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Feline chronic kidney disease: Pathophysiology and diagnosis

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Chronic kidney disease (CKD), defined as the structural or functional impairment of the kidneys lasting a period of at least three months,[47] is an increasingly common medical condition in domestic cats. While its exact prevalence is unknown, it is estimated that up to 20% of all cats will be affected at some point in their lifetime.[5] CKD is more commonly found in older cats, with approximately 30% of cats over the age of 15 years being diagnosed.[4] A recent study of mortality in cats found that 12.1% of deaths were attributed to renal disorders, making it the second most common cause of death in cats of all ages and the leading cause of death in cats of five years or older.[42]

The kidneys are involved in maintaining homeostasis of extracellular fluid and normally carry out a wide variety of functions. One of the most notable of these functions is the filtration of blood. Blood filtration occurs at the renal corpuscle in the cortex of the kidney, which consists of the glomerulus and Bowman's capsule. Here, fluid and solutes are filtered from blood. This ultrafiltrate is delivered to the nephron where selective reabsorption of necessary components occurs. The majority of reabsorption occurs in the proximal tubules. Filtrate moves from the proximal tubule into the loop of Henle, where water and sodium chloride are reabsorbed, then into the distal tubule and collecting duct, where the concentration of the filtrate is adjusted.[4]

In addition to carrying out blood filtration, the kidneys are involved in other essential activities including regulating acid-base balance, secreting hormones, and regulating blood pressure. Through the reabsorption of bicarbonate and excretion of ammonia and acids in urine,[21] the kidneys help to maintain a blood pH of approximately 7.4. The kidneys are also responsible for the production of the hormones erythropoietin and calcitriol, and play an important role in blood pressure regulation by releasing renin and stimulating the adrenal release of aldosterone.[4]

Because the kidneys are so deeply involved in whole-body homeostasis, a decrease in renal function can potentially result in a broad array of symptoms which are generally caused by the failure of the kidneys to reabsorb desirable compounds (such as water and proteins) and excrete undesirable ones (including phosphorus and creatinine). Often the first clinical signs noticed by cat owners are increased water intake and urinary output, followed by loss of body weight and muscle mass, lethargy, and an unkempt appearance. Although symptoms vary among individuals, many cats will also experience anorexia, vomiting, and ulcerative stomatitis. Upon physical examination and diagnostic imaging, the kidneys are often found to be small and irregularly shaped, though some cases may present with renomegaly due to renal neoplasia, pyelonephritis, or ureteral obstruction.[4]

CKD is a progressive and irreversible condition. As a result, the goal of treatment is to slow the advancement of the disease and to maintain the glomerular filtration rate of the patient at a level where clinical symptoms are tolerable or can be minimized using conservative methods.[4] The International Renal Interest Society (IRIS) has developed guidelines for the diagnosis and staging of feline CKD from stages 1 through 4, where stage 4 is the most advanced case of renal disease. Stage 1 and early stage 2 are diagnosed by the presence of abnormal kidney imaging or persistent increased serum creatinine, symmetric dimethylarginine or proteinuria. Diagnosis of late stage 2 through stage 4 require increased creatinine and symmetric dimethylarginine as well as decreased urine specific gravity.[25]

Treatments are recommended by IRIS based on the stage of the disease and generally fall into two categories: those that slow the advancement of CKD and those that address the clinical signs of CKD and seek to improve the patient's quality of life.[63] Beginning in CKD stage 2 and continuing through end-stage renal failure, IRIS recommends dietary modification; specifically,

a clinical renal diet with decreased protein, phosphorus, and sodium content and increased B-vitamin and soluble fiber content and caloric density.[47] Feeding a renal diet is believed to be the single most beneficial treatment option currently available, and many owners report higher quality of life after transitioning their cats to the new diet.[47] However, dietary modification is not without its disadvantages: changing a cat's diet can be difficult under normal circumstances, and more so when anorexia, vomiting, and other gastrointestinal symptoms are already present.

Similarly, other common treatment options present their own difficulties. Fluid therapy may be necessary to manage dehydration. IRIS recommends the intravenous or subcutaneous administration of replacement fluid solutions, such as lactated Ringer's solution, as needed.[63] Some cats will require fluid administration on a fixed schedule as many as two to three times daily, requiring owners to either give fluids at home (a problematic task for those uncomfortable with needles) or transport their cat to a veterinary clinic, which increases the stress placed on the already unwell cat. Regardless, the emotional and financial burdens can quickly add up.

Because of the limited treatment options available, particularly for end-stage renal failure, CKD may best be managed by prevention and early diagnosis. Little is known about the exact causes of renal disease. Studies of veterinary medical records have identified several potential risk factors including advanced age, certain breeds, and the presence of a pre-existing infection or injury;[51] however, the pathophysiology of CKD in cats is not well characterized despite its high prevalence. The current diagnostic methods recommended by IRIS rely on measurements of blood urea and creatinine levels,[63] which are known to be insensitive markers of kidney function and do not increase above the reference range until function has been reduced by 75%.[3] Recently symmetric dimethylarginine (SDMA) has been investigated as an earlier marker of glomerular filtration rate and has been added to the diagnostic methods recommended

by IRIS, but further research is needed to determine if any non-renal influences on SDMA exist.[55]

CKD is chronic and irreversible in nature and appears to be rapidly increasing in prevalence.[51] There is little knowledge regarding the pathology and progression of the disease, particularly in veterinary literature, where much of what is known has been extrapolated from research in human nephrology, and the limitations of the current diagnostic recommendations make it difficult to diagnose during its early stages when preventative measures would be most effective. Further research into the pathophysiology of CKD may reveal novel markers that could indicate the disease earlier and would thus be beneficial in our ability both to predict the progression of the disease and to increase the length and quality of life for CKD patients.

