Single-slice dynamic computed tomographic
determination of glomerular filtration rate by use
of Patlak plot analysis in anesthetized pigs

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Objective—To compare glomerular filtration rate (GFR) as estimated from Patlak plot analysis by use of single-slice computed tomography (CT) with that obtained from clearance of plasma inulin in pigs.

Animals—8 healthy anesthetized juvenile pigs.

Procedures—All pigs underwent precontrast, whole-kidney, helical CT; postcontrast single-slice dynamic CT; and postcontrast, whole-kidney CT for volume determination. On dynamic images, corrected Hounsfield unit values were determined for each kidney and the aorta. A Patlak plot for each kidney was generated, and plasma clearance per unit volume was multiplied by renal volume to obtain whole-renal contrast clearance. Mean GFR determined via inulin clearance (Inu-GFR) was measured from each kidney and correlated to mean GFR determined via CT (CT-GFR) for the left kidney, right kidney, and both kidneys by use of linear regression and Bland-Altman analyses.

Results—CT-GFR results from 7 pigs were valid. Total and right kidney Inu-GFR were correlated with total and right kidney CT-GFR (total, $R^2 = 0.85$; right kidney, $R^2 = 0.86$). However, left kidney CT-GFR was poorly correlated with left kidney Inu-GFR ($R^2 = 0.47$). Bland-Altman analysis revealed no significant bias between Inu-GFR and CT-GFR for the left kidney, right kidney, or both kidneys.

Conclusions and Clinical Relevance—CT-GFR as determined by use of a single-slice acquisition technique, low-dose of iohexol, and Patlak plot analysis correlated without bias with Inu-GFR for the right kidney and both kidneys (combined). This technique has promise as an accurate CT-GFR method that can be combined with renal morphologic evaluation.

The GFR was 22 ml/min/1.73 m². Therefore, iohexol clearance should represent the GFR value. Computed tomography can precisely detect changes in the relative attenuation (density) characteristics of tissue caused by the presence of iodinated contrast medium. These attenuation changes are linearly related to tissue iodine concentration after a clinical dose of contrast medium is administered IV. On the basis of this principle, methods of GFR determination via CT have been developed in humans, pigs, and dogs, and several acquisition techniques and methods of calculation exist. Absolute CT-GFR correlates well with inulin clearance, iodine (iohexol) clearance, creatinine clearance, and scintigraphic methods of GFR determination. The most accurate CT-GFR methods use the Patlak plot to calculate GFR. The Patlak plot was originally developed as a method to determine the permeability of the blood-brain barrier and was first applied to determine CT-GFR in 1993. The plot is linear, and its slope represents whole-blood clearance (mL/min/100 g of tissue).

The CT-GFR has many advantages over other methods of GFR determination, with the primary advantage being the ability to evaluate renal morphologic and functional features during the same imaging session. Other variables, such as renal blood flow, fractional vascular volume, and tubular dynamics, can potentially be determined during the same imaging session. The CT-GFR is rapid (requiring a few minutes), and it avoids the need for urinary catheterization or multiple samples. Contrary to scintigraphic GFR determination methods, CT-GFR can be repeated after a short delay during the same imaging session. Also, CT obviates the need for isolation of the patient after the procedure because of exposure to radiation.

A single-slice, low-dose iohexol Patlak plot method of CT-GFR has never been validated in anesthetized pigs. Such validation would be useful both as a research tool that could possibly replace invasive Inu-GFR determination and to extend the technique to other animal species for clinical veterinary use. The purpose of the study reported here was to validate Patlak plot CT-GFR in healthy anesthetized pigs by use of a low dose of iodinated contrast medium (iohexol) and single-slice CT acquisition by comparing CT-GFR with Inu-GFR. We hypothesized that the Patlak plot CT-GFR could provide an accurate and unbiased estimation of GFR in swine, compared with the cumbersome gold-standard procedure.

