PROTEINURIA UPDATE – THE DIAGNOSTIC WORK-UP

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Keystone Veterinary Conference, August 17, 2018

Checking for proteinuria should be part of the annual wellness examination in all dogs and cats.[1] Here we briefly address these common questions: Why should we bother checking for proteinuria, how often should we check for it, and with which test? What other information helps to localize the source of proteinuria? If protein-losing nephropathy (PLN) is suspected, when is renal biopsy indicated? What are the current recommendations for intervention?

WHY SHOULD WE BOTHER CHECKING FOR PROTEINURIA?

Proteinuria is relatively easy to check for and is one of those potential "silent killer" markers that can be associated with simmering damage which might otherwise go unnoticed by even the most observant owner. If proteinuria continues unrecognized, a variety of clinical signs could occur down the road, including severe and dramatic signs, with less chance of reversal or stabilization and more risk of morbidity and mortality.

Large amounts of protein in the urine which are associated with glomerular disease[2] can lead to the nephrotic syndrome which can cause serious sequellae including death, even before azotemia or polyuria/polydipsia (PU/PD) and signs of renal failure occur. Significant proteinuria may lead to hypoalbuminemia and loss of antithrombin, with the attendant risk for effusions, edema, and thromboembolic events. Even small amounts of protein leaking into the glomerular filtrate may cause tubular damage further down the nephron, and eventually renal function may decline due to decreased renal reserve, leading to chronic kidney disease (CKD) and renal failure. Hypertension (another silent killer) can be either a cause or effect of renal disease, especially glomerular disease. If we detect proteinuria, it is a reminder to check the blood pressure measurement (BPM) and potentially avoid target organ damage from hypertension, including changes to the eye (blindness due to retinal hemorrhage or detachment), cardiovascular system (hypertrophic cardiomyopathy, arteriosclerosis, epistaxis), kidney (nephrosclerosis), and/or cerebrovascular system (cerebrovascular accidents or "strokes"). Other underlying diseases causing proteinuria, such as amyloidosis, infectious/inflammatory/ immune-mediated, neoplastic, toxic, or genetic diseases triggering immune-mediated glomerulonephritis or glomerulopathy, may be first recognized by detection of proteinuria, a warning flag to look further. And vice versa: animals with infections, cancer, or immune-mediated disease affecting other organs (e.g., polyarthropathy, vasculitis, uveitis, hemolytic anemia, thrombocytopenia, pemphigus, myositis, myasthenia gravis, etc.) should be closely monitored for the development of proteinuria. Screening for occult proteinuria in breeding stock may help future generations avoid familial PLN[3,4] and screening non-breeding individuals of such predisposed breeds will help spot those that may benefit from early therapeutic intervention.

WHEN AND HOW OFTEN SHOULD WE CHECK FOR PROTEINURIA?

The ACVIM Consensus Statement on Proteinuria[1] recommended that healthy dogs and cats be checked annually, as part of their wellness exam. Urine testing is also an important part of the database for sick pets. If a pet is proteinuric, guidelines (see Table 1 below) are given regarding monitoring (for magnitude, persistence, and trend), investigation (for localization and diagnostic work-up for cause), and therapeutic intervention (specific, supportive, and symptomatic).

Mild proteinuria on urinalysis or microalbuminuria testing (MA) should be monitored over time to study progression or stability, and to notice any trend. Moderate to severe proteinuria should be monitored even more closely as well as investigated with other testing, for instance, urine protein/creatinine ratio (UPC), BPM, CBC, biochemical profile (an SDMA test result may show earliest warning of decreased GFR), urine culture/sensitivity, chest radiographs, abdominal ultrasound, serologic tests (and possibly PCR) for infectious diseases in the area, and possibly renal biopsy and/or urine SDS-PAGE[5] testing. All dogs which are seropositive for antibodies against *Ehrlichia*, *Anaplasma*, or Lyme or positive for heartworm antigen should be checked for proteinuria. Whether or not seropositive animals are treated with doxycycline (and the current consensus[6,7,8] is that non-clinical animals do not all need to be treated), when should they be rechecked for proteinuria? Since the carrier state may not always be cleared even if they are treated, rechecking for proteinuria is recommended 2-3 times/year for seropositive animals.[6]

WHICH TEST SHOULD WE USE?