Pathophysiology

With feline CKD, cat owners often first seek veterinary treatment when they notice polyuria and polydipsia. These symptoms are due in large part to the kidneys' decreased ability to regulate fluid balance. Damage to nephrons prevents adequate reabsorption of water and electrolytes, resulting in large amounts of inappropriately dilute urine and a compensatory increase in drinking behavior.[47] Furthermore, up to 30% of cats with stages 2 to 4 CKD develop hypokalemia due in part to excessive renal loss of potassium. Hypokalemia often manifests first as polymyopathy (Fig. 1). The generalized muscle weakness may prevent the performance of normal behaviors such as grooming, leading to poor coat quality and matting, giving the cat an unkempt appearance.[4]

Figure 1: Hypokalemic polymyopathy in an 18-year-old domestic shorthair cat with CKD, resulting in an inability to raise the head. (4)

Biochemical symptoms of hypokalemia include metabolic acidosis, which can lead to vomiting and anorexia.[21] The resulting decrease in the amount of dietary potassium absorbed may lead to further metabolic acidosis and loss of kidney function, resulting in a hard-to-break cycle.[51] Nausea and anorexia may also be caused by other biochemical imbalances (such as azotemia or mineral imbalances), retention of toxins that would normally be excreted in urine, or comorbid conditions including anemia and hyperthyroidism.[4]

Proteinuria is often encountered in feline CKD and is considered to be a negative prognostic factor (Fig. 2).[51] Small proteins are filtered by glomeruli, then reabsorbed in the proximal tubules. Excessive protein content in the urine may be caused by impairment of one or both of these processes.[4] While proteinuria in feline CKD is typically milder than in humans, increased severity of proteinuria is strongly correlated with a decrease in survival time, though whether proteinuria contributes to the progression of CKD or is the result of a confounding variable is unknown.[51]

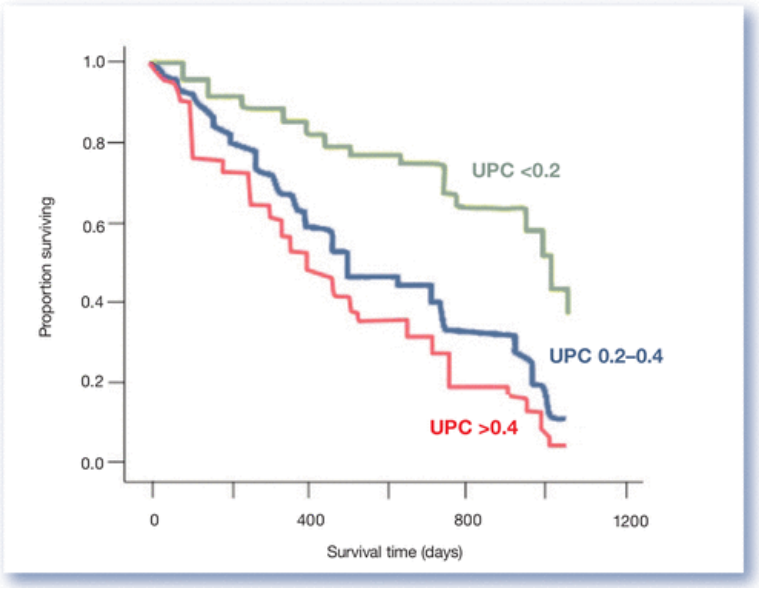

Figure 2: Effect of proteinuria on survival of cats with CKD. (51)

Blood creatinine level and urine protein:creatinine ratio (UPC) are often elevated in cats with CKD. Renal damage decreases the kidney's ability to excrete creatinine, resulting in high levels of creatinine in the blood and low levels in urine. Blood urea nitrogen (BUN) levels are similarly elevated in CKD patients due to a decrease in renal clearance.[31]

While the common symptoms of CKD are well-documented, relatively little is known about either the initial onset of feline CKD or its progression. Previous studies have been limited by multiple factors, including the unpredictability of the timeline of feline CKD, the financial and emotional costs of treatment, and the tendency of pet owners to elect for euthanasia before the end stages of renal failure. However, several potential risk factors for the development of CKD have been identified. Of these, one of the most strongly supported is acute kidney injury.

Acute kidney injury (AKI) differs from CKD in that it is often reversible and is not always associated with a decline in renal function.[12] CKD is believed to decrease the kidney's ability to recover from insults, but in some cases may develop in response to AKI.[9] The most common cause of feline AKI is thought to be nephrotoxicosis, often the result of lily, ethylene glycol (found in antifreeze), or NSAID ingestion;[56] however, in most cases the exact cause is unknown, and AKI can also be induced by traumatic injury, ureteral obstruction, and renal ischemia.[12]

The effects of renal ischemia were observed in a study involving twenty-one intact male adult cats in which the left renal artery and vein of each cat were clamped for one hour. The cats were euthanized after the procedure at time intervals ranging from three to seventy days. In the days immediately following the procedure, the affected kidneys showed necrosis of the tubular epithelium with some rapid regeneration. At day forty-two, interstitial fibrosis and tubular atrophy similar to lesions in CKD were observed and were still present at day seventy, indicating chronic and likely irreversible damage. Kidney function was reduced by 49% on average.[56] It is

estimated that up to 75% loss of function can occur before any clinical signs of renal damage appear, so unsurprisingly the cats did not display symptoms typical of chronic renal failure; however, the study demonstrates that AKI may potentially predispose cats to CKD.

