# CLINICAL EVALUATION OF GLOMERULAR FILTRATION

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**SUMMARY**

Glomerular filtration is the one of the most important functions of the kidney, and glomerular filtration rate (GFR) is a strong indicator of the patient's renal health because GFR is directly related to functional renal mass. Glomerular filtration rate decreases as chronic kidney disease (CKD) progresses, and ability to determine the patient's GFR allows the clinician to assess the success of treatment interventions (such as "renal" diets) intended to slow the progression of CKD. Ideally, a substance used to measure glomerular filtration rate (GFR) would have the following characteristics: it should be freely filtered by the glomeruli, not bound to plasma proteins, not metabolized, excreted only by the kidneys, and neither reabsorbed nor secreted by the renal tubules. It should be non-toxic, stable in both blood and urine, and easily measured in the laboratory.

Serum creatinine (SCr) and blood urea nitrogen (BUN) concentrations are commonly used (but relatively insensitive) screening tests of renal function whereas creatinine clearance can be determined to provide an actual estimate of GFR. Iohexol clearance also estimates GFR and has the advantage of not requiring collection of urine samples. Recently, new tests such as serum cystatin C and symmetric dimethylarginine (SDMA) concentrations have been introduced into veterinary medicine as tools to evaluate GFR.

***Blood urea nitrogen concentration***

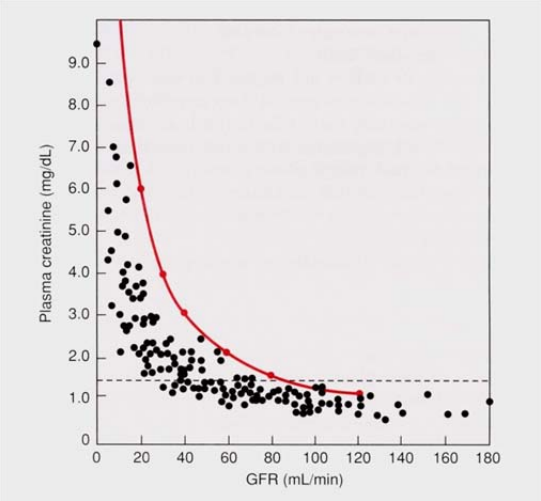
Urea is synthesized in the liver via the ornithine cycle using ammonia derived from amino acid catabolism. Amino acids used in the production of urea arise from the catabolism of exogenous (i.e. dietary) and endogenous proteins. Renal excretion of urea occurs by glomerular filtration, and BUN concentrations are inversely proportional to GFR. Urea is subject to passive reabsorption in the tubules, and this occurs to a greater extent at slower tubular flow rates which occur during dehydration and volume depletion. The production and excretion of urea do not proceed at a constant rate. Urea production and excretion increase after a high protein meal, and an 8-12 hour fast is recommended before measuring BUN concentrations to avoid the effect of feeding on urea production. Gastrointestinal bleeding can increase BUN concentrations because blood represents an endogenous protein load. Clinical conditions characterized by increased catabolism (e.g., starvation, infection, fever) also can increase BUN concentrations. Some drugs may increase BUN concentrations by increasing tissue catabolism (e.g., glucocorticoids, azathioprine) or decreasing protein synthesis (e.g., tetracyclines) but these effects usually are minimal. On the other hand, BUN concentrations can be decreased by low protein diets, anabolic steroids, severe hepatic insufficiency, or portosystemic shunting. These non-renal variables limit the usefulness of the BUN concentration as an indicator of GFR.

***Serum creatinine concentration***

Creatinine is a non-enzymatic breakdown product of phosphocreatine in muscle, and daily production of creatinine in the body is determined largely by the muscle mass of the individual. Young animals have lower concentrations whereas males and well-muscled individuals have higher concentrations. Serum creatinine concentration is not affected appreciably by diet. Creatinine is not

metabolized and is excreted by the kidneys almost entirely by glomerular filtration. Its rate of excretion is relatively constant in the steady state and SCr varies inversely with GFR. Thus, *determination of creatinine clearance provides an estimate of GFR* (see below). Greyhounds have slightly higher SCr than non-Greyhound dogs, and this difference is attributable to increased muscle mass and not to any decrease in GFR.