Searching for protein in the urine by dipstick can give false negative or false positive results. The dipstick square showing shades of green can be misinterpreted because of ambient lighting conditions, outdated sticks, dipstick bottles left open, etc. Almost 10% of men have some form of colorblindness and may find reading the dipstick problematic. False positives may be due to alkaline urine (due to respiratory alkalosis in excited, hyperventilating dogs; post-prandial sampling; or an old urine sample left out on the counter too long), pigmenturia (which turns all dipstick squares darker), or highly concentrated urine. False negatives may be due to dilute urine or old sticks. One study showed that +1 protein dipstick results in USG >1.012 is probably insignificant. But a +2 protein dipstick result with any USG result or a +1 protein dipstick result with a USG ≤ 1.012 should probably be further assessed my microalbuminuria or UPC tests.

Microalbuminuria (MA) testing is more sensitive and specific than the urine dipstick for proteinuria due to albuminuria.[9-12] The MA test is often mildly positive in elderly individuals.[2] Monitoring for persistence and following the trend is important for low or medium high results. Whenever MA results are very high, switching over to UPC testing is more helpful. In the rare cases where MA is normal but UPC is high, proteinuria may be due to non-albumin small molecular weight (MW) proteins, such as is seen with Bence Jones proteinuria or tubular proteinuria.

Urine protein levels are not seriously impacted by the presence of microscopic amounts of blood in the sample (commonly occurring with cystocentesis samples),[13] however, urine samples which are grossly bloody will have increased protein levels which interfere with diagnostic interpretations. Likewise, urine samples taken soon after ejaculation may be contaminated with semen and prostatic fluid, yielding higher urine protein dipstick values.[14]

Normal UPC is 0.2 (0.2-0.4 is borderline in cats; 0.2-0.5 is borderline dogs). Proteinuria is defined as >0.4 in cats and > 0.5 in dogs. To check for persistence, proteinuric cases should be rechecked in 2-4 weeks (borderline cases should be rechecked in 2 weeks to 2 months). Guideline recommendations for UPC levels warranting monitoring, diagnostic investigation, and intervention are given by the ACVIM Consensus on Proteinuria,[1] although many clinicians would intervene when UPC is much lower than 2.0. Intervention may begin immediately if UPC>2 if there is hypoalbuminemia or CKD, without waiting to demonstrate persistence.[15]

Table 1

	Non-Azotemic Dogs and Cats	**Azotemic Dogs**	**Azotemic Cats**
Monitor	UPC 0.5	UPC < 0.5	UPC < 0.4
Investigate	UPC 1.0	UPC < 0.5	UPC < 0.4
Intervene	UPC 2.0	UPC 0.5	UPC 0.4

Because of daily variations (up to 80% for UPC 0.5, and 35% for UPC of 12.0), when monitoring the UPC, consider testing a pooled sample of equal aliquots of 3 samples.[16] Each sample can be saved at the same time daily, separately in the refrigerator, and brought to the veterinarian at one time, when the aliquots can be mixed and submitted as one sample. Of note is that for proteinuric dogs, UPC values were greater on urine samples obtained in the clinic vs. from home.[17]

WHAT OTHER INFORMATION HELPS TO LOCALIZE THE SOURCE OF PROTEINURIA?

Persistent proteinuria may have pre-renal, renal, or post-renal sources. The cause of proteinuria needs to be localized.[1,2] Infection, inflammation, calculi, or neoplasia of the lower urogenital tract (ureter, bladder, prostate, uterus, urethra, prepuce/vagina, etc.) is common and needs to be ruled out so that proteinuria is not misinterpreted as a glomerular leak. Examination of urinary sediment for evidence of inflammatory cells is important, since the urine dipstick square for leukesterase often gives false negative results. A history of clinical signs may suggest lower urogenital tract disease causing post-renal proteinuria, for which urine cultures, imaging, or uroendoscopy may be helpful. Pre-renal causes may be suggested by abnormal serum globulins, or if UPC is high while MA results are normal, suggesting small molecular weight proteins such as Bence Jones proteins (immunoglobulin light chains, e.g., with multiple myeloma, having MW of 22-24 kDa) may be in the urine. Albumin, associated with glomerular proteinuria, has a MW of 60 kDa. The SDS-PAGE[5] urine test also helps to differentiate small and large MW proteinuria.

IF RENAL PROTEINURIA IS SUSPECTED, WHAT OTHER TESTS SHOULD WE DO?

