Glomerular Filtration Rate Determined from a Single Plasma Sample after Intravenous Iohexol Injection: Is It Reliable?[1]

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ABSTRACT

The iohexol injection plasma clearance method is a good alternative to the inulin clearance method for determination of GFR, but requires multiple blood samples. To avoid this, methods have been developed which derive GFR from a formula that uses a single plasma concentration of the tracer and anthropometric data. The aim of this study was to evaluate whether a single plasma sample taken after iohexol injection allows reliable estimation of GFR. In this study, results of single-point determination were compared with those obtained by multiple-point plasma clearance. The GFR of 686 outpatients with different degrees of renal function were recalculated by use of the Jacobsson formula. The optimum time for sampling was found at 10 h after injection of the marker for clearances <40 mL/min per 1.73 m^2, 4 h for clearances between 40 and 99 mL/min per 1.73 m^2, and 3 h for clearances >100 mL/min per 1.73 m^2. Results documented that for 75% of the patients, the simplified technique gave an error between −5% to +5% in the evaluation of GFR; for the remaining 25% of the patients, prediction error ranged from −22% to +40%. Furthermore, despite a highly significant correlation between multiple-point iohexol clearance (six plasma samples) and the single-point method ($Y = 0.968X + 1.704$, $r^2 = 0.988$), the regression intercept was statistically different from 0 and the standard error of the slope estimate established that 95% confidence interval did not include 1.0 (the line of identity), thus indicating that the model can be rejected by the data at a significance level of 0.05. Thus the single-plasma-sample method to determine GFR after radiocontrast injection does not represent a real advantage over the multiple-point method and may lead to unacceptable errors in GFR calculation.

Key Words: *GFR, iohexol, single injection, single-point method*

Rigorous assessement of GFR requires the measurement of the renal clearance of an exogenous marker that fulfills the criteria for an ideal filtration substance (1,2). Inulin clearance has become the standard for measuring GFR because this fructose polymer is freely filtered at the glomerulus but is not acted on by the tubules, is not synthetized or degraded by the body, and is physiologically inert. However, the inulin clearance method requires continuous intravenous infusion and urine sampling. To overcome the need of constant infusion and urine collection, alternatives based only on plasma data were sought. Among these plasma methods, the single-injection techniques utilizing either radiolabeled or nonlabeled compounds are widely used; however, both of these techniques require multiple blood samples. Thus, the use of either a very limited number of samples or of even a single sampling point has become a valuable goal. We recently validated a method for GFR determination after intravenous administration of iohexol, which requires only six plasma samples (3). This method is currently utilized for GFR evaluation in multicentric studies with patients with different forms of renal disease. Because of the huge number of GFR determinations to be performed, we studied the possibility of using further simplified methods that require a single plasma sampling. Single-point methods have been suggested (4–9) that allow calculation of GFR usually by means of a formula that uses a single plasma concentration of the filtration marker and anthropometric data. The major drawback of these studies was the limited number of subjects considered, thus making the applicability of the proposed methods for GFR prediction questionable.

The aim of our study was to evaluate in a very large number of patients (approximately 700) with a wide range of renal function the reliability of GFR determination with a single plasma sample by comparing the results with multiple-point plasma clearance technique data.

METHODS

The clearance rates of 686 outpatients (456 men, 130 women) aged 22 to 71 yr enrolled in three different clinical

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trials were re-evaluated according to Brown and O'Reilly (6) by using the formula described by Jacobsson (4):

$$CL = \frac{1}{t/V + 0.0016} \times \ln \frac{Dose}{V \times C_t}$$

$$V_{male} = 166 \times W + 2490$$

$$V_{female} = 95 \times W + 6170$$

where V is the apparent volume of distribution (mL), C_t is the sample concentration at time t, and W is the body weight (kg).

The optimum time (t) for GFR calculation was established by calculating the clearances, using the two latest points for both clearance ranges considered, *i.e.*, 450 and 600 min for GFR ≤ 40 mL/min, 180 and 240 min for GFR > 40 mL/min, and comparing the parameters of regression lines obtained, the percentage of error between the predicted and the measured GFR, and by plotting the data according to Bland and Altman (10).