Creatinine usually is measured by the alkaline picrate reaction, which is not entirely specific for creatinine and measures another group of substances in plasma collectively known as non-creatinine chromagens. Non-creatinine chromagens may constitute up to 50% of the measured creatinine at normal SCr, but non-creatinine chromagens do not appear in the urine. Special methods have been designed to circumvent the problem of non-creatinine chromagens but these methods are difficult and not routinely used by commercial laboratories. As SCr increases as a result of the progression of renal disease and decrease in GFR, the amount of non-creatinine chromagens in plasma remains unchanged and contributes proportionately less to the total measured SCr. This can result in creatinine clearance being less reliable as an indicator of GFR.




As shown above, the relationship of SCr (or BUN) to GFR is a rectangular hyperbola. The slope of the curve is small when GFR is mildly or moderately decreased but large when GFR is severely decreased. Thus, large changes in GFR early in the course of renal disease cause small increases in BUN or SCr that may be difficult to appreciate clinically whereas small changes in GFR in advanced renal disease cause large changes in BUN or SCr. The inverse relationship between SCr and GFR is valid only in the steady state.

When non-renal variables have been eliminated from consideration, an increase in BUN or SCr above normal implies that at least 75% of the nephrons are not functioning. Neither the cause nor the reversibility of this malfunction can be predicted from the magnitude of BUN or SCr. Although pre-renal azotemia frequently is mild and post-renal azotemia often is severe, *the magnitude of the BUN or SCr cannot be used to predict whether azotemia is pre-renal, primary renal, or post-renal in origin.* Furthermore, the magnitude of azotemia cannot be used to distinguish between acute and chronic, reversible and irreversible, or progressive and non-progressive processes. The BUN/creatinine ratio in pre-renal and post-renal azotemia may be increased due to increased tubular reabsorption of urea at lower tubular flow rates or easier absorption of urea than creatinine across peritoneal membranes in animals with uroabdomen. A decrease in the BUN/creatinine ratio often follows fluid therapy and reflects decreased tubular reabsorption of urea rather than increased GFR.

Both BUN and SCr are relatively insensitive indicators of renal function and one is not more sensitive than the other. Serum creatinine concentration, however, is affected by fewer non-renal variables (primarily lean body mass). Localization of azotemia (i.e. differentiation of pre-renal, renal, and post-renal causes) requires consideration of the history and physical examination, the patient's urine specific gravity before any fluid therapy or drugs that may interfere with concentrating ability (e.g. glucocorticoids, diuretics), and the patient's response to fluid therapy.

***Concept of renal clearance and use of creatinine clearance to estimate GFR***

The renal clearance of a substance is that volume of plasma that would have to be filtered by the glomeruli in one minute to account for the amount of that substance appearing in the urine each minute under steady state conditions. Put differently, it is that volume of plasma that contains the same amount of the substance that is excreted in the urine in one minute under steady state conditions. The general clearance formula (ml/min) is $U_xV/P_x$. Where,

$U_x$ = Urine concentration of x (mg/dL)
$P_x$ = Plasma concentration of x (mg/dL)
V = Urine output (mL/min)

The renal clearance of a substance that is neither reabsorbed nor secreted by the tubules is equal to the GFR. For such a substance in a steady state, the amount being filtered equals the amount being excreted. Thus, GFR × $P_x$ = $U_x$ × V. Dividing both sides of the equation by $P_x$ gives the general clearance formula ($U_xV/P_x$) which in this case is equal to the GFR. If x is reabsorbed from the tubules, $C_x$ will be less than GFR because $U_x$ will be decreased and $P_x$ will be increased. If x is secreted into the tubules, $C_x$ will be greater than GFR because $U_x$ will be increased and $P_x$ will be decreased. Clearance values usually are expressed relative to body weight (ml/min/kg) or body surface area (ml/min/$m^2$).