Another widely-accepted risk factor for CKD is advanced age, though whether aging itself can be considered a primary renal disease or the kidneys of older cats are simply more sensitive to acute injury is still unknown.[9] Several age-related factors have been hypothesized to play a role in the development or progression of CKD, such as shortened telomere length in renal epithelial cells[49] and increased prevalence of comorbid conditions including hypertension, hyperthyroidism, and severe dental disease.[9]

A recent study of cellular senescence in the feline kidney suggests that shortened telomeres may be related to loss of renal function.[49] Telomeres, the protective caps on chromosome ends which prevent damage to DNA during replication, are normally shortened with each replicative cycle. Once they have been shortened to a length insufficient for protection during further cycles, telomeres are detected as DNA damage, triggering cellular senescence, a state of permanent cell cycle arrest.[39] An assessment of feline kidney, liver, and skin cells found that cats with CKD had significantly shorter telomeres in the proximal and distal tubular epithelia of the kidney than did either young or geriatric cats without CKD. Additionally, telomere length in the kidneys of cats with CKD was significantly shorter than in the liver and skin cells. While a causal relationship has not been demonstrated, this study provides evidence that the shortening of telomeres in the kidney may play a role in the loss of kidney function in CKD patients.[49]

The difference in telomere length between cats with CKD and normal geriatric cats suggests that aging alone does not cause CKD; however, age-related changes may increase the

likelihood of developing renal disease and may speed its progression.[9] Oxidative stress is known to contribute to renal fibrosis in humans and is suspected to play a similar role in CKD development in aged cats. Furthermore, oxidative stress is associated with common comorbid conditions such as malnutrition and anemia in humans[39] and may contribute to these conditions in cats with CKD as well. Measurements of blood levels of reduced and oxidized glutathione were compared between healthy cats and age-matched cats with CKD. The ratio of reduced to oxidized glutathione was found to be significantly higher in the cats with renal failure, suggesting a state of increased activation of antioxidant defense mechanisms. The same study also investigated the association between inflammation and the production of superoxide anion, one of the free radicals known to contribute to the progression of renal failure in humans.[28] Chronic renal inflammation is in part a result of the production of free radicals by neutrophils[29] and is thought to be an important factor in the development of glomerulosclerosis and interstitial fibrosis.[57] When a solution containing *Escherichia coli* was added to whole blood samples to create an *E. coli* to neutrophil ratio of 30:1, the neutrophils from cats with CKD showed a greater respiratory burst than those from healthy cats despite having similar percentages of neutrophils present in blood, indicating that the increased activity may contribute to renal inflammation. Interestingly, the cats with CKD were also found to be deficient in vitamin E. Previous research into potential CKD treatments found that dietary antioxidant supplements of beta-carotene and vitamins C and E may be beneficial in cats with renal failure;[72] thus, oxidative stress is a potential focus for further research.

Feline CKD is known to be associated with systemic hypertension. The overall prevalence of hypertension among cats with CKD is reported to be as high as 65%,[26] and the results of longitudinal studies indicate that there is a positive correlation between age and blood

pressure even in otherwise healthy cats.[9] While systemic blood pressure is generally under control of the central nervous system, which receives feedback from baroreceptors in the carotid sinus and aortic arch, it is also subject to influence from the renin-angiotensin-aldosterone system (RAAS). In healthy animals, a decrease in arterial pressure is detected by the juxtaglomerular apparatus in nephrons. This change in blood pressure stimulates the release of renin, which cleaves angiotensinogen (produced by the liver), converting it to angiotensin I. Angiotensin I is then converted to angiotensin II by angiotensin-converting enzyme (ACE).[8] Angiotensin II is a potent vasoconstrictor and causes increased sodium and water retention, leading to an increase in blood pressure. In humans, hypertension is a well-known factor which can lead to the development of CKD as well as a potential consequence of renal disease: chronic systemic hypertension can contribute to glomerular damage, which is associated with a further decrease in the ability of the kidneys to regulate both systemic and intrarenal blood pressure.[34]

In contrast to renal disease in humans, research has yet to identify a cause-and-effect relationship between feline CKD and systemic hypertension.[51] Longitudinal studies have found that the prevalence of both conditions is positively correlated with increasing age,[9] but hypertension is not predictive of survival time in cats with CKD.[27,61] Furthermore, in humans, chronic renal diseases including hypertension are primarily associated with glomerular damage. In cats, substantial glomerular lesions are rarely seen and are comparatively milder than in other species.[51] Instead, the majority of damage is tubulointerstitial.[4] A study of markers of renal disease in cats found that systemic hypertension is not associated with interstitial fibrosis or inflammation and is thus unlikely to be a critical factor in the progression of CKD. However, the hypertensive cats in the study were being treated with amlodipine, an antihypertensive drug.[11] Had the hypertension not been treated, the results may have been more significant. Given the

difficulty of diagnosing CKD in its early stages, it is also possible that subclinical CKD is present in some hypertensive cats that appear to be otherwise healthy.[9] The known importance of the kidneys in regulating blood pressure and the high incidence of comorbidity suggest a potential target for further research into the both the initial development and progression of CKD.

Hyperthyroidism is the most frequently diagnosed endocrine disease in geriatric cats and has also been studied in connection with CKD. Thyroid hormones decrease systemic vascular resistance, and when produced in excess also decrease the resistance of renal afferent arterioles. This leads to an increase in glomerular hydrostatic pressure.[64] If CKD is already present in hyperthyroid cats, the decreased number of functional nephrons means that intraglomerular pressure is further increased, which may cause damage to the remaining nephrons over time and further exacerbate renal disease.[4] Hyperthyroidism has been known to complicate diagnosis of CKD, as both conditions have similar symptoms. Increased thyroid hormone levels are relatively easily detected in cats, and it is only after treatment, which can take several months, that renal failure becomes apparent.[51] Given the unpredictable rate of progression of feline CKD, an extended delay in diagnosis and treatment of kidney failure could potentially have devastating consequences for the patient's prognosis.