Once renal proteinuria is suspected, tests to identify the cause and to stage the disease are indicated. Some of these tests may have already been done, e.g., urine culture and imaging, during the localization process. In December 2013 the International Renal Interest Society (IRIS) Canine Glomerulonephritis Study Group published a group of consensus papers regarding the diagnosis and treatment of suspected glomerular disease.[18-25] Recommendations were made by the Diagnosis Subgroup and accepted by a consensus of veterinary nephrologists, including a prioritization of the diagnostic tests with recommendations based on each individual's clinicopathologic circumstances, with attention to the clinical severity, resources, expertise, finances, and client willingness.[7] See Table 2.

Renal proteinuria may be further characterized as originating from glomerular and/or tubular sources. Diseases which primarily target tubules (e.g., leptospirosis, pyelonephritis) or whole nephron disease (e.g., juvenile renal disease) may mimic primary glomerular diseases. Tests for leptospirosis include PCR tests on blood and urine, and serologic testing for antibodies, especially convalescent titers. A urine SDS-PAGE[5] test helps to differentiate high molecular weight (albumin/glomerular disease) from low molecular weight proteinuria (tubular disease), as does cortical renal biopsy.

Suspected glomerular disease may be due to endocrine, genetic, immune-mediated, infectious, inflammatory, neoplastic, toxic, or vascular insults.[2] Diagnostic testing is warranted to find the underlying cause of suspected glomerular disease and stage disease so that symptomatic, supportive and specific therapy may be targeted for the individual. Categorization and development of criteria for inclusion for clinical trials is important to find the best treatment protocol for each subtype. When studying familial glomerulopathy, identification of the subtype is important to match the appropriate phenotype with its genotype for affected animals and properly chosen control animals, in order to utilize genomic tools (e.g., genome-wide association studies or gene sequencing of candidate genes) to discover the underlying molecular basis for the disease and to develop a DNA test to identify dogs at-risk and help breeders with genetic counselling.

TABLE 2: Diagnostic priorities based on canine glomerular disease tier

	Tier I Uncomplicated Renal Proteinuria (a)	**Tier II Renal Proteinuria With Hypoalbuminemia Without Azotemia**	**Tier III Renal Proteinuria With Azotemia**						
	E	R	PH	E	R	PH	E	R	PH
Hx, PE, BPM, CBC, Chemistry, Urinalysis, Urine culture, UPCs (a)	X			X			X		
Investigate any evident extrarenal disease		(X)			(X)			(X)	
Abdominal ultrasound	(b)	X		X			X		
Chest radiographs	(b)		X		X			X	
Work-up for hypertension	(X)			(X)			(X)		
Work-up for infectious diseases	X			X			X		
Work-up for hypoalbuminemia				X			(X)		

Classify and work-up for azotemia						X		
Renal biopsy		(b)	X		X		X	
Antithrombin, TEG, SDS-PAGE[5], bank DNA samples, etc.			X			X		X

*Adapted[7] available at https://onlinelibrary.wiley.com/toc/19391676/27/s1
(a)=proteinuria needs to be evaluated for localization, persistence, and magnitude; (b)=a small number of respondents made this recommendation; BPM=blood pressure measurement; CBC=complete blood count; E= essential; Hx=history; PE=physical examination; PH=potentially helpful; R= recommended; TEG=thromboelastography; UPCs=urine protein/ creatinine ratios; (X)=if appropriate.

The minimal (essential) assessment recommended for all tiers includes history, physical examination, complete blood count including platelets, biochemical profile, urinalysis, urine culture, UPC, BPM, and serologic testing for exposure to common infectious diseases associated with proteinuria in the individual's environs or travel history (e.g., heartworm, Lyme disease, Ehrlichiosis, and leptospirosis in endemic regions of the United States, and, depending on the case, Ehrlichiosis, Babesiosis, RMSF, Hepatozoonosis, Brucellosis, and in southern Europe, Leishmaniasis. Some tests may have already been done to localize proteinuria (e.g., blood tests, urine culture, abdominal ultrasound). The essential evaluation helps stage the severity of disease and prognosticate using the IRIS classification of CKD (history, anemia, hypoalbuminemia, hypercholesterolemia, azotemia, isosthenuria, electrolyte/water/nitrogen balance), detects possible sequellae (hypertension with target organ damage; thromboembolic events associated with hypercoagulopathy; nephrotic syndrome associated with edema or effusions, hypoalbuminemia and hypercholesterolemia; or severe azotemia with possible hyperphosphatemia, anemia, etc.), and may suggest an underlying cause (e.g., history of lameness, oculoneural signs, hyperadrenocorticism, cytopenias, gammopathy, etc.) which may have a specific treatment (e.g., for Lyme nephritis, Leishmaniasis, or Babesiosis). Some specialists include imaging (chest radiographs and abdominal ultrasound) as part of a minimal assessment, e.g., to screen for neoplasia. Others recommend imaging but with slightly lower priority than essential for otherwise healthy mildly proteinuric dogs (Tier I, see Table 2). Depending on initial findings, further testing may be indicated, e.g., a more in-depth search for less common infectious diseases, further diagnostic testing for hypoalbuminemia (to rule out liver disease, gastrointestinal losses, etc.) or for extra-renal abnormalities discovered. Other tests may be helpful, e.g., thromboelastography, DNA testing for familial disease,[3,4] or SDS-PAGE[5] testing on the urine to help differentiate glomerular from tubular proteinuria.