Inulin is a polymer of fructose that meets all of the criteria for the ideal substance to measure GFR (see above), and inulin clearance is considered the "gold standard" for GFR determination. Inulin does not occur naturally in the body and must be infused into the animal at a constant rate to determine its renal clearance. Consequently, inulin is not used clinically to estimate GFR. Creatinine however is produced endogenously and excreted from the body by glomerular filtration without significant tubular reabsorption or secretion. Thus, its clearance can be used to estimate GFR in the steady state. Normal *endogenous* creatinine clearance in dogs and cats is 2 to 5 ml/min/kg. The only requirements for determination of *endogenous* creatinine clearance are an accurately timed collection of urine (ideally 24 hours), determination of the patient's body weight, and serum and urine creatinine concentrations. Failure to collect all urine produced will erroneously decrease the calculated clearance value. *Endogenous* creatinine clearance slightly underestimates GFR as measured by inulin clearance because the plasma creatinine concentration includes non-creatinine chromagens when the alkaline picrate technique is used to measure creatinine but the urine creatinine concentration does not (i.e. non-creatinine chromagens are found in plasma but not urine). In the dog, this error is somewhat offset by a weak secretory mechanism for creatinine in the renal tubules. To eliminate inaccuracy caused by non-creatinine chromagens, some investigators have advocated use of *exogenous* creatinine clearance. In this procedure, creatinine is administered subcutaneously or intravenously to increase SCr approximately 10-fold and decrease the relative contribution of non-creatinine chromagens. *Exogenous* creatinine clearance exceeds *endogenous* creatinine clearance and closely approximates inulin clearance.

In chronic progressive renal disease, urinary concentrating ability is impaired after 67% of the nephron population has become non-functional whereas azotemia does not develop until 75% of the nephrons have become non-functional. Thus, the main indication for determination of creatinine clearance is the clinical suspicion of renal disease in a patient with polyuria and polydipsia but normal BUN and

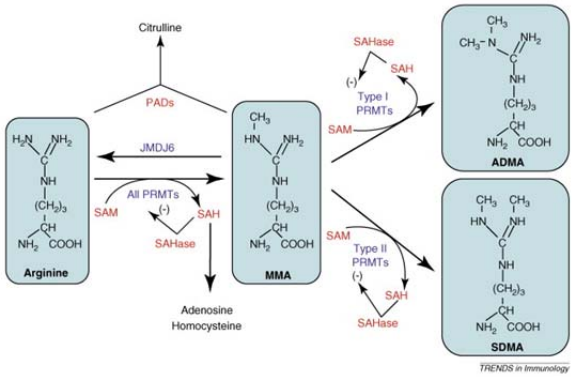
SCr. This test is not helpful in hydrated patients that are already azotemic. Variation in creatinine clearance determinations in normal animals can arise from many factors and attempts should be made to control as many of them as possible. Factors that can affect the results obtained include different methods of urine collection (i.e. voided versus catheterized samples), fasted or fed condition (i.e. protein feeding can augment GFR for several hours), state of hydration, and use of anesthesia.

***Iohexol clearance***

Iohexol is an iodinated, water-soluble contrast agent that can be used to estimate GFR in humans and domestic animals. It is non-toxic, confined to the extracellular space, not metabolized, experiences negligible binding to plasma proteins, and is excreted solely by the kidneys. Determination of iohexol clearance allows estimation of GFR with a limited number of plasma samples and without need for urine collection. Other advantages include no need for radioactivity and the stability of iodine in plasma, which allows samples to be shipped to remote laboratories. The clearance of iohexol is calculated as the dose administered divided by the area under the plasma disappearance curve. A 2-sample method with plasma samples collected at 5 and 120 minutes in dogs or 20 and 180 minutes in cats is sufficient for clinical use. Normal values for iohexol clearance are 1.7-4.1 ml/min/kg or 44-96 ml/min/$m^2$ in dogs and 1.3-4.2 ml/min/kg or 22-65 ml/min/$m^2$ in cats.

