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# **Chronic Kidney Disease: Management of Proteinuria**

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## **Introduction**

Chronic kidney disease (CKD) is defined as a chronic sustained reduction in renal function and/or structural change. CKD therefore, by this definition, includes patients with tubulointerstitial nephritis considered the typical finding in CKD but also patients with primary glomerular disease. The distinction between these two is important as they may differ in aetiology and also management. In patients with CKD, proteinuria can develop as a result of tubular or glomerular injury. In addition, proteinuria may cause renal injury and contribute to the progression of CKD. Proteinuria has been associated with all cause mortality and hence diagnosis and management is considered important. This article will review the pathophysiology of proteinuria in CKD, diagnostic workup and appropriate management.

## **Chronic Kidney Disease**

Chronic Kidney Disease is not a single disease entity, but a heterogeneous syndrome caused by a plethora of congenital, familial or acquired factors resulting in loss of functioning renal mass. CKD is a significant cause of morbidity and mortality in cats and dogs with the UK prevalence reported to be 4% (O'Neill et al., 2013) and 0.37% (O'Neill et al., 2014) in the respective populations. Disease prevalence increases with age in both species and familial nephropathies are recognised in certain breeds.

## **Diagnosis and Staging of CKD**

Chronic kidney disease is suspected when consistent historical or clinical findings are

present. There should be evidence of disease chronicity, functional and/or structural change and/or renal damage. Diagnostic work-up generally involves complete physical examination, blood and urine testing as well as imaging.

Following the diagnosis of CKD, staging is performed. The International Renal Interest Society (IRIS) have established a staging system that is applied to patients with normal hydration status and stable renal function and is based on fasting serum/plasma creatinine concentration (Table 1).

<b>Stage</b>	<b>Blood creatinine concentration</b>	
	<b>Cats</b>	<b>Dogs</b>
<b>1</b>	<140	<125
<b>2</b>	140-250	125-180
<b>3</b>	251-440	181-440
<b>4</b>	>440	>440

Substaging is performed for urine protein: creatinine ratio (UPC; Table 2) and systolic blood pressure (SBP; Table 3). This is important as proteinuria and hypertension are therapeutic targets for which management is aimed at slowing disease progression.

<b>Substage</b>	<b>Cats<sup>a</sup></b>	<b>Dogs</b>
Non proteinuric	<0.2	<0.2
Borderline proteinuric	0.2-0.4	0.2-0.5

**Table 2: IRIS Substaging by Proteinuria (UPC)**

Proteinuric	>0.4	>0.5
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<sup>a</sup>Normal intact male cats can have a UPC ≤ 0.6.

**Table 3: IRIS Substaging by Systolic Blood Pressure (mmHg)**

Substage	Systolic Blood Pressure	Risk of Future Target Organ Damage
Normotensive	<150	Minimal
Borderline hypertensive	150 – 159	Low
Hypertensive	160 – 179	Moderate
Severely hypertensive	≥ 180	High

### **Proteinuria in CKD: cause or effect?**

Proteinuria contributes to the progression of CKD by promoting tubulointerstitial inflammation, fibrosis and atrophy. Although its role is not fully understood, proteinuria has been shown to be a negative prognostic indicator in cats and dogs with CKD and it is suggested that it may play a role in disease progression. The mechanisms by which proteinuria may cause renal injury have not yet been fully elucidated, but may include direct toxicity to tubular cells, inciting inflammatory responses, formation of proteinaceous casts resulting in tubular obstruction or it may be the presence of other solutes that are filtered through a damaged glomerulus alongside protein that are damaging to the tubules.

## **Glomerular proteinuria**

The functioning unit of the kidney (nephron) consists of the glomerulus with surrounding Bowman's capsule, and renal tubules (Figure 1). The glomerular filtration barrier filters water and small solutes from plasma through a pressure driven process. It is charge and size selective, thereby restricting the filtration of large (>60KDa) negatively charged solutes. The glomerular filtration barrier comprises the fenestrated endothelium, glomerular basement membrane and the podocytes. (Figure 2).

As the glomerulus is considered to be the sole filtration barrier to protein, the degree of proteinuria is considered to be greater with glomerular injury than tubulointerstitial injury. Glomerular proteinuria is often the result of primary glomerular disease. It is generally accepted that most dogs with a UPC >2.0 will have glomerular disease, whilst a UPC <2.0 can be associated with both glomerular and tubulointerstitial disease. Tubulointerstitial disease is more closely associated with decreased glomerular filtration rate (GFR). Primary glomerular disease is rarely recognised in cats since secondary changes are quite common in CKD, especially when hypertension is present.

## **Tubulointerstitial proteinuria**

Tubulointerstitial proteinuria can develop as a result of defects in protein reabsorption in damaged renal tubular cells or a tubulopathy (e.g. Fanconi syndrome) and is also likely to be exacerbated by accelerated tubular flow rates. Tubulointerstitial inflammation may cause exudation of protein into urine. Tubular damage, tubulointerstitial inflammation and fibrosis may also be induced by direct toxicity from filtered proteins.

Lesions initially localised to one portion of the nephron may result in the formation of lesions in other portions and proteinuria itself may contribute to tubulointerstitial disease.

Injured tubular cells can lose their capacity to regenerate and, as a result, undergo apoptosis. This can lead to tubular atrophy and, consequently, non-functional glomeruli.

### **Differentiating glomerular and tubular proteinuria**

The magnitude of proteinuria may be suggestive of whether it is glomerular or tubular in origin, but cannot be used definitively. The use of urine electrophoresis to determine the presence of low or high molecular weight proteins or a mixed pattern has been suggested as a useful method to differentiate between glomerular and tubulointerstitial proteinuria. High molecular weight proteinuria may be expected in glomerular damage, whilst low molecular weight proteinuria may be expected with tubular damage. In addition, the measurement of certain urinary biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL) may indicate tubular injury. These methods are largely research tools and further research is required to evaluate their clinical utility in proteinuric patients.

### **Proteinuria in hypertension**

The kidney is susceptible to target organ hypertensive damage. The presence of hypertension will result in transmission of high systemic arterial pressure to the glomerulus. This in turn will increase hydrostatic pressure at the glomerular filtration barrier, resulting in the development of or worsening of pre-existing proteinuria. In addition, hypertension can contribute to renal injury.

Management to restore normotension will reduce proteinuria and thus specific therapy for proteinuria is not generally required as well. Renin-angiotensin-aldosterone-system

(RAAS) inhibition has been shown to cause only a small reduction in blood pressure (approximately 15%) and therefore doses in the high end of the dose range may be required for the management of hypertensive patients. In canine patients, therapy using angiotensin-converting enzyme inhibitors (ACEi) should be initiated if the patient is not already receiving this. However, if there is severe hypertension (SBP >200mmHg) or evidence of ocular or neurological target organ damage, administration of both an ACEi and a second agent is recommended. There are no studies or consensus demonstrating the most effective second agent and the authors generally recommend amlodipine. However, some clinicians may prefer the use of ARBs. The calcium channel blocker amlodipine as a sole agent is the recommended initial therapy of choice for hypertensive cats. The goal of treatment should be a SBP between 120-150mmHg in dogs and 120-160mmHg in cats.

### **Prevalence of proteinuria in cats and dogs with CKD**

Approximately 20% of cats with CKD will be overtly proteinuric (UPC >0.4). There is less data available in dogs that can be used to estimate the prevalence of proteinuria. Nevertheless, a higher prevalence than in cats should be expected since primary glomerular disease is more common in this species (Jacob et al,2005).

### **Clinical assessment of proteinuria in cats and dogs with CKD**

Following diagnosis of CKD, an important part of disease staging is to determine if a patient is proteinuric. Evaluation of proteinuria includes assessment of its localisation, persistence and magnitude.

The **localisation** of proteinuria can be categorised as pre-renal, renal or post-renal.

- **Pre-renal proteinuria** results from the inability of the tubules to reabsorb the high plasma content of small proteins that can freely pass across the glomerular barrier. Examples include immunoglobulin light chains in multiple myeloma, myoglobin in rhabdomyolysis and haemoglobin in intravascular haemolysis. Serum protein electrophoresis or other diagnostics are useful in excluding pre-renal proteinuria.
- **Renal proteinuria** results from glomerular or tubulointerstitial pathology as discussed above. Functional proteinuria as a result of fever, seizure activity or strenuous exercise is possible, although it is rarely recognised in clinical patients.
- **Post-renal proteinuria** results from entry of proteins into the urine derived from exudative or haemorrhagic processes in the lower urinary tract or genital tract. Urine sediment examination should be performed to exclude post-renal proteinuria.

Proteinuria is characterised as **persistent** if confirmed on three or more occasions, two or more weeks apart.

The **magnitude** of proteinuria can be assessed using quantitative methods to determine urine protein concentration.

### **Diagnostic testing for proteinuria**

#### **Semi-quantitative methods**

- The reagent pad colorimetric method ("Dipstick") is a widely available simple to perform in-house test. However, it is associated with both false positives and false negatives in patients with highly concentrated urine, pigmenturia or very acidic or alkaline urine and it has poor sensitivity and specificity particularly in the cat. The urine dipstick primarily detects albumin in the urine. The sensitivity of conventional urine protein dipsticks for albuminuria in canine and feline urine (a trace positive



reaction or greater) was 81% and 90%, respectively, but the specificity was only 48% and 11%(Lyon et al., 2010).

- The Sulfosalicylic acid turbidimetric method (SSA) is more reliable than the dipstick, however, the sample requires sending to a reference laboratory for analysis. Similar to the dipstick test, false positives or negatives can also occur. The SSA test detects both albumin and globulin in the urine.

Both dipstick and SSA results need to be interpreted in the light of USG and sediment examination.

### Quantitative methods

- UPC is considered the most clinically appropriate method for quantification of proteinuria and should be performed in any patient with a positive dipstick or SSA or when investigating renal proteinuria. Both in-house testing and analysis at reference laboratories are available. A single UPC measurement (spot sample) measured from either a free catch or cystocentesis sample correlates with 24-hour urine quantification in both cats (Adams et al., 1992) and dogs (Grauer et al, 1985). There is also excellent correlation between free catch and samples obtained via cystocentesis in both cats and dogs (Vilhena et al., 2015; Beatrice et al., 2010). Measuring UPC in a pooled sample from 2-3 urine collections is a reliable and cost-effective alternative to assessing 2-3 serial UPC, but cannot be used for demonstrating persistence (LeVine et al, 2010). A normal dog, female cat or neutered male cat would be expected to have a UPC <0.2 (see Table 2). However, in an intact cat the UPC may be <0.6, most likely due to the presence of urinary cauxin.
- Urine albumin can be measured using a species-specific assay. When performing analysis of spot samples, urine albumin can be normalised to a standard USG or, more commonly, to creatinine (urine albumin creatinine ratio; UAC). In human

patients, microalbuminuria is defined as 30-300mg/day of urinary albumin excretion. Microalbuminuria is an important risk factor for cardiovascular disease in humans and therefore is routinely measured in many patient groups. However, it does not appear to be a similar risk factor in cats and dogs. The clinical benefit of measuring urine albumin in cats and dogs remains unclear. Indeed, measurement of UAC offered no advantage over UPC in predicting the onset of azotaemic CKD within 12 months in cats (Jepson et al, 2009). .

### **Additional screening**

Patients with glomerular level proteinuria should be evaluated to determine if there are any potential infectious, inflammatory or neoplastic diseases that may serve as a trigger for glomerulonephritis or amyloidosis.

### **The renin-angiotensin-aldosterone-system in CKD**

In patients with CKD, there is RAAS activation (Jensen et al, 1997) (Figure 3). Within the glomerulus, the effect of this is vasoconstriction of the efferent arteriole with an increase in the glomerular capillary hydrostatic pressure. This results in an initial improvement in GFR. However, in the long term, RAAS activation is a mediator of progressive renal injury via increasing glomerular capillary pressure and associated filtration of plasma proteins. RAAS activation and in particular aldosterone are also considered to be important mediators of pro-inflammatory and pro-fibrotic pathways.

Passage of proteins across the glomerular filtration barrier is influenced by haemodynamic factors and it is thus logical that altering renal haemodynamics should reduce proteinuria.

RAAS inhibition has been the main therapeutic target in the approach to reducing proteinuria. Agents that target RAAS include ACEi (e.g. benazepril, enalapril), angiotensin-receptor blockers (ARB; e.g. telmisartan, losartan) and aldosterone-receptor antagonists (e.g. spironolactone). Renin inhibitors are used in humans, but have not been used to any great extent in dogs or cats.

### **Management of Proteinuria**

Current IRIS guidelines suggest initiating management for persistent proteinuria in cats with a UPC >0.4 and dogs with a UPC >0.5. Some clinicians have advocated treatment for cats with borderline proteinuria, since these cats have been shown to have a poorer prognosis compared to cats with non-proteinuric CKD.

### **Angiotensin-converting enzyme inhibitors (ACEi)**

The most important mechanism by which ACEi reduce proteinuria is through preferential dilation of the efferent arteriole in the glomerulus to decrease glomerular capillary hydrostatic pressure. ACEi inhibit the conversion of angiotensin I (AT-I) to angiotensin II (AT-II) (Figure 3).

The use of ACEi has been shown to increase survival and delay progression of CKD in dogs and humans. Despite a significant reduction in proteinuria, no survival benefit has been shown in proteinuric cats, however studies to date have included small numbers of patients. In one study, there was no correlation between the intra-renal expression of renin and angiotensin II and tubulointerstitial fibrosis in cats, however a positive correlation was found in dogs (Mitani et al., 2013). These findings may suggest that intra-renal RAAS

activation is not a significant mediator of interstitial fibrosis in cats compared to dogs and could explain why ACEi appear to be less effective in cats.

When administered to dogs with glomerulonephritis, enalapril delayed the onset and/or progression of azotaemia. Despite this, enalapril is only licensed for the treatment of congestive heart failure in dogs and is not licensed in cats in the UK. Benazepril decreases proteinuria in both dogs and cats with proteinuric CKD. It is licensed in the UK for the management of proteinuric CKD in cats. The authors currently recommend its use only for proteinuric (UPC >0.5) patients, however, some clinicians advocate its use in borderline proteinuric patients (UPC 0.2-0.5). Benazepril undergoes both renal and hepatic elimination, which could offer a potential advantage over enalapril in terms of safety when administered to animals with late IRIS stage CKD. There are no other ACEi licensed for use in cats and dogs in the UK. Studies evaluating the use of other ACEi such as lisinopril, ramipril and imidapril in cats and dogs with proteinuric CKD are lacking (Table 4). There is no current evidence to suggest the efficacy of one ACEi to be greater than any other. It is likely to be variable between patients due to pharmacodynamic effects and, consequently, therapy should be tailored to the individual.

Administration of ACEi can result in hypotension, hyperkalaemia or a decrease in GFR that can worsen pre-existing azotaemia. Therefore, patients should be stable, adequately hydrated and normotensive prior to initiating ACEi therapy.

**Table 4: RAAS inhibitors licensed for the management of proteinuria in CKD in UK**

<b>Class</b>	<b>Drug</b>	<b>Formulation</b>	<b>Licensed Indication</b>	<b>Initial dose</b>	<b>Dose adjustments</b>
Angiotensin-	Benazepril	Tablet	Cats: Reduction of	0.5-1.0	Increase to a

**Table 4: RAAS inhibitors licensed for the management of proteinuria in CKD in UK**

<p>converting enzyme inhibitors</p>			<p>proteinuria associated with CKD</p> <p>Dogs: Treatment of congestive heart failure</p>	<p>mg/kg PO q24hrs (C)*  0.25-0.5 mg/kg PO q24hrs (D)*</p>	<p>maximum daily dose of 1mg/kg PO q24hrs (D)</p>
	<p>Enalapril</p>	<p>Tablet</p>	<p>Dogs: Treatment of congestive heart failure</p>	<p>0.5 mg/kg PO q24hrs</p>	<p>Increase up to a maximum of 0.5mg/kg q12hrs</p>
<p>Angiotensin receptor blockers</p>	<p>Telmisartan</p>	<p>Oral solution</p>	<p>Cats: Reduction of proteinuria associated with CKD</p>	<p>1 mg/kg PO q24hrs)</p>	<p>Not applicable</p>
<p>Aldosterone receptor blockers</p>	<p>Spironolactone</p>	<p>Tablet</p>	<p>Dogs: Treatment of congestive heart failure caused by valvular regurgitation</p>	<p>2 mg/kg PO q12- 24hrs</p>	<p>Not applicable</p>

PO; per os, C; cats, D; dogs, q24hrs; once daily

\* A lower starting dose is recommended in patients with late IRIS stage 3 or stage 4 CKD.

### **Angiotensin receptor blockers (ARB)**

Most of the functions of AT-II are mediated through the AT-II type-1 receptor (AT<sub>1</sub>). One of the effects of AT-II binding to AT<sub>1</sub> includes vasoconstriction and this can exacerbate systemic hypertension and proteinuria. The AT-II type-2 receptor (AT<sub>2</sub>) is considered to have renoprotective actions by promoting vasodilation and natriuresis, inhibiting renin secretion and exerting anti-inflammatory and anti-fibrotic effects on the kidneys. Angiotensin receptor blockers selectively inhibit AT<sub>1</sub>, preventing AT-II binding.

Several ARB, including telmisartan and losartan (Table 4), have been studied in humans with proteinuria and were shown to result in a similar reduction in proteinuria compared to ACEi. Losartan was effective in reducing proteinuria in dogs, but not in cats (Jenkins et al, 2015). However, this study was conducted in response to Angiotensin II infusion and therefore may not extrapolate to patients with naturally occurring disease. It is not licensed in the UK in either species. Telmisartan is licensed in the UK for reducing proteinuria in cats with CKD. The decrease in UPC in cats in which telmisartan was administered appeared to be greater than that of benazepril at all assessment points for the 6-month duration of a UK study although this did not reach statistical significance. However, there was a significant decrease in UPC over time compared to baseline in cats receiving telmisartan but not those receiving benazepril (Sent et al, 2015). Studies in humans have shown that telmisartan decreased blood pressure as effectively as amlodipine, suggesting that it might be the drug of choice for concurrent hypertension and proteinuria. A study in healthy cats comparing the administration of benazepril, irbesartan, losartan and various doses of telmisartan found that telmisartan had a longer duration of action than benazepril and, at a higher dose (3mg/kg), telmisartan attenuated systolic blood pressure to a

significantly greater degree than benazepril and all other treatments (Jenkins et al, 2015). However, there was no dose escalation of benazepril and therefore with higher doses it is possible that this would have also been effective. There are no systematic studies evaluating the efficacy of telmisartan administration in dogs with proteinuric CKD, although individual reports have been published and there are anecdotal reports of its efficacy.

### **Aldosterone breakthrough**

Serum aldosterone concentration increases over time in a subset of human patients receiving even maximal doses of RAAS inhibitors. This phenomenon is known as aldosterone breakthrough. In addition, ACE-escape, in which there is incomplete inhibition of conversion of AT-I to AT-II in patients administered ACEi resulting in ongoing AT-II production, is also recognised. In humans, the most favoured explanation for aldosterone breakthrough and ACE-escape is that non-ACE enzymes such as chymase and cathepsin G can cleave ATI to form AT-II. This mechanism for aldosterone breakthrough mediated by AT-II might suggest that the phenomenon occurs less often in patients receiving ARBs than in those receiving ACEi. However, this does not appear to hold true in human patients. The mechanisms of aldosterone breakthrough and ACE-escape in cats and dogs are poorly understood and the incidence is unclear. It has been suggested that aldosterone breakthrough may develop in up to 33% of dogs with proteinuric renal disease receiving RAAS inhibitors (Vaden and Elliott, 2016). Persistently elevated aldosterone concentration is of concern as it may have adverse effects on the heart, systemic vasculature and kidneys.

### **Combination therapy**

In human patients, combination therapy with an ACEi and an ARB may be recommended if monotherapy is not effective in reducing proteinuria. There are no published studies in

cats or dogs evaluating combination therapy. However, it is potentially very dangerous given the results of a human study that found increased risk of kidney failure and death in elderly patients prescribed combination therapy.

### **Aldosterone receptor antagonists**

Aldosterone receptor antagonists such as spironolactone have been used in humans to counter aldosterone breakthrough and also in the management of proteinuric kidney disease. There are no published studies exploring the use of aldosterone receptor antagonists in dogs or cats with proteinuric CKD. Currently, spironolactone (Table 4) is licensed in the UK for the treatment of congestive heart failure in dogs. It may be considered in a patient with high serum aldosterone concentration and persistent proteinuria despite treatment with ACEi and/or ARB; however, its use would be off license.

### **Renal Biopsy**

Renal biopsy can potentially provide a definitive diagnosis, but can also provide information about the severity of the underlying renal injury. Renal biopsy is indicated in patients with suspected glomerular proteinuria of high magnitude (UPC  $\geq 3.5$  in dogs) that have not responded to standard therapy, such as RAAS inhibition, and that do not have any contraindications to a renal biopsy being performed. Such contraindications include moderate or marked azotaemia, uncontrolled hypertension, renal cystic disease, hydronephrosis, pyelonephritis, coagulopathy or severe anaemia. Renal biopsy is indicated in similar scenarios in cats, although glomerular-level proteinuria is seen less commonly. Furthermore, over 50% of cats with CKD have been shown to have tubulointerstitial nephritis (Chakrabarti et al, 2012; DiBartola et al, 1987) for which results of a renal biopsy are unlikely to alter long-term management.



Renal biopsies should be obtained under sedation or general anaesthesia using ultrasound guidance by someone experienced with the procedure and should be handled with extreme care to ensure a diagnosis is made. A practical guide to obtaining a renal biopsy is beyond the scope of this review article and it is recommended that a veterinary nephropathology centre is contacted prior to obtaining a biopsy to ensure the correct fixatives can be provided and to provide further instruction regarding collection. Samples are obtained from the renal cortex rather than medulla as the cortex contains the glomeruli and also there can be damage to large vessels resulting in haemorrhage or infarction if the corticomedullary junction is crossed. Samples should be submitted to a veterinary nephropathology service to ensure appropriate processing and provide a full diagnosis. A regular pathologist will be able to identify diseases such as lymphoma, amyloidosis or pyelonephritis. An experienced nephropathologist, however, will visualise the tissue using light microscopy and will be able to identify any ultrastructural changes using electron microscopy. Immunostaining techniques can also be applied to the sample. The primary aim of obtaining a renal biopsy to investigate glomerular disease in dogs is to determine if there is an immunopathogenesis present requiring immunosuppressive therapy. This can be identified by the finding of electron-dense immune deposits in the subepithelium, subendothelium intramembranous or mesangium of the glomerulus when visualising the biopsy using electron microscopy or by the presence of positive immunostaining when visualised using immunofluorescence microscopy.

Specific treatment can be tailored based on renal biopsy findings. In dogs with glomerular disease, consensus guidelines have been produced by IRIS for the management of glomerular disease using data obtained from the WSAVA Renal Standardization Project. No similar Renal Standardization Projects have been undertaken in cats to date.

It is important to consider that the collection of a renal biopsy should only be performed when it is believed that the identification of pathological changes may be useful for modifying therapeutic options for the patient. However, the risk of the procedure must always be weighed up against any potential benefit.

**Table 5: Glomerular diseases in dogs and associated findings on renal biopsy**

	Diagnostic findings on renal biopsy	Glomerulopathy
Amyloidosis	<p>Specific findings</p> <ul style="list-style-type: none"> <li>• Green birefringent material when stained with Congo red and viewed using polarised light</li> <li>• Fibrillar material present in the mesangium and glomerular basement membrane using electron microscopy</li> </ul> <p>Non-specific findings</p> <ul style="list-style-type: none"> <li>• Eosinophilic material on haematoxylin and eosin staining using light microscopy</li> </ul>	n/a
Immune complex glomerulonephritis	<p>Specific findings</p> <ul style="list-style-type: none"> <li>• Electron-dense immune deposits in the subepithelium, subendothelium</li> </ul>	n/a

	<p>intramembranous or mesangium of the glomerulus using electron microscopy</p> <ul style="list-style-type: none"> <li>• Positive immunostaining using immunofluorescence microscopy</li> </ul> <p>Non-specific findings</p> <ul style="list-style-type: none"> <li>• Immune complexes detected using Masson's trichrome on light microscopy</li> <li>• Glomerular basement membrane remodelling (spikes, holes, double and irregular contours) using Jones Methenamine silver staining on light microscopy</li> <li>• Glomerular endocapillary hypercellularity using light microscopy</li> </ul>	
<p>Non-immunecomplex glomerulonephritis</p>	<p>Specific findings</p> <ul style="list-style-type: none"> <li>• Ruling out of findings consistent with immunecomplex glomerulonephritis using electron microscopy and immunofluorescence</li> </ul>	<ul style="list-style-type: none"> <li>• Focal segmental or global glomerulosclerosis</li> <li>• Glomerular basement membrane abnormalities</li> <li>• Glomerular lipidosis</li> </ul>

	<p>Non-specific findings</p> <ul style="list-style-type: none"> <li>• Smooth contours of the glomerular basement membrane using light microscopy</li> </ul> <p>Note: Glomeruli that are reported to be normal using light and electron microscopy and immunofluorescence should not be considered to have non-immunecomplex glomerulonephritis and an alternative cause of the proteinuria should be investigated.</p>	<ul style="list-style-type: none"> <li>• Congenital or developmental nephropathies</li> </ul>
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**Immunosuppressive therapy**

Immunosuppressive treatment should be reserved for patients in which a renal biopsy has been performed and there is evidence of immune-mediated disease. Furthermore, any underlying infectious disease should be excluded and underlying disease for which immunosuppression would be contraindicated should not be present. There is currently a lack of controlled clinical trials evaluating immunosuppressive therapies in glomerular disease in dogs and this makes evidence-based recommendations difficult. The immunosuppressive agent or agents prescribed are dependent on the severity of disease and its progression. IRIS consensus guidelines for the management of confirmed

immunocomplex glomerulonephritis have suggested the following agents may be effective in dogs with acute or rapidly progressive disease: glucocorticoids, mycophenolate, cyclosporine, cyclophosphamide and azathioprine (Table 6). For dogs with stable or slowly progressive immunocomplex glomerulonephritis, similar immunosuppressive therapies can be prescribed; however, agents with a rapid onset of action may be less important in these cases.

Approximately 50% of dogs from which renal biopsies have been evaluated had evidence of immunocomplex glomerulonephritis. Therefore, when obtaining a renal biopsy is not possible due to contraindications, lack of experienced clinician or owner constraints, initiating immunosuppressive therapy may be a consideration as one in two dogs are likely to be candidates for this. Nevertheless, the potential benefits and risks should be carefully discussed with the owner and, if no response to therapy is seen, it should be discontinued after 8-12 weeks. IRIS consensus guidelines for glomerular disease in dogs recommend that immunosuppressive therapy should be considered without performing a renal biopsy when a patient is markedly azotaemic (IRIS stage 3 or 4 CKD), has rapidly progressive azotaemia or severe hypoalbuminaemia (<20g/dL).

It is important to remember that immunosuppressive therapy should be prescribed in combination with standard therapy such as RAAS blockade. If considering performing a renal biopsy or initiating immunosuppressive therapy it may be worth considering consulting a veterinary specialist with an interest in nephrology for advice.

Currently, there is a lack of published literature exploring glomerular disease in cats; however, clinically this does not appear to be a common diagnosis. When that occurs, the most common underlying diagnosis is membranous nephropathy and, interestingly, a

greater proportion of cats will have immune-mediated glomerular disease. Therefore, immunecomplex glomerulonephritis is considered to be rare in cats and immunosuppressive therapy would not be recommended in the management of a proteinuric cat unless there is a biopsy-confirmed immunopathogenesis.

**Table 6: Immunosuppressive therapy options in the management of canine immunecomplex glomerulonephritis**

Immunosuppressive drug	Advantages	Disadvantages	Evidence for use
Glucocorticoids	Cheap, readily available	Adverse effects e.g polyuria and polydipsia, increased proteinuria, systemic hypertension, polyphagia, risk of thromboembolism, adrenal suppression Risk of development of infection e.g urinary tract infection	IRIS consensus guidelines for glomerular disease recommend short-term administration in fulminant cases requiring immediate immunosuppression (usually in combination with other drugs) or in cases of multisystemic immune-mediated disease
Mycophenolate	Less toxicity e.g myelotoxicity, hepatotoxicity than other alkylating	Adverse effects (predominantly gastrointestinal) are dose-dependent and reversible following withdrawal	Single case report describing its use in a dog with glomerulonephritis of unknown pathology IRIS consensus guidelines for glomerular disease

	agents, rapid onset of action		recommends this as the first drug of choice for managing dogs with acute or rapidly progressive glomerular disease
Cyclosporine	Readily available	Expensive  Adverse effects e.g gastrointestinal signs, gingival hyperplasia	Little clinical benefit in a randomised controlled clinical trial of dogs with glomerular disease  IRIS consensus does not currently recommend the use of cyclosporine.
Cyclophosphamide	Rapid onset of action	Adverse effects e.g gastrointestinal signs, myelosuppression, haemorrhagic cystitis  Strict regular monitoring required	IRIS consensus guidelines for glomerular disease recommends this as a drug to consider for managing dogs with acute or rapidly progressive glomerular disease
Azathioprine	Cheap	Delayed onset of action (2-5 weeks)  Adverse effects e.g gastrointestinal signs, myelosuppression, hepatotoxicity, acute pancreatitis	IRIS consensus guidelines for glomerular disease describes uncontrolled anecdotal clinical experience supporting its efficacy usually when combined with

			chlorambucil
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### **Adjunctive therapies**

**Dietary therapy:** Renal diets are protein restricted and the feeding of such diets is considered to decrease proteinuria not only through decreasing the amount of protein presented to the glomerular filtration barrier, but also through reducing intraglomerular capillary pressure. However, they are rarely useful as a monotherapy, as they only marginally reduce proteinuria.

**Omega 3 (n3) and Omega 6 (n6) Polyunsaturated fatty acids (PUFA):** Dietary supplementation with n3 PUFA decreases the magnitude of proteinuria, whilst supplementation with n6 PUFA increases GFR in dogs. The recommended n6/n3 ratio is 5:1 and this is found in most formulated renal diets. For proteinuric patients that are not fed a renal diet, supplementation with n3 PUFA and specifically docosahexaenoic acid and eicosapentaenoic acid can be recommended. The dosage is 0.25–0.5g/kg of docosahexaenoic acid and eicosapentaenoic acid. In an uncontrolled retrospective study evaluating various renal diets fed to cats with CKD, the diet with the highest eicosapentaenoic acid (n3) content was associated with the longest survival compared to control cats, although all diets studied improved survival. However, a causal relationship could not be established and proteinuria was not evaluated. Survival studies in dogs with naturally occurring proteinuric CKD receiving PUFA supplementation have not been performed.

**Antithrombotic therapy:** Thromboembolism is recognised in both humans and canine proteinuric patients with the prevalence reported to be up to 25% of dogs with glomerular



disease. The pathophysiological mechanism remains to be fully elucidated, but factors that are likely to be involved include loss of antithrombin into the urine, an increase in plasma procoagulant factors and fibrinogen, an increase in platelet reactivity and endothelial dysfunction. Unfortunately, there is a lack of evidence for the optimal drug and dosage and when to institute prophylactic treatment in dogs. The IRIS consensus guidelines for managing canine glomerular disease recommends the administration of low-dose aspirin (1-5mg/kg daily) to all dogs with proteinuric glomerular disease, provided that they are normotensive and well-hydrated. Clopidogrel may also be an effective anti-thrombotic therapy; however, there is no evidence that it is superior to aspirin and it is significantly more expensive.

Thromboembolism is not generally recognised in cats with proteinuric CKD and to the authors' knowledge there are no studies exploring antithrombotic therapy in these patients.

### **Targets and monitoring of treatment**

The optimal therapeutic target for proteinuric patients receiving standard therapy is to decrease UPC to <0.5 in dogs and <0.4 in cats. However, this is not achieved in many patients. Serial UPC measurements differ by at least 80% in dogs with low UPC (approximately 0.5) and 35% in dogs with high UPC (approximately 12). Therefore, a reduction in UPC close to this magnitude is probably required to conclude that treatment is effective. The IRIS consensus guidelines for canine glomerular disease recommend a reduction in UPC of >50% as an alternative target. Whether achieving any of these therapeutic targets offers any survival benefit is still unknown. It is important to note that in the later stages of disease when the number of remaining functioning nephrons through which protein can be lost has decreased, a decrease in UPC may be seen.

Following initiation or a change in dose of RAAS blockade therapy, UPC, SBP, serum creatinine and potassium should be measured after one to two weeks (see Figure 4). Hyperkalaemia can be a common side effect of RAAS inhibition in dogs but not cats with CKD. Treatment modification consisting of ACEi or ARB dose reduction, discontinuation of spironolactone or feeding a potassium-deficient diet formulated by a veterinary nutritionist, is indicated if serum potassium concentrations  $>6\text{mmol/l}$  are seen. An increase in serum creatinine  $>30\%$  would suggest worsening renal function requiring cessation of treatment or a dose reduction.

The IRIS consensus guidelines for monitoring of dogs receiving standard therapy as management for glomerular disease recommend measuring UPC, SBP, performing biochemistry (to include albumin, creatinine and potassium) and urinalysis at least every 3 months. This would also be similar to the International Society of Feline Medicine consensus guidelines for monitoring of cats with stable CKD. Recommendations for the monitoring of dogs receiving immunosuppressive therapy include re-evaluating the patients one to two weeks after initiating treatment followed by every two weeks for the first 6 weeks and then monthly for three months extending to every 3 months for long term monitoring. Evaluation of dogs receiving immunosuppressive therapy should include SBP, haematology, biochemistry (to include serum albumin, creatinine, urea, phosphorous, electrolytes, hepatic markers and cholesterol), UPC and urinalysis.

### **Nephrotic syndrome**

Nephrotic syndrome is characterised by the presence of hypoalbuminemia, proteinuria, hypercholesterolaemia and fluid accumulation in interstitial spaces and/or body cavities. Fluid formation is attributed to decreased plasma colloid osmotic pressure resulting from hypoalbuminaemia associated with glomerular protein loss. Intravenous fluid therapy

should be administered cautiously to nephrotic patients as they are predisposed to fluid overload. Fluid therapy is only indicated for haemodynamic stabilisation. Colloid use may be considered when crystalloid fluids alone have not stabilised the patient. Colloids and plasma/albumin are only indicated for haemodynamic stabilisation rather than to increase oncotic pressure or treat hypoalbuminaemia. The use of diuretics in dogs with oedema should be limited to situations where organ function is critically impaired, such as in patients with dyspnoea due to pleural effusion. Another indication would be for transient reduction in oedema and/or ascites to facilitate renal biopsy. Furosemide is the agent of choice in dogs with hyperkalaemia and spironolactone for dogs with pleural or abdominal effusion.

### **Prognosis of cats and dogs with proteinuria associated with CKD**

Proteinuria has been shown to be a negative prognostic indicator in both dogs and cats with CKD in multiple studies. In humans and dogs, reducing proteinuria has been shown to improve survival, delay disease progression and improve quality of life. In cats, multiple studies have concluded that the magnitude of proteinuria is inversely proportional to survival and that even mild proteinuria is strongly associated with and predictive of onset of azotaemia and progression of CKD. However, a survival benefit has failed to be shown in cats receiving therapy to reduce proteinuria.

### **Conclusion**

Management of proteinuria is primarily achieved by administering drugs that inhibit the RAAS system. Adjunctive treatments such as dietary therapy and n3 PUFA administration may also be beneficial in proteinuric patients with CKD. Approximately 50% of dogs with glomerular disease may have an immune-mediated pathogenesis and immunosuppressive therapies may be indicated in these patients. Management of proteinuria should always be tailored to the individual patient and on-going treatment should be based on regular

assessment of clinical and laboratory parameters. Reducing proteinuria is an important therapeutic target with the aim of delaying progression of CKD and potentially improving survival.

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## **FURTHER READING**

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**Figure 1: The structure of the glomerulus.** The glomerulus is a network of capillaries interposed between the afferent and efferent arterioles enclosed within an epithelial capsule (Bowman's capsule) and separated by the Bowman's space. Glomerular filtration is a pressure-driven process governed by hydrostatic and oncotic pressures in the glomerular capillaries and Bowman's capsule, and by glomerular conductivity and the capillary surface area. Taken from Finch, N. (2014). Measurement of glomerular filtration rate in cats. *Journal of Feline Medicine and Surgery*, 16(9), pp.736-748

**Figure 2 A (lower magnification) and B (higher magnification): Transmission electron micrograph of the glomerular filtration barrier comprising the fenestrated endothelial cells, basement membrane and podocytes.** P, podocyte; GBM, glomerular basement membrane; E, glomerular endothelial cell; FP, podocyte foot process; F, glomerular endothelial cell fenestration; SD, podocyte slit diaphragm

**Figure 3. The renin-angiotensin-aldosterone-system and its inhibitors.** ACE, angiotensin-converting enzyme, AT<sub>1</sub>; angiotensin II type-1 receptor, AT<sub>2</sub>, angiotensin II type-2 receptor.

- Renin inhibitors are not currently used in cats or dogs
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- **Figure 4. Protocol for adjusting RAAS inhibition therapy in dogs with glomerular disease**
- SCr; serum creatinine, K; potassium, BP; blood pressure, UPC; urine: protein creatinine ratio
- \*Tolerable limits: SCr change generally considered tolerable in CKD stage 1 or 2 if <30% above baseline; in CKD stage 3 <10% but in stage 4 no increase in SCr may be tolerable. K tolerable when <6mmol/l. SBP should be ≤160-179mmHg or lower; BP decline acceptable if SBP >120mmHg. Adapted from Brown, S., Elliott, J., Francey, T., Polzin, D. and Vaden, S. (2013). Consensus Recommendations for Standard Therapy of Glomerular Disease in Dogs. *Journal of Veterinary Internal Medicine*, 27, pp.S27-S43.