Renal Scintigraphy in Dogs

Evaluation of Glomerular Filtration Rate Measurement by ^{99m}Tc-DTPA Renogram

Naruepon Kampa
Faculty of Veterinary Medicine and Animal Science
Department of Biomedical Sciences and Public Health
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2006

Acta Universitatis Agriculturae Sueciae
2006:9

ISSN 1652-6880
ISBN 91-576-7058-7

Tryck: SLU Service/Repro, Uppsala 2006

Abstract

Kampa, N. 2006. *Renal scintigraphy in dogs: Evaluation of glomerular filtration rate measurement by ^{99m}Tc-DTPA renogram.* Doctor's dissertation.
ISSN: 1652-6880, ISBN: 91-576-7058-7

Renal scintigraphy has been used widely to measure the individual kidney glomerular filtration rate (IKGFR). In dogs, the estimated GFR is calculated by a regression equation relating the percent of the injected dose of radiopharmaceutical, technetium-99m DTPA (dietylene-triaminepentaacetic acid), taken up by the kidneys to the known GFR by standard (plasma) clearance normalized to bodyweight (BW). Uptake is calculated by the integral (area under the curve) of time activity curve (TAC) of the renogram, which is corrected for attenuation of radiation in the body from kidneys to body surface of the back and background activity. A more physiologically correct method is to normalize GFR to plasma volume (GFR/PV), which requires a region of interest (ROI) of the left ventricle (LV). The first aim of the thesis was to determine the variations within the standard method and minimize them to improve the accuracy of the methods. Physiological variability of GFR within and among dogs on different days was also studied. The integral method was more stable than a slope method and recommended to used for calculating IKGFR/BW. The best methods for measuring were: semi-automatic drawing of kidney ROI with 20% threshold, a perirenal background ROI at one pixel wide and one pixel out from kidney ROI, a threshold color scale rather than continuous for measuring kidney depth to calculate attenuation, and a time interval between 30 – 120 seconds from the start of the TAC. Measurement variation was also caused by significant observer variability, which indicates that methods must be standardized and the same person should measure all compared results. Physiologic day-to-day variability of GFR in normal dogs was mostly found between rather than within dogs, and accounted for most of the variability. GFR/PV was not affected by LV ROI sizes. Subtracting extravascular activity from the LV ROI did not improve precision, but increased variability due to different LV ROI sizes and time intervals chosen for LV plot. Manual LV ROI, without extravascular subtraction and a time interval for LV input between 1 and 4 minutes are recommended.

Keywords: GFR, renal scintigraphy, dogs, ^{99m}Tc-DTPA, day-to-day variability, observer variability, plasma volume.

Author's address: Naruepon Kampa, Department of Biomedical Sciences and Public Health, Division of Diagnostic Imaging and Clinical Pathology, Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, Box 7029, 750 07 Uppsala Sweden. On leave from the Faculty of Veterinary Medicine, Khonkaen University, Khonkaen, 40002 Thailand.
E-mail: Naruepon.Kampa@bvf.slu.se, Naruepon.Kampa@gmail.com

To my parents and my family

Contents

Appendix

Papers I–IV

This thesis is based on the following 4 papers, which will be referred to by their Roman numerals:

I. Kampa, N., Wennstrom, U., Lord, P., Twardock, R., Maripuu, E., Eksell, P. & Fredriksson, S.O. 2002. Effect of region of interest selection and uptake measurement on glomerular filtration rate measured by ^{99m}Tc-DTPA scintigraphy in dogs. *Veterinary Radiology and Ultrasound 43,* 383-391.

II. Kampa, N., Bostrom, I., Lord, P., Wennstrom, U., Ohagen, P. & Maripuu, E. 2003. Day-to-day variability in glomerular filtration rate in normal dogs by scintigraphic technique. *Journal of Veterinary Medicine Series A, Physiology, Pathology, Clinical Medicine 50,* 37-41.

III. Kampa, N., Lord, P. & Maripuu, E. 2006. Effect of observer variability on glomerular filtration rate measurement by renal scintigraphy in dogs. *Veterinary Radiology and Ultrasound (in press).*

IV. Kampa, N., Lord, P., Maripuu, E. & Hoppe, A. 2006. Glomerular filtration rate normalized to plasma volume by a scintigraphic method in dogs: effects of measurements of plasma activity input. *Manuscript.*

Abbreviations

Abbreviations used in the thesis are presented in alphabetical order:

^{51}Cr-EDTA	Chromium-51 ethylenediaminetetraacetic acid
^{99m}Tc-DTPA	Technetiumdietylene-99m triaminepentaacetic acid
^{99m}Tc-MAG3	Technetiumdietylene-99m mercaptoacetyltriglycine
BSA	Body surface area
BUN	Blood urea nitrogen
BW	Body weight
ECV	Extracellular fluid volume
ERBF	Effective renal blood flow
EV	Extravascular fluid
GFR	Glomerular filtration rate
IKGFR	Individual kidney glomerular filtration rate
LK	Left kidney
LV	Left ventricle
MBq	Megabecquerel
NK	Observer, Naruepon Kampa
PMT	Photomultiplier tubes
PV	Plasma volume
RK	Right kidney
ROI	Region of interest
TAC	Time-activity curve

Introduction

Although imaging modalities can be used to evaluate renal morphology: size, shape and internal architecture, and doppler ultrasound provides a relative assessment of blood flow velocity (renal vascular resistance) in systole and diastole (Nyland et al., 2002), functional information of the kidney such as glomerular filtration rate (GFR) and effective renal blood flow are routinely derived from scintigraphy (gamma camera based method) or from blood samples after injecting a marker for glomerular filtration.

Renal anatomy and physiology

In the dog, the kidneys are located in the retroperitoneal space at the level of the upper three lumbar vertebrae. The kidneys lie in an oblique position, tilted cranioventrally. The left kidney is usually located caudal and ventral to the right one (Evans & Christensen, 1993). The right kidney is firmly attached to the dorsal body wall, adjacent to the caudate lobe of the liver and has a correspondingly larger retroperitoneal contact area. They are not rigidly fixed, particularly the left kidney and may move during respiration or may be displaced by a full stomach or by the dog assuming different positions.

The functional unit of the kidney is the nephron. A million or more of these tiny complex structures are found in each kidney. Each nephron is hollow and comprises a renal corpuscle and renal tubule. The renal corpuscle is composed of two parts, a vascular structure, the glomerulus, and a hollow cup, Bowman's capsule, surrounding it. The kidney receives 20-25% of cardiac output and the average renal plasma flow is 15 ml/min/kg of body weight (Daniel et al., 1999; Guyton & Hall, 2000). About 20% of this volume is filtered through the glomerulus, but the percentage changes because of autoregulatory mechanisms. The glomerular filtration of plasma controls the volume and composition of the body fluids. GFR is the volume of fluid filtered from the renal glomerular capillaries into Bowman's capsule per unit time.

GFR is considered to be the best single parameter for assessing renal function (Levey, 1989) because it is directly proportional to the number of functioning nephrons (Liedtke & Duarte, 1980; Ross, 1995). Many different kinds of diseases can cause swelling or scarring of the nephron or glomerulus: as a direct result of infection, a toxic drug, autoimmune diseases etc., damaging the glomeruli which reduces the filtering capacity of the kidney. Blood urea nitrogen (BUN) and serum creatinine concentration, which most commonly are used as the standard method of measuring renal function, are relatively insensitive in detecting renal dysfunction in that they do not accumulate significantly in the blood until there is severe renal insufficiency. About 70-75% of the nephrons must be nonfunctional before these values rise above the normal range (Finco, Coulter & Barsanti, 1981; Krawiec et al., 1986; Ross, 1995). Thus, the limitations of BUN and serum

creatinine must be recognized as they cannot determine renal reserve or subclinical decrease in function.

Control of GFR

The GFR is directly determined by the glomerular hydrostatic pressure and the glomerular capillary colloid osmotic pressure, which are mediated by the sympathetic nervous system, hormones, autacoid (vasoactive substances that are released in the kidneys and act locally, such as angiotensin, prostaglandins etc.), and other feedback controls that are intrinsic to the kidneys (Guyton & Hall, 2000). The GFR is controlled by autoregulatory mechanisms (intrinsic feedback mechanisms). The major effect of autoregulation is to maintain a relatively constant GFR in the face of decreased renal blood flow or a decrease in systemic blood pressure, and to precisely control renal excretion of water and solutes. Blood pressure within the glomerulus determines GFR controlled by vasoconstriction of the afferent or efferent arteriole, which are controlled by hormones or autacoid.

Glomerular filtration rate measurement

The GFR is determined by the sum of the hydrostatic and colloid osmotic forces across the glomerular membrane, which gives the net filtration pressure, and the glomerular capillary coefficient (Guyton & Hall, 2000), but these cannot be directly measured; therefore determination of total GFR by laboratory methods relies on the concept of clearance.

Clearance studies

Clearance is defined as an the theoretical volume of fluid from which an indicator or tracer is completely extracted a given interval of time. It has the units of ml/min (or ml/min/kg or ml/min/ml when normalized to tissue mass or fluid volume).

Measurement of GFR is based on measurement of tracers that are cleared from plasma exclusively by glomerular filtration. Renal (urinary) clearance is the volume of blood or plasma cleared of tracer that has passed through the kidney and ends up in the urine (Chew & DiBartola, 1989). For GFR, plasma clearance of tracers equals renal clearance if the tracer is excreted only through the kidney. A tracer should ideally meet the following criteria:

- It is excreted only through the kidney, being freely filterable through the glomerular capillary membranes.
- It must be neither secreted nor absorbed by renal tubules.
- It must by physiologically inert and not metabolized by kidneys.
- It must not be bound to plasma proteins or to red cells, and not sifted in the ultrafiltration process.
- It must be nontoxic.
- It must be measurable with a high degree of accuracy in body fluids.

Clearance can be measured under steady state or non-steady state conditions. The gold standard of GFR measurement is to measure the urinary clearance of inulin at steady state. Under such circumstance, the product of urine flow rate and urinary concentration is equal to the product of GFR and plasma concentration. This technique, however, has practical limitations for clinical cases: it is time consuming (24 hours); it is invasive in nature as the bladder must be catheterized and completely emptied to collect all urine. It is rarely performed outside research laboratories (Finco et al., 1981; Krawiec et al., 1986; Chew & DiBartola, 1989). The single injection of tracer (bolus injection technique) with measurement of plasma clearance under non-steady conditions has been used instead. Plasma clearance is measured by dividing the administered dose of tracer by area under the plasma time-concentration curve.

The iodine-containing tracer, Iohexol® which is used for radiographic contrast studies can be used as a filtration marker measuring GFR by plasma clearance method (Effersoe et al., 1990; Moe & Heiene, 1995; Brown et al., 1996; Gleadhill & Michell, 1996). The disadvantages are the large volume of the injection and samples because of required high concentration for accurate measurement in plasma, with risk of toxicity and blood loss in small animals.

Radionuclide clearance

A radiopharmaceutical is a chemical substance that contains a radioactive atom (radionuclide) bound to it, usually by chelation, and is suitable for in vivo use in the diagnosis or treatment of disease. Radionuclide clearance with a single intravenous injection is simple, accurate, and reproducible (Dubovsky & Russell, 1982).The quantification of renal function by radionuclide clearance has been widely used because plasma concentration of a radionuclide can be measured accurately in a well counter at very high and very low levels. The disadvantage is that the measurements must be corrected for radioactive decay.

The radionuclide agent of choice for an extremely accurate measurement of GFR is ^{51}Cr-EDTA (chromium ethylenediaminetetraacetic acid) (Stacy & Thorburn, 1966). The clearance of ^{51}Cr-EDTA is virtually identical with the clearance of inulin (Blaufox et al., 1996); therefore, it is a true GFR marker.

At the present time, technetium-99m DTPA (dietylene-triaminepentaacetic acid) (^{99m}Tc-DTPA) comes closest to being the radiopharmaceutical of choice for GFR determination in clinical practice because of its properties and the ease of preparation of for either imaging or external counting of plasma samples (Twardock, Krawiec & Lamb, 1991; Peters, 2004). It has been shown to give equivalent results to inulin clearance in dogs (McAfee et al., 1981; Krawiec et al., 1986; Barthez et al., 1998). It is inexpensive, has low radiation, and has a short half life of 6 hours and most importantly, a low degree of binding to plasma protein, in dogs, 10% , which does not significantly influence the measured GFR (Twardock et al., 1991; Uribe et al., 1992). Commercially available kits use stannous reduction to bind ^{99m}Tc to the DTPA molecule. After intravenous injection of a bolus of ^{99m}Tc-DTPA, the first pass extraction of ^{99m}Tc-DTPA is

approximately 20%, resulting in rapid blood clearance with normal kidney function. The biologic half-life in man is 2.5 hours with 95% of the ^{99m}Tc-DTPA within the urine by 24 hours (Kim et al., 1996).

The accuracy of plasma clearance estimation with a two compartment model depends on the number of samples used to fit the data. Using 8 blood plasma samples, a two compartment model of ^{99m}Tc-DTPA has a very high correlation (r=0.98) with the gold standard of inulin clearance (Barthez et al., 1998). For GFR measurement by plasma clearance of ^{99m}Tc-DTPA, collection of at least 6 samples has been recommended, at 5, 15, 30, 45, 60, 90, 120 and 180 minutes (Daniel et al., 1999). But others (Barthez, Chew & DiBartola, 2000) showed that accurate GFR can be obtained by use of 4 blood plasma samples taken at specific times.

The main disadvantages of the DTPA plasma clearance method is that it requires multiple blood sampling over 4 hours, some laboratory skill for sample analysis, and computer analysis. Mutiple blood sampling may be stressful for the patient and is time consuming (Barthez et al., 1998).

Imaging methods (scintigraphic method, gamma camera based method)

Scintigraphy is a diagnostic technique in which a two-dimensional picture of internal body tissue is produced through the detection of the gamma radiation emitted by radioactive substances injected into the body. The image is obtained with a gamma camera (Figure 1). ^{99m}Tc-DTPA is the radiopharmaceutical of choice for GFR study by scintigraphy, because DTPA meets the criteria for GFR measurement and energy of the emitted radiation of ^{99m}Tc is at 140 Kev, which is ideal for effective detection by the gamma camera. The uptake as a fraction of the injected activity of ^{99m}Tc-DTPA by filtration within the kidney is directly related to GFR (Gates, 1982; Twardock, Krawiec & Itkin, 1996).

Gamma camera

The gamma camera is a scintillation detector that counts the number of photons over a period of time, and detects their position, creating a functional image of the passage of the injected tracer. It detects gamma rays within a given energy window around the energy of the gamma photon emitted by the tracer (Figure 1).

