A novel simple hemoglobin dilution technique to measure hemodialysis vascular access flow

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Measurement of the vascular access flow rate (Q_a) is a widely accepted method for surveillance and predicting access failure. Among current practical methods, the ultrasound dilution technique is standard, but this requires a costly device available in few hemodialysis (HD) centers. Here, we devised a simple hemoglobin dilution technique to accurately measure Q_a without the need for any special machines. Before HD, values of Q_a were determined in each of 30 patients by hemoglobin dilution and then, in the same session, by ultrasound dilution. There was a significant correlation between the two techniques using automated hemoglobin and hematocrit or centrifuge-measured hematocrit levels to calculate HD fluid-derived Q_a values. Our study shows that the HD dilution technique, using no special device, is economical, highly accurate, and easy to perform, and can be used as an alternative to standard ultrasound dilution for vascular access surveillance.

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KEYWORDS: hemodialysis; vascular access flow rate; hemoglobin dilution technique; ultrasound dilution technique

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A well-functioning vascular access is crucial in achieving adequacy of hemodialysis (HD).[1] Failure to detect access dysfunction increases consequences on morbidity and mortality. Regular measurement of the vascular access flow rate (Q_a) is the most widely accepted method for surveillance and predicting access failure.[2] At present, various indicator dilution techniques are widely used in determining Q_a during HD.[2–7] The most extensive validation as well as the most precise standardization is ultrasound dilution technique (UDT).[6–10] The indicator used in the UDT is normal saline, whereas the detector is an ultrasound dilution sensor (Figure 1a). Thus, the UDT requires a specific instrument that is available in limited HD centers.

To overcome the limitations of UDT, a novel simple method for measuring the Q_a is created, and this is based on a red blood cell (RBC) concentration dilution principle. In this novel hemoglobin (Hb) dilution technique (HDT), only the standard HD machine and routine HD materials are required. To determine the Q_a by the HDT, the HD circuit had also been reversed (Figures 1b and 2) as in the UDT. In the HDT, only two samples of either Hb or Hct at the baseline before beginning HD session and 12 seconds after starting blood pump for infusion of the priming saline in the reverse manner are used for calculating the Q_a.

This study was conducted to analyze the correlation between the values of Q_a measured by the HDT, using automated Hb (Hb_a), automated Hct (Hct_a), or manual centrifuged Hct (Hct_m), and those determined by the standard UDT in the same HD session.

RESULTS

Demographic and baseline laboratory data

Of the 30 participating patients, there were 12 male and 18 female subjects who underwent thrice-a-week high flux HD for 4.2 ± 3.1 years. All patients were nonsmokers and had no significant residual renal function with daily urine output below 100 ml day^{-1}. The mean values of the patients' age and dry weights were 58.7 ± 14.4 years and 55.4 ± 11.9 kg, respectively. The types of vascular access were native arteriovenous fistula (AVF) and loop graft (AVG) in 20 (all at forearm) and 10 (all at forearm) participating patients, respectively (Table 1).

The mean arterial blood pressure values before measuring Q_a were not different between the HDT and the UDT (100.2 ± 8.6 vs 97.1 ± 10.5 mm Hg, NS).

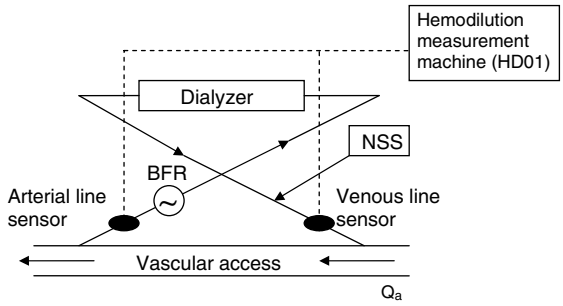

Figure 1 | HD circuit during vascular access flow rate measurement by the transonic HD01 machine. The HD circuit was reversed and arterial/venous line sensors were placed as picture.