Materials and Methods

Animals—The pigs were treated according to the Canadian Council on Animal Care guidelines. Eight healthy young (3- to 4-month-old) male pigs were included. By random allocation, CT-GFR was acquired for clinical veterinary use. The purpose of the study reported here was to validate Patlak plot CT-GFR in healthy anesthetized pigs by use of a low dose of iodinated contrast medium (iohexol) and single-slice CT acquisition by comparing CT-GFR with Inu-GFR. We hypothesized that the Patlak plot CT-GFR could provide an accurate and unbiased estimation of GFR in swine, compared with the cumbersome gold-standard procedure.

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Renal clearance of plasma inulin—The GFR was determined by means of inulin clearance. An initial bolus of inulin was administered. The bolus consisted of 2.6 g of inulin dissolved in 10 mL of sterile water for injection and 10 mL of PBS solution and saline (0.9% NaCl) solution (up to 50 mL); pH was adjusted to 7.4 by use of NaOH. Immediately after bolus administration, a CRI of inulin was started. The rate of infusion was set at 1 mL/min by use of a pump. The CRI was prepared as follows: 0.5 g of inulin was dissolved in 2.5 mL of sterile water for injection, 6.0 mL of phosphate buffer and saline solution (up to 125 mL) was added, and pH was adjusted to 7.4 with NaOH. The total time of infusion was 110 minutes.

Approximately 30 minutes after the inulin CRI was started, after steady state was reached, the residual urine production of each kidney was collected and disposed of (time 0). Blood and urine sampling for determination of inulin concentration was begun. Starting at time = 5 minutes, venous and arterial blood was collected at 10-minute intervals initially for 3 intervals, then at 20-minute intervals for 2 intervals. Alternating with this and starting at time = 10 minutes, urine was collected in separate containers for the left and right kidney initially also at 10-minute intervals for 3 intervals and then at 20-minute intervals for 2 intervals. Total urine flow was measured at each interval.

Inulin concentration was measured in urine and plasma by use of an enzymatic assay; Inu-GFR was considered equal to inulin clearance and was calculated by use of the standard clearance formula as follows:

\[ \text{Inu-GFR} = \text{Cl}_{\text{i}} / \text{C}_{\text{iu}} = \text{UF} (\text{C}_i / \text{C}_u) \]

where Clᵢ is the renal inulin clearance, UF is urine flow (mL/min⁻¹), Ci is inulin urinary concentration, and Cᵢ is arterial inulin plasma concentration. Five consecutive inulin excretion rates were obtained, and the mean rate was calculated for comparison with CT-GFR results.

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CT image acquisition—The CT-GFR was performed with a single-slice helical scanner. The acquisition involved 3 steps and included baseline precontrast imaging, dynamic postcontrast single-slice imaging, and postcontrast imaging for determination of renal volume. Initial precontrast baseline imaging of both kidneys and the abdominal aorta was performed with a helical acquisition, 10-mm slice thickness, pitch of 1, and matrix of 512 × 512 (display field of view, 30 cm; scan field of view, medium, 120 kVp, and 200 mAs). By use of a pressure-injector and associated software, iohexol (0.25 mL·kg⁻¹ [300 mg·mL⁻¹; 75 mg of I·kg⁻¹]) was injected as a bolus (rate, 4 mL·s⁻¹). Dynamic CT acquisition was initiated simultaneously to the beginning of injection. A 10-mm-thick slice centered at the hilus of both kidneys was scanned repetitively every 4 seconds for 120 seconds. Manual breath hold was used to arrest abdominal motion, and pauses for breath were allowed at approximately 30 seconds and every 15 seconds thereafter between slice acquisitions.

Following the dynamic acquisition, both kidneys were again scanned in their entirety for determination of renal volume. Scan variables were unchanged, except for slice thickness, which was reduced to 5 mm.

Image analysis and CT-GFR calculation—The CT-GFR determination was based on Patlak plot analysis. Regions of interest were manually drawn on an image-processing workstation around the entire kidney on each dynamic slice and on the equivalent slice using the precontrast baseline acquisition, excluding the renal hilus and main vessels. Edge detection software was not used because occasional silhouetting between the renal contour and adjacent organs was present. Image window width and level were set at 150 and 20 HUs, respectively. This was repeated separately for the right and left kidneys. An ROI of constant size (22 mm²) was centered in the abdominal aorta on each dynamic and precontrast slice (Figure 1).

