difference*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>to week 7-10 (mean, 95% CI)</td>
<td>-9.6% (-17.3; -1.8%)</td>
<td>+2.4% (-2.3; +7.2%)</td>
<td>10.5% (1.3; 19.8%)</td>
<td>0.026</td>
</tr>
<tr>
<td>to week 9-10 (mean, 95% CI)</td>
<td>-12.7% (-21.5; -3.8%)</td>
<td>+4.9% (-2.8; +11.3%)</td>
<td>16.3% (5.1; 27.6%)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Adjusted for baseline incidence, the use of antihypertensive drugs, systolic and diastolic blood pressure.

Table 3: Incidence of IHAAE

<table>
<thead>
<tr>
<th>IHAAE incidence 1-4 (mean, SD)</th>
<th>Total study population</th>
<th>Intervention group (N = 29)</th>
<th>Control group (N = 27)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHAEE incidence week 1-4</td>
<td>26.0% ± 25.1</td>
<td>28.9% ± 23.4</td>
<td>23.0% ± 26.8</td>
<td>0.39</td>
</tr>
<tr>
<td>IHAEE incidence week 7-10</td>
<td>23.4% ± 24.0</td>
<td>21.5% ± 22.5</td>
<td>25.4% ± 25.8</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Table 4: Frequency and relation of IHAAE at least once during baseline period

<table>
<thead>
<tr>
<th>Dizziness</th>
<th>Light-headedness</th>
<th>Sweating</th>
<th>Nausea or vomiting</th>
<th>Cramps</th>
<th>Visual disturbances</th>
<th>Altered motor, verbal or cognitive functions</th>
<th>Loss of consciousness</th>
<th>Systolic blood pressure &lt;90 mmHg</th>
<th>Drop in systolic blood pressure &gt;20 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dizziness</td>
<td>25</td>
<td>24</td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>16</td>
<td>8</td>
</tr>
</tbody>
</table>
Light-headedness | 24 | 35 | 0 | 8 | 16 | 7 | 6 | 1 | 20 | 10
Sweating | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0
Nausea or vomiting | 6 | 8 | 0 | 9 | 4 | 3 | 1 | 0 | 5 | 2
Cramps | 9 | 16 | 0 | 4 | 23 | 2 | 3 | 0 | 10 | 2
Visual disturbances | 5 | 7 | 0 | 3 | 2 | 7 | 3 | 0 | 7 | 4
Altered motor, verbal or cognitive functions | 5 | 6 | 0 | 1 | 3 | 3 | 9 | 0 | 6 | 1
Loss of consciousness | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0
Systolic blood pressure <90 mmHg | 16 | 20 | 0 | 5 | 10 | 7 | 6 | 0 | 26 | 9
Drop in systolic blood pressure ≥20 mmHg | 8 | 10 | 0 | 2 | 5 | 4 | 1 | 0 | 9 | 12

Shaded cells show the number of patients that experienced a single symptom at least once during the 4-week baseline period. The frequency of concomitant symptoms is shown in the same column and row. For example: 25 patients developed dizziness at least once during the baseline period, while 16 of them experienced both dizziness and hypotension.

Table 5: Measurement of absolute and normalised blood volume using different methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Predialysis ABV (l)</th>
<th>Postdialysis ABV (l)</th>
<th>Postdialysis Vs (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First ABV measurement</td>
<td>5.8 ± 1.6</td>
<td>5.2 ± 1.5</td>
<td>63.8 ± 16.8</td>
</tr>
<tr>
<td>Second ABV measurement</td>
<td>5.9 ± 1.5</td>
<td>5.3 ± 1.3</td>
<td>65.2 ± 16.1</td>
</tr>
<tr>
<td>Mean of two consecutive ABV measurements</td>
<td>5.9 ± 1.4</td>
<td>5.3 ± 1.3</td>
<td>64.6 ± 15.3</td>
</tr>
<tr>
<td>Nadler's formula</td>
<td>5.0 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isotope dilution technique</td>
<td>4.8 ± 0.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Differences tested with paired samples T test: First versus second ABV measurement (p = 0.518). Nadler’s formula versus post-dialysis ABV (p = 0.085). Isotope dilution versus pre-dialysis ABV (p = 0.002).

Table 6: Contingency table with a cut-off of 65 ml/kg for Vs and incidence of IHAAE above or below the median incidence of IHAAE in the total study population.

<table>
<thead>
<tr>
<th>IHAAE incidence</th>
<th>Condition positive: frequency of IHAAE</th>
<th>Condition negative: frequency of IHAAE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vs of 65 ml/kg</td>
<td>Condition positive: frequency of IHAAE</td>
<td>Condition negative: frequency of IHAAE</td>
<td>Total</td>
</tr>
<tr>
<td>Test positive (Vs &lt; 65 ml/kg)</td>
<td>20</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>Test negative (Vs ≥ 65 ml/kg)</td>
<td>7</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>28</td>
<td>55</td>
</tr>
</tbody>
</table>
Sensitivity 74%, Specificity 50%, Positive predictive value 63%, Negative predictive value 61%.

*Median incidence: 17.4% [IQR 8.3 – 41.6%].

**Figure 1:** schematic representation of the trial procedure.

**Figure 2:** CONSORT 2010 Flow Diagram
Figure 3: Bland-Altman plot of (A) two subsequent ABV measurements, (B) anthropomorphometric versus ABV method, (C) Isotope dilution versus ABV method.

A Bias: -1.13ml/kg, 95% CI -4.61; 2.35
LLoA -26.15, ULoA 23.89

B Bias: -0.29 litres, 95% CI -0.63; 0.04
LLoA -2.56, ULoA 1.97

C Bias: -1.17 litre, 95% CI -1.71; -0.63
LLoA -2.31, ULoA -0.05

Bias is the mean difference between the two sets of measurements. This provides an estimate of the systematic error. Lower and upper limits of agreement (LLoA and ULoA) represent the range within 95% of differences between the two methods are expected to fall.

Interpretation: Plot A and B indicate that mean difference between two measurements were not significantly different from zero, without an indication for varying agreement across the range of measurements (heteroscedasticity). Plot C reveals the presence of significant bias; all isotope dilution measurements were lower than ABV measurement.
Figure 4: Receiver Operating Characteristic curve of ABV measurement to predict the incidence of IHAAE above the median value of 17.4%

The optimal threshold, with the highest sum of sensitivity and specificity, was found to be at 67.5 ml/kg, with a sensitivity of 50% and a specificity of 85.2%. Area under the curve: 0.624 (0.473 – 0.776)