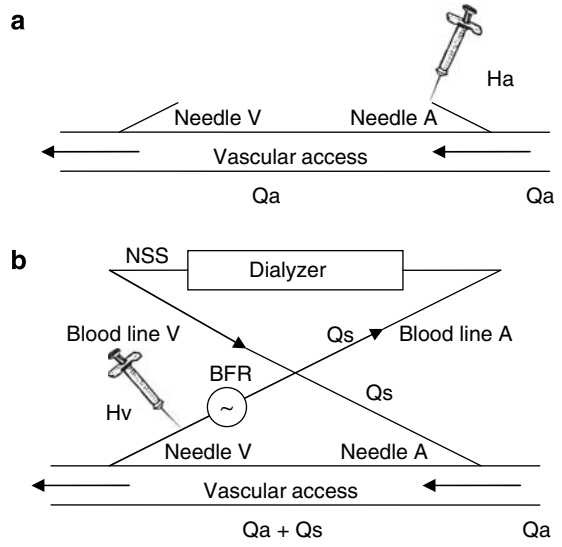

Figure 2 | Step by step during vascular access flow rate measurement by the novel HDT. (**a**) The first blood sample was withdrawn from the arterial AVF needle (needle A) for Ha determination. (**b**) Connection between needles A and V and blood lines A and V was set in a reversed position. Then, the blood pump was started at 300 ml min^{-1} (BFR). The second blood sample was taken from the sample port of the arterial blood line (blood line A) for Hv determination. H_a = hemoglobin or hematocrit in vascular access, before saline dilution; H_v = hemoglobin or hematocrit in vascular access, after saline dilution; Q_a = vascular access flow rate, Q_s = saline infusion flow or adjusted pump flow (ml min^{-1}) = [1−(BFR−200)/2000] × BFR; BFR = blood pump setting flow rate (ml min^{-1}), NSS = normal saline preprimed in the HD circuit at the initial setup.

Vascular access flow rate

The values of standard UDT-derived Q_a were derived from the average of the duplicated measurement, which demonstrated a good repeatability (coefficient of variation = 6.4%). To test the repeatability of the HDT-derived Q_a values, the second measurement using the remaining priming saline in the blood line was consequently repeated at 1 min after the first one in the same session. The coefficient of variation of these repeated measurements were 8.3, 6.9, and 9.7% for the HDT-derived Q_a values using Hb_a, Hct_a, and Hct_m, respectively, all of which represented the good repeatability.

The values of standard UDT-derived Q_a were 886.50 ± 439.17 ml min^{-1} and were utilized as the reference values. The values of Q_a determined by the HDT, calculated by using the RBC concentration (Hb_a, Hct_a, and Hct_m), are demonstrated in Table 2. In all 30 patients, there were no significant differences in the mean values of the standard UDT-derived Q_a and HDT-derived Q_a using any RBC concentrations, Hb_a (924.06 ± 415.31 ml min^{-1}, NS), Hct_a (983.51 ± 512.68 ml min^{-1}, NS), or Hct_m (954.86 ± 431.81 ml min^{-1}, NS). Importantly, they were highly significantly correlated with the values of UDT (all, $r > 0.90$, $P < 0.01$) (Table 2). The HDT determined by Hb_a had the lowest mean absolute difference (37.56 ± 147.25 ml min^{-1}) and the highest significant correlation ($r = 0.94$, $P < 0.01$). This trend was also shown in both subgroups of AVF ($r = 0.93$, 0.92, and 0.89 for HDT-derived Q_a using Hb_a, Hct_a, and Hct_m, respectively) and AVG patients ($r = 0.98$, 0.95, and 0.90 for HDT-derived Q_a using Hb_a, Hct_a, and Hct_m, respectively). Moreover, the better correlations were shown in the AVG than the AVF subgroups. By regression analysis, Figure 3 shows the correlation between the values of Q_a from the HD01 UDT and all three HDT methods. The regression equation of the HDT using Hb_a, which provides the highest correlation was $UDT-Q_a = [1.00 \times HDT(Hb_a) - Q_a] - 34.10$. A Bland–Altman plot comparing the HDT using Hb_a and UDT is displayed in Figure 4.

DISCUSSION

This study demonstrated the novel 'Hemoglobin Dilution Technique (HDT)' for measuring vascular access flow rate (Q_a). The HDT using any RBC concentration values for calculation could provide an excellent correlation and minimal mean absolute difference with the standard UDT method (Table 2). Among all HDT methods, the Hb_a-derived Q_a showed the best correlation and the lowest mean absolute difference when compared with the standard UDT (Table 2, Figure 4). This might be explained by the high accuracy of

Table 1 | Demographic data

	Total (30 patients)		AVF (20 patients)		AVG (10 patients)	
	Range	Mean ± s.d.	Range	Mean ± s.d.	Range	Mean ± s.d.
Age (years)	34–93	58.7 ± 14.4	34–81	55.6 ± 12.5	38–93	64.8 ± 16.8
Duration of hemodialysis (years)	0.3–11.0	4.2 ± 3.1	0.6–9.4	4.9 ± 4.5	0.3–11.0	2.9 ± 3.3
Dry weight (kg)	38–81	55.4 ± 11.9	38–81	57.9 ± 12.2	38–70	50.5 ± 10.1

