Glucose infusion test (GIT) compared with the saline dilution technology in recirculation measurements

Alberto Magnasco1 and Sandro Alloatti2

1Renal Unit G. Gaslini Institute, Largo Gaslini 4, 16148 Genoa, Italy and 2Renal Unit, Aosta

Abstract

Background. Glucose infusion test (GIT) is a new method to measure vascular access recirculation (R) based on basal glucose increase in the arterial blood line after a 20% glucose bolus (5 ml) into the venous chamber. Methods. We compared GIT with the ultrasound dilution method (HD01, Transonic Systems Inc., USA) in a circuit reproducing in vitro the phenomenon of R. We repeated the comparison in 162 chronic haemodialysis patients (133 fistulae, 17 central venous catheters, 12 prosthetic grafts).

Results. In vitro, we determined the timing for C2 sampling: QB 200 ml/min, C2 16–20 s; QB 300 ml/min, C2 13–17 s; QB 400 ml/min, C2 9–12 s. GIT showed no false positives nor false negatives (100% specificity and sensitivity) while HD01 did not recognize three cases with R = 5% (91% sensitivity) and it yielded no false positive (100% specificity). The Bland–Altman analysis showed a bias of 0.2 ± 1.3% and 1.3 ± 2.9% for GIT and HD01, respectively. In vivo, only 16 out of 162 patients were found positive with both methods (GIT 13.5 ± 13%; HD01 16.3 ± 15%; P = NS) while three patients with minimal R (GIT 3.2%) were not recognized by HD01 although a low R peak was clearly evident and repeatable on the laptop plot. The Bland–Altman analysis showed an overall bias of 0.2 ± 1.7% to the limits of agreement = −3.1 and 3.6% (n = 162) and no correlation between the difference and the mean of positive tests. The pooled coefficient of variation of positive cases was 13.3 and 18.1% for GIT and HD01, respectively.

Discussion. Our in vitro study showed a good performance of GIT and its better sensitivity compared to HD01. These results were confirmed in vivo with only 3/162 discordant results due to a low R under the HD01 limit of detection (R = 5%). In conclusion, the GIT proved to be a very accurate screening test for R, with a very low threshold of detection. In addition, it is simple, user-friendly and inexpensive.

Keywords: dialysis; dialysis adequacy; dialysis efficiency; recirculation; vascular access

Introduction

Vascular access recirculation (R) contributes significantly to reduce dialysis efficacy and can increase the relative risk of mortality of chronic dialysis patients [1]. R can also indicate impaired blood flow in vascular access at the risk of thrombosis [2]. R measurement is a simple and useful step in the dialysis surveillance programme [3]. It is simple because it is a fast and easy bedside measurement. It is crucial because it allows an immediate and accurate evaluation of the efficiency of dialysis treatment and indirectly of the vascular access. The urea recirculation test (URT) [4] is the oldest and most common method because it does not require dedicated devices. However, it has some limitations such as low specificity and sensitivity, and it gives delayed results. Another method proposed as gold standard is based on the dilution of blood with a saline bolus (ultrasound saline dilution technology, HD01 Transonic Inc., Itacha, USA) [5]. It has the advantages of giving immediate results and of measuring blood flow but it requires a specific and expensive device.

Recently, we proposed a novel method based on a glucose bolus as a tracer for R, the glucose infusion test (GIT) [6], which is simple, inexpensive and accurate. Several studies on a large dialysis population showed the clinical reliability of GIT in comparison with the classic stop-flow URT [6,7]. The GIT protocol [6] required a fixed QB at 300 ml/min. This was an important limitation of the method because it did not make it possible to verify the efficacy of scheduled dialysis with blood flows other than 300 ml/min. The aim of this study is to broaden the usability of GIT by finding different sample timing for different QB and to complete its validation comparing it to the ultrasound technique both in vitro and in vivo.

Methods

The GIT protocol [6] briefly consists of the injection of 5 ml of 20% glucose bolus (or 2 ml of 50% glucose + 3 ml saline or...
3 ml of 33% glucose + 2 ml saline into the venous chamber and of two blood samplings from the arterial blood line, the basal one (C1) before the test and the second are (C2) taken slowly from 13 to 17 s after the start of the bolus (with the blood pump at QB 300 ml/min). We measured blood glucose expressed as mg/dl (C1 and C2) at the bedside using a glucometer (Accu-Chek Active, Roche Diagnostics GmbH, Mannheim, Germany), then we calculated R as follows:

\[ R\% = \left( \frac{C2 - C1}{C0} \right) \times 100 \] (1)

This equation derives from previous correlations between the glucose increase and the R values showing that \( R\% = [(C2 - C1) 0.05\%], [6,7] \).

From Equation (1), it follows that if there is no glucose increase (C2 = C1), R will be 0.