A gamma ray photon interacts with the detector by means of the photoelectric effect or Compton scattering with the iodide ions of the crystal. This interaction causes the release of electrons which in turn interact with the crystal lattice to produce light, in a process known as scintillation. Only a very small amount of light is given off from the scintillation detector. Light photons enter the photocathode which produces electrons that are proportional in numbers to the intensity of the light flash. Because the small number of electrons is not enough to generate an electrical signal, the electrons need to be amplified by photomultiplier tubes (PMT). The output signal from each PMT is analyzed to determine the point of origin (Figure 1). The output of the gamma camera is digitized and can be displayed, manipulated or stored.

The digital image is composed of rows and columns of square individual picture elements called pixels. The number of rows and columns is called the image matrix size. The matrix size determines the number of discrete points (pixels) of which the image is composed. For example, an image displayed in a 64 x 64 matrix has 64 rows and 64 columns of pixels for a total of 4,096 pixels. Each pixel contains data recording the number of detected counts collected during the time interval of the picture. In dynamic studies, a series of pictures is taken. Each picture is called a frame, and the intervals for each are described as the frame rate, or time interval per frame (e.g. 10 frames/second or 6 seconds/frame). The activity of each pixel in each frame is the data for the calculation of the camera-based method of GFR determination. A functional image of the passage of the radiolabelled tracer (radiopharmaceutical drug) can be obtained by taking a sequence of pictures of the passage of the tracer through the kidney.

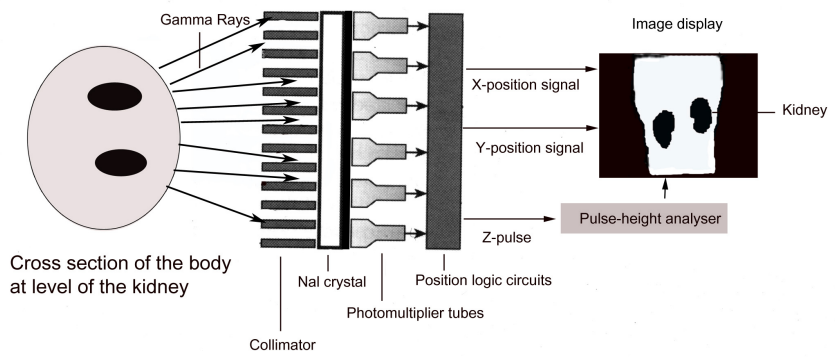


Figure 1. The illustration shows the basic components of the gamma camera. The patient would be placed against the collimator. The collimator is next to the NaI crystal (scintillation detector), which is light-coupled to the photomultiplier tubes. The X and Y positions are determined as each absorbed gamma ray is detected. The pulse height analyzer discriminates the Z pulse energy level for each photon absorbed and if the energy value is in the predefined window centered on the photopeak of the radionuclide being imaged, the event is recorded along with its X and Y position and stored in a digital format on the computer.

The gamma camera method has many advantages over the plasma clearance method. Firstly, renal scintigraphy is a quick, noninvasive method, requiring only the placement of a venous catheter and a bolus injection. The entire procedure takes only 15 minutes to complete. Urine or blood samples are not required. Secondly, serial evaluation can be used to follow the response to therapy and can provide prognostic information. Because of the short half-life and rapid extraction of ^{99m}Tc-DTPA, serial measurement can be made at intervals of a few hours, so the method can be used to study the effects of interventions such as therapeutic drug monitoring or anesthesia on renal function (Newell et al., 1997; Bostrom et al., 2002; Bostrom et al., 2003). Thirdly, the gamma camera based technique determines GFR individually for each kidney. This is very important when a diseased kidney is being considered for removal.

The ^{99m}Tc-DTPA renogram

Region of interest (ROI) selection

Once a scintigraphic image is created, actual activity (counts) can be quantified in any area of the image by the computer program looking up the number of counts within a pixel or within a designated group of pixels called a region of interest (ROI). A ROI is defined by drawing with the mouse or other cursor manually or automatically on a static image of an organ made by summing the frames of the dynamic study to get sufficient count density to define the limits of the organ.

Time activity curve (TAC)

The sum of the counts of each pixel within the ROI on each the frames of the dynamic image can be plotted against time, giving the time-activity curve (TAC) for the ROI. The renogram is the renal TAC recorded after tracer administration in a ROI delineating the kidney. A normal renogram can be described by three phases which partially overlap (Figure 2).

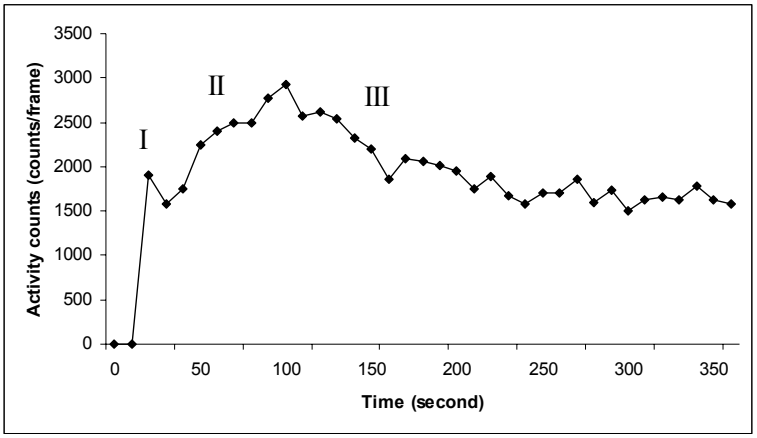


Figure 2. ^{99m}Tc-DTPA renogram of a normal kidney composed of three phases.

1. Circulation phase (bolus phase, vascular phase or perfusion phase). A rapid initial rise within 15–20 seconds after the injection of radionuclide, a peak is followed by a down slope, which at about 20–40 seconds reaches an inflection point. This bolus peak is typical for the DTPA renogram as this phase reflects the renal blood circulation since the first pass extraction of only approximately 20% of the renal plasma flow passes though the glomerular membrane, and 80% remains in the blood.

2. Uptake phase (secretary phase or functional phase). The kidney accumulates the radiopharmaceutical represented by a gradual increase in activity within the nephron through glomerular filtration. Peak renal activity in the dog was stated to be at 2.5–3.5 minutes after injection (Twardock et al., 1996). This phase expresses the functional capacity of the renal parenchyma and is therefore the most suitable for GFR determination by gamma camera.

3. Outflow phase (excretory phase). This is represented by falling slope as kidney activity decreases as the radiopharmaceutical passes out of the collecting system into the lower urinary tract. The peak of the renogram between phase 2 and phase 3 corresponds to the state when the amount of indicator leaving the kidney through the renal pelvis to the bladder exceeds the amount of tracer taken up by the kidney. This phase is of no practical relevance in the determination of renal uptake function, but is useful for evaluating obstruction to outflow, such as by pelvic or ureteral calculi.

This renogram is the net renogram, the true counts from the kidney. The actual counts from the kidney ROIs also include counts from the surrounding tissues, the background counts, which must be subtracted. These background activities are measured by drawing ROIs adjacent to the kidney. In addition, the kidney activity has to be corrected for attenuation of radiation by the tissue of the body between the kidney and the body surface.

Methods for calculation of GFR

GFR can be measured by gamma camera renography with the following techniques:

1. The integral method (Gates, 1982) (Gates' method): the parameter determined is the area under the TAC of kidney after correcting for kidney background and attenuation. The cumulative uptake (percent dose uptake) is obtained during the selected time interval. The estimated GFR is calculated by a regression equation relating the percent dose uptake of tracer to known GFR by plasma clearance. The regression is based on the percent of the injected dose of radiopharmaceutical taken up by the kidneys, related to the known GFR by plasma clearance normalized to bodyweight. (Gates, 1982; Krawiec et al., 1986). In veterinary medicine, this is the standard method (Krawiec et al., 1986; Barthez et al., 1998; Daniel et al., 1999).

2. The mean slope method (Shore et al., 1984) (uptake method): the parameter determined is the mean slope of the TAC of kidney after kidney background and depth correction. The estimated GFR is calculated by a regression equation relating the rate of uptake of tracer during the selected time interval to the known GFR by plasma clearance.

3. The normalized slope method (Piepsz, Dobbeleir & Erbsmann, 1977): the TAC of each kidney is not only corrected for external background activity but also corrected for the tracer concentration in the blood, which creates intrarenal background activity, and declines during renogram. The plasma concentration activity is obtained by drawing a ROI over the heart. The mean slope of the uptake phase of TAC of kidney after background correction is divided by the corresponding cardiac counts against time.

4. The uptake index (Patlak-Rutland plot) method (Rehling et al., 1985; Moonen et al., 1994b; Peters, 1994): This method aims to correct for the vascular activity

in the kidney ROI. The method of determining GFR is the Patlak-Rutland plot. This plot is the mean slope of the kidney uptake curve (after background correction) divided by cardiac counts as a function of the integral of cardiac counts divided by cardiac counts, which is an equivalent of time.

Factors affecting the GFR measurement by scintigraphy

The gamma camera method has been stated as less accurate than the plasma clearance method of radionuclides (Russell & Dubovsky, 1989; Barthez et al., 1998; De Santo et al., 1999; Itoh, 2003). The gamma camera is more complex than the well counter used for the plasma clearance method and factors such as field uniformly, linearity, and spatial resolution can affect the image quantification. The variations and the lower accuracy in the results by the gamma camera method compared to the plasma clearance methods could be due to the above camera factors, the accuracy of the regression equation and variabilities of the measurements made.

Quality control of scintigraphic method

Gamma camera

Routine weekly quality control of the camera is necessary to assure that imaging studies will be at the highest quality (Daniel, Poteet & Kowalsky, 1996).

Checking of full amount of injection

The radiopharmaceutical may leak at the site of injection. This needs to be checked to make sure that the amount of activity administered into the body is correct. This can be checked by obtaining an image of the injection site.

Patient motion

Movement of the dog during the dynamic acquisition period may affect kidney counts and kidney ROIs appearance (Newell et al., 1997; Barthez et al., 1998). The patient may move in some cases without it being noticed. If there is movement, the margins of the kidney will be soft, ROIs difficult to determine, and the curve will show deviations of the data points below the straight line if part of the kidney moves out of the ROI. Slight movement can be accommodated by drawing kidney ROIs large enough to encompass the motion (Cosgriff, Lawson & Nimmon, 1992). However, this may affect the background correction. Sedation may be used to prevent patient movement during acquisition. In one study in dogs, commonly used sedative protocols had no significant effect of on measured GFR values (Newell et al., 1997).

Dose of injected activity

It has been shown in humans that the error induced by statistical noise is generally very low (<3%) when using 100 MBq ^{99m}Tc-DTPA (Moonen & Jacobsson, 1997)

when measuring split renal function. For adults, a relative large range of activity for ^{99m}Tc-DTPA between 70-200 MBq has been recommended (Prigent et al., 1999). The dose of 99mTc-DTPA recommended for dogs ranges between 37-148 MBq (Twardock et al., 1996; Daniel et al., 1999).

Data acquisition technique

Collimator

A low-energy, general purpose collimator is recommended. High or ultra-high resolution collimations are not recommended for ^{99m}Tc-DTPA, as the gain in resolution provides no significant clinical gain, whereas loss in sensitivity require higher doses and higher radiation exposure (Prigent et al., 1999).

Matrix and acquisition mode

The matrix size should be matched to the particular study being done. A matrix which is too large has few counts in each pixel causing poor count statistics. A matrix which is too small has good count statistics of each pixel value but the resolution of image is less. An image matrix of 64 X 64 pixels with 16 bits of image depth (word mode) has been the standard for dynamic acquisition in dogs (Krawiec et al., 1986; Twardock et al., 1996; Barthez et al., 1998; Daniel et al., 1999). In humans, 128 X 128 pixels is recommended as the first choice; 64 X 64 is the second choice (Prigent et al., 1999). Modern computers can process the larger amount of data with no delay.

Frame rate and acquisition time

Frame rates from 5 to 15 second per frame have been recommended for dogs (Twardock et al., 1996; Daniel et al., 1999). In humans, either 10 second or 20 second frames are acceptable and equivalent for relative renal function quantitative such as GFR study (Prigent et al., 1999). Only 3 minutes of data of dynamic renography is required for GFR measurement in dogs (Twardock et al., 1996; Daniel et al., 1999). Longer acquisitions have been recommended for other specific renographic tests such as diuresis renography (O'Reilly et al., 1996).

Correction of attenuation in soft tissue

A proportion of the gamma rays emitted from the kidney are absorbed by the tissue between the kidney and camera (Figure 3). The deeper the kidney, the lower the recorded kidney counts by the gamma camera because of the greater attenuation. In human, depth corrected data are significantly different from non-depth corrected data. Correlations with creatinine clearance GFR improved dramatically with depth correction (Gates, 1982). In dogs, depth-correcting the data improved the correlations but not as much as in human (Krawiec et al., 1986) as the thickness of tissue between the camera and kidney in dogs is less than in humans.

The method of measurement may affect kidney depth. Kidney depth can be measured by ultrasound (Gruenewald, Collins & Fawdry, 1985), computed tomography (Taylor et al., 1993; Inoue et al., 2000) or a lateral image after the dynamic acquisition (Gruenewald et al., 1985; Krawiec et al., 1986; Twardock et al., 1996) (Steinmetz et al., 1998; Daniel et al., 1999). In humans, formulas based on patient characteristics such as height, weight and age have been used (Gates, 1982) (Taylor et al., 1993). In dogs, the standard method for calculating attenuation is by drawing on a single lateral image the depth of each kidney from its center to the dorsal body surface using cursors calibrated to measured pixel size (Krawiec et al., 1986; Twardock et al., 1996; Daniel et al., 1999). In adult human studies, the lateral static image has proved to be a reliable method for depth measurement, with good correlation with ultrasound (Gruenewald et al., 1985).

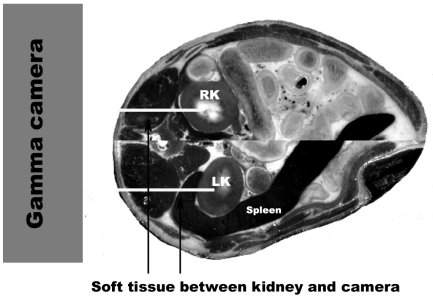


Figure 3 Cross section of abdomen of a dog at the level the center of each kidney showing that the kidneys are surrounded by soft tissue and abdominal organs. Some radioactivity from the kidney is absorbed within tissue before reaching the gamma camera. At the same time, the kidney ROIs also detect the activity from the organs in front of and behind the kidney.

The kidney depth measurement causes errors in the estimated GFR (Gruenewald et al., 1985; Awdeh et al., 1990; Moonen & Granerus, 1992; Barthez et al., 1998; Delpassand et al., 2000). Variations in depth measurement for attenuation correction are a significant problem: a 1 cm error in the estimation of true kidney depth in humans leads to 14-16% difference in the calculated GFR (Gruenewald et al., 1985; Awdeh et al., 1990).