The original evaluation of GFR in these patients was performed as follows: after an overnight fast, a polyethylene catheter was inserted into a cubital vein in both arms for iohexol injection and blood-sampling collection, respectively. Exactly 5 mL of iohexol solution (Omnipaque 300, 647 mg of iohexol per milliliter corresponding to 300 mg of iodine per milliliter; Schering, Milan, Italy) was injected over 2 min, followed by 10 mL of saline solution. Blood samples were then taken from the contralateral arm at 120, 180, 240, 300, 450, and 600 min for patients with an expected GFR ≤ 40 mL/min, and at 120, 150, 180, 210, 240 min for patients with an expected GFR > 40 mL/min. After centrifugation at room temperature (2000× *g* for 20 min), plasma was separated and stored at −20°C for HPLC determination of iohexol concentration.

The plasma profiles were analyzed by a one-compartment open model system. All data were fitted by a non-linear regression iterative program (Carl Peck, Uniformed Services University, Bethesda, MD) and flooded according to Sacchi-Landriani, Guardabasso, and Rocchetti (11) on a LC 475 computer (Macintosh, Cupertino, CA) and the coefficient of correlation of the fittings were always > 0.99. The clearance of iohexol was estimated using the measurements from the timed period 120 min after the injection to the last sampling point, according to a one-compartment model (CL_1) by the formula: CL_1 = Dose/AUC, where AUC is the area under the plasma concentration-time curve. According to Brõchner-Mortensen (12), plasma clearances were then estimated by the formula

$$CL = (0.990778 \times CL_1) - (0.001218 \times CL_1)^2.$$

The agreement between the single-point and the multiple-point plasma clearance of iohexol was estimated by using linear regression analysis. A further estimate of agreement between the methods was performed by plotting the difference between the two methods against the average of the two methods for each patient (10). Limits of agreement were calculated as mean difference ± 1.96 × SD of the differences. To investigate a possible increase of the difference as the GFR increases, a logarithmic transformation of the data was also performed.

Laboratory Procedures

Plasma concentrations of iohexol were determined by HPLC as previously reported (13), with minor modification. Plasma samples were deproteinized by adding 4 vol 5% perchloric acid and centrifuging. Twenty microliters of the supernatant were chromatographed using a System Gold HPLC with a Model 160 UV detector set at 254 nm (Beckman, Fullerton, CA) and a 250 × 4 mm column packed with Lichrosorb C-18 (Merck, Darmstadt, Germany). Iohexol was eluted by a mixture of deionized water/acetonitrile (96:4 by volume, adjusted to pH 2.5 with phosphoric acid), pumped at a rate of 1.5 mL/min. Internal calibration curves of iohexol were prepared for each set of samples. The accuracy of the method was calculated as the percentage error from the true value by processing daily three samples containing a known amount of compound and was usually less than 3%. The precision of the assay was good and the coefficient of variation never exceeded 2.2%.

Statistical Analysis

Results were analyzed by t test and linear regression analysis, as appropriate. Regression estimates were also performed after log transformation of the clearance values. P values less than 0.05 were considered significant. In addition, 95% confidence intervals of the regression estimate were calculated to test the hypothesis of the slope = 1 and intercept = 0.

RESULTS

The slope of the regression lines for GFR calculated by using multiple-sampling point and single-sampling point were statistically different from the unity (except when considering 3 h as sampling point and GFR between 40 and 99 mL/min), and the intercepts statistically different from zero. For patients with GFR > 40 mL/min, an improvement of the results was observed by splitting the data in two ranges: clearances between 40 and 99 mL/min, and clearances ≥ 100 mL/min.

The reliability of GFR calculation by the single-point method was also assessed considering the error in clearance prediction. Using 10 h as optimum sampling point for GFR ≤ 40 mL/min, 49% of the values were predicted with an absolute error ≤ 2.5%, and 76% of the values were predicted with an absolute error ≤ 5%. The corresponding figures for 7.5 h as sampling point were 25% (absolute error ≤ 2.5%) and 52% (absolute error ≤ 5%).