### In vitro circuit

**In vitro**, we used an appropriate circuit filled with outdated human blood (haematocrit 37 ± 3%) reproducing the phenomenon of R (Figure 1). A short bypass (priming volume of 0.5 ml) with an additional peristaltic pump (QB) (BL 705; Belloco, Mirandola, Italy) created R between the venous and the arterial blood lines of a standard dialysis set according to the following ratio:

\[ R = \left( \frac{Q_R \times 100}{Q_B} \right) \] (2)

We set the QB in a stepwise manner at 200, 300, 400 ml/min with a negative arterial pressure at -100, -150, -200 mm Hg, respectively. By varying QB from 0 to 80 ml/min we obtained an R range from 0 to 20%. We repeated the sequence GIT–HD01 five times for every R (at 20% R, we repeated it ten times). We used the Transonic’s dilution curves to study the kinetic of the glucose bolus in the dialysis circuit. In addition, we did several C2 samples at different intervals to identify the exact timing for C2.

Finally, we repeated some Transonic tests by circulating 4.5% hypertonic saline instead of blood according to the indications of Krivitski [5].

### In vivo validation

This study completes the validation of GIT vs ultrasound saline dilution method in 162 chronic haemodialysis patients (133 fistulae, 17 central venous catheters, 12 prosthetic grafts). After obtaining informed consent, we performed GIT and HD01 tests twice and in sequence in each patient (first HD01–first GIT; second HD01–second GIT).

### Statistical analysis

Data were reported as mean ± SD. The two data groups were compared by the paired Student’s t-test, significance was set at \( P < 0.05 \). The relationship between groups was analysed by plotting differences against means (Bland–Altman test) [8] and calculating the bias (mean difference) and the limits of agreement (=bias ± 2SD). The relationships between glucose increase (C2 – C1) and **in vitro** recirculation and **in vivo** HD01 Transonic results were obtained by linear regression analysis.

The sensitivity was calculated from the ratio: 100× true-positive test results/all in vitro cases with R. The specificity was obtained from the ratio: 100× true-negative test results/all in vitro cases without R.

We tested the reproducibility of GIT and HD01 by repeating each test twice in all cases. The SD of paired GIT was determined and then we averaged SD\(_{GIT}\) by pooling the individual standard deviation (SD\(_i\)) of the N individual patients (\(N\)):

\[ \text{Pooled SD} = \sqrt{\frac{\sum (SD_i)^2}{N}} \] (3)

From the pooled SD\(_{GIT}\), we obtained the coefficient of variation (CV = pooled SD/mean) for GIT. Similar analysis was done for the saline dilution technology.

### Results

**In vitro**

The **in vitro** circuit and the application of the HD01 allowed us to verify the transit time of the glucose bolus. We confirmed our previous results [6], showing a glucose peak at the 10th second with a QB at 300 ml/min (5 ml/s) at the top of the venous needle; an intra-access delay for the R for about a couple of seconds and finally an arrival time to the arterial port depending on the position of it. The arterial port is usually between two extreme positions, close to the top of the arterial line (about 50 cm, priming volume of 7 ml) or close to the arterial blood pump (about 200 cm, priming volume of 25 ml) (personal observations). From these data, it is easy to obtain the correct timing for C2 samples with different QB or port positions (Table 1). It is worth nothing that the glucose peak at QB 400 ml/min is tighter compared to a lower pump rate, requiring a faster C2 sample time (only 3 s rather than 4 s).

**In vitro**, GIT confirmed previous data showing no false positives nor false negatives (specificity and sensitivity 100%) in all the 40 tests performed. The regression equation between glucose increase and artificial R was

\[ R = 0.048(C2 - C1) + 0.14 \] (4)

very similar to the previous equations derived both in vitro \([R = 0.046(C2 - C1) + 0.07]\) and in vivo with
the classic URT \( R_{URT} = 0.048(C_2 - C_1) + 1.3 \) [6]. The pooled coefficient of variation of repeated GIT was 9.5%.

The HD01 failed 7/40 tests (giving no result) and six of them had low \( R \) (<10%). There were no errors during the infusion and in three failed tests, an \( R \) curve was evident on the laptop plot but it was not detected by the software. In addition, the HD01 yielded three false negative results (no \( R \) for HD01 with an actual artificial \( R \) of 5%). Finally, HD01 showed no false-positive results (sensitivity 91%, specificity 100%, not considering the seven failed tests). The regression equation with the artificial \( R \) was \( R = 0.86 \text{HD01} + 2.7 \); the pooled coefficient of variation of repeated HD01 was 28.3%.

By circulating 4.5% hypertonic saline instead of blood, we obtained a better baseline and no failed tests with HD01, which yielded only one false-negative result at \( R = 5\% \). The coefficient of variation was 23% and it improved to 12% not considering the minimal \( R = 5\% \).