Among the less well-documented risk factors of CKD are dental disease, various viral infections, and frequency of certain vaccines (Fig. 3B). Chronic periodontitis is associated with declining kidney function in humans. Periodontitis, a bacterial infection of the gums which may destroy the bone and soft tissue around the teeth, can elicit systemic inflammatory responses when bacteria enter circulation through ulcerated gingival tissue.[19] Although the relationship between inflammation and CKD has not been extensively studied in cats, multiple inflammatory

markers are associated with the development of CKD in human patients.[20] A bidirectional relationship may exist between dental disease and feline CKD. Gastrointestinal complications such as uremic ulcerative stomatitis and halitosis occur in up to 40% of cats.[51] While the exact reason for these symptoms is undetermined, stomatitis is thought to develop as a result of high levels of ammonia[1] and often resolves with a reduction in blood urea nitrogen levels.[47] However, it is also worth noting that cats must be anesthetized in order to perform dental work, and the effects of general anesthesia on renal function may be a potential confound.

Various viral diseases may also play a role in predisposing cats to CKD. Nephropathies are frequently encountered in people with human immunodeficiency virus; though the precise mechanisms are still unknown, it is suspected that the response of renal cells to HIV proteins contribute to glomerular disease with nonspecific tubulointerstitial lesions.[9,46] The closely related feline immunodeficiency virus (FIV) in cats is causes a similar infection and is thought to adversely affect kidney function. FIV is not uncommon in cats, particularly in feral colonies, and is most often transmitted between cats through bite wounds. Many individual cats, after an initial acute phase of infection shortly after the virus is acquired, remain asymptomatic for a period of years or even throughout their lifespan. In approximately 30% of cats, the infection proceeds to a stage resembling AIDS in humans in which they develop immunodeficiency resulting in secondary infections. A study of cats infected naturally and experimentally with FIV discovered alterations in kidney structure and function that closely mimic those found in humans with HIV. After euthanasia either two or three years post-infection (approximated in naturally-infected cats), 85% of the cats were found to have glomerular nephropathy, and 76% had tubulointerstitial damage.[46] The high frequency of glomerular alterations in FIV-infected cats does not reflect findings in the general population of cats with CKD, in which less than 10% are believed to

develop glomerulonephritis.[51] However, FIV-positive cats may not represent a significant portion of cats diagnosed with CKD, whether because of an overall low rate of occurrence of the virus or because they are more likely to die of secondary infections prior to developing renal disease. It is also possible that glomerular disease is slightly more common than reported due to the higher frequency of FIV in feral colonies where cats are unlikely to receive diagnosis of or live long enough to develop CKD. Regardless, the significant association between CKD and both HIV and FIV warrants further study.

An epidemiological study on potential risk factors found a potential correlation between CKD and the frequency of vaccinations (Fig. 3B).[17] Vaccines protecting against feline herpesvirus 1, calicivirus, and panleukopenia, including the commonly used FVRCP combination vaccine, are produced using viral cultures on Crandell-Rees feline kidney (CRFK) cells. The manufacturing process used does not prevent CRFK proteins from being incorporated into the vaccines.[67] Exposure to CRFK antigens upon administration of the vaccine may induce an immune response and the production of autoantibodies due to the similarity of CRFK cells to endogenous kidney cells. Results of the

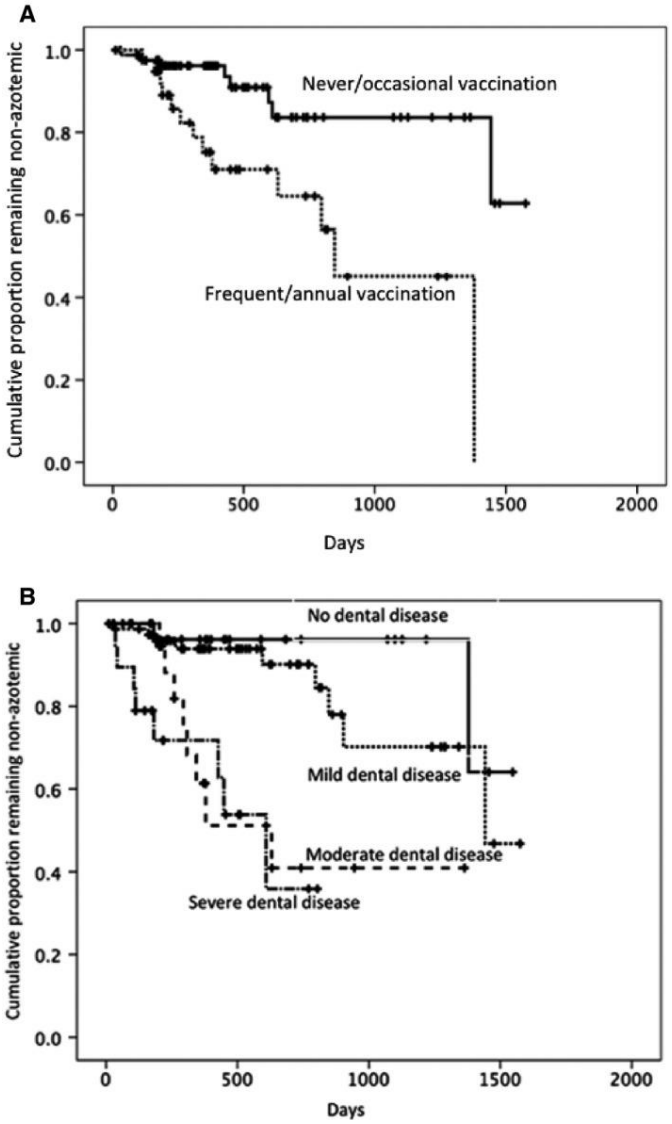

Figure 3: Risk of developing azotemia. (A) Vaccination status. (B) Dental disease category. (17)