Using 4 h as optimum sampling point for GFR > 40 mL/min, 44% of the values were predicted with an absolute error ≤ 2.5%, and 64% of the values were predicted with an absoluter error ≤ 5%. The corresponding figures for 3 h as sampling point were 26% (absolute error ≤ 2.5%) and 51% (absolute error between ≤ 5%). When we split the data into two ranges, we obtained an improvement of the results: for clearances between 40 and 99 mL/min, the optimum sampling time was 4 h, with an absolute error ≤ 5% for 73% of the values; and for clearances ≥ 100 mL/min, the sampling time was 3 h, with an absolute error ≤ 5% for 72% of the values. These results were confirmed by examining the agreement between the multiple-point iohexol clearance and the single-point clearance by plotting the difference between the two methods against the mean and by calculating the SD

TABLE 1. Mean difference and 95% limits of agreement between multiple- and single-point methods of GFR calculation

Sampling Point	Mean Difference ± SD	Limits of Agreement		Logarithmic Transformed Data			
				Mean Difference ± SD	Limits of Agreement		Antilogarithm
		Lower	Upper		Lower	Upper	
3 h	−1.730 ± 5.872	−13.239	9.779	−0.033 ± 0.092	−0.213	0.147	0.81 1.16
4 h	1.162 ± 6.972	−12.503	14.827	0.002 ± 0.079	−0.153	0.157	0.86 1.17
3 h, GFR between 40 and 99 mL/min	−2.947 ± 5.269	−13.274	7.380	−0.047 ± 0.095	−0.233	0.139	0.79 1.15
4 h, GFR between 40 and 99 mL/min	−1.249 ± 3.856	−8.807	6.309	−0.025 ± 0.064	−0.150	0.100	0.86 1.11
3 h, GFR > 100 mL/min	3.242 ± 5.609	−7.752	14.236	0.025 ± 0.046	−0.065	0.115	0.94 1.12
4 h, GFR > 100 mL/min	11.078 ± 8.100	−4.798	26.954	0.092 ± 0.066	−0.037	0.221	0.96 1.25
7.5 h	−1.071 ± 1.920	−4.834	2.692	−0.044 ± 0.089	−0.218	0.130	0.80 1.14
10 h	0.245 ± 1.353	−2.407	2.897	0.004 ± 0.058	−0.110	0.118	0.90 1.12
Overall	−0.095 ± 3.552	−7.057	6.867	−0.007 ± 0.062	−0.128	0.115	0.88 1.12

of the differences. Indeed, after logarithmic transformation of the data, the antilogarithm of the limits of agreement indicated that for approximately 95% of the cases, the single-point GFR determination may differ from multiple-point GFR by values 12% above to 10% below when using 10 h as the optimum sampling point for GFR < 40 mL/min, by values 11% above to 14% below when using 4 h as the optimum sampling point for GFR between 40 and 99 mL/min, and by values 12% above to 6% below when using 3 h as the optimum sampling point for GFR > 100 mL/min. Indeed, these sampling points had the narrowest range in percentage of error prediction (Table 1).

Taking these results all together, we established, as the optimum sampling time for single-point GFR calculation, 10 h after iohexol injection for clearances ≤ 40 mL/min, 4 h for clearances between 40 and 99 mL/min, and 3 h for clearances ≥ 100 mL/min. Thus, we calculated the agreement between the two methods by considering the entire range of the data.

Figure 1 shows the correlation between the multiple-sample point clearance and the single-point clearance calculated using the optimum sampling point derived. Despite the highly significant correlation observed (r^2 = 0.988), the slope of the regression was statistically different from the unity (95% confidence interval for the slope, 0.960 to 0.976). The Deming regression analysis confirmed that the slope was statistically different from the unity: y = 0.974x + 1.422. The logarithmic tansformation of the data gave essentially the same results: y = 0.987x + 0.054, r^2 =