The mean HU value within the ROI of each kidney before administration of contrast medium was subtracted from each value after administration of contrast medium, giving a corrected kidney HU value (c[t]). The same was done for the abdominal aorta, to give a cor-
Statistical analysis—Statistical analysis was performed with statistical software. The mean and SD of CT-GFR and Inu-GFR were obtained. Because of the small sample size, Wilcoxon signed rank tests were used to compare the CT-GFR values for the right kidney versus left kidney as well as Inu-GFR values for the right kidney versus left kidney. Then, linear regression was applied to compare CT-GFR with Inu-GFR for the right kidney, left kidney, and the sum of both kidneys (total GFR). Agreement was evaluated by use of Bland-Altman analysis. The Bland-Altman method is used to plot the difference between 2 diagnostic tests (eg, CT-GFR and Inu-GFR) against the mean of results of both tests together. This technique estimates the degree to which 1 test agrees with another, can detect whether a consistent over- or underestimation is present, and determines the upper and lower values for the difference of agreement. When 2 tests are in perfect agreement, points in the Bland-Altman plot will be clustered around a line representing 0 on the y-axis. These tests can then be used interchangeably in a clinical situation. Significance was set at P < 0.05 for all tests.

Results

The pigs ranged in weight from 17.5 to 37.0 kg (mean 26.5 kg), and PCV ranged from 26% to 31% (mean 26.5 kg). The pigs ranged in weight from 17.5 to 37.0 kg (mean 26.5 kg), and PCV ranged from 26% to 31% (mean 26.5 kg).
(mean, 28%). The Patlak plot for 1 pig had large variability (right kidney Patlak, $R^2 = 0.64$; left kidney Patlak, $R^2 = 0.27$). With such high variability, CT-GFR results for this pig were considered invalid and results from this pig were eliminated from all statistical analysis and results.

Peak aortic enhancement occurred at 4 seconds ($n = 2$ pigs) or 8 seconds (5) after injection of contrast medium. The aortic time attenuation curves revealed an initial high enhancement peak, with a second much lower peak at 20 to 35 seconds. Progressively smaller peaks occurred approximately every 20 seconds thereafter (Figure 2).

Initial peak renal parenchymal enhancement occurred at 8 to 20 seconds (mean, 14 seconds) and thus occurred 4 to 8 seconds after maximal aortic enhancement. In 5 pigs, both kidneys were enhanced maximally simultaneously. In the remaining 2 pigs, a 4-second delay occurred between the kidneys; in 1 pig, the right kidney was enhanced first, and in the other, the left kidney was enhanced first. After this initial enhancement peak, much smaller peaks occurred approximately every 20 seconds. Despite these small undulations in the renal TAC, an overall gradual increase in renal parenchymal HU values followed the initial enhancement peak until the end of data acquisition at 120 seconds. In 4 pigs, this overall increase in renal parenchymal at-

Table 3—Results of linear regression analysis of the relationship between Inu-GFR and CT-GFR for the left kidney, right kidney, and total CT-GFR in 7 pigs.

<table>
<thead>
<tr>
<th>Kidney</th>
<th>$R^2$</th>
<th>Slope (SE)</th>
<th>$P$ value</th>
<th>Intercept (SE)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>0.47</td>
<td>0.77 (0.36)</td>
<td>0.090</td>
<td>2.98 (15.48)</td>
<td>0.855</td>
</tr>
<tr>
<td>Right</td>
<td>0.86</td>
<td>0.82 (0.15)</td>
<td>0.002</td>
<td>3.43 (6.45)</td>
<td>0.618</td>
</tr>
<tr>
<td>Total</td>
<td>0.85</td>
<td>0.94 (0.18)</td>
<td>0.003</td>
<td>-6.43 (15.28)</td>
<td>0.691</td>
</tr>
</tbody>
</table>

Figure 4—Linear regression graphs of Inu-GFR versus CT-GFR (mL/min) for the LK (A), RK (B), and total of both kidneys (C) in 7 pigs. Straight line represents the linear regression between CT-GFR and Inu-GFR for the LK, RK, and total of both kidneys.