study indicate that cats that were vaccinated annually were more likely to develop CKD. However, data on vaccination history was collected using an online survey rather than veterinary records, introducing some potential confounds. The researchers note that owners who were willing to volunteer their time and complete the survey may have been more inclined to maintain a consistent vaccine schedule. It is also possible that participants answered the questions in a way that they believed would cause others to view them more favorably.[17]

Regardless of the cause of feline CKD in individuals, tubulointerstitial fibrosis appears to be the most common final outcome.[51] Like many organs, the kidney has the ability to repair itself to some extent following acute injury. Successful repairs occur following epithelial cell proliferation along a scaffold formed by the basement membrane. It is speculated that in patients with CKD, the kidneys fail to terminate the healing response, leading to expansion of the extracellular matrix (ECM) which destroys the healthy tissue.[35] The reason for this failure is unclear, though it may be related to an arrest in the epithelial cell cycle during the G2/M phase.[70] Normally, the ECM is maintained by interstitial fibroblasts through the synthesis of ECM as well as ECM-degrading proteases. It is believed that activation of fibroblasts causes them to undergo a phenotypic change into myofibroblasts, which play a critical role in the excessive production of ECM.[38] Several mechanisms through which fibroblast activation occurs have been proposed, perhaps most notably those involving transforming growth factor beta (TGF-β) and the RAAS.

TGF-β is a family of cytokines expressed by all cell types in the kidney,[35] though epithelial cells appear to be the main source.[68] Once secreted, TGF-β must be activated through proteolytic cleavage.[35] Researchers have identified multiple potential activation triggers, including oxidative stress and an acidic environment.[2] Following activation, TGF-β regulates gene transcription by binding to TGF-β receptor II, resulting in myofibroblast formation from

multiple cell types; increased production of ECM by tubular and endothelial cells; decreased ECM degradation; and increased production of connective tissue growth factor, a pro-fibrotic cytokine.[35]

While hypertension itself has not been definitively linked to either the development or progression of feline CKD, the RAAS is known to have proinflammatory and profibrotic effects through the upregulation of TGF-β signaling. Renin increases TGF-β production,[35] and aldosterone, which is released from the adrenal glands in response to stimulation by angiotensin II (AngII), acts on a number of renal cell types to increase reactive oxygen species production and upregulate expression of epithelial growth factor and angiotensin type 1 receptors.[58]

AngII is increasingly believed to mediate fibrosis through its interactions with TGF-β and Smad signaling pathways. Smad proteins make up a family of signal transducers for TGF-β-related responses.[53] Binding of AngII to the angiotensin type 1 receptor activates the ERK/p38/MAPK signal cascade, resulting in the phosphorylation and activation of Smad2 and Smad3, which form a complex with Smad4. The complex enters the nucleus, allowing transcription of the genes regulating TGF-β expression.[40] Overexpression of Smad in response to AngII has also been shown to be associated with an increase in connective tissue growth factor followed by fibrosis, indicating that AngII has a role in CKD progression independent of its effects on systemic blood pressure.[53] The contributions of a number of other factors associated with AngII- and TGF-β-related pathways have also been investigated. Transglutaminase 2 (TG-2) is directly involved in ECM stabilization through the cross-linking of ECM proteins.[35] It also plays a role in activating TGF-β as well as cross-linking TGF-β to the ECM.[59] A study of postmortem feline kidney tissue found that tubulointerstitial TG-2 was upregulated in cats with

CKD and positively correlated with both tubulointerstitial fibrosis and decreased renal function.[54]

Chronic inflammation has been implicated in fibrotic tissue formation. In an effort to determine whether inflammatory processes are causal factors or results of fibrosis, researchers investigated the roles of macrophages and T-cells following AKI in mice and rats.[32,62] Macrophages have well-described anti-inflammatory properties, undergoing a change from a proinflammatory to a suppressive phenotype.[37] However, macrophages may be involved in fibrosis through direct or indirect interactions with TGF-β. In rodent models of CKD following AKI, TGF-β expression decreases in response to macrophage depletion.[32] The precise mechanisms are unknown, but TGF-β may be produced or activated in response to proinflammatory cytokines released by macrophages.[59]

Decreased expression of the protein Klotho may also be related to fibrosis. The Klotho gene has been referred to as an aging-suppressor; Klotho-deficient mice are known to display accelerated aging phenotypes, while mice in which the gene is overexpressed have extended lifespans. Three forms of the protein are known: membrane Klotho, which complexes with fibroblast growth factor receptors; secreted Klotho, which regulates growth factor signaling, oxidative stress, and ion homeostasis; and intracellular Klotho, which suppresses cellular senescence due to inflammation.[30] The membrane form of Klotho in particular is thought to be involved in pathways leading to renal fibrosis. The membrane form of Klotho is an obligate co-receptor for fibroblast growth factor (FGF) receptors and acts to increase receptor affinity for FGF23. A decrease in Klotho expression is believed to be linked to elevated levels of circulating FGF23, which has been shown to activate fibroblasts and may play a role in the activation of TGF-β signaling, thereby mediating profibrotic pathways.[60]