Figure 2 shows Bland–Altman analysis for both methods. The bias was 0.2 ± 1.3% and 1.3 ± 2.9% for GIT and HD01, respectively.

**Discussion**

In the literature, the role of \( R \) measurement in the surveillance of access dysfunction is still debated. The DOQI recommendations [3] state that \( R \) is a reliable

**Table 1.** Time for C2 sample

<table>
<thead>
<tr>
<th>( Q_b ) (ml/min)</th>
<th>C2 time (s)</th>
<th>Port 50 cm</th>
<th>Port 200 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>16–20</td>
<td>21–25</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>13–17</td>
<td>17–21</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>9–12</td>
<td>12–15</td>
<td></td>
</tr>
</tbody>
</table>

The timing of C2 withdrawal varies with the variation of the blood pump speed (\( Q_b \)) and with the position of the arterial port.

**Table 2.** GIT and HD01 recirculation results

<table>
<thead>
<tr>
<th></th>
<th>GIT%</th>
<th>HD01%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases without R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVF (n = 120)</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>CVC (n = 11)</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>Graft (n = 12)</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>Cases with R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVF (n = 13)</td>
<td>15.1</td>
<td>17.8*</td>
</tr>
<tr>
<td>CVC (n = 4)</td>
<td>5.4</td>
<td>7.4*</td>
</tr>
<tr>
<td>Discordant (n = 3)</td>
<td>3.2</td>
<td>0</td>
</tr>
<tr>
<td>All cases (n = 162)</td>
<td>1.4</td>
<td>1.6*</td>
</tr>
</tbody>
</table>

Comparison of GIT and HD01 results separated for a kind of vascular access: AVF, arteriovenous fistula; CVC, central venous catheter. *\( P = NS \).
but late indicator of a dysfunctional vascular access. Recently, Tessitore et al. [9] showed that R measurements with URT are good predictors of impending thrombosis in native fistulae with an area under the ROC curve of 0.9, similar to the area obtained with the Transonic HD01 of 0.86. Nevertheless, the usefulness of R is reduced in grafts where the underlying pathological process is faster than in native fistulae and R usually provides ‘late warning’ [3].

The first validation of the new glucose–based method was obtained both in vitro and in vivo in a small single–centre dialysis population [6], a fact that reduced the impact of its results to an almost anecdotal level. A subsequent multicentre validation study [7] confirmed the preliminary results in a large number of haemodialysis patients (n = 623). We used URT as a reference in that study because it is still a very good and universally available screening test for access recirculation and many dialysis units do not have access to the Transonic device. This latter study indicated that the GIT protocol was simple and easy to use. However, several criticisms remained. These included lack of head-to-head comparison with the ultrasound dilution technology and lack of evaluation of R at prescribed Qb lower or higher than 300 ml/min. The aim of the present study is to provide answers to these questions. We performed a direct comparison of GIT with HD01 first in vitro using an artificial recirculation circuit and subsequently in vivo in a large number of haemodialysis patients.

Using the artificial circuit we determined the correct timing for three Qb levels (at 200, 300, 400 ml/min, Table 1). We chose to use the set Qb (i.e. the blood flow set by the pump speed) rather than the effective one because not all dialysis machines provide data on the latter. This problem may be trivial since in usual conditions the difference between set and effective Qb is largely reproducible. In cases with very negative arterial pressure, the difference between the set blood flow rate and the effective blood flow rate becomes higher and very variable. In such instances, it is better to perform GIT at the lowest Qb level minimizing the negative arterial pressure. However, a very negative arterial pressure may suggest vascular access dysfunction requiring ultrasound Doppler assessment irrespective of the presence of R. In our in vitro experiments, the GIT showed a close correlation with the artificial recirculation without any false-positive, false-negative or failed test. As expected, the reproducibility of GIT was better in vitro than in vivo because the artificial circuit provided stable conditions (R, Qb, sample timing) ideal for GIT. On the contrary, the artificial model is not ideal for HD01 because of blood density variations due to glucose infusions and haemolysis. Nevertheless, by filling the circuit with hypertonic saline, we overcame some of these limitations with only slight improvement in HD01 performance. Finally, the good concordance between R results obtained in vitro with both methods confirms the reliability of our artificial R circuit.