Variation of kidney depth measurement on a lateral scintigraphic image can be caused by the appearance of the image. The display of the scintigraphic images is one of the greatest variables among departments (Prigent et al., 1999). Understanding image manipulations is essential for correct processing and interpretation of nuclear medicine studies. In dogs, a grey scale lateral image, not stated if it was linear or of other type, has been used for kidney depth measurement (Krawiec et al., 1986; Barthez et al., 1998; Daniel et al., 1999). Different color scales, particularly pseudocolor displays affect the perception of the position of the edge of the body, thus cause different kidney depth values and result in variation of correction of soft tissue attenuation. Therefore the degrees of

variability of the effects on uptake and GFR of different color scale for measuring depth need to be evaluated.

Kidney ROI selection and drawing

Times for selection of frames

In dynamic renal scintigraphy with ^{99m}Tc-DTPA, the physiological behavior of the parenchyma is not uniform as the glomeruli are located in the cortex, and with time, during the renogram (1-5 minutes) the concentration of activity moves from the cortex to the medulla as urine moves from the cortex to the tubules and collecting ducts and then the pelvis. Selecting frames after 3 minutes records more activity from the pelvis and less activity from in the cortex. Therefore to ensure that the entire cortex is included in the kidney ROI, and as little of the pelvis as possible, the frames selected for drawing the ROIs should be from the uptake period when activity is maximal in the cortex.

In dogs, the kidney image for drawing of ROIs is stated as the summed frames in the 1–3 minute acquisition interval (Daniel et al., 1999). In humans, the consensus is to assign the kidney ROIs using several minutes of summed images (Prigent et al., 1999). However, when renal function is decreased, a later summed image may be selected to obtain the best signal-to-noise ratio after the background activity has decreased (Sennewald & Taylor, 1993).

Method of drawing ROI

The choice of kidney ROI drawing is important for reproducibility and accuracy of camera-based absolute function measurement (Prigent et al., 1999). Different methods of kidney ROI drawing have been used in humans; manual freehand kidney ROI (Piepsz et al., 1977; Gates, 1982; Rehling et al., 1985; Moonen et al., 1994b), rectangular kidney ROI (Inoue et al., 1994), semi-automatic kidney ROI using a threshold technique (Tomaru et al., 1998) and polar edge search (Hornof et al., 1988), fully automatic kidney ROI methods using spatial information for two- and three- dimensional images or based on artificial neural networks (Houston et al., 1998); factor analysis of dynamic renal study using fuzzy ROIs for the extraction of TAC corresponding to renal parenchyma, renal pelvis, vascular and spatially homogeneous background (Bergmann et al., 1999).

In dogs, the standard method of kidney ROI drawing is to draw the region manually (Lourens, Dormehl & Goosen, 1982; Krawiec et al., 1986; Barthez et al., 1998; Daniel et al., 1999). Manual kidney ROI drawing has been stated likely to be the most accurate method if done by a skilled user (Hornof, 1996). However, error may occur because it depends on an operator's skill to draw the region accurately (White et al., 1999). In addition, subjective assessment of image contours can be strongly influenced by the display, particularly with pseudocolor displays as previously mentioned in section of attenuation correction (kidney depth measurement) (Hornof, 1996). This affects the perception of the edge of the

kidney and thus the size of the ROIs. The other methods of kidney ROI drawing have not been tested in dogs.

Automatic kidney ROI drawing methods should minimize the error (Houston et al., 1998; Bergmann et al., 1999; White et al., 1999). These different ROIs methods perform probably equally well in humans for relative split renal function determination as least as long as renal function is not severely decreased (Tomaru et al., 1998). No single method is recommended for use in humans (Prigent et al., 1999).

Correction of background activity

The kidney lies in the abdomen, surrounded by other organs, which contain differing amounts of radioactivity (Figure 3). Background activity is radioactivity which adds to the true activity signals from the kidney filtrate measured by the gamma camera. The background activity can be separated into extrarenal and intrarenal components. The extrarenal background activity is the scatter activity from the organs around the kidney such as liver, spleen and from tissues lying in front of and behind the kidney. The intrarenal background activity is all activity within the kidney that is not filtered. It originates from the renal blood pool activity, interstitial activity and renal pelvis activity, and decreases at a different rate from the extrarenal background (Moonen & Granerus, 1992). The intrarenal interstitial tissue background activity cannot be separated as it is already included in the kidney ROI itself. Within the first minutes after the injection, the intravascular component is rapidly falling while the interstitial part of the intrarenal background is slowly increasing. The relative activity of intrarenal and extrarenal background may change depending on kidney dysfunction. When kidney function is severely impaired, intrarenal blood volume is reduced and extrarenal background activity is high. Vascular renal neoplasms increase intrarenal blood volume (Moonen, 1994).

Correction of extrarenal background

Extrarenal background is usually corrected by finding an area outside the organ which is representative of the background in the organ ROI. The counts in this region divided by the number of pixels represents the background counts in each pixel of the organ ROI. This value is then subtracted from every pixel in the ROI.

The extrarenal background activity level is very dependent on the position of the kidney in relation to other abdominal organs (Figure 3), whose size, shape and position may vary considerably from one individual to another. For ^{99m}Tc-DTPA, with its low extraction fraction and high background activity, the choice of location of the kidney background ROIs is important. Many regions around the kidneys can be chosen as background, for example: semilunar under kidney, rectangular around kidney, perirenal ring (Houston & Sampson, 1989; Moonen & Granerus, 1992; Granerus, 2000). The published studies in dogs used manually drawn backgrounds as small areas at the cranial and caudal poles of the kidneys (Krawiec et al., 1986; Twardock et al., 1996; Barthez et al., 1998; Daniel et al.,

1999) and at only the caudal pole of the kidneys (Lora-Michiels et al., 2001). Small ROIs are not likely to be representative of the entire background (Figure 4). In humans, a two pixel wide circumferential (perirenal) kidney ROI background, surrounding the whole kidney and one pixel out from the kidney ROI was the best approximation of the extrarenal background because it incorporates the variations caused by the position and activity of the other abdominal organs (Moonen & Granerus, 1992). The most accurate size and position of perirenal ring background in dogs need to be evaluated.

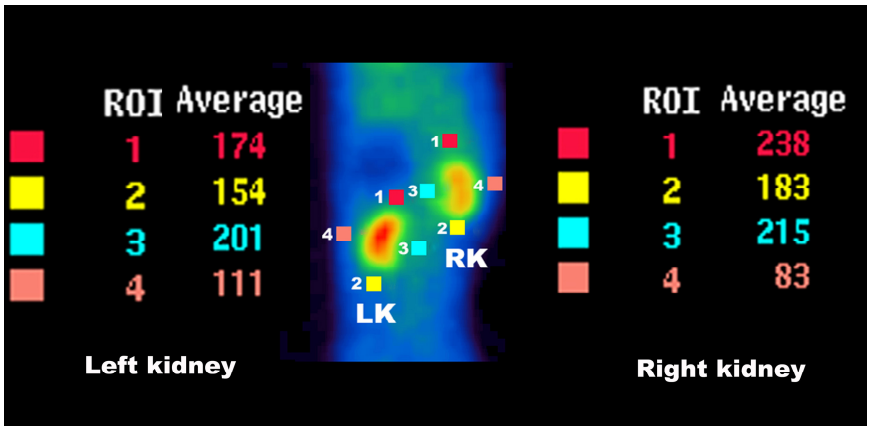


Figure 4. Showing the different activity counts around each kidney at different positions of a small rectangular ROI. The average counts per pixel ranges from 83 to 235.

Correction of intravascular intrarenal background

Methods which correct for decline of plasma activity such as normalized slope Patlak-Rutland plot (uptake index) methods, use a cardiac ROI to correct for the intrarenal (mainly intravascular) part (Piepsz et al., 1977) (Rehling et al., 1985; Moonen et al., 1994b). As the cardiac ROI is a standard size, this method is unsuitable for application to dogs as different sized ROIs to accommodate all size of dogs would require a formula for each size of ROI.

Time interval for measuring uptake

Currently with the standard method for dogs, GFR estimation using percentage kidney uptake of ^{99m}Tc-DTPA, it is presumed that during the first 3 min after injection ^{99m}Tc-DTPA has not left the kidney as the percentage uptake of ^{99m}Tc-DTPA using the integral method from 1 to 3 minute gave the best correlation with inulin clearance (Krawiec et al., 1986). However in one dog study, ^{99m}Tc-DTPA appeared in the bladder routinely within 3 min during renal scintigraphy (Barthez et al., 1998). In our routine clinical cases, we and Lourens et al. (1982) also observed that many renograms had peak activity between one and three minutes. After the peak, more activity is leaving the kidney than is being filtered and activity measured in the kidney between 1 and 3 min after the injection often underestimates kidney uptake, because the radiotracer may have begun to leave the kidney and not be available for counting. The most accurate time intervals for

physiological validity in dogs should be determined. In humans, the recommended time interval for processing is between 60 and 150 seconds postinjection, unless the time to peak has been reached (Prigent et al., 1999).

Physiological variability of dogs

Reference values, not only the absolute values but also normal ranges for GFR varied considerably between studies (Heiene & Moe, 1998). Differences in technique for measuring GFR is one possible contributor to the variations in the reported values. The GFR may vary from dog to dog and among the dogs used to represent the population at different times, as the variation of GFR in dogs is influenced by nonrenal factors such as protein intake, hydration status, sodium balance, and gender. Because kidney scintigraphy is based on sampling of data in a short time (2 minutes) compared with plasma clearance techniques that measure GFR over a longer time interval (hours), GFR values may vary considerably in an animal with a normal renal reserve capacity. These short term physiological variations within a certain range for each dog are averaged in measurements made by clearance methods over hours. The variability of GFR between and within dogs has not been investigated.

Normalization of GFR measurement

GFR measured by scintigraphy was reported as ml/min/kg in dogs with the assumption that the renal uptake or clearance is linearly related to bodyweight (BW) (Krawiec et al., 1986; Barthez et al., 1998; Daniel et al., 1999). Expressing GFR in terms of BW may not be the most physiologically correct relationship. The relationship between BW and GFR becomes non-linear in very small and very large dogs (van den Brom & Biewenga, 1981). One study in dogs has taken into consideration that metabolic rate is more closely related to body surface area (BSA) than to BW (Moe & Heiene, 1995). In humans GFR is usually expressed to BSA (ml/min/m2) (Rehling et al., 1985; Inoue et al., 1998) and this also controversial (Peters, 1992). It was pointed out long ago that such correlations are physiologically invalid (Tanner, 1949), and recently, a poor relationship between echocardiographic measurements of heart size and BW and BSA was found (Cornell et al., 2004). Heart size is related to body fluid volumes and oxygen needs.

A primary function of the kidneys is to maintain homeostasis of fluid balance (Guyton & Hall, 2000). The two major body fluid compartments are the intracellular compartment and the extracellular fluid volume (ECV). Approximately 2/3 of body fluid is intracellular and 1/3 is extracellular. The ECV is further divided into plasma volume (PV, intravascular space), and interstitial fluid volume that which is found in the microscopic spaces between cells (extravascular space, EV). Of the ECV approximately 80% is interstitial fluid and 20% is blood plasma (PV) (Guyton & Hall, 2000).

Since the function of the kidneys is homeostasis of these fluid volumes, GFR may be normalized to volumes of body fluid compartments: ECV, PV and total body water (White & Strydom, 1991; Peters, 1992). In humans, the physiological validity of expressing GFR in relation to ECV or PV has been demonstrated in a large pediatric population (Peters, Gordon & Sixt, 1994b). This normalization has been applied to dogs (Gleadhill, Peters & Michell, 1995). Its advantage over normalization to body weight or body surface area is that physiologic changes in GFR in response to changes in fluid volume rather than disease are automatically corrected for by the ratio. If GFR changes are not in proportion to the change in fluid volume, the GFR is abnormal (Peters, 1992; Heiene & Moe, 1998).

GFR is more sensitive to PV than to ECV, because volume receptors are present within the blood vessel walls. PV would also be more relevant for normalizing GFR if the interstitial fluid were to serve as a reservoir into which fluid was transferred from plasma, such as a result of renal dysfunction (Peters, Allison & Ussov, 1994a). Normalizing GFR to PV should take into account these variations, and this in theory makes it a better index as it would not be affected by state of hydration or fluid retention caused by disease or pregnancy.

A method of normalizing GFR to PV (GFR/PV) has been introduced, using the ^{99m}Tc-DTPA gamma camera renogram and without requiring a blood sample (Peters et al., 1994a). A ratio of GFR/PV to GFR/ECV of 4.0, which is the ratio of ECV to PV, was found in humans (Peters et al., 1994a), and is the same ratio according to Guyton & Hall (2000). Theoretical advantages of relating GFR to plasma volume over the standard method of scintigraphic GFR measurement used in dogs are that:

- It is a physiological correct.
- It corrects for declining plasma activity.
- It is potentially more reliable as it uses the stable Patlak plot to measure uptake of activity.
- It does not require a regression equation relating uptake to GFR by plasma clearance.

However, the validity of these principles have not been determined in practice. Many factors that can affect the reliability of this measurement, including the sizes and types of left ventricle ROI (LV ROI), and the effects of extravascular background activity and time interval chosen for the LV TAC used to measure plasma activity.

Principle of GFR/PV measurement

The principle of this measurement is based on a technique of graphical analysis of dynamic data, the Patlak plot (Rutland, 1979; Patlak, Blasberg & Fenstermacher, 1983; Peters, 1994). The Patlak technique is the plotting of one mathematical term against another. The slope of the plot is proportional to the clearance of tracer from blood to a tissue compartment, in this case, the kidney. Provided no tracer

leaves the tissue compartment, either into a third compartment or back into plasma, the rate of tissue uptake of tracer is proportional to plasma concentration.

The rate of ^{99m}Tc-DTPA that accumulates in the kidney following IV injection is equal to the product of GFR and simultaneous plasma concentration of DTPA. Therefore, the rate of increase of activity in the kidney, count rate (R, units of counts/min/min) (after background and attenuation correction) recorded from a ROI over the kidney at any time (t), before any filtered tracer has left the ROI, is equal to the product of individual kidney GFR (IKGFR, units of ml/min), and the plasma concentration (C, units of injected counts/min/ml).

$$dR(t)/dt = IKGFR \times C(t)$$

At t = 0, when all tracer is theoretically confined to intravascular fluid (plasma volume, PV),

$$dR(0)/dt = IKGFR \times C(0)$$

Since

$$C(0) = \frac{injected\ counts/min}{PV}$$

$$\frac{dR(0)/dt}{injected\ counts/min} = \frac{IKGFR}{PV}$$

$$dR(0)/dt = \alpha \bullet LV\ (0)$$

An indirect method is used to derive *dR(0)/dt*.