Tubulointerstitial hypoxia is thought to be the final common pathway in the progression of CKD to end-stage renal failure. Tubular epithelial cells have a high metabolic rate and are therefore vulnerable to a reduction of oxygen supply through ischemia, vasoconstriction resulting from RAAS overactivation, and increased distance between capillaries and epithelial cells due to extracellular matrix (ECM) expansion.[35] Moreover, partial loss of function is followed by a compensatory increase in single nephron GFR,[24] resulting in an up to threefold increase in the oxygen consumption of the remaining healthy nephrons and subsequent further damage due to the increase in metabolic rate.[23] Direct relationships also appear to exist between hypoxia and fibrosis: decreased oxygen supply causes tubular cells to transition to myofibroblasts, stimulates inflammatory cell recruitment, and increases ECM production through its effects on fibroblasts.[35]

Diagnosis

The current diagnostic guidelines set by the International Renal Interest Society are based on several biochemical factors (Fig. 4). Abnormal kidney imaging, an increase of either blood creatinine or symmetric dimethylarginine (SDMA) levels within the reference interval, or persistently increased SDMA (>14 µg/dL) or renal proteinuria (UPC>0.4) are indicative of stage 1 or early stage 2 CKD. More severe renal disease, considered late stage 2 to stage 4, is indicated if both creatinine and SDMA are above the reference interval and urine specific gravity is below 1.035.[14] After a diagnosis is made, the stage is decided based on at least two measurements, taken at least two weeks apart, of fasting blood creatinine and SDMA levels when the cat is well hydrated. Further substaging is based on proteinuria and blood pressure.[25]

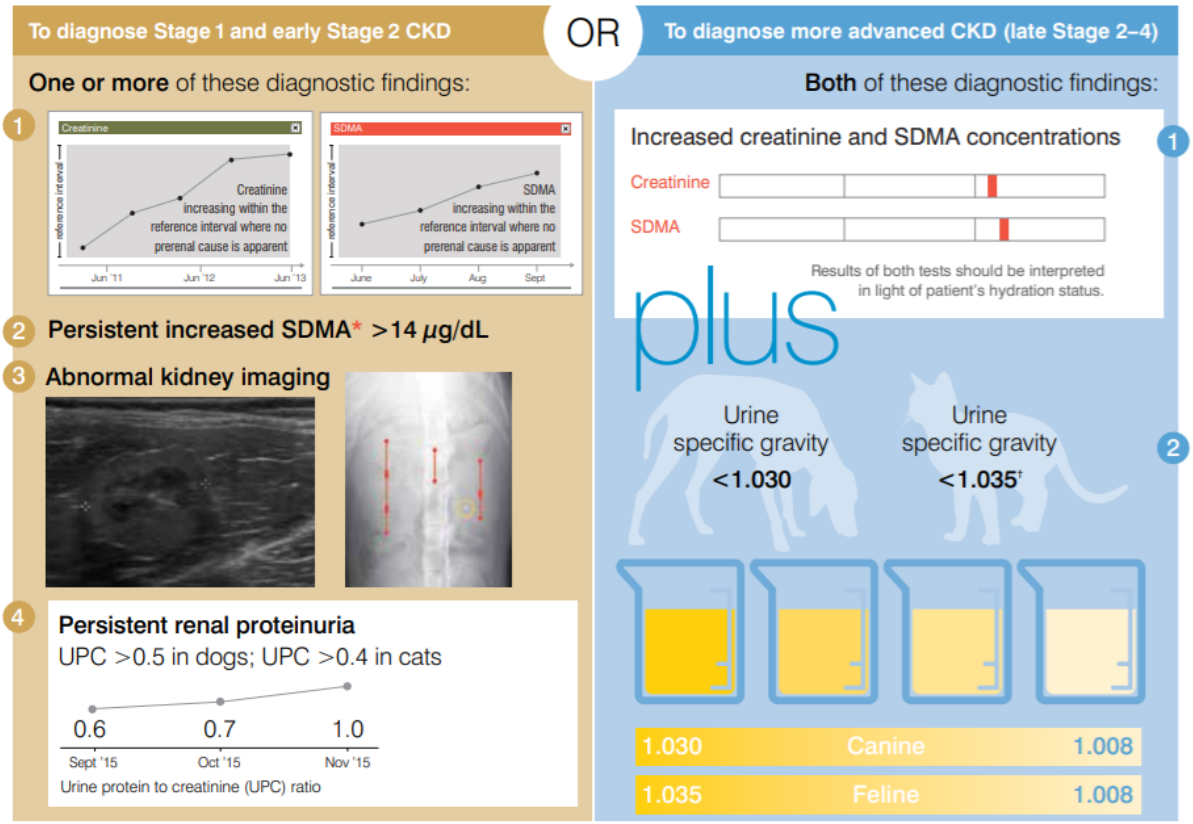

Figure 4: IRIS CKD diagnostic guidelines for dogs and cats. (14)

Measurement of glomerular filtration rate (GFR) is considered the gold standard for assessing renal function but is impractical for clinical use in veterinary medicine. The most accurate methods of GFR measurement necessitate complete urine collection over a period of 24 hours, a difficult task in cats which requires catheterization at regular intervals.[65] It is important to note that repeated sampling and extended time spent in the veterinary clinic can be stressful to the patient, and multiple analyses may not be possible due to financial cost. As a result, endogenous markers such as urine specific gravity (USG), blood urea nitrogen (BUN), and creatinine have previously been recommended and widely used for diagnosis of CKD.[15]