Check for hypertension

Hypertension can be the cause or effect of renal disease and proteinuria.[26-28] Persistence of elevated BPM or even a single reliable high BPM warrants assessment for target organ damage (ocular, cardiovascular, renal, cerebrovascular), testing for a possible underlying non-renal cause of hypertension (hyperadrenocorticism, hyperaldosteronism, pheochromocytoma, exposure to alpha-agonists, high salt diet, oversupplementation of thyroid medication, etc.), and appropriate monitoring and treatment of hypertension.

Systemic hypertension (from any cause) increases glomerular filtration pressure and may cause proteinuria. Currently in veterinary medicine, measuring blood pressure is not done as routinely as is screening for proteinuria. Whenever proteinuria is found, a BPM and retinal examination should be done. If proteinuria screening were not included in the wellness exam, occult target organ damage from hypertension may be missed as well, and possibly progress unchecked until gross clinical signs (e.g., blindness, epistaxis, neurologic signs) could occur.

Some causes of systemic hypertension include renal disease (especially glomerular disease), adrenal disease (Cushing's, hyperaldosteronism, pheochromocytoma), and thyroid disease (hyperthyroidism in cats and hypothyroidism in dogs). Examples of iatrogenic hypertension include exogenous steroids, high dietary salt intake in the rare salt sensitive individual, and phenylpropanolamine (PPA) treatment for canine urinary incontinence. Blood pressure measurement and screening for proteinuria should be monitored in cases so treated. Normal systolic BPM is <150 mmHg or <10 mmHg over the reference range for the breed, when minimal risk for target organ damage occurs; there is

intermediate risk for such damage at 150-179 mmHg (or 10-40 mmHg over the reference range for the breed), and high risk for damage at ≥180 mmHg (or >40 over the average BPM for the breed).[29]

Check for infectious diseases

Infectious diseases may cause immune-complex glomerulonephritis (ICGN), vasculitis, or minimal change disease with proteinuria.[2,6,7,18,22] Proteinuria may be associated with acute infections (for which convalescent tests may be needed), for instance with Rocky Mountain Spotted Fever or adenovirus-1 (infectious canine hepatitis), or chronic immune stimulation and glomerular immune-complex deposition may occur in carriers of many other infections such as Anaplasmosis, Babesiosis, bacterial infections, Bartonellosis, Borreliosis (Lyme disease), Brucellosis, Dirofilariasis (heartworm), Ehrlichiosis, FeLV/FIV, fungal infections, Hepatozoonosis, Leishmaniasis, Mycoplasmosis, etc. Some of these diseases may also cause vasculitis, polyarthropathy (synovitis), meningitis, uveitis, endocarditis, and/or bone marrow damage or immune-mediated cytopenias, such as thrombocytopenia, anemia, and/or neutropenia. If an infectious disease is causing proteinuria and is allowed to progress, more damage to the kidney will be ongoing, leading potentially to nephrotic syndrome, hypertensive target organ damage, and/or renal failure. Animals found to have infectious disease should be checked for proteinuria, and vice versa (animals with proteinuria should be checked for infectious diseases in their area). The commonly used in-house screening test, SNAP 4Dx Plus (IDEXX) for heartworm antigen and antibodies against *Borrelia* (Lyme), *Anaplasma*, and *Ehrlichia* spp. may identify seropositive animals. This test shows excellent sensitivity/specificity and repeatability. It is very rewarding to be able to detect and treat occult proteinuria in seemingly normal non-clinical (asymptomatic) animals at a time when early intervention may make a real impact, and before presenting with a more severe stage of illness, with anorexia, vomiting, and azotemia, by which time prognosis may be guarded.