α in units of min-1 is the slope of the Patlak plot of uptake curve/cardiac counts as a function of the integral of cardiac counts/cardiac counts, applied to the renal and left ventricular ROI.

$$\frac{R(t)}{LV(t)} = \frac{\alpha \int LV(t) \bullet dt}{LV(t)}$$

LV (0) is derived by extrapolation of LV (t) to LV (t = 0) from a least squares fit to semilogarthmical LV (t). Details are in Paper IV.

In this method, the actual activity in the LV ROI does not need to be known, as this factor is removed during the calculations since its count rate is, respectively, inversely and directly proportional to α and LV activity (Peters et al., 1994a). However in practice a large ROI may be affected by extravascular activity and a too small ROI by low counts and statistical noise.

Extravascular activity rapidly accumulates in the thoracic wall tissues and may affect the slope and intercept of the semilogarithmic plot of LV activity (Bell & Peters, 1991).

Aims of the study

The overall purpose of the thesis was to improve the GFR measurement by scintigraphy. The specific objectives were the following:

1. To improve the accuracy of the standard method by determining sources of variation and minimize them.
    i. To compare manual and semi-automatic kidney ROIs and to investigate the variability within and between observers caused by kidney ROI drawing (Papers I and III).
    ii. To determine the most accurate size and position of perirenal kidney background ROI (Paper I).
    iii. To evaluate two different color scales for accuracy of measuring kidney depth for attenuation correction (Paper III).
    iv. To determine the most accurate time interval for calculations (Paper I).
    v. To compare slope method with integral method (Paper I).
    vi. To test the overall variability of GFR measured by scintigraphy using the integral method (Papers II and III).
2. To determine the day-to-day variability of GFR within and between dogs in a group of normal dogs (Paper II).
3. To adapt the method to dogs for measuring GFR/PV as an alternative to the standard integral method/body weight and to evaluate (Paper IV):
    i. The effect of different LV ROI size
    ii. The effect of extravascular activity background
    iii. The effect of different time intervals for LV ROI curve

Material and methods

Experimental beagle dogs were borrowed from the Department of Small Animal Sciences. They were fed a standard diet and water was accessible ad lib. They were trained to accept procedures such as blood and urine sampling. Clinical cases were examined collected from 1994 to 2004. All studies were performed at the Department of Clinical Radiology, Swedish University of Agricultural Sciences.

Dogs used

Paper I: 10 healthy laboratory female beagles, age 2-9 years, weight 10-21 kg.

Paper II: 8 healthy laboratory beagles (4 males + 4 females), age 1-6 years, weight 10-16 kg and the dogs in paper I.

Paper III: 60 clinical cases referred for evaluation of renal function during 1994-2004.

Paper IV: The dogs in paper I and paper III.

Plasma clearance method (Paper I)

The exact activity of radionuclide was measured in a well counter before injection. Heparinized blood samples were collected at 10, 20, 30, 60, 120, 180, and 240 minute intervals, after the intravenous injection for the scintigraphic method using a separate iv cannula. A standard dose was diluted (1:1000) in a flask for calibration of the activity measured in a well counter to correct for counting efficiency and dead time losses. Activity of each sample was measured from a 500uL aliquot of plasma in a gamma counter (Nuclear Chicago 1186 with a well-type NaI detector, Searle Analytic Inc., Des Plaines, Iillinois, USA) for 2 minutes. Activity was corrected for physical decay and a decay curve was plotted using the program (JMP®, SAS Institute Inc. Cary, NC, USA). The decay curve was fitted to a double exponential function and the area under the curve measured by a trapezoidal model (Daniel et al., 1999). The clearance was calculated as the injected dose divided by the area under the curve.

Scintigraphic methods (Papers I-IV)

Acquisition of data

A standard preparation of ^{99m}Tc-DTPA was used (TechneScan®; Mallinckrodt Medical B.V., Petten, The Netherlands). A low energy all purpose (LEGP) collimator on a gamma camera (Picker 300 SX®; Picker International Inc., Cleveland, OH, USA) were used. A 64 x 64 pixel matrix was used for the dynamic study and 128 x 128 matrix was used for static study. The exact amount of radioactivity injected, approximately 70 MBq, was measured by counting the

activity in front of the gamma camera before and after injection, and correcting for radioactive decay during the time interval. The dog was positioned in left lateral recumbency and the gamma camera was positioned dorsally to include the kidneys and the thorax if possible. A dynamic acquisition was started of six frames per minute for five minutes. Immediately after staring acquisition, ^{99m}Tc-DTPA was injected intravenously as a bolus flushed by 4 ml saline solution via catheter in the vein. Immediately after the dynamic acquisition period, the camera was rotated 90 degree above the dog and a static lateral 30 second image was made to measure the kidney depth. The camera was then returned to its original position and the injection tubing, cannula and syringe were counted on a stand. All data were kept in the computer and calculated using a program written for the nuclear medicine software (Hermes®; Nuclear Diagnostics, Hägersten, Sweden). If necessary the dynamic study was corrected for motion (Lord, Makela & Maripuu, 1999).

Kidney ROI drawing

In normal dogs (Paper I, II and IV) and in most clinical (unknown condition) dogs (Paper III and IV), the images from 1-2 min were summed to create a single image of the kidneys with sufficient counts to define the edges of the kidneys. In instances of poor kidney function with slow uptake and high background activity, the summed imaging time had to be extended to get sufficient count density to locate and outline the kidney.

Two different methods of kidney ROIs drawing were tested, the manual and a semi-automatic ROI method (Papers I and III). The semi-automatic ROI method was called the automatic ROI method in paper I and II. This was changed to semi-automatic ROIs in paper III and IV since this technique is not fully automatic. In paper I, the manual ROIs were all made by the same person to eliminate observer variability. ROIs were drawn semi-automatically around each kidney at threshold of 15, 20, 25, 30, 35 and 40% of the maximum pixel activity within the background subtracted kidney (Figure 5). In papers II, III and IV, only the threshold of 20% of the maximum was used as this was determined in Paper I to be the best.

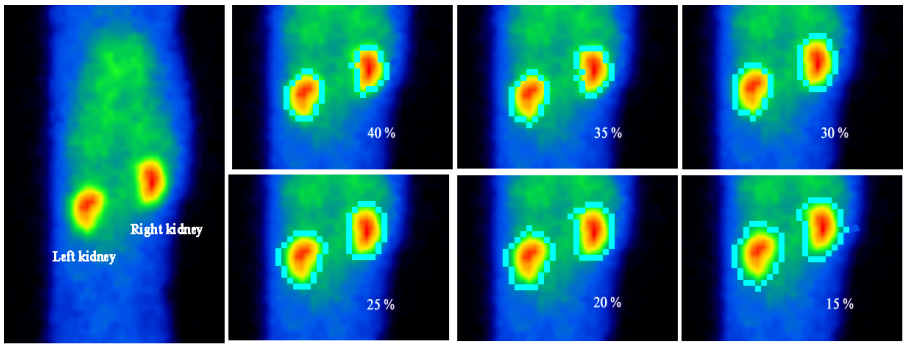


Figure 5 Kidney ROI drawing using the semi-automatic technique at the different thresholds (in percent of maximum pixel count).

The variability of kidney ROI drawing

The effect on the percentage uptake of ^{99m}Tc-DTPA of the individual kidney of the different kidney ROIs methods was tested in clinical dogs (unknown conditions) in paper III. The variabilities within and between observers of semi-automatic and manual kidney ROI drawing were investigated. Because the GFR values were calculated using regression equations which were slightly different between semi-automatic and manual ROI drawing technique, the true variability was presented as percentage uptake of ^{99m}Tc-DTPA rather that individual kidney GFR. However, to express the results as GFR units for clinical use, when possible the percentage uptake of each kidney was converted to the estimated GFR using the regressions previously derived in Paper I.

Background activity correction

For correction of background activity, only the perirenal (circumferential) background ROI was used (Moonen & Granerus, 1992). Perirenal kidney background ROIs were drawn automatically one pixel wide and zero, one, and two pixels out from the kidney ROI (Figure 6) in paper I and at one pixel out for the kidney ROI in papers II, III and IV. Background was subtracted from the kidney ROIs. We did not test the small background ROI at the pole of the kidneys compared to perirenal background ROI in Paper I. Since this paper was published, we compared the effects of three kinds of background ROIs as percentage uptake on the 58 normal kidneys.

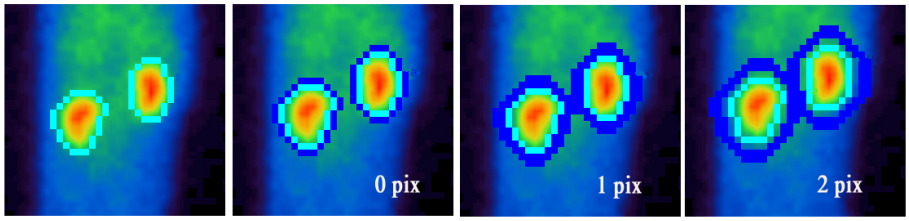


Figure 6 Background ROIs placed 0, 1 and 2 pixels out from the kidney ROIs

Kidney depth measurement and the effect of color tables on kidney depth for attenuation correction

The distance from the center of each kidney to the skin (kidney depth) was measured on the lateral image using cursors calibrated to measured pixel size with a threshold (duotone) color display (Paper I, II, III and IV) and with a continuous red-green-blue color display (Paper III) (Figure 7). The threshold table gave a sharp transition at the edge of the body, set at 1% of maximum activity in the image. The continuous red-green-blue color scale resembled the continuous grey scale color used in the original paper (Krawiec et al., 1986). The correction for soft tissue attenuation was calculated using the known attenuation coefficient for ^{99m}Tc in soft tissue (linear absorption coefficient in soft tissue = 0.153/cm).

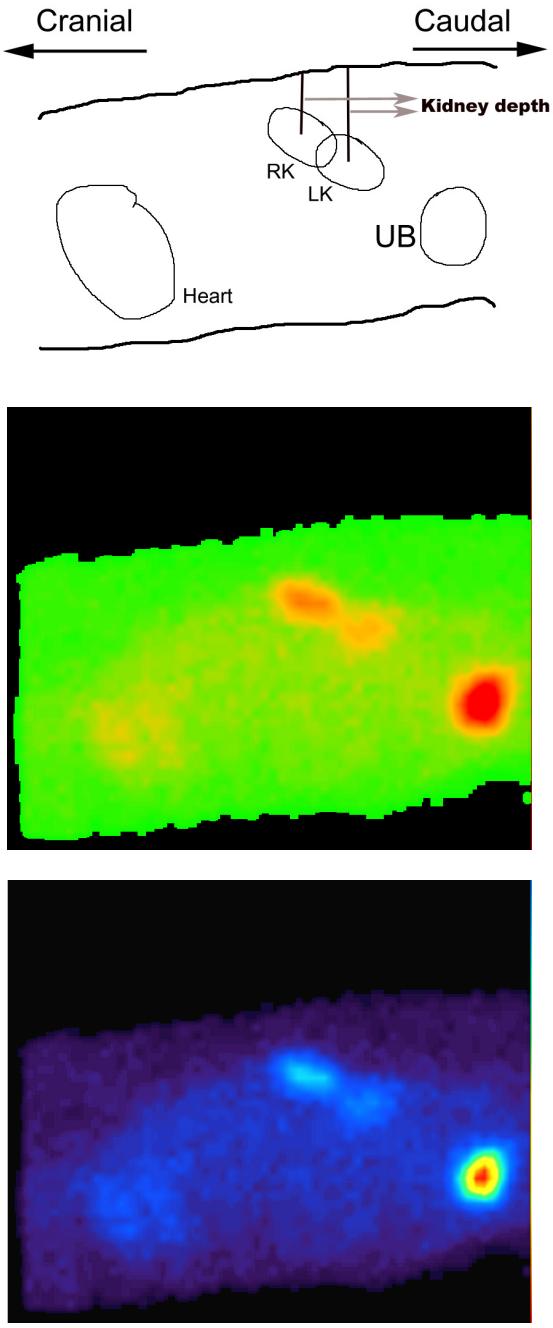


Figure 7 (a) Schematic diagram of the lateral scintigraphic image of a dog for kidney depth measurement, the kidney depth of each kidney is measured from the dog's back to the center of the kidney. (b) The threshold color table. (c) The continuous red-green-blue color table.

The effects of the two different color scales on observer variability of kidney depth were tested (Paper III). The within observer variations of the individual kidney depth and percent dose uptake of ^{99m}Tc-DTPA caused by each color table were calculated. For between observers variation, only kidney depth variation was calculated because the program did not allow calculation of percent uptake with saved ROIs necessary for standardization of all other factors.

Time interval selection of renogram

Different time intervals of TAC were tested in paper I. Cursors were used to define portions of the curve. The following time periods were tested: 30–120, 60–120, 60–180, 30–peak (unit as second) and only the straight line part of the uptake curve after 30 seconds.

Methods of calculation

For the integral method, from each time period, the total counts accumulated after background correction were measured as area under the curve and normalized to counts/min. For the mean slope method, the slope of the curve was measured as counts per sec/sec/100MBq injected, after background correction. All methods (Paper I) of measuring kidney ROIs, background ROIs, and uptake (integral and slope method) were evaluated by a regression equation with global GFR by the plasma clearance method. All calculations were made in a program written for the Hermes® nuclear medicine software.

In Papers II and III, the best correlation obtained from Paper I was incorporated into the program and the GFRs were measured using it and the corresponding best thresholds for kidney ROIs and the background ROIs. In addition, the difference between calculated GFR by renography and GFR measured by plasma clearance in Paper I was calculated by plotting the differences between the two methods are plotted against the averages of the two methods (Bland & Altman, 1986; Bland & Altman, 1999).

The overall variability

The overall variability of the integral method caused by the effects of kidney ROI drawing technique and kidney depth measurement together was evaluated (Paper II and III). In paper II, using normal dogs, the repeatability of method of the observer was tested using the regression equation obtained from Paper I and the kidney depth value was measured from the threshold table. The global estimated GFR value was average of 3 measurements of each study. In Paper III, two observers measured all studies again approximately 3-6 months after the first measurement to ensure that they did not remember any previous values. Both manual and semi-automatic kidney ROIs drawing were used and the kidney depth value was measured from different color scales in both observers.

Day-to-day variability within and between dogs

The variations of GFR in the individual normal dogs and on three different days at intervals of 5 to 26 days were studied (Paper II). The global GFR of each dog was measured by one person (NK) three times using the best method obtained from Paper I.

Normalization of GFR to plasma volume (GFR/PV measurement)

For GFR/PV measurement in Paper IV, another ROI representing plasma activity is required. The ROI was located over the heart (left ventricle, LV). Manual and three different sized automatically-drawn ROIs were evaluated. The plasma concentration curve was calculated with and without subtracting EV activity. The EV activity was measured by drawing a ROI, the same size as the LV ROI, over the right lung symmetrically (Figure 8). Two different time intervals for LV curve were evaluated: at 30 –120 seconds and 60 – 240 seconds. All compared measurements and ROI drawings were made by one person. GFR/PV was calculated by the method of Peters et al. (1994a), using a program written for the Hermes® nuclear medicine software.