Measurement of USG is relatively simple using a refractometer, which requires only a drop of urine. However, it is not by itself considered an accurate tool for diagnostic purposes as

it is an insensitive marker of kidney function.[15] When used in conjunction with other measurements, a specific gravity of less than 1.035 may be indicative of CKD,[14] but cats with moderate to severe renal disease have been shown to retain their urine-concentrating ability with the loss of as much as two-thirds of renal mass.[52] USG is also influenced by non-renal factors such as dehydration and a high-protein diet.[15]

BUN was widely used in the past as an indicator of renal function but has been found to be significantly influenced by a variety of both renal non-renal factors. A study of dogs and cats with varying degrees of azotemia evaluated BUN-creatinine ratios before and after treatment revealed that the ratios were highest in animals with mild azotemia, despite a significant association between degree of azotemia and severity of CKD. BUN-creatinine ratios varied widely with all degrees of azotemia and decreased significantly following treatment for azotemia, indicating that BUN is more susceptible to influence of extrarenal factors than is creatinine. As a result, researchers concluded that BUN alone is not a reliable indicator of renal function.[17]

Creatinine also is often considered to be a non-specific marker. It is more reliable than BUN as it is not subject to influence from factors such as liver function or dietary protein content.[43] However, creatinine is produced during the breakdown of muscle cells. While loss of muscle mass is a symptom of CKD, it is also common in geriatric cats with no evidence of loss of renal function. Creatinine levels have also been shown to be affected by breed as well as environmental factors such as housing situation.[50] However, despite its variability between individuals and its susceptibility to extrarenal influence, creatinine can be useful as a marker of GFR over extended periods of time if baseline levels are obtained while the cat is healthy.[48]

Estimation of GFR through measurements of USG, BUN, and creatinine, therefore, are useful when CKD is already suspected based on the presence of symptoms of renal failure. However, due to compensatory increases in single nephron GFR following renal damage, most commonly used markers of GFR do not change until permanent and significant impairment, an approximately 75% loss of renal function, has occurred.[35]

There is also a possibility of breed-related influences on plasma markers. A study of purebred Birman, Chartreux, Maine coon, and Persian cats determined that creatinine, glucose, and total protein concentrations differed from the averages of the global cat population used to calculate reference intervals for diagnostic purposes (Fig. 5). In particular, Birmans had higher

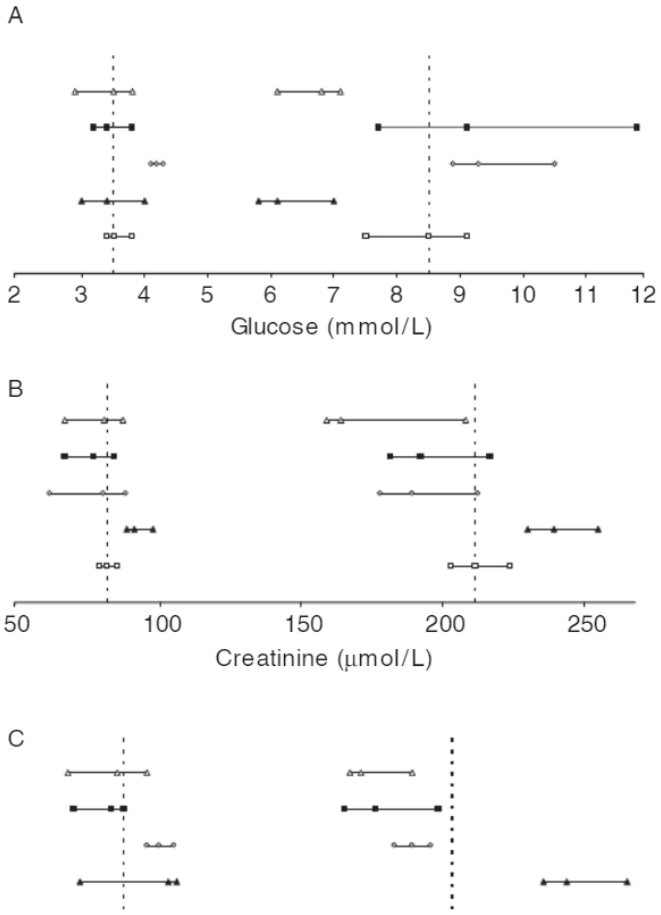

Figure 5: Lower and upper limits with 90% confidence intervals of reference intervals for the global population (white squares), Birmans (black triangles), Chartreux (white circles), Maine coons (black squares), and Persians (white triangles) for (A) plasma glucose, (B) creatinine, and (C) total proteins.

serum creatinine levels.[50] Further studies have found that Siberian, Siamese, and Somali cats also have higher serum creatinine as well as urea, indicating a need for breed-specific reference intervals.[45]

The recently developed SDMA test represents a promising step toward early diagnosis. Symmetric dimethylarginine is a byproduct of protein methylation which is released into circulation.[6] More than 90% of SDMA in blood is cleared through renal excretion,[71] and the results of several studies support its usefulness as an early indicator of reduced GFR.[22]

Measurement of GFR via iohexol plasma clearance is considered to be an accurate method but is rarely used in veterinary medicine due to its impracticability in the clinical setting. A retroactive study used stored samples to compare serum SDMA concentration with GFR estimations via iohexol clearance. The two measurements were found to have a significant inverse correlation, indicating that SDMA levels can be used as an accurate marker of renal function. The same study found that SDMA concentration increased before serum creatinine concentration by an average of seventeen months.[65]