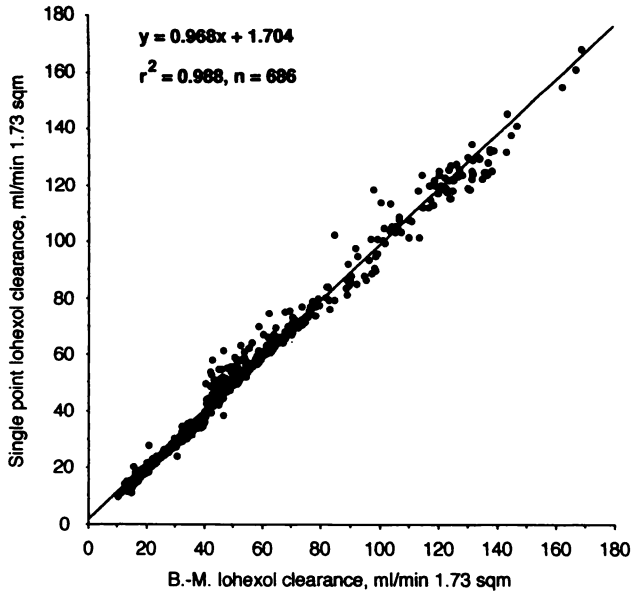

Figure 1. Correlation between plasma clearance of iohexol calculated by the one-compartment open model system then corrected by the Brochner-Mortensen equation and plasma clearance of iohexol calculated by using a single point sampling and the Jacobsson equation.

0.989, with the slope different from the unity (95% confidence interval, 0.980 to 0.995).

The frequency distribution of the error value between the multiple- and single-point techniques indicated that for the 49.6% of the predicted GFR, the absolute error was < 2.5% (22.3% negative, 27.3% positive), and for the 24.4% of the predicted GFR, the absolute error was between 2.5% and 5% (10.6% negative, 13.8% positive), suggesting that the single-point method permits the GFR calculation with an acceptable error in about 74% of the cases.

The agreement between the multiple-point iohexol clearance and the single-point clearance is shown in Figure 2A. The mean difference between the two methods was −0.095 mL/min per 1.73 m^2 (95% confidence interval for the difference from −0.367 to 0.177 mL/min per 1.73 m^2) and the 95% limits of agreement varied from −7.057 mL/min per 1.73 m^2 and 6.867 mL/min per 1.73 m^2. A logarithmic transformation of the data confirmed a scattered distribution of the differences over the range of GFR considered (Figure 2B). The mean difference was −0.0066 on the logarithmic scale, and the limits of agreement were −0.128 and 0.115. Taking the antilogarithm of these limits, we calculated 0.88 and 1.12, indicating that for approximately 95% of the cases the single-point GFR determinations are between 0.88 and 1.12 times the multiple-point GFR values. Thus, the single-point GFR may differ from multiple-point GFR by values 12% below to 12% above.

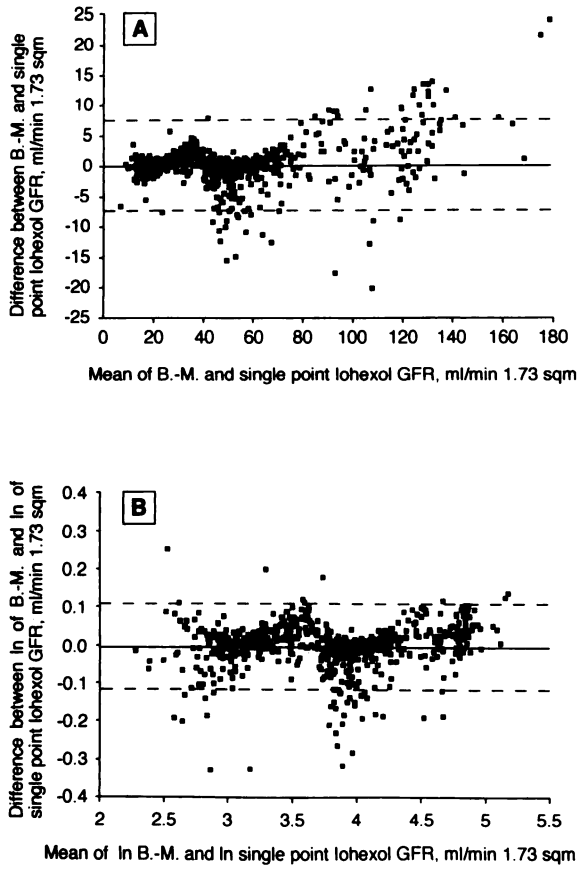

Figure 2. Difference between the plasma clearance of iohexol one-compartment, open model system, Bröchner-Mortensen equation and single point, Jacobsson equation versus the mean of the two methods in all patient studied (A); data after logarithmic transformation (B). Mean difference (straight line) and 95% limits of agreement (dashed lines) are shown.

To explore the reasons for the large discrepancy (> ±10%) in some of the patients, an in-depth analysis of characteristics of these "outliers" was performed. These discrepancies could not have been explained by anthropometric variables (which make the calculation of volume of distribution difficult, *i.e.*, patients grossly obese or thin) or by presence of edema, and no relationship with age and sex was observed.