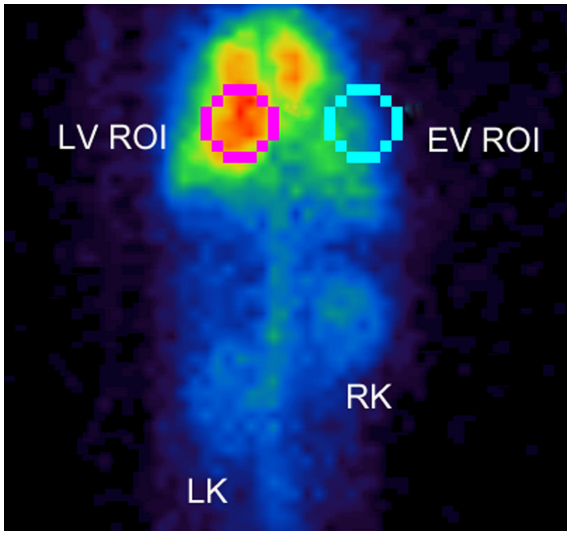


Figure 8 LV ROI (left over heart) and EV subtraction ROI (symmetrically opposite) over the right lung.

Statistical analyses

In Paper I, GFR calculated by plasma clearance was correlated and related by a regression equation to the percent uptake and the slope of the uptake phase for each of the different time intervals, for manual and various thresholds of semi-automatic kidney ROIs, and the various background ROIs tested. Linear regression analysis was used to determine the correlation and the predicted

equations. The difference between calculated GFR by renography and GFR measured by plasma clearance was calculated by a Bland-Altman plot (Bland & Altman, 1986; Bland & Altman, 1999), and 95% limits of agreement were also calculated.

In Paper II, the mean and standard deviation of GFR for the three different studies from each dog were calculated. Analysis of variance (ANOVA), using general linear model (GLM) was used to test the variation of GFR both in individual dogs and in the same dog on different days, and to test the repeatability of the method, the intra-observer variability. The coefficient of variation (CV) (the ratio of the standard deviation to the mean) of the observer variability and the GFR of the same dog on the different days were then calculated. The repeatability coefficient is $1.96\ \sqrt{2}Sw$, where Sw is the standard deviation within subject and is the square root of residual mean square obtained by one-way ANOVA with subject as a factor (Bland & Altman, 1999). The relation between weight and GFR was analyzed by correlation coefficient. Significance was tested at a 5% limit.

In Paper III, the within and between observer variability of kidney depth by the two different color tables (threshold and continuous red-green-blue table), the effect of ROI drawing (semi-automatic & manual), and the repeatability of the method were analyzed by Bland-Altman plots of the mean and mean difference. The 95% limits of agreement, 95% of the differences lie between $\bar{d} - 1.96SD$ and $\bar{d} + 1.96SD$, was then calculated (Bland & Altman, 1986; Bland & Altman, 1999). These values define the range within which most differences between measurements by the two studies/observers lie.

In Paper IV, one way ANOVA using GLM was used to determine if different sizes of LV ROI, effects of EV subtraction and different LV time intervals affected the GFR/PV value. Significance was tested at a 5% limit. SD and CV were used to evaluate the variability of the method. Bland-Altman plots (Bland & Altman, 1986) were used to determine the effect of these parameters on GFR/PV values as the absolute difference and percentage of differences values (Bland & Altman, 2002; Dewitte et al., 2002).

Results and discussion

The mean GFR (± SD) of 29 measurements of 10 normal dogs in Paper I obtained by ^{99m}Tc-DTPA plasma clearance method with 7 collected blood samples was 3.44 ± 0.62 ml/min/kg, ranging between 2.53 and 5.34 ml/min/kg. As the plasma clearance method is considered to be accurate, the range is due to difference among and within dogs, physiologic variations discussed in Paper II.

Kidney ROI drawing (Paper I)

In our studies, we did not sedate any of the dogs. Fewer than 20 percent of the studies showed some movement, which was corrected by a program realigning the frames from 30 seconds onward. Sedation may be used to prevent patient movement during acquisition (Newell et al., 1997). However, not every department uses the same sedative protocols and sedation with some agents may affect GFR.

With manual kidney ROI drawing using the integral method, the best correlation coefficient (r = 0.81) was derived from time intervals 30–120 seconds with the background ROI at 2 pixels out from the kidney. The best example of the slope uptake method was slightly better, the best correlation (r = 0.83) with ^{99m}Tc-DTPA plasma clearance found at the time interval of 30 seconds to peak of TAC using a background ROI one pixel out from the kidney ROI.

With the semi-automatic kidney ROI drawing using the integral method, the best correlation of the percentage uptake of ^{99m}Tc-DTPA of both kidneys with the GFR by ^{99m}Tc-DTPA plasma clearance (r = 0.84) was found at the threshold of 20 % maximum pixel activity using kidney background ROIs 1 or 2 pixels out from the kidney ROIs and the time interval 30–120 seconds. With the slope uptake method, the best correlation (r = 0.85) with ^{99m}Tc-DTPA plasma clearance was found at the threshold of 35 % maximum pixel activity using a background ROI 2 pixels out from the kidney ROI with the time interval of 30 seconds to peak of TAC.

The semi-automatic kidney ROIs were slightly more accurate than the manual ROIs in relation to the ^{99m}Tc-DTPA plasma clearance. The use of automatic background ROIs is likely to have minimized the variability between semi-automatic and manual kidney ROIs, compared with manual drawing of both.

Effect of observer variability on kidney ROI (Paper III)

The within observer (NK) variability of kidney ROI drawing (repeated measurement). The ^{99m}Tc-DTPA percentage uptake by semi-automatic kidney ROI was slightly higher than by manual kidney ROI in both kidneys. The 95% limits of agreement of ^{99m}Tc-DTPA percentage uptake between the first and second measurement by the same observer was wider with the manual kidney ROI than with the semi-automatic one. The GFR values could vary up to 10 % for the right kidney (RK) and 9 % for the left kidney (LK) using semi-automatic kidney ROI, and up to 14 % for the RK and 11 % for the LK for manual kidney ROI.

Between observer variability of kidney ROI drawing. The range of 95% limits of agreement of ^{99m}Tc-DTPA percentage uptake was wider using manual kidney ROI than using semi-automatic one. It was greater than the within-observer variability for manual kidney ROI. The GFR value between observers caused by semi-automatic ROI could vary up to 6 % for the RK and 8 % for the LK. Manual ROI could vary up to 15 % for the RK and 15 % for the LK.

The within observer variability of the different kidney ROI methods (semi-automatic and manual ROI). The range of the 95% limits of agreement of the second observer was nearly twice that of the first observer. For the first observer, the different kidney ROI methods could cause variation in GFR up to 7 % for both kidneys. For the second observer, the GFR value could vary up to 12% for the RK and 11 % for the LK. As the difference in variation of GFR from the different regression equations for the semi-automatic and manual ROIs was only 1 %, using the semi-automatic equation caused only 1% of variation of GFR in these results.

The variation was presented as how much of the individual kidney ROI variability affected the global GFR in term of the percentage of GFR value, which could be varied due to the kidney ROI method and observers. The variability of each kidney may be additive or subtractive in their effects on global GFR because drawing each individual kidney ROI is independent. The results indicate that manual kidney ROI causes more variation than semi-automatic one, both between and within observers. The variation between observers of semi-automatic kidney ROI was not equal. The semi-automatic method might be expected have no variability, but it is not fully automatic. Inconsistencies in drawing the size of the box around the kidney are a source of small variability. Each observer was not equally consistent at placing the box around kidneys at the same size and position.

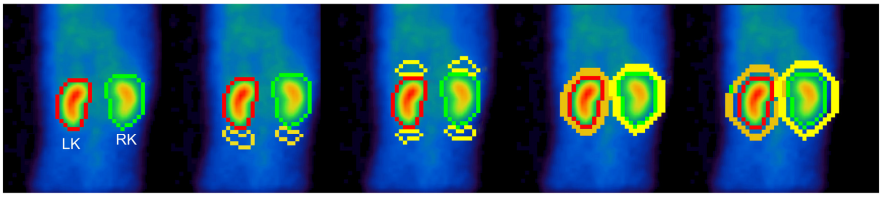
The disadvantage of the semi-automatic ROI technique is that it does not work with severely diseased kidneys, because the algorithm cannot separate kidney from the high background level. In these instances the manual ROI drawing has to be used instead. In 5 of 65 clinical dogs, due to very poor uptake, the algorithm did not work, and the semi-automatic kidney ROI drawing could not be used. In practice this is not important unless subtle improvements due to treatment are being measured.

Background activity correction (Paper I)

In Paper I, the perirenal kidney background ROIs at 1 or 2 pixels from the kidney ROI gave the best correlation coefficients using both manual ROI and semi-automatic ROI. Placement 1 or 2 pixels out from the kidney ROI ensures that no renal activity is included in the background ROI. Two pixels out from manual kidney ROIs would ensure no kidney activity in the background if the ROIs were drawn too tightly around the kidney, but particularly in small dogs, these background ROIs may overlap the opposite kidney. If the area facing the renal hilus is included in the background ROIs, a large amount of radiotracer excreted into the renal pelvis may appear in the ROI at the late phase, overestimating the background activity, and thus decreasing the net kidney counts in the late phase. This could be a substantial source of error in hydronephosis, but can be reduced by drawing the kidney ROIs from the early phase of the renogram (60- 120 seconds summed image), and only using the early part of the TAC for calculation, as we did.

The manually drawn backgrounds as small areas at the cranial and caudal poles of the kidneys (Krawiec et al., 1986; Twardock et al., 1996; Barthez et al., 1998;

Daniel et al., 1999), or only the caudal pole of the kidneys (Lora-Michiels et al., 2001) used in previous studies in dogs are probably not as accurate as the perirenal background as they sample only small parts of the background (Figure 9). The percentage uptake using a ROI at only the caudal pole or at both poles of the kidney was 33% and 5% higher respectively than with perirenal ring background ROI. The correlation coefficients using perirenal background (r = 0.80) was higher than with these two background ROI methods (r=0.67 and r=0.58) (in preparation). These small ROIs would be subject to greater individual variation than the larger perirenal ROI (Figure 4).



Kidney background ROI	Caudal pole	Cranial & caudal poles	Perirenal	Perirenal one pixel out
% Uptake of LK and RK	8.3, 11.7	6.3, 6.1	6.1, 4.5	6.7, 5.2

Figure 9. Showing different types of kidney background ROI in a dog. Note the percentage uptake of kidney ROIs after correction with background ROI are different.

The effect of color tables on kidney depth measurement (Paper III)

For the first observer, the within observer variation of the depth measurement between threshold and continuous red-green-blue color caused variation of the estimated GFR up to 4 % and 6 % of the RK and LK respectively. For the second observer, the variation was higher, approximately 7% for both kidneys. The variability between the two different colors of the second observer was higher than that of the first observer. The different variation of observers could be explained by the observers selecting different points on the slope of the edge of the profile of counts on the line between kidney and body surface. The continuous red-green-blue table caused more between-observer variation than the threshold table, both in the mean difference and 95% limits of agreement.

The threshold table measured a greater kidney depth value than the continuous red-green-blue table, causing higher percentage uptake and higher estimated GFR. With continuous red-green-blue table, the measurers apparently consistently selected a point on the body surface at the high side of the count curve of the gradual transition at the edge of the continuous tone margin. The systematic relatively greater depth with the threshold method was adjusted for in the regression equation between uptake and plasma clearance GFR.

To minimize the variation of GFR affecting by kidney depth measurement, a threshold scale which produces a consistent sharp edge should be used rather than a continuous color or grey scale. The choice of threshold percent should be

determined by testing. To ensure an accurate result, the same threshold color scale must be used for the examination as for the derivation of the regression equation.

Errors in measuring kidney depth could also be caused when selecting the center of the kidney. The center of the kidney may be erroneously located in the renal pelvis because most of the tracer has left the renal parenchyma by the time of making the lateral static view (Prigent et al., 1999). The pelvis may not be in the same plane for measuring as the center of the kidney. Placing the dog on its back may reduce this effect. Another inherent disadvantage of the single lateral view for attenuation correction is that in some instances, the location of the center of each kidney is imprecise when the two are superimposed on each other. In addition, in dogs with very poor kidney function and high background activity, the kidneys sometimes cannot be located accurately. The kidney further from the camera (left in our studies) is less well defined due to greater attenuation. This could be corrected by taking a static view of the opposite side, without moving the dog. Determination of a geometric mean of renal activity using a dual-detector gamma camera obviates the need to measure kidney depth for calculating the percentage uptake for estimating GFR and results in improved accuracy and reduced the time required for acquisition compared with single-detector cameras (Delpassand et al., 2000). However, dual-headed cameras are not universally available.

Attenuation coefficient factor

The attenuation coefficient is also considered to have a substantial effect on the accuracy of the attenuation correction (Prigent et al., 1999; Inoue et al., 2000). The linear attenuation coefficient for ^{99m}Tc in water is 0.153/cm, and this value is routinely used to correct for soft tissue attenuation in renography with ^{99m}Tc-DTPA as (Gates, 1982; Krawiec et al., 1986). We followed these examples. The effective attenuation coefficient should be lower because of scattering photons, and reported values from phantom experiments have a wide range from 0.10 to 0.14/cm (Taylor et al., 1995; Prigent et al., 1999). However, phantom experiments may not provide a sufficiently reliable attenuation coefficient for radionuclide renography. The optimal attenuation coefficient for the estimation of GFR by renography in humans was determined to be 0.087/cm in one study (Inoue et al., 2000). At the present, there is no agreement on the correct ^{99m}Tc linear attenuation coefficient; different values in different laboratories may lead to an apparent change in a patient examined in different departments (Prigent et al., 1999). But one study suggested that the theoretical value of linear attenuation coefficient (0.153) is accurate when renal activity is measured using background subtraction as most scatter will be removed by the subtraction (Hindie et al., 1999).

Time interval for measuring uptake (Paper I)

Integral method

In paper I, 50 of 58 individual renograms of normal beagles peaked before 180 sec. The mean of the peak time of TAC was 131± 30 (SD) seconds following injection. With the integral method, the correlation coefficients derived from

linear regression analysis of the GFR by ^{99m}Tc-DTPA plasma clearance and the percent dose uptake using different time intervals are shown in Table 1.

Time intervals (sec)	*Manual kidney ROIs drawing*	*Semi-automatic kidney ROIs drawing*
60-180	*0.60*	*0.59*
60-120	*0.77*	*0.77*
30-120	*0.78*	*0.80*
30-peak	*0.71*	*0.71*
straight line part	*0.74*	*0.74*

Table 1. The average of correlation coefficients derived from linear regression analysis of ^{99m}Tc-DTPA plasma clearance method and with the integral methods of measuring ^{99m}Tc-DTPA uptake at different time intervals using different distance of background ROI. With semi-automatic kidney ROIs, the value was the average from the different percent thresholds of kidney ROIs.