Figure 5—Bland-Altman graphs of Inu-GFR and CT-GFR for the LK (A), RK (B), and total (total) kidneys (C) in 7 pigs. Straight line represents the Bland-Altman relationship between the difference between CT-GFR and Inu-GFR (y-axis) and the mean of CT-GFR and Inu-GFR.
tennuation was equivalent to or surpassed the HU value of the initial enhancement peak (Table 1).

In 6 pigs, contrast medium was seen in the collecting system of both kidneys by the time the renal volume scan was obtained (6 to 7 minutes after injection). In 1 pig, contrast medium was not seen in the collecting system. Moderate hydrenephrosis was present in the 2 first-tested pigs, probably as a result of Foley catheter inflation. Foley catheters were left uninfilled for the remaining pigs; no hydrenephrosis occurred in those pigs.

For all pigs, outlier values were seen in the Patlak plots at points corresponding with initial aortic or renal attenuation peaks (Figure 3). These outlier points were removed for calculation of the Patlak regression equations.

The CT-GFR and Inu-GFR data distribution for the left kidney, right kidney, and total kidneys was normal (Table 2). One pig had lower GFR than all of the others, particularly when evaluated via CT.

No significant ($P = 0.938$) differences were found between the left kidney and right kidney Inu-GFR. Although not significant ($P = 0.156$), left kidney function was often slightly lower than right kidney function as measured via CT.

For linear regression, Inu-GFR (gold standard) was designated as the independent variable and CT-GFR as the dependent variable (Table 3; Figure 4). For the left kidney, CT-GFR was a poor predictor of Inu-GFR, with $R^2$ of 0.47 and a slope that was not significantly different from 0 ($P = 0.09$). Right kidney Inu-GFR was more predictable ($R^2 = 0.86$), with a slope that was significantly ($P = 0.002$) different from 0 and not significantly different from 1 ($P = 0.2$). Total Inu-GFR was almost as closely predicted ($R^2 = 0.85$), and the slope was significantly ($P = 0.003$) different from 0 and not significantly different from 1 ($P = 0.7$).

The mean difference between Inu-GFR and CT-GFR was 6.63 mL/min (95% CI, ± 20.8 mL/min) for the left kidney, 3.9 mL/min (95% CI, ± 13.2 mL/min) for the right kidney, and 10.54 mL/min (95% CI, ± 22.4 mL/min) for the total. Although CT-GFR often underestimated Inu-GFR (Figure 5), there was no significant relationship (bias) between the difference in Inu-GFR and CT-GFR and the mean of these 2 measurements for the left kidney ($P = 0.73$), right kidney ($P = 0.49$), and total ($P = 0.87$).

Linear regression statistics were repeated with 1 pig eliminated from the data set because its GFR was below the reference limit value (Table 4). Good prediction of right kidney Inu-GFR ($R^2 = 0.83$) and total Inu-GFR ($R^2 = 0.81$) was still evident via CT-GFR. Although the slopes of both of the regression equations were farther from 1.0, they remained significantly different from 0. The intercepts were still not significantly different from 0. Poor prediction of left kidney Inu-GFR by CT-GFR remained ($R^2 = 0.21$). Bland-Altman analysis revealed no significant bias for the left kidney ($P = 0.61$), right kidney ($P = 0.22$), and total ($P = 0.40$).

### Discussion

For the present study, a single-slice CT acquisition technique, Patlak plot analysis, and a low IV dose of contrast medium were selected. The single-slice technique is limited in that it requires extrapolation of GFR information from a single slice to that of the entire kidney. Therefore, in diseased kidneys in which there is a large variability in function, the single-slice technique may be inaccurate. However, the data generated from the single-slice acquisition contain a greater number of time points and thus allows generation of more complete Patlak plots and TACs. In addition, this acquisition technique allows other mathematical models to be applied to the data and for separate data to be acquired from the cortex and medulla.