Table 2 | Vascular access flow rates determined by the UDT and the HDT

Method	Mean ± s.d. (ml min^{-1})	*r*	Mean absolute difference ± s.d. (ml min^{-1})
UDT	886.50 ± 439.17		
HDT			
Using Hb_a	924.06 ± 415.31[a]	0.94[b]	37.56 ± 147.25
Using Hct_a	983.51 ± 512.68[a]	0.93[b]	97.01 ± 191.42
Using Hct_m	954.86 ± 431.81[a]	0.91[b]	68.36 ± 190.45

AVF, arteriovenous fistula; AVG, arteriovenous graft; Hb, hemoglobin; Hct, hematocrit; *r*, correlation from linear regression analysis.
[a]Not significantly different ($P > 0.05$) with UDT values from paired Student's *t*-test.
[b]Significant correlation ($P < 0.01$) with UDT values from linear regression analysis.

directed determination of RBC concentration by Hb_a than both Hct_a, which was derived from the calculation, and Hct_m. However, the Hct_m-derived Q_a, which also provided a good correlation, could be a reliable alternative to Hb_a-derived Q_a in the situation that Hct_m is more practical to determine than the automated values.

The HDT is a dilution method based on saline infusion into vascular access via the arterial needle to create hemodilution and determination of two Hb (or Hct) values, the basal one before saline infusion and the other during saline infusion. Using isotonic saline injection to cause hemodilution is advantageous in that it is safe, available, and easy to mix with blood. The priming saline in HDT also offers several additional benefits, and these included the following: the exact saline flow rate can be known without any blood mixing; there is no need for additional infusion materials; and there is no change in blood volume because of simultaneous arterial and venous blood line connection. This HDT is simple to set up and can be used in all HD centers. Both are superior advantages when compared to other previously reported hemodilution techniques, all of which required special sensor to detect the changes in hematocrit.[5]

In this study, all pitfalls of indicator dilution method were considerably prevented. Ultrafiltration was kept off during the measurement by both HDT and UDT to ensure that the blood flow is identical in the circuit. The duration of saline infusion in HDT was set at 12 s to avoid the presence of cardiopulmonary recirculation and to allow the complete mixing with blood. The insertion of the venous or return needle in the direction opposite to access flow, placing the two needles at least 3 cm apart and setting blood pump setting flow rate (BFR) at 300 ml min^{-1}, also facilitated mixing, as Q_a is influenced by blood pressure, which usually falls during HD. This error was minimized by performing this HDT before the start of dialysis session, and there was no change in blood volume due to simultaneous arterial and venous blood line connection. Also, the standard UDT was conducted as early as possible, during the first 60 min of dialysis session.

The countercurrent peristaltic flow, created by the blood pump in the reversal blood line position, may potentially decrease the Q_a in both methods. Indeed, the rapid injection