With both manual and semi-automatic kidney ROI drawing, the percentage dose of ^{99m}Tc-DTPA between 30–120 seconds gave the most accurate estimation of GFR compared to GFR by plasma clearance method. A histogram (Figure 10) shows the distribution of peak time of the TACs of the 58 normal kidneys (Paper I).

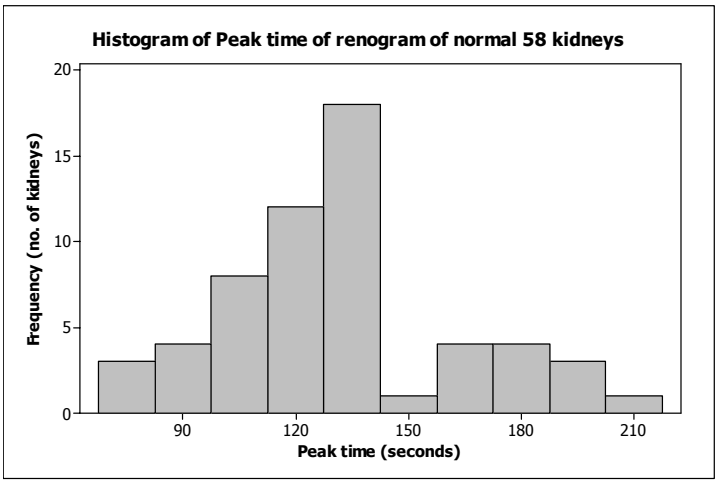


Figure 10. A histogram of peak time of 58 normal renograms.

The vascular phase was always completed by 30 seconds and the peak time of TAC was 131± 30 (SD) second after injection. Within the published time interval of 60 –180 seconds (Krawiec et al., 1986; Twardock et al., 1996), most curves had started to decline particularly when function and diuresis were good (Kunze, Bahr & Lees, 2006), and GFR values would have been underestimated because this time interval would not have been physiological valid. Therefore using the time interval of 30–120 seconds fulfilled in all but a few cases the physiologic requirement that

no activity left the kidney (Figure 10). The time to peak of excretion of ^{99m}Tc-DTPA variability can be influenced by the degree of kidney function, hydration, diuresis and pelvic volume (Granerus, 2000; Kunze et al., 2006). Slight underestimation of GFR of dogs with good diuresis would not be clinically important.

By its definition the integral method must have a fixed and constant time interval for all kidneys. The start point should be after vascular transit time. The last point should be selected before any significant escape of the tracer from the renal ROI occurs. The short time interval 30–120 seconds gives a low number of data points. This is however unavoidable, as a fixed time interval that suits almost all TACs must be used. The time interval 60–120 seconds is a even shorter curve with even fewer data points and thus more variability.

Slope method

With the slope uptake method, 60–180 seconds and 60–120 seconds time intervals had many negative values because the curve often ended lower than when it started at 60 seconds. Even 30–120 seconds gave a poor result because the slopes were highly variable. Only the variable interval of 30 seconds to peak gave reliable slopes but which were more sensitive to kidney and background threshold choices than the integral method.

Method of calculation of uptake (Paper I)

With the slope uptake method, the best correlation with known GFR by ^{99m}Tc-DTPA plasma clearance using semi-automatic ROI and manual ROI was found at the time interval of 30 second to peak of TAC with correlation coefficient (r) = 0.85 for semi-automatic ROI and r = 0.83 for manual ROI. The best correlation coefficient using slope uptake method was slightly higher than with the integral method using both kidney ROI drawing methods. With the integral method, the best correlation using semi-automatic ROI and manual ROI was found at the time interval of 30–120 seconds of TAC with r = 0.84 and 0.81 respectively.

However, the slope method was less stable than the integral method, as changes of time intervals caused a big drop in correlation coefficient, and the offset (Y-intercept) was also high, at 1.85 ml/min/kg. The much higher offset of the regression with the slope method was probably caused by the few data point of the kidney uptake TAC as in some dogs had a very short uptake time. It may also reflect a greater effect during the uptake period of declining plasma activity on the slope than on the total counts. To correct for this directly requires a plasma curve from a heart ROI, of standard size and consistent placement, in itself a source of error (Rehling et al., 1985; Peters, 1991; Moonen & Granerus, 1992).

When using DTPA as the tracer with low renal extraction fraction of 20%, the correction of the intravascular component of the background is important. This may be achieved with the uptake index method (Rutland-Patlak plot) (Prigent et

al., 1999). Neither of the methods we used for calculating uptake takes into account the decrease in plasma concentration during the time interval since it was not planned at that time. In humans, the mean slope method is not recommended because it is not validated in the literature (Prigent et al., 1999). According to this European Consensus report, the two recommended methods, which probably perform similarly well for GFR meansurement, are the integral method and the uptake index method (Rutland-Patlak plot) (Prigent et al., 1999). Because of its greater stability with different ROIs, we recommend the integral method over the slope method. The uptake index method was not tested.

Regression equation for GFR calculation (Paper I)

The regression equation by the integral method used to predict the GFR was slightly different between manual and semi-automatic kidney ROIs drawing.

For semi-automatic ROI drawing:

predicted GFR = 0.44 x percentage uptake of injected radioactivity+0.87

For manual ROI drawing:

predicted GFR = 0.45 x percentage uptake of injected radioactivity+0.90

Our regression equation was different from the two previous published equations for dogs (Krawiec et al., 1986; Barthez et al., 1998). Many differences between laboratories can cause the regression equations to be different: labeling efficiency, camera sensitivity, methods of ROI drawing and attenuation determination, and the range of GFRs used for the regression equation. The sample population would not affect the regression equation, as long as it includes a wide range of GFR down to severely decreased function. A deficiency of our regression equation is that it did not include low GFRs. This inclusion may have changed the slope and intercept of regression.

The Bland-Altman plot, comparing GFR estimated by scintigraphy using the best factors for the integral method (Paper I) with GFR by plasma clearance in the normal dogs (Figure 11), gave 95% limits of agreement of ± 0.67 ml/min/kg. This means that any measurement by this scintigraphic method has 95% likelihood of truly being with these limits, assuming the conditions are the same as in Paper I. In renal failure the estimate is not likely to be as accurate.

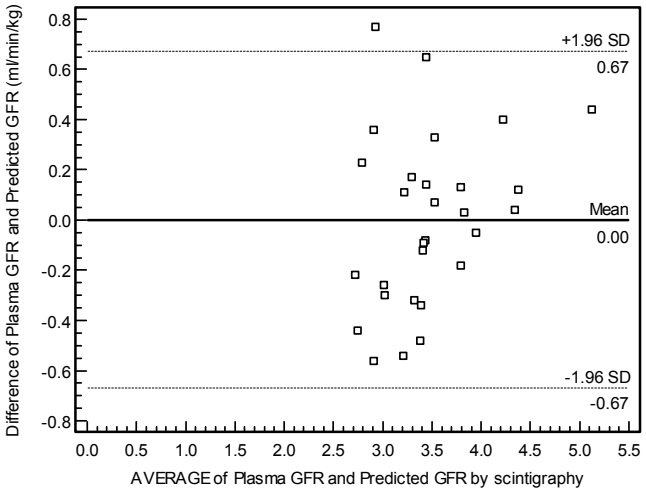


Figure 10 Bland-Altman plot of the GFR comparing between GFR by plasma clearance method and scintigraphy

The overall repeatability variation of the integral method (Papers I and III)

There was no significant effect on the repeatability of measurement in normal dogs by the same observer in Paper II. The CV% of the global estimated GFR of this observer for the repeated measurement was 1.74% ranging from 0.37 to 4.13 ± 0.9% (SD).The within-observer repeatability coefficient was 0.21 ml/min/kg, which means that the variation of the global estimated GFR was up to 5%.

In paper III, the overall repeatability variation combined the effects of different color tables for measuring kidney depth and the effects of kidney ROI drawing technique. The choice of color scale for measuring kidney depth and ROI drawing affected the overall variability. The repeatability using the semi-automatic ROI drawing was higher than with the manual one with both color scales, but was not equal between observers. The threshold table gave slightly less intra-observer variation.

The variations found in Paper II, using semi-automatic ROI and threshold table were less than in Paper III. The kidneys of Paper III were of clinical patients with a wide range of kidney function, whereas in the Paper II only healthy kidneys were studied. Moreover, in Paper II the variability of measurement was evaluated for the global GFR, not for the individual kidney. The variability of each kidney may be additive or subtractive in their effects on global GFR. The combination of variation due to kidney depth measurement and kidney ROI drawing in Paper III could cause a variation of estimated GFR up to 10 % compared to 5% in Paper II. When comparing the overall reproducibility due to color tables and ROIs on GFR with their effects on individual kidneys, the results were less than the additive effects of both factors, indicating that overall the effects were partially additive.

However, this result does not eliminate the possibility in the individual patient that the variability of the kidneys is fully additive.

In some circumstances one individual factor may be eliminated to reduce variability. For example, when monitoring how an intervention affects GFR under anesthesia, the dogs do not move, and the kidney depth is constant, therefore only kidney ROI drawing is a cause of variability. In clinical patients in which only one kidney is functioning, the variability arises from only that kidney.

The physiological variability of GFR (Paper II)

The variation of GFR on the individual dogs and on the different days was measured by one person (NK) by scintigraphy in the normal dogs (Paper II). There was a significant difference of GFR between dogs and in the same dog on different days (day to day variation). The lowest total GFR value of these healthy dogs was 2.66 ml/min/kg and the highest was 5.67 ml/min/kg. The mean total GFR was 3.97 ± 0.72 (SD) ml/min/kg, slightly different and higher than the value in normal dogs by plasma clearance method (Paper I). The variation in characteristics of dogs and the physiologic variability may affect the normal values obtained by different laboratories by different methods (Heiene & Moe, 1998). The 95 % confidence interval for the difference of GFR measurement on the same dog but on different days was ± 1.6 ml/min/kg. The mean percent coefficient of variation (CV %) of all measurements of all dogs on different days of GFR (difference between the three day to day values) was 8.45%, close to the value in humans using a gamma camera and uptake index method, in which the normal day to day variation in GFR was 8.8% (Rehling et al., 1986).

The day to day differences in GFR within and between dogs in our study may be influenced by several nonrenal factors such as protein intake, hydration status, sodium balance, and gender. In humans, GFR and renal plasma flow increase 20-30% within one or two hours after a person has eaten a high protein meal (Guyton & Hall, 2000). In our study the dogs were fed using the same food for the entire study period but the amounts eaten were not controlled as we wished to replicate the conditions of practical use as mush as possible.

The state of hydration has been shown to affect the GFR value. Because the state of hydration of a dog cannot be precisely determined by inspection, the effect of dehydration should be minimized by administration of fluids. Usually, GFR values above 3 ml/min/kg have been considered as normal (Daniel et al., 1999). In paper II, the water intake was not controlled, but was available ad lib. In some examinations, the GFRs were below 3 ml/min/kg indicating the dogs may have been slightly dehydrated on these occasions. However, no dog in paper II had a GFR value below 3 ml/min/kg at more than once of the three measurements. False interpretations of reduced kidney function in dogs because the GFR measurement was lower than normal range has been reported (Kampa & Lord, 2004).

The regression of body weight on GFR was a horizontal line, showing no systematic effect of bodyweight on the GFR (Figure 11). Among our dogs, the

body composition was quite variable, some dogs were fat and others rather thin, with a range of body weight from 10 to 21 kg for the same breed. Fat has a low metabolic rate and low fluid content, contributing little to GFR and thus the assumption that GFR is directly proportional to body weight is untrue and almost certainly contributes to the variability.

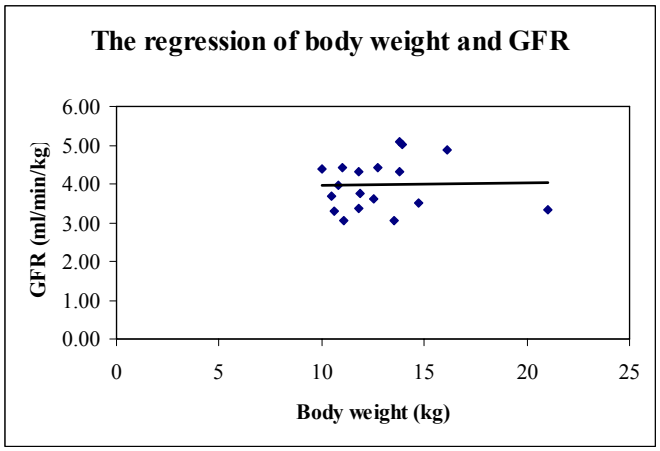


Figure 10. The correlation of body weight and GFR in 18 normal dogs, the GFR of each dog was averaged from the three measurements.

Normalization of GFR to plasma volume (GFR/PV measurement) (Paper IV)

The effect of LV ROI sizes

The results were similar both normal and clinical (unknown condition) dogs. The difference was only in the value, as the clinical material included many kidneys with decreased function, and in many dogs, total GFR was low and consequencely, background activity was high. There was no significant difference in the results using the different types of the LV ROI in normal and clinical dogs, confirming the hypothesis that the theoretical irrelevance of size was correct. Although the sizes of LV ROI did not significant affect the GFR/PV, we recommend using the manual LV ROI. In practice the fixed automatic size of LV ROI may not be suitable for different sizes of dogs. Using a small LV ROI on a large heart may increase statistical errors or the LV ROI may not be not centered on LV consistently. Although a small heart ROI includes less EV background activity from the lung than a large one, the count rate is lower, thus decreasing the statistical confidence (Moonen et al., 1994a). Using a large LV ROI in a small dog may include much lung and background activity in the chest wall relative to LV activity.

The effect of EV activity

Subtraction of EV background activity over the LV caused a significant difference in GFR/PV values in both normal and clinical groups. The assumption that a ROI over the heart following injection of ^{99m}Tc-DTPA, even a small one confined to the LV, records only the activity which originates from blood (Peters et al 1994), is not exactly true (Bell & Peters, 1991). The contribution of EV activity to the total activity recorded over the heart was calculated to be 11% at 1.5 minutes after DTPA injection, rising to 35.1% at 15 minutes (Bell & Peters, 1991). In our study, the average of the total GFR/PV of normal dogs and clinical dogs with EV subtraction were approximately 17% higher than without EV subtraction using the time interval for the plasma activity curve between 1–4 minutes, which corresponds to an interpolation of the above results in humans over the same time period.