Patlak plot analysis is a 2-compartment model based on the assumption that the GFR is equivalent to the influx constant (K) of a solute (ie, contrast medium) across a barrier (ie, the glomerular membrane). The model may be applied when solute flow is unidirectional, which is true when evaluating GFR because back diffusion across the glomerulus does not occur. Linearity of the Patlak plot is related to the fact that solute flow is unidirectional. The entire amount of diffused solute must be present within the organ parenchyma at the time of sampling, making it important to perform the analysis prior to the presence of contrast medium within the ureters. For this reason, our analysis included only the data obtained up to 120 seconds. Too short of an acquisition period (10 to 15 seconds) results in inaccurate CT-GFR determination. Several other conditions must also be met. The Patlak model accounts for the fact that there may be a change in time of the test solute in plasma, which is clearly true with a bolus of contrast medium within the aorta. A limitation in the model is that it does not account for interstitial space as a third compartment; therefore, CT-GFR in patients with increased interstitial space may be inaccurate. The low dose of contrast medium (0.25 mg.kg$^{-1}$) allowed calculation of GFR while limiting the risk of CMIN. This dose was chosen on the basis of another CT-GFR study. The CMIN has become a major concern in human medicine, in which frequency rates of severe CMIN can be as high as 18%. Contrast medium dose is recognized to be one of the major contributing factors for CMIN in humans, especially when doses as high as 900 to 1,800 mg of I/kg are used.

The shape of the renal time attenuation curves was similar among all individuals in the study reported here and similar to that seen with camera-based radiopharmaceutical plasma clearance methods and 2 other CT-GFR studies. It is likely that the time to maximal enhancement, maximal HU value, and shape of the renal TAC would be altered in animals with decreased renal function.
function and that the alteration of these variables could be related to specific disease processes. In this study, for 1 pig that had the lowest CT-GFR function, the renal TACs were flatter with the longest time to maximal enhancement (20 seconds) and the lowest corrected peak enhancement. More complete renal function curves could be constructed with a longer data acquisition period (ie, 5 to 10 minutes).

A recirculation effect was seen in the renal and aortic TACs and consisted of a series of small undulations after the initial maximal enhancement peak. This indicated that iohexol was not homogeneously mixed in blood after injection. Because the Patlak model assumes that homogeneous mixing of iohexol in plasma has occurred, this recirculation effect has been mentioned as proof that the model may not be strictly applicable to GFR within the first few seconds after bolus injection. For this reason, it has been suggested that Patlak plot analysis should be delayed until after 2 or 3 circulation peaks. The outlier data points seen in Patlak plots of pigs of the present study seemed to represent these early recirculation peaks and were consequently removed from the Patlak plot. In doing this, Patlak plot analysis performed as of time 0 in the study group was successful in this study as well as in another study.

As expected, no difference in GFR was found between the left and right kidneys by use of either inulin plasma clearance or CT. This was also consistent with CT-GFR studies evaluating relative renal function. However, left kidney CT-GFR was a poor predictor of left kidney Inu-GFR. No clear explanation for this discrepancy was found, but this relationship indicated a larger and more variable discrepancy between left kidney Inu-GFR and CT-GFR. Renal function, image analysis, image acquisition, and sample analysis factors were identical for both kidneys. A similar discrepancy between right renal CT-GFR and right renal scintigraphic GFR has been explained by temporal differences in attenuation of the kidneys. In pigs, the left kidney is generally slightly more caudally located, which could result in a slight delay in contrast medium arriving at this kidney and a subsequent delay in the time attenuation curve for this kidney. This slight delay would affect the CT-GFR results but not the Inu-GFR results. However, no consistent delay in the left renal TAC was observed in our pigs. Another explanation is that extrapolation of CT-GFR from a single slice of kidney led to a consistently greater degree of error for the left kidney. The percentage of nonfunctioning renal tissue may have been overrepresented in the slices acquired in the left kidneys, which led to significantly lower left kidney CT-GFR values.