***Symmetric Dimethylarginine (SDMA)***

Asymmetric and symmetric dimethylarginine (ADMA, SDMA) are by-products of post-translational intranuclear protein methylation. Asymmetric dimethylarginine functions as an endogenous inhibitor of nitric oxide (NO) synthesis by preventing NO production from l-arginine. Symmetric dimethylarginine may act as an indirect inhibitor of NO synthesis by competing with l-arginine for transport across cell membranes. Asymmetric dimethyl arginine (ADMA) is metabolized to dimethylamine and citrulline (> 80%) whereas SDMA is eliminated unchanged by the kidneys (> 90%). Consequently, SDMA, measured by liquid chromatography-mass spectroscopy, has been evaluated as a potential indicator of GFR. Normal concentrations of SDMA are < 14 µg/dL in dogs and cats. One important advantage of SDMA is that its concentration is not affected by lean body mass as is the case for SCr. Older dogs and cats may have decreased muscle mass which can result in lower SCr, potentially delaying identification of CKD.




In one study that used iohexol clearance as the "gold standard" for GFR, 21 normal geriatric cats were compared to 21 cats with CKD (Hall et al, 2014). Median GFR in healthy geriatric cats was determined to be 1.94 ml/min/kg with 1.34 ml/min/kg being the lower 2.5 percentile. Cats with GFR < 1.34 ml/min/kg were considered to have CKD. In this study, SDMA increased a median of 17 months (range, 1.5-48 months) before SCr in cats with CKD. An SDMA concentration < 14 µg/dL had 100% sensitivity and negative predictive value compared to SCr < 2.1 mg/dL (17% sensitivity and 70%

negative predictive value). The specificity and positive predictive value of SDMA were 91% and 86% compared to 100% for SCr. In another study (Nabity et al, 2015), SDMA was compared to SCr in 8 dogs with X-linked hereditary nephropathy and 4 healthy unaffected littermates from 7 to 37 weeks of age, at which time the affected dogs had developed moderately severe azotemia (SCr 5.4-6.7 mg/dL). SDMA increased 4.1 weeks earlier than decreased GFR and 4.8 weeks earlier than increased SCr in the dogs with rapidly progressive juvenile nephropathy.

***Cystatin C***

Cystatin C is a low molecular weight cysteine proteinase inhibitor encoded by a housekeeping gene and produced at a constant rate. It is not bound to plasma proteins and is freely filtered by the glomeruli. It does undergo some tubular reabsorption but is completely catabolized within the tubular cells and none is returned to the circulation. It is not secreted by the renal tubular cells and no extra-renal clearance occurs. Thus, the serum cystatin C concentration potentially is a useful indicator of GFR.

Studies of cystatin C in dogs and cats have relied upon assays used in human medicine, but the homology between human and dog and cat cystatin C is 50 to 80%. Normal serum cystatin C concentration in dogs is approximately 1 mg/dL (range, 0.4-1.4 mg/dL) and concentrations may be lower in adult dogs than in very young or very old dogs. Its concentration also may be lower in small breed dogs (< 15 kg). No sex differences have been identified. Cystatin C increases markedly 1 hour after feeding; it remains increased for approximately 9 hours and then returns to baseline. Thus, a 12-hour fast is recommended before measuring cystatin C. The reciprocal of the serum cystatin C concentration (1/sCysC) is better correlated with GFR than is the reciprocal of the SCr (1/SCr) in humans and dogs, and it has higher sensitivity and negative predictive value. Conflicting results have been obtained in cats. Cystatin C concentration also may be affected by thyroid disease, some types of neoplasia, inflammation, and corticosteroids. Thus, additional studies must be performed before serum cystatin C concentration can be recommended as a useful indicator of GFR in dogs and cats.

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