It is also found that the effect of EV activity was greater with the shorter time intervals (30 – 120s) than the longer time intervals (60 –240s) for LV plot as the GFR/PV value measured with EV, 25% higher than without EV subtraction. EV subtraction increased both the mean difference and the 95% limits of agreement of the small compared to the large LV ROI, and of the short (30 –120s) and long (60 –240s) time intervals for the LV (t) plot. These effect of EV subtraction can be explained.

The measured plasma activity of ^{99m}Tc-DTPA in the intravascular space obtained by LV ROI is decreased using EV subtraction, and this effect is greater later in the acquisition as EV activity rises. The subtraction of the EV signal causes the slope of the plasma activity curve to be steeper than without subtraction as during the time interval some activity moves from intravascular space to EV space, and the value of LV (0) and slope α are increased increasing GFR/PV. After EV subtraction, the LV curves were often very noisy compared to those without EV subtraction, because the total counts became very low. This was due to the EV ROI representing not only EV activity alone but also including the activity in the lung blood pool, which means blood activity is being subtracted from blood activity. In our study, the EV ROI was placed over the right lung to try to avoid the large vessels such as aorta. But sometime it was difficult to avoid these vessels. Thus the EV ROI activity, which actually has a large inadvertent intravascular component, becomes a large proportion of the LV ROI activity and the net counts are low and the curve noisy.

The effect of different time intervals for LV curve

Different time intervals for the LV TAC significantly affected the results in normal and clinical dogs. The mean of the total GFR/PV of clinical dogs with time intervals of plasma activity curve at 30 –120 seconds time interval was 14% higher than using the time interval between 1-4 minutes measured without EV subtraction. In normal dogs, the difference in GFR/PV value was greater at 24%. The effect of time interval on LV plot is greater when subtracting EV activity. The lower difference in the clinical group is because the shorter time intervals of the

diseased kidneys were often long (3–4 minutes) whereas the peak time of TACs in normal kidney were usually 2-3 minutes. LV semilogarithmic plots with long time interval have more EV activity, less slope and intercept, (LV(0)), and lower GFR/PV value than that with a short time interval.

In principle, it is more physiologically correct to use the time interval from only the kidney uptake phase as there is less EV activity earlier than later. But in practice, some kidneys have a very short time of kidney uptake phase, no more than 30 – 90 seconds after injection, therefore only a few data points of LV curve are obtained and used, which makes the slope and intercept susceptible to statistical error, especially when the curve is noisy after subtracting EV background activity. Although the time intervals between 1 and 4 minutes may not physiological correct in that EV activity becomes an increasing fraction of the activity, to minimize the variation due to few data points, we recommend using a longer time such as 1 to 4 minutes.

Since there is no reference value as the gold standard, the normal value ranges of GFR/PV depend on which method or parameters are chosen. The GFR/PV value in normal dogs obtained by manual heart ROI and no EV subtraction and time interval for LV plot between 1 and 4 minutes was the following: the mean GFR/PV ± SD of the left kidney was 25.3 ±8.5 ml/min/L, ranging 14.4–51.0 ml/min/L. The right kidney was 23.3 ± 6.9 ml/min/L ranging 14.8 –42.6 ml/min/L. The global GFR/PV was 48.5 ± 14.4 ml/min/L ranging 33.5– 99.6 ml/min/L. These values are slightly higher than those obtained in humans by Peter et al. (1994a).

It is to be realized that the normalized GFR is not a measure of kidney function alone (as the absolute GFR is) but instead is a measure of kidney function in relation to the environment, in this study, PV. From our data, it cannot say that normalizing GFR to PV is more accurate than absolute GFR related to BW, because there is no objective method of comparing these two methods. Change in body fluid volume and PV influence absolute GFR and GFR related to fixed body parameters such as BW. GFR normalized to PV adjusts for these variations which do not affect intrinsic kidney function, and thus is a physiologic unit. One possible factor limiting measurement of GFR/PV is that the heart in large dogs may not always be included in the field of view of the camera; therefore the LV ROI cannot be obtained. In these cases, drawing ROI over aorta may be used instead and this needs to be tested.

Conclusions

The following conclusions can be drawn from the studies:

1. Determination of sources of variability and improvement

   i. The semi-automatic kidney ROI drawing was slightly better than manual kidney ROI drawing in term of accuracy and variation within and between observers. A semi-automatic kidney ROI drawing should be used whenever possible. 20% threshold percent of the maximum pixel activity is recommended for semi-automatic kidney ROI drawing.

   ii. The most suitable perirenal background ROI is one pixel out from the kidney ROI, and one pixel wide with a 64x64 pixel matrix.

   iii. The choice of color scales for measuring kidney depth affects the calculated attenuation and causes variations in the estimated GFR. The kidney depth measurement using continuous red-green-blue color scale causes greater variation than a threshold scale. A threshold color scale is recommended.

   iv. The time interval between 30 – 120 seconds is recommended as being the most accurate and physiological for calculating the percent dose uptake by the integral method.

   v. The integral method was less affected than the slope method by variations in kidney and background ROIs, and is recommended.

   vi. The repeatability of the method is high and acceptable in the clinic. However, the variability of each observer is not equal. It is recommended that in sequential examinations the same person should make all the measurements.

2. Physiological variability of GFR: the normal range of GFR is wide, which reflects changes in response to physiological needs. The variability of GFR between dogs is higher than day to day variability within dogs and the measurement variability indicating that these physiological changes are different from dog to dog. They may be affected by the method of normalization to BW.

3. Normalizing GFR to plasma volume:

   i. Different LV ROI sizes had no significant effect on the results, but as manual LV ROI is more flexible in adapting to different sized dogs, it is recommended.

   ii. There was significant effect of EV activity subtraction on the results and the variation was greater than without EV subtraction. Therefore, it is not recommended.

iii. Different time interval of LV plot had significant effect on the values. Shorter time intervals, had greater variability, thus LV plot time intervals of 1–3 or 4 minutes are recommended.

Future perspectives

It would be of interest to continue study further:

1. Improvement of the methods
   - It would be of interest to include dogs with impaired kidney function with known GFR by plasma clearance method in the study and see if there is better correlation coefficient.
   - A new method of semi-automatic kidney ROI drawing using smoothing technique and threashold has been developed in our department. This should also be tested if there is improving of the accuracy and reducing the variability either of method or observer.
   - The dynamic study acquisition at higher resolution, 128 x 128 matrix sizes should be used and tested to obtain the new regression equation.

2. Comparing variation of GFR related to BW and GFR/PV
   - It would be of interest to investigate the accuracy and sensitivity of the gamma camera method (GFR and GFR/PV measurement), which method is the least sensitive to variations in kidney & background ROI drawing and kidney depth measurement.
   - It would be of interest to test the effect of fluid on GFR/BW (kg) compared to GFR/PV measurement. Increased plasma volume should not affect GFR/PV.

3. Other interesting perspectives would be to for evaluating kidney function compared to the finding from ultrasound and pathology as kidney biopsy in the clinic.

4. The GFR/PV method may be adaptable to use in horse. Because of the large size of the animal, the heart would not be in the field of view. A plasma input ROI over the aorta would have to be used.

5. The comparison of renal scintigraphy between ^{99m}Tc-DTPA and ^{99m}Tc-MAG3 (mercaptoacetyltriglycine) would also be tested. MAG3 is tracer with much greater renal extraction than DTPA.

References

Awdeh, M., Kouris, K., Hassan, I.M. & Abdel-Dayem, H.M. 1990. Factors affecting the Gates' measurement of glomerular filtration rate. *American Journal of Physiologic Imaging 5*, 36-41.

Barthez, P.Y., Chew, D.J. & DiBartola, S.P. 2000. Effect of sample number and time on determination of plasma clearance of technetium Tc 99m pentetate and orthoiodohippurate sodium I 131 in dogs and cats. *American Journal of Veterinary Research 61*, 280-285.

Barthez, P.Y., Hornof, W.J., Cowgill, L.D., Neal, L.A. & Mickel, P. 1998. Comparison between the scintigraphic uptake and plasma clearance of 99mTc-diethylenetriaminepentacetic acid (DTPA) for the evaluation of the glomerular filtration rate in dogs. *Veterinary Radiology and Ultrasound 39*, 470-474.

Bell, S.D. & Peters, A.M. 1991. Extravascular chest wall technetium 99m diethylene triamine penta-acetic acid: implications for the measurement of renal function during renography. *European journal of nuclear medicine 18*, 87-90.

Bergmann, H., Dworak, E., Konig, B., Mostbeck, A. & Samal, M. 1999. Improved automatic separation of renal parenchyma and pelvis in dynamic renal scintigraphy using fuzzy regions of interest. *European journal of nuclear medicine 26*, 837-843.

Bland, J.M. & Altman, D.G. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet 1*, 307-310.

Bland, J.M. & Altman, D.G. 1999. Measuring agreement in method comparison studies. *Statistical Methods in Medical Research 8*, 135-160. 56.

Bland, J.M. & Altman, D.G. 2002. Commentary on quantifying agreement between two methods of measurement. *Clinical Chemistry 48*, 801-802.

Blaufox, M.D. et al. 1996. Report of the Radionuclides in Nephrourology Committee on renal clearance. *Journal of Nuclear Medicine 37*, 1883-1890.

Bostrom, I., Nyman, G., Kampa, N., Haggstrom, J. & Lord, P. 2003. Effects of acepromazine on renal function in anesthetized dogs. *American Journal of Veterinary Research 64*, 590-598.

Bostrom, I.M. et al. 2002. Effects of carprofen on renal function and results of serum biochemical and hematologic analyses in anesthetized dogs that had low blood pressure during anesthesia. *American Journal of Veterinary Research 63*, 712-721.

Brown, S.A. et al. 1996. Evaluation of a single injection method, using iohexol, for estimating glomerular filtration rate in cats and dogs. *American Journal of Veterinary Research 57*, 105-110.

Chew, D.J. & DiBartola, S. 1989. Diagnosis and pathophysiology of renal disease. In: Ettinger, S.J. (Ed.) *Textbook of Veterinary Internal Medicine*. WB Saunders, Philadephia. 1893-1962 pp.

Cornell, C.C. et al. 2004. Allometric scaling of M-mode cardiac measurements in normal adult dogs. *Journal of Veterinary Internal Medicine 18*, 311-321.

Cosgriff, P.S., Lawson, R.S. & Nimmon, C.C. 1992. Towards standardization in gamma camera renography. *Nuclear Medicine Communications 13*, 580-585.

Daniel, G.B., Mitchell, S.K., Mawby, D., Sackman, J.E. & Schmidt, D. 1999. Renal nuclear medicine: a review. *Veterinary Radiology and Ultrasound 40*, 572-587.

Daniel, G.B., Poteet, B. & Kowalsky, R.D. 1996. Image artifacts and quality control. In: Berry, C.R., Daniel, G.B. (Eds.) *Handbook of Veterinary Nuclear Medicine*. North Carolina State University, North Carolina. 36-44 pp.

De Santo, N.G. et al. 1999. Measurement of glomerular filtration rate by the 99mTc-DTPA renogram is less precise than measured and predicted creatinine clearance. *Nephron 81*, 136-140.

Delpassand, E.S., Homayoon, K., Madden, T., Mathai, M. & Podoloff, D.A. 2000. Determination of glomerular filtration rate using a dual-detector gamma camera and the geometric mean of renal activity: correlation with the Tc-99m DTPA plasma clearance method. *Clinical Nuclear Medicine 25*, 258-262.

Dewitte, K., Fierens, C., Stockl, D. & Thienpont, L.M. 2002. Application of the Bland-Altman plot for interpretation of method-comparison studies: a critical investigation of its practice. *Clinical Chemistry 48*, 799-801; author reply 801-792.

Dubovsky, E.V. & Russell, C.D. 1982. Quantitation of renal function with glomerular and tubular agents. *Seminars in Nuclear Medicine 12*, 308-329.

Effersoe, H., Rosenkilde, P., Groth, S., Jensen, L.I. & Golman, K. 1990. Measurement of renal function with iohexol. A comparison of iohexol, 99mTc-DTPA, and 51Cr-EDTA clearance. *Investigative Radiology 25*, 778-782.

Evans, H.E. & Christensen, G.C. 1993. The urogenital system. In: Evans, H.E. (Ed.) *Miller's anatomay of the dogs*. W.B. Saunders company, Philadephia. 3rd edition. 494-558 pp.

Finco, D.R., Coulter, D.B. & Barsanti, J.A. 1981. Simple, accurate method for clinical estimation of glomerular filtration rate in the dog. *American Journal of Veterinary Research 42*, 1874-1877.

Gates, G.F. 1982. Glomerular filtration rate: estimation from fractional renal accumulation of 99mTc-DTPA (stannous). *American Journal of Roentgenology 138*, 565-570.

Gleadhill, A. & Michell, A.R. 1996. Evaluation of iohexol as a marker for the clinical measurement of glomerular filtration rate in dogs. *Research in Veterinary Science 60*, 117-121.

Gleadhill, A., Peters, A.M. & Michell, A.R. 1995. A simple method for measuring glomerular filtration rate in dogs. *Research in Veterinary Science 59*, 118-123.

Granerus, G. 2000. Bestämning av separat njurfunktion (Determination of separate kidney function) *Njurarna och övre urinvägarna: Metoder använda inom klinisk fysiologi för diagnostik och funktionsvärdering (The kidney and upper urinary tract: methods used in clinical physiology for diagnosis and functional evaluation)*. Studentlitteratur, Lund, Sweden. 78-137 pp.

Gruenewald, S.M., Collins, L.T. & Fawdry, R.M. 1985. Kidney depth measurement and its influence on quantitation of function from gamma camera renography. *Clinical Nuclear Medicine 10*, 398-401.

Guyton, A.C. & Hall, J.E. 2000. *Textbook of Medical Physiology*. 10 edition. W.B. Saunders Company, Philadephia, Pennsylvania. 1064 pp.

Heiene, R. & Moe, L. 1998. Pharmacokinetic aspects of measurement of glomerular filtration rate in the dog: a review. *Journal of Veterinary Internal Medicine 12*, 401-414.

Hindie, E., Buvat, I., Jeanguillaume, C., Prigent, A. & Galle, P. 1999. Quantitation in planar renal scintigraphy: which mu value should be used? *European Journal of Nuclear Medicine 26*, 1610-1613.

Hornof, W.J. 1996. An introduction to computer processing of planar scintigraphic images. In: Berry, C.R., Daniel, G.B. (Eds.) *The handbook of veterinary nuclear medicine*. North Carolina State Univeristy, North Carolina. 25-35 pp.

Hornof, W.J., Cowgill, L.D., Conrad, G.R. & Fisher, P. 1988. Semiautomated renal region-of-interest selection method: validation in a dog model. *American Journal of Physiologic Imaging 3*, 133-138.

Houston, A.S. & Sampson, W.F. 1989. Comparison of two interpolative background subtraction methods using phantom and clinical data. *Nuclear Medicine Communications 10*, 121-132.