However, a more recent study found that both serum creatinine and SDMA levels were equally correlated with GFR. Based on these results, SDMA does not appear to have any additional diagnostic value when compared with creatinine. The researchers noted that this conclusion may have been affected by differing serum creatinine reference intervals across studies. Additionally, this study did not examine SDMA or creatinine levels across an extended period of time. It is therefore likely that while SDMA has little added value in diagnosing cats with advanced stages of CKD, it is useful as an early indicator of decreasing renal function.[7]

Despite these promising results, SDMA does not appear to be an accurate marker of renal function in all cats. SDMA was not significantly associated with GFR in a study of hyperthyroid

cats, indicating that it is subject to non-renal influences.[10] Hyperthyroidism presents with many of the same symptoms as CKD and the two conditions are often comorbid; SDMA test results must therefore be interpreted in the context of other markers such as thyroid hormone levels. There is also a possibility of breed-related influence on SDMA levels. Birman cats are known to have higher serum creatinine levels,[50] and a recent study found that SDMA is also significantly elevated in this breed.[44] While it can be argued that a false positive diagnosis of renal disease is safer than delaying treatment due to a false negative, these results suggest that further research into breed differences is warranted.

Despite the need for further investigation into individual variations, SDMA is, at present, the most reliable commercially available marker for early renal damage in cats. Currently, many veterinarians recommend annual examinations with routine bloodwork including creatinine, BUN, and SDMA for aging cats, with more frequent lab work for cats over fifteen years or those at higher risk. Further research into the risk factors for renal disease as well as the mechanisms by which those factors influence kidney function may prove beneficial in deciding how often screening for CKD should be done. Client education of early symptoms such as polydipsia, polyuria, weight loss, lethargy, and other changes in health or behavior is also essential to early detection.

Few other diagnostic techniques are used in the veterinary clinical setting. Renal biopsy may be considered, but due to its relatively high level of invasiveness and increased cost compared to blood and urine testing, it is only recommended for use in cats already displaying symptoms of CKD in cases where treatment may be changed with knowledge of altered renal morphology, such as renal lymphoma.[43] Early diagnostic methods should therefore focus on markers in blood or urine.

While markers such as creatinine and BUN do not typically change until 75% of renal function has been lost, fibrosis is known to be present in the earliest stages of CKD.[51] Specific markers of fibrosis provide a more ideal method of early detection. Because of its known proinflammatory and profibrotic effects, blood and urinary levels of AngII have been proposed as potential indicators. While hormone levels are relatively easily detectable, AngII, although usually formed in circulation by conversion of AngI, has also been found to be produced by organs such as the kidney and heart.[13] Local production of AngII appears to be unrelated to systemic release and its effects on renal function are unknown. Furthermore, urinary AngII has not been consistently found to accurately reflect GFR. Direct evaluation of intrarenal AngII is difficult in a clinical setting due to the need for renal biopsy.[69] However, angiotensinogen, a precursor of AngII, is believed to provide a better indication of renal function. A study evaluating urinary angiotensinogen levels found that it is positively correlated with intrarenal AngII and the severity of fibrosis, suggesting that it may be used as both an initial diagnostic tool and a prognostic indicator (Fig. 6).[69]

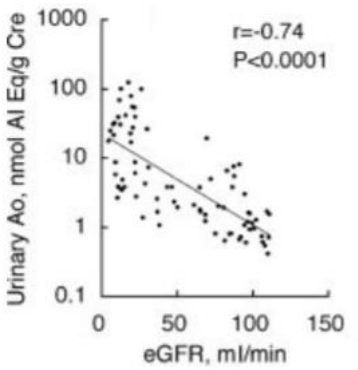

Figure 6: Scatter diagram of correlations between urinary angiotensinogen and estimated GFR. (69)

Urinary TGF-β has also been investigated for diagnostic purposes. A Free Active TGF-β1 ELISA was demonstrated to produce acceptably precise and repeatable results when testing levels of the active peptide (aTGF-β) in feline urine.[36] The active form is known to undergo hepatic clearance rather than renal clearance and has a short plasma half-life;[66] thus, aTGF-β in urine is likely to have renal origin.[36] A cross-sectional study of cats diagnosed with CKD did not detect differences in urinary aTGF-β concentration between individuals with different stages of

CKD. However, a longitudinal study found that aTGF-β levels became elevated an average of six months prior to a decrease in GFR and were positively correlated with renal inflammation and fibrosis detected through histopathology.[36] More research is needed to validate these results, including the effects of variables such as breed, but urinary aTGF-β may present an alternative method of early CKD diagnosis in the future.

Measurements of serum and urinary Klotho are being investigated as a potential diagnostic tool in human medicine. Soluble Klotho levels are known to decline in stage 2 of human CKD, and urinary Klotho declines even earlier in stage 1.[41] Further studies have found that the decrease in urinary Klotho closely mirrors the loss of nephrons and the subsequent decrease in GFR.[33] The use of Klotho measurement for diagnosis of CKD is still in development and requires much further study, but represents a promising step toward early detection of renal failure as well as a possible target for treatment in human medicine. It has not been researched extensively with regard to feline CKD but based on positive correlations found between FGF23 and GFR in cats, feline Klotho appears to play a similar role in fibrosis as in humans[16] and may be a more precise and reliable method of detecting CKD in its earliest stages than those methods currently used.

The increasing prevalence of feline CKD in recent years is of great concern. Given the irreversible nature of the disease, CKD may best be managed through prevention and early treatment. Further research into potential risk factors is essential for improved screening of individuals likely to develop renal failure, and the mechanisms by which those factors mediate the progression of interstitial fibrosis may reveal potential biomarkers for early diagnosis.

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