The in vitro study showed a good concordance between the two tests, with an overall bias of 0.2% and only 3 out of 162 discordant results, all with a minimal R close to the HD01 limit of detection (3–5%) [10]. An R < 5% is of trivial importance in the clinical practice because of the minimal reduction of the delivered dialysis dose. However, the presence of even such a minimal R may be a warning sign of impending vascular access failure as the access may already have low blood flow or venous stenosis requiring further assessment with appropriate diagnostic tests (colour Doppler ultrasound or venography). The GIT detection limit depends on errors of the glucose measurements. The error of glucose measurements can be calculated from the mean values and SDs of repeated determinations, and C1 and C2 samples are significantly different when the 95% confidence intervals did not overlap. Similarly, considering the coefficient of variation of a glucometer device (we used the Accu-Check Active, Roche Diagnostics GmbH, Mannheim, Germany, with a CV of 2%), it is possible to estimate a theoretical 95% CI for glucose values ranging from 100 to 200 mg/dl, obtaining 4 mg/dl and 8 mg/dl, respectively. This means that a minimal R of 0.5% calculated from a C2 – C1 value of 10 mg/dl is significant and it is the theoretical detection limit of GIT. In this study, we had a mean (C2 – C1) = –0.4 ± 2.7 mg/dl in all cases without R; a mean (C2 – C1) = 252 ± 185 mg/dl in R cases and the minimal R of 1.25% with a (C2 – C1) value of 25 mg/dl, significantly above the 95% CI of 8 mg/dl. These data confirm the very low threshold of GIT. The identity between C2 and C1 glucose results in 146 no R patients shows the glucose stability during such a short lag of time (only 20 s) of GIT performance. In addition, this identity indirectly shows that C2 timing is unaffected by glucose return via the cardiopulmonary R. This crucial issue has been further assessed by the

Fig. 4. Conflicting Transonic results in one patient with minimal recirculation (GIT = 3.2%). Nessun ricircolo, no recirculation; Per conferma, ripeti; Qb, blood pump rate.
HD01 continuous measurements. In fact, the HD01 separates the vascular access R from the cardiopulmonary R. In this study, the HD01 showed no overlap between vascular access R from the cardiopulmonary R (on an average, the latter occurring 15–20 s after the tracer bolus) indirectly proving the safety of GIT timing.

GIT can be safely used in diabetic patients because the glucose bolus is quickly dialysed. In these patients, the only relative limitation is a basal glucose >300 mg/dl for the risk to have an over range C2 sample in case of minimal R. In 21 diabetic patients studied, we only had two cases with high basal glucose (>300 mg/dl). We did GIT after having obtained a better glucose control using small insulin doses.

Both tests are easy to do but GIT requires blood handling for glucose measurements, a precise protocol varying with blood pump rate and arterial port positions, whereas HD01 does not (Table 3). However, HD01 might not be appropriate during dialysis techniques like haemodiafiltration, haemofiltration or acetate-free biofiltration because the venous reinfusion influences the blood density disturbing the ultrasound probes reading. This interference may persist several minutes after the interruption of reinfusion due to the slow re-equilibrium across the dialyser requiring to postpone the test (personal observation). On the contrary, the fluid reinfusion into the bubble trap increases the glucose mixing by improving the reproducibility of the venous peak of GIT. Another important difference is the need of an expensive dedicated device for HD01, whereas GIT only requires an inexpensive glucometer.

This study has some limitations. First, we only did two intrasession tests without repeating them in a separate dialysis to complete the proper evaluation of the intersession variability. This reduces the power of comparison between the two tests but, at the same time, it reduced the patients’ bother. Second, we studied a small number of grafts in comparison with the native fistulae because this was the actual situation studied a small number of grafts in comparison with our dialysis facilities with only a minimal number of grafts. However, the native fistulae are the best model for R because they keep patency until very low blood flows allowing the start and increase of R. On the contrary, the grafts are unlikely to tolerate the blood flows allowing the start and increase of R (on the contrary, the grafts are unlikely to tolerate the blood flows allowing the start and increase of R). Third, we simply compared the two R tests without performing further investigations (ultrasound assessment or flebography) to calculate the sensitivity and specificity in vivo, and the accuracy of the R results in predicting vascular access failure. In conclusion, the dependence of GIT protocol on Qb levels and arterial port positions are the main pitfalls of the method. In addition, the C2 sample could partly fail to catch the flowing glucose peak, especially with high R and high Qb. Nevertheless, this study and the previous ones show that in the clinical practice, GIT has a good reproducibility and also accuracy in comparison with the Transonic HD01. The GITs main valuable point is its high sensitivity as a cheap screening test.

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References


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Table 3. GIT and HD01 comparison

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>GIT</th>
<th>HD01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection limit</td>
<td>0.5%</td>
<td>5%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>91%</td>
</tr>
<tr>
<td>Pooled CV</td>
<td>16%</td>
<td>18%</td>
</tr>
<tr>
<td>Immediacy</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ease of use</td>
<td>Good</td>
<td>Better</td>
</tr>
<tr>
<td>Test time (min)</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Blood handling</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Pump rate (ml/min)</td>
<td>200–300–400</td>
<td>All</td>
</tr>
<tr>
<td>Cost</td>
<td>Low</td>
<td>High</td>
</tr>
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</table>

CV, coefficient of variation.