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How to