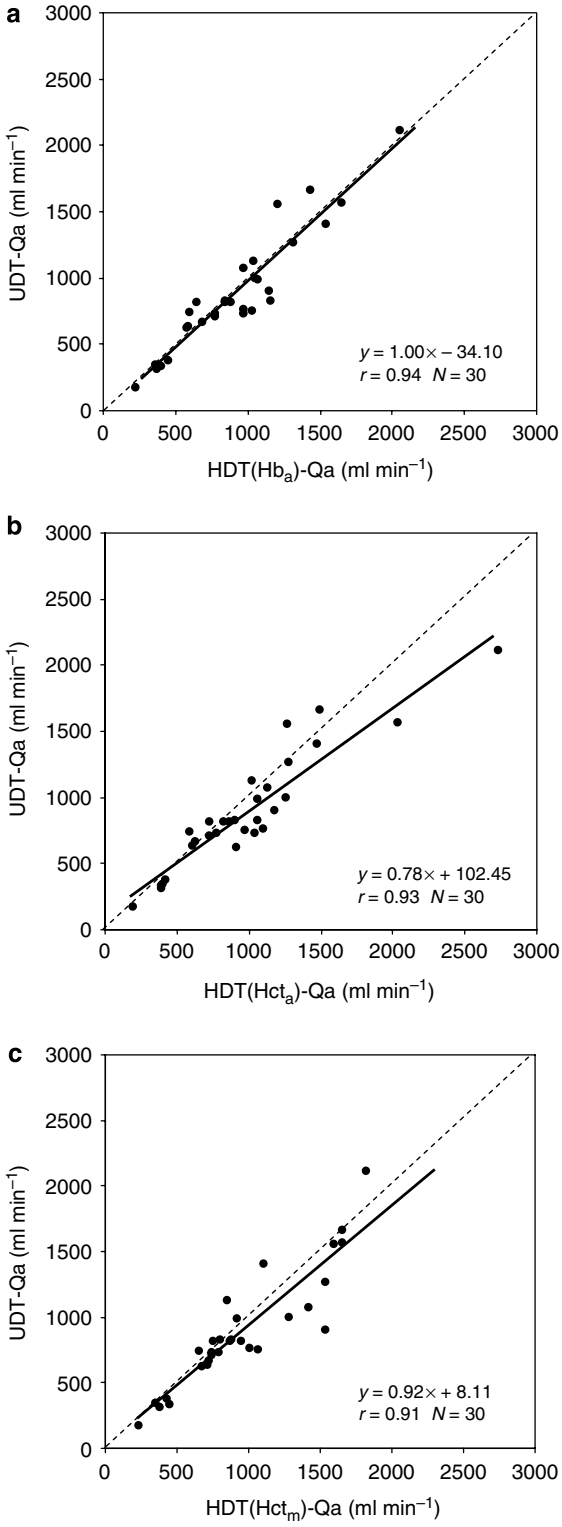

Figure 3 | Correlation between the transonic HD01 UDT and the HDT. (**a**) Automated Hb, (**b**) automated Hct, and (**c**) manual centrifuged Hct.

of saline into the vascular access in UDT may cause substantial increase in the saline injection flow that could reduce the Q_a more than saline infusion in HDT. This may partially explain the higher mean values of Q_a in HDT

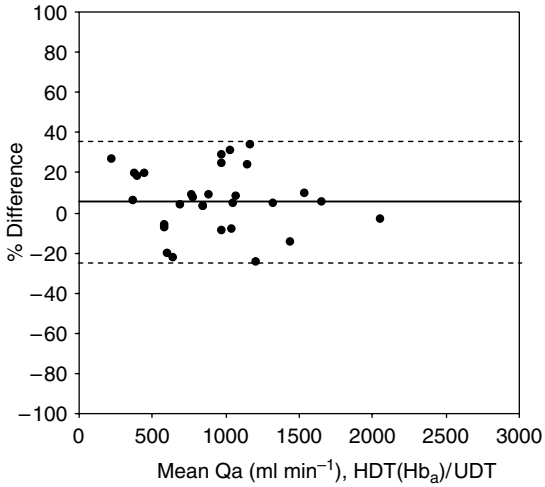

Figure 4 | Bland–Altman HDT (Hb_a)/UDT. Q_a = vascular access flow rate; HDT (Hb_a) = hemoglobin dilution technique using automated hemoglobin; UDT = transonic HD01 UDT.

(Table 2). Alternatively, the earlier measurement by HDT might possibly be less affected by the decrease in blood pressure when compared with UDT.

There are several other practical methods to determine the Q_a, including the thermodilution[11] and conductivity/ionic dialysance method;[12,13] however, both are time consuming and require special function in the dialysis machine. Recently, the temperature gradient method[14] was introduced. This interesting method was not as much time consuming as the original thermodilution technique, but the HD machine with additional blood temperature monitoring configuration was required. The glucose pump infusion technique, which does not require any special devices, also needs the specific procedure setup.[15,16]

In conclusion, the HDT, using no special device, is highly accurate, easy to perform, and economical. This maneuver could be routinely used to measure and follow the vascular access flow in a surveillance protocol as a reliable alternative to the ultrasound dilution method.

MATERIALS AND METHODS

Patients

The HDT in measuring Q_a was conducted in 30 end-stage renal disease patients who, for at least 3 months, underwent thrice-a-week high flux HD, using Fresenius 4008 HD machine, blood pump flow rate of which was regularly calibrated. All patients were more than 18 years and had no severe cardiovascular diseases. The study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University Hospital, Bangkok, Thailand. Informed consent was obtained from each patient.