Right kidney CT-GFR predicted right kidney Inu-GFR accurately and without observable bias in this small population of swine. The CT-GFR of only the right kidney may therefore be a sufficient estimate of GFR when CT-GFR is used as a research tool to evaluate normal renal function. This would reduce the amount of necessary image analysis by half. Total CT-GFR also predicted total Inu-GFR accurately and without bias. This strong positive correlation detected between the right kidney and total kidneys was comparable to other studies that used the same CT-GFR analysis method in which total CT-GFR had a correlation of $R = 0.87$ to 0.92 with blood clearance of creatinine in humans with and without diabetes. An underestimation of GFR by use of CT has been reported by others. Transient CMIN, vascular effects related to general anesthesia, and the Faraeus effect have all been used to explain the lower GFR value obtained by use of CT. Underestimation of renal volume would also lead to an underestimation with CT-GFR. In patients with increased interstitial space, CT-GFR overestimates the value obtained by use of the test standard.

Although no such over or underestimation was detected in the present study via Bland-Altman analysis, it is possible that had a larger test population been used, a significant bias between CT-GFR and Inu-GFR would have been found.

One pig was eliminated from the study because of invalidation of the Patlak plot analysis and CT-GFR results. A breath-hold misregistration error, resulting in excessive patient motion, occurred at 20 seconds after injection. This caused an early gap in the aortic and renal TACs and likely caused a calculation error within the Patlak plot analysis. Because of the 2-minute image acquisition period, 1 or more breath-hold misregistration errors occurred in every pig in this study; however, most were late in the image acquisition and had no apparent effect on the results.

Total GFR as determined via Inu-GFR and technetium 99m-Tc-pentetate plasma clearance in healthy juvenile anesthetized pigs ranges from $40$ to $100$ mL·min$^{-1}$ for pigs with a similar weight as our study population. The Inu-GFR and CT-GFR results were within this range for all pigs in this study, except 1, in which Inu-GFR was near the lower limit of the range at $42.6$ mL·min$^{-1}$ and CT-GFR was low at $19.5$ mL·min$^{-1}$. Because of this finding, it was unclear whether or not this pig should be excluded; thus, results were presented both with and without this pig. The discrepancy in function between Inu-GFR and CT-GFR in this pig may indicate that in individuals with renal function near the lower limit of the range in healthy animals, CT-GFR may underestimate renal function. Another explanation is a temporal discrepancy between the CT-GFR results and the Inu-GFR results, which were obtained at least 1 hour from the CT-GFR results and over a longer period.

A temporal reduction in GFR during the CT acquisition could have been the result of a transient reduction in renal blood flow or glomerular functionsecondary to contrast medium vascular effects, hypotension, hypovolemia, or transient excessive anesthetic depth.

The CT-GFR performed with a single-slice acquisition technique, low dose of iohexol, and Patlak plot analysis correlated highly and without bias with inulin plasma clearance GFR for the right kidney and total kidneys. This technique has promising clinical relevance in the development of an accurate CT-GFR method that can be combined with renal morphologic evaluation. Further studies on a larger number of individuals, in other species, and in animals with abnormal renal function are needed to investigate the clinical usefulness of this technique.

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b. Frennby B. Use of iohexol clearance to determine the glomerular


d. MRF-065, Brain Tree Scientific, Braintree, Mass.

e. VPFCF:55, Cook Veterinary Products, Bloomington, Ind.

f. 13754, Sigma-Aldrich Canada Ltd, Oakville, ON, Canada.

g. 55-2222, Harvard Apparatus, Holliston, Mass.

h. Hi-speed XZI, General Electric, Milwaukee, Wis.

i. 402 syringe pump, Gilson, Middleton, Wis.

j. Gilson, Serial Input Output Channel (GSIOC). Gilson, Middleton, Wis.

k. Omnipaque 300, Amersham Health Inc, Oakville, ON, Canada.

l. Advantage (AW), version 4.0, General Electric, Milwaukee, Wis.

m. SAS, version 8.02, SAS Institute Inc, Cary, NC.

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