DISCUSSION

Our routine method for determination of total plasma clearance usually utilizes an abbreviated serial blood sampling to estimate GFR from the slope of the disappearance concentration profile. Because this method was previously validated against the classic technique of inulin clearance, the "gold standard" for GFR determination, and the results confirmed a highly significant correlation between the methods (3), it was deemed adequate, for the comparison described in the study presented here, to consider our iohexol method for GFR determination as the "reference method."

However, although this technique has the advantage of avoiding the need for urine collection and continuous infusion of the marker of glomerular filtration, it requires analysis of multiple plasma samples.

Reduction of the number of plasma drawings for each patient would result in an important advantage in terms of safety and comfort for the patient and in lowered analysis costs. Thus we pondered whether this goal could be achieved by determining GFR by means of a single-sampling-point analysis.

Indeed our results indicated that this simplified technique gave good GFR determination with an error ranging between −5% to 5% for approximately 75% of the patients observed; however, for some of the remaining 25% of the patients, the error in prediction was as large as 40%.

Both demographic and anthropometric data of the patients failed to offer a possible explanation of the poor correlation between GFR values calculated with single-point and multiple-point techniques found in some of the patients, thus no guidelines for a correct and optimal design for the single-point method could be drawn from our results.

Because of the good performance of the chromatographic method, the discrepancies found in some patients seem not to derive from analytical impreci-

sion; however, it seems reasonable that an analytical error may play a major role when a single concentration point is used for GFR calculation rather than when five to six concentration points are fitted by means of a non-linear equation model. In this case, possible errors on a single concentration point could be nullified. Indeed, a good interpolation of the concentration points was confirmed by the absence of "outlier" concentrations and by the coefficient of correlation of the fittings >0.99.

As previously proposed by other authors (7,9), a different optimum sampling time was found, depending on the GFR value.

However, Boijsen *et al.* (5) investigated the applicability of the Jacobsson's formula using sampling times from 180 to 240 min after marker injection in 26 patients with GFR ranging from about 30 to 130 mL/min. They found a total error below 5% for clearance values above 70 mL/min at 180 min and above 50 mL/min at the 240-min sampling time, whereas for lower GFR values it rose dramatically.

Indeed, as recently proposed by Frennby *et al.* (9) and by Nilsson-Ehle and Grubb (7), a variable sampling time should be used for GFR calculation, depending on the expected clearance range.

Thus, according to this approach for GFR calculation using a single point, we search *a posteriori* for the optimum sampling point between those normally utilized with our routine technique.

However, in clinical practice, one has to decide *a priori* the optimum sampling time for each patient on the basis of a predefined criterion, usually the expected GFR calculated as the creatinine clearance. Thus, to avoid performing a different sampling time for each single patient (for practical reasons), the patients were divided into ranges of GFR, and sampling times were selected according to this division to give the best estimate of GFR.

Our results confirmed that is not possible to select a unique sampling time independently from the renal function for GFR determination, and the finding of a greatest deviation occurring close to the breakpoints (40 and 100 mL/min) seems to indicate that it is difficult to define *a priori* a criterion to decide the optimum sampling point.

A closer inspection of Figure 2 showed that GFR values slightly higher than 40 mL/min were well predicted and those between 50 and 70 mL/min were underestimated: we do not have an obvious explanation for these findings because, changing the sampling point considered for GFR calculation, the results were not improved.

In the highest range of GFR values, a tendency to overestimate the predicted clearances was found: to improve the results, we had to split the GFR values > 40 mL/min further into two ranges: the data indicated that GFR > 100 mL/min were best predicted by using the sampling point at 3 h rather than at 4 h. Even using this method, the GFRs were still overestimated, although to at a lesser extent.

This finding contrasts with the statement that the optimum sampling time should be as high as possible to allow a more accurate clearance determination; this seems to hold true only for very low GFR values.

However, even though sampling times as long as 24 h and even 72 h have been proposed for very low GFR values (7,8), it should be considered that non-hospitalized patients have to return to the medical center the day after the marker injection.

Thus, the real advantage of the single-point GFR calculation should be accurately evaluated because neither an improvement of precision in GFR calculation nor a reduced time of patient observation after filtration marker injection is achieved.

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