Technique

Ultrasound dilution technique. The gold standard values of the Q_a were determined by the standard UDT using HD01 device (Transonic System Inc., Ithaca, New York, USA) as previously described.[4,9] The values of Q_a were derived from the average of the duplicated measurement during the first 60 min of dialysis session according to the manufacturer's recommendation. As illustrated in Figure 1a, the direction of the blood flow in the HD circuit had to be reversed. The blood pump was rolled at 300 ml min^{-1} without ultrafiltration. The indicator, sterile isotonic saline, was injected into the venous bubble trap before ultrasound dilution sensor, mixed with the blood flow in the extracorporeal circuit (Qb), then passed to the arterial access, and was detected by arterial sensor.[10] These duplicated measurement was also analyzed for the repeatability.

Novel HDT. To determine the Q_a by the HDT, only two samples of either Hb or Hct at the baseline before beginning of HD session and at 12 s after infusion of priming saline using the blood pump in the reverse manner (Figure 2b) were required. When the regular blood circulation circuit was filled with priming saline and the vascular access was cannulated with arterial (needle A) and venous (needle V) AV access needles, the first blood sample (H_a) was collected from needle A (Figure 2a) for determination of Hb or Hct. Then, the needles A and V were connected in a reversed position with the venous blood line (blood line V) and the arterial blood line (blood line A), respectively (Figure 2b). The BFR was set up at 300 ml min^{-1} and ultrafiltration rate was set up at zero. Twelve seconds after the blood pump was started and the priming saline in the blood line was constantly infused into the upstream of the AV access, the second downstream blood sample (H_v) was taken from the sampling port of the arterial blood line (Figure 2b) and was also determined for the values of Hb or Hct. Indeed, the actually effective Q_s was not the same as the set-up BFR value of 300 ml min^{-1} because blood lines reduce the stroke volume from prepump negative pressure.[17] The values could be obtained from the formula.[17]

$$Q_s = [1 - (\mathrm{BFR} - 200)/2000] \times \mathrm{BFR}$$

To test the repeatability of this HDT, the repeated measurement was determined consequently after the first one. After the second blood sample was drawn in the first Q_a measurement, the blood pump was stopped for 30 s to allow the saline to completely mix with the blood in the body. Then, the second Q_a measurement was performed in the same manner as the first one and was started by collecting the first blood sample (H_a) from needle A, which was temporally disconnected from the blood line. The second blood sample was taken from the sampling port of the arterial blood line after 12 s of infusion of the remaining priming saline left over in the blood line at the 300 ml min^{-1} setting rate.

At the end of the test procedures, the blood lines A and B were reconnected in the regular nonreversed position.

By the basis of RBC balance,[16] thus

$$\begin{aligned} Q_a \cdot H_a &= (Q_a + Q_s) \cdot H_v \\ &= Q_a \cdot H_v + Q_s \cdot H_v \\ Q_a \cdot (H_a - H_v) &= Q_s \cdot H_v \\ Q_a &= \frac{Q_s \cdot H_v}{H_a - H_v} \end{aligned}$$

Where, Q_a = vascular access blood flow (ml/ min)

Q_s = effective saline flow rate (ml/ min)

$= [1 - (\mathrm{BFR} - 200)/2000] \times \mathrm{BFR}$

BFR = blood pump flow rate (ml/ min)

H_a = Hb or Hct in the access flow before saline infusion

H_v = Hb or Hct in the access flow twelve seconds after starting saline infusion

This study was conducted to analyze the correlation between the values of Q_a measured by this novel HDT, using Hb_a, Hct_a, or Hct_m, and those determined by the standard UDT in the same HD session.

The values of Hb_a and Hct_a were measured by the automated complete blood count analysis in the hospital's central laboratory using automated Bayer Advia 120 hematology analyzer (Bayer Diagnostics, Tarrytown, NY, USA). The Hct_m levels were determined by manual centrifugation technique at 10 000 r.p.m. for 5 min. The processes took about 30 min after the sample collection for automated method and 10 min for manual centrifugation method to receive the results for HDT-derived Q_a calculation.

Study design

The values of Q_a in each participating patient were determined before the start of HD session by the novel HDT and then during the first 60 min of the same dialysis session, by the standard UDT.

Statistical analysis

All data were expressed as mean ± s.d. The data from UDT and HDT were compared by the paired Student's *t*-test, whereas the relationship between them was obtained by Pearson correlation and linear regression analysis. The Bland–Altman plot was used to visually assess agreement between the different methods. The repeatability of duplicated measurements is shown as the coefficient of variation for UDT and HDT. The statistical significance was defined when $P<0.05$.

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