Houston, A.S., White, D.R., Sampson, W.F., Macleod, M.A. & Pilkington, J.B. 1998. An assessment of two methods for generating automatic regions of interest. *Nuclear Medicine Communications 19*, 1005-1016.

Inoue, Y., Machida, K., Honda, N., Takahashi, T. & Mamiya, T. 1994. Background correction in estimating initial renal uptake. Comparison between Tc-99m MAG3 and Tc-99m DTPA. *Clinical Nuclear Medicine 19*, 1049-1054.

Inoue, Y. et al. 1998. Evaluation of glomerular filtration rate by camera-based method in both children and adults. *Journal of Nuclear Medicine 39*, 1784-1788.

Inoue, Y. et al. 2000. Attenuation correction in evaluating renal function in children and adults by a camera-based method. *Journal of Nuclear Medicine 41*, 823-829.

Itoh, K. 2003. Comparison of methods for determination of glomerular filtration rate: Tc-99m-DTPA renography, predicted creatinine clearance method and plasma sample method. *Annals of Nuclear Medicine 17*, 561-565.

Kampa, N. & Lord, P. 2004. *The effect of fluid administration on glomerular filtration rate measured by scintigraphy in dogs*, European College of Veterinary Diagnostic Imaging meeting 11th, Ghent, Belgium. pp 32.

Kim, E.E., Barron, B.J., Lamki, L.M. & Podoloff, D.A. 1996. Genitourinary Nuclear Medicine I. In: Sandlers, M.P., Coleman, R.E., Wackers, F.J., Patton, J.A., Gottschalk, A., Hoffer, P.B. (Eds.) *Diagnostic Nuclear Medicine*. Williams&Wilkins, Baltimore. 1191-1208 pp.

Krawiec, D.R., Badertscher, R.R., 2nd, Twardock, A.R., Rubin, S.I. & Gelberg, H.B. 1986. Evaluation of 99mTc-diethylenetriaminepentaacetic acid nuclear imaging for quantitative determination of the glomerular filtration rate of dogs. *American Journal of Veterinary Research 47*, 2175-2179.

Kunze, C., Bahr, A. & Lees, G.E. 2006. Evaluation of 99mTc -diethylenetriamine-pentaacetic acid renal scintigram curves in normal dogs after induction of diuresis. *Veterinary Radiology & Ultrasound 47*, 103-107.

Levey, A.S. 1989. Use of glomerular filtration rate measurements to assess the progression of renal disease. *Seminars in Nephrology 9*, 370-379.

Liedtke, R. & Duarte, C. 1980. Laboratory protocols and methods for measurement of glomerular filtration rate and renal plasma flow. In: CG, D. (Ed.) *Renal Function Test*. Little, Brown&Co., Boston. 49-63 pp.

Lora-Michiels, M., Anzola, K., Amaya, G. & Solano, M. 2001. Quantitative and qualitative scintigraphic measurement of renal function in dogs exposed to toxic doses of Gentamicin. *Veterinary Radiology and Ultrasound 42*, 553-561.

Lord, P., Makela, O. & Maripuu, E. 1999. Evaluation of a motion correction program to improve resolution of equine scintigraphic images. 1998 EAVDI abstract. *Veterinary Radiology and Ultrasound 40*, 203. 83.

Lourens, D.C., Dormehl, I. & Goosen, D.J. 1982. The feasibility of a renogram study in dogs with radiopharmaceutical 99mTc-DTPA. *Journal of the South African Veterinary Association 53*, 243-248.

McAfee, J.G. et al. 1981. Comparison of renal extraction efficiencies for radioactive agents in the normal dog. *Journal of Nuclear Medicine 22*, 333-338.

Moe, L. & Heiene, R. 1995. Estimation of glomerular filtration rate in dogs with 99M-Tc-DTPA and iohexol. *Res Vet Sci 58*, 138-143.

Moonen, M. 1994. *Gamma Camera Renography with ^{99m}Tc-DTPA: Assessment of Total and Split Renal Function*, Division of Clinical Physiology, Göteborg Univeristy, Göteborg. 71 pp.

Moonen, M. & Granerus, G. 1992. Subtraction of extra-renal background in 99mTc-DTPA renography: comparison of various regions of interest. *Clinical Physiology 12*, 453-461.

Moonen, M. & Jacobsson, L. 1997. Effect of administered activity on precision in the assessment of renal function using gamma camera renography. *Nuclear Medicine Communications 18*, 346-351.

Moonen, M., Jacobsson, L. & Granerus, G. 1994a. Gamma camera renography with 99Tcm-DTPA: the impact of variations in input plasma curve on estimated GFR. *Nuclear Medicine Communications 15*, 673-679.

Moonen, M., Jacobsson, L., Granerus, G., Friberg, P. & Volkmann, R. 1994b. Determination of split renal function from gamma camera renography: a study of three methods. *Nuclear Medicine Communications 15*, 704-711.

Newell, S.M. et al. 1997. Effects of three sedative protocols on glomerular filtration rate in clinically normal dogs. *American Journal of Veterinary Research 58*, 446-450.

Nyland, T.G., Mattoon, J.S., Herrgesell, E.J. & Wisner, E.R. 2002. Urinary tract. In: Nyland, T.G., Mattoon, J.S. (Eds.) *Small animal diagnostic ultrasound*. W.B. Saunders company, Philadelphia. 2nd edition. 158-206 pp.

O'Reilly, P. et al. 1996. Consensus on diuresis renography for investigating the dilated upper urinary tract. Radionuclides in Nephrourology Group. Consensus Committee on Diuresis Renography. *Journal of Nuclear Medicine 37*, 1872-1876.

Patlak, C.S., Blasberg, R.G. & Fenstermacher, J.D. 1983. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. *Journal of Cerebral Blood Flow and Metabolism 3*, 1-7.

Peters, A.M. 1991. Quantification of renal haemodynamics with radionuclides. *Eur J Nucl Med 18*, 274-286.

Peters, A.M. 1992. Expressing glomerular filtration rate in terms of extracellular fluid volume. *Nephrology, Dialysis, Transplantation 7*, 205-210.

Peters, A.M. 1994. Graphical analysis of daynamic data: the Patlak-Rutland plot. *Nuclear Medicine Communications 15*, 669-672.

Peters, A.M. 2004. The kinetic basis of glomerular filtration rate measurement and new concepts of indexation to body size. *European Journal of Nuclear Medicine and Molecular Imaging 31*, 137-149.

Peters, A.M., Allison, H. & Ussov, W.Y. 1994a. Measurement of the ratio of glomerular filtration rate to plasma volume from the technetium-99m diethylene triamine pentaacetic acid renogram: comparison with glomerular filtration rate in relation to extracellular fluid volume. *European journal of nuclear medicine 21*, 322-327.

Peters, A.M., Gordon, I. & Sixt, R. 1994b. Normalization of glomerular filtration rate in children: body surface area, body weight or extracellular fluid volume? *Journal of Nuclear Medicine 35*, 438-444.

Piepsz, A., Dobbeleir, A. & Erbsmann, F. 1977. Measurement of separate kidney clearance by means of 99mTc-DTPA complex and a scintillation camera. *European Journal of Nuclear Medicine 2*, 173-177.

Prigent, A. et al. 1999. Consensus report on quality control of quantitative measurements of renal function obtained from the renogram: International Consensus Committee from the Scientific Committee of Radionuclides in Nephrourology. *Seminars in Nuclear Medicine 29*, 146-159.

Rehling, M. et al. 1985. 99mTc-DTPA gamma-camera renography: normal values and rapid determination of single-kidney glomerular filtration rate. *European Journal of Nuclear Medicine 11*, 1-6.

Rehling, M., Moller, M.L., Thamdrup, B., Lund, J.O. & Trap-Jensen, J. 1986. Reliability of a 99mTc-DTPA gamma camera technique for determination of single kidney glomerular filtration rate. A comparison to plasma clearance of 51Cr-EDTA in one-kidney patients, using the renal clearance of inulin as a reference. *Scandinavian journal of urology and nephrology. 20*, 57-62.

Ross, L.A. 1995. Assessment of renal function in the dog and cat. In: RW, K. (Ed.) *Current Veterinary Therapy IX*. W.B. Saunders, Philadelphia. 1103-1108 pp.

Russell, C.D. & Dubovsky, E.V. 1989. Measurement of renal function with radionuclide. *Journal of Nuclear Medicine 30*, 2053-2057.

Rutland, M.D. 1979. A single injection technique for subtraction of blood background in 131I-hippuran renograms. *British Journal of Radiology 52*, 134-137.

Sennewald, K. & Taylor, A., Jr. 1993. A pitfall in calculating differential renal function in patients with renal failure. *Clinical Nuclear Medicine 18*, 377-381.

Shore, R.M. et al. 1984. Glomerular filtration rate in children: determination from the Tc-99m-DTPA renogram. *Radiology 151*, 627-633.

Stacy, B.D. & Thorburn, G.D. 1966. Chromium-51 ethylenediaminetetraacetate for estimation of globerular filtration rate. *Science 152*, 1076-1077.

Steinmetz, A.P., Zwas, S.T., Macadziob, S., Rotemberg, G. & Shrem, Y. 1998. Renal depth estimates to improve the accuracy of glomerular filtration rate. *Journal of Nuclear Medicine 39*, 1822-1825.

Tanner, J.M. 1949. Fallacy of per-weight and per-surface area standard, and their relation to spurious correlation. *Journal of Applied Physiology 2*, 1-15.

Taylor, A., Jr. et al. 1995. Measuring technetium-99m-MAG3 clearance with an improved camera-based method. *Journal of Nuclear Medicine 36*, 1689-1695.

Taylor, A., Lewis, C., Giacometti, A., Hall, E.C. & Barefield, K.P. 1993. Improved formulas for the estimation of renal depth in adults. *Journal of Nuclear Medicine 34*, 1766-1769.

Tomaru, Y., Inoue, T., Oriuchi, N., Takahashi, K. & Endo, K. 1998. Semi-automated renal region of interest selection method using the double-threshold technique: inter-operator variability in quantitating 99mTc-MAG3 renal uptake. *European Journal of Nuclear Medicine 25*, 55-59.

Twardock, A.R., Krawiec, D.R. & Itkin, R.J. 1996. Renal imaging I: Functional Renal Scintigraphy. In: Berry, C.R., Daniel, G.B. (Eds.) *Handbook of Veterinary Nuclear Medicine*. North Carolina State University, North Carolina. 122-130 pp.

Twardock, A.R., Krawiec, D.R. & Lamb, C.R. 1991. Kidney scintigraphy. *Seminars in Veterinary Medicine and Surgery (Small Animal) 6*, 164-169.

Uribe, D., Krawiec, D.R., Twardock, A.R. & Gelberg, H.B. 1992. Quantitative renal scintigraphic determination of the glomerular filtration rate in cats with normal and abnormal kidney function, using 99mTc-diethylenetriaminepentaacetic acid. *American Journal of Veterinary Research 53*, 1101-1107.

van den Brom, W.E. & Biewenga, W.J. 1981. Assessment of glomerular filtration rate in normal dog: analysis of the 51Cr-EDTA clearance and its relation to several endogenous parameters of glomerular filtration. *Research in Veterinary Science 30*, 152-157.

White, A.J. & Strydom, W.J. 1991. Normalisation of glomerular filtration rate measurements. *Eur J Nucl Med 18*, 385-390.

White, D.R., Houston, A.S., Sampson, W.F. & Wilkins, G.P. 1999. Intra- and interoperator variations in region-of-interest drawing and their effect on the measurement of glomerular filtration rates. *Clinical Nuclear Medicine 24*, 177-181.

Acknowledgements

This study was carried out at the former Department of Clinical Radiology, presently at Department of Biomedical Sciences and Public Health, Division of Diagnostic Imaging and Clinical Chemistry, Faculty of Veterinary Medicine and Animal Sciences, University of Agricultural Sciences (SLU), Uppsala, Sweden.

The Royal Thai government and Khonkaen University are acknowledged for granting study leave. The research was financial supported by the Swedish Foundation for International Cooperation in Research and Higher Education (STINT), Forsbergs Stiftelse, and Agria Djurförsäkring.

I would like to express my sincere gratitude to all of you who have directly or indirectly contributed to this thesis, and especially to the following;

Professor Peter Lord, the former head of the Department of Clinical Radiology, my main supervisor, for giving me an opportunity to study here, for guidance and introducing me to the world of diagnostic imaging, and encouraging me in the academic and research world. I also would like to thank you for sharing the knowledge and experience, and taking your time for inspiring discussions on my PhD work.

Enn Maripuu, for writing many versions and upgrades of the programs for this work, thank you very much. It would not have been possible without you.

Professor Jens Häggström and Associate professor Astrid Hoppe, my co-supervisors, for taking your time with me, and also very useful guidance in the research filed.

Mieth Berger, for your technical assistance for the lab as well as for being nice friend to me. Thanks for everything that we have done together here.

Ursula Wennström, for starting the first part of work before I came, Thanks a lots.

My colleagues in the Department of Small Animal Sciences who referred the clinical cases, and especially Astrid Hoppe, urologist in this department, for her support of the project.

Per Eksell, my college, friend and roommate, for all kind of your help and thanks for the conversations (back pain issue) filled with a lot of valuable advice.

Ewa Thebo, for always helping me in any kind of problem and for your friendship.

Everybody in our department all present and former members, Kerstin, Maggi, Estelle, Charles, Ina, Judit, Hege, Anders, Susi, Lotte, Jenni, Peter, Anna & Anna, Marita, Vivan and other persons, for being extremely kind to me, sharing experiences and friendships.

My colleges at Department of Surgery, Faculty of Veterinary Medicine, KKU, in Thailand, for working hard during my absence in Sweden to study.
P'Slil, P'Tassanee, P'Aew and Khun Per, my second Thai family here, for being very kind to me and my family. Your kindness and valuable advice are definitely appreciated. Thanks for make our family feel like being at home with many nice relatives.

All of my present Thai friends here, Aran, Nok, P'Jate, P'Or, Thanks for sharing and spending a nice time with me, and also everything that we have done together. It's been a lot of fun for me.

All of my friends (who used to be here), especially some of them, who have become as my sisters and brothers, P'Lee & Padet, Nong&Seng, Boyd, Tui, P'Ma, and other nice friends, Pim, Hao, Nort, Chain, Alice, Kampon, Hun, Tui, and more…Thank you very much to you all.

All of friends at Thai association in Uppsala, TFU, P'On, P'Jang, P'Tim, K. Kongtub, P'Tuk, Nouy, and more ..for sharing a great time together.

The former ambassador of Thailand, Poksak and his wife, Supajee, for being very kind to me and my family.

My parents, Nibondh and Ampha, my grand mom, Yai-Mat, My sisters, P'Tukta and Nong Tor, for their love and encouragement.

Last and especially, my wife Nong Ae (Jaruwan) and my daughter Ten (Yada) for always sharing with love and happiness life of our family here. Thanks for love, understanding and endless support.