Check for neoplasia

If not done already, a complete physical examination, CBC, biochemical profile, urinalysis, and imaging (chest radiographs and preferably abdominal ultrasound) are recommended to check for gross evidence of neoplasia, which can be associated with ICGN and proteinuria.

Check for genetic diseases

In breeds with familial glomerulopathy or amyloidosis, if genetic marker tests are as yet unavailable, screening for proteinuria before animals are used for breeding (several times a year), or annually for non-breeding pets, is advocated by conscientious breed clubs and owners. Examples of breeds predisposed to proteinuria include Bernese Mountain Dog, Bull Mastiff, Bull Terrier, English Cocker Spaniel, Samoyed, Soft Coated Wheaten Terrier, Shar pei, etc. Breeds that are predisposed to Lyme nephritis may also be monitored more closely, including Labrador and Golden Retrievers.

WHEN IS RENAL BIOPSY INDICATED?

Diagnostic tests for dogs with greater magnitude proteinuria (UPC is 3.5 or greater) or those with progressive proteinuria, hypertension, hypoalbuminemia, or azotemia

Additional testing is recommended as essential in these cases, such as imaging, a more in-depth search for infectious diseases, and renal cortical biopsy, if the dog is a candidate. It should be stable to undergo the biopsy procedure, with hypertension controlled, with adequate platelets and without end stage renal disease (when renal biopsy findings would be unlikely to help). Experienced personnel are needed to both procure the sample and to interpret it. Although examination of renal biopsies by light microscopy alone may differentiate amyloidosis from other glomerular diseases, it is recommended to have the renal biopsies prepared for more thorough examination, including transmission electron microscopy, immunofluorescence, and thin section (3 micron) light microscopy, in order to characterize the subtype of glomerular disease, e.g., ICGN, glomerulosclerosis, amyloid or non-amyloid fibrillary deposition, non-immune complex glomerulopathy or nephropathy, ultrastructural glomerular basement membrane defects, or to reveal primary tubulointerstitial disease as the cause of renal proteinuria.[23,24] Such biopsy samples should be sent in special media, on ice, expedited overnight, and sent to the International Veterinary Renal Pathology Service for interpretation by veterinary nephropathologists[30] (in the United States: IVRPS, Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210, contact Dr Rachel Cianciolo at rachel.cianciolo@cvm.osu.edu ; in Europe: IVRPS, Utrecht Veterinary Nephropathology Service, Utrecht University, Utrecht, The Netherlands, contact Dr Astrid M. van Dongen at a.m.vandongen@uu.nl). Treatment can be better guided once correct characterization is done.

Until veterinary validation studies are done, we can get clues for which treatments may help in a particular PLN subtype by studying what is known for human cases.

WHAT ARE THE CURRENT RECOMMENDATIONS FOR INTERVENTION?

Intervention for PLN usually includes an RAAS (renin-angiotensin-aldosterone system) inhibitor, e.g., an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin-receptor blocker (ARB) to decrease proteinuria; an anti-thrombotic low dose of baby aspirin or clopidogrel (if hypoalbuminemic), to decrease risk of thromboembolic events; omega-3 fatty acid supplementation; diet modifications (low protein, phosphorus, salt); possibly immunosuppressants (depending on the biopsy findings); amlodipine or other antihypertensives (if still hypertensive despite RAAS inhibitor therapy); anti-aldosterone or anti-renin medications (if UPC is non-responsive to other RAAS inhibitors alone); anti-emetics; antacids; phosphate binders; colloid and/or crystalloid supportive therapy; and other treatments, e.g., for chronic renal failure, as indicated. Immunosuppressives are generally not recommended unless there is documentation of ICGN by biopsy results, however, if a case on standard PLN protocol (without immunosuppressives) is deteriorating and renal biopsy is not able to be done, immunosuppressives may be started in view that about half of glomerular disease is found to be immune-mediated.[24] The BPM, UPC, serum albumin, creatinine, BUN, phosphorus, bicarbonate, PCV, and other variables should be monitored as needed. Re-evaluation for decreased Lyme Quant C6 (IDEXX) in Lyme nephritis cases is usually done 3-6 months after starting treatment with doxycycline and standard PLN treatments, to get a new baseline and check for indicators of clearance of carrier status. In other situations, PCR for infectious agents may be done 1-2 months after antibiotics are stopped, however negative PCR status does not guarantee clearance of infection. Further detailed information regarding guidelines for PLN diagnosis and treatment, with and without renal biopsy, are available in the J Vet Intern Med 2013 December supplement papers, found at https://onlinelibrary.wiley.com/toc/19391676/27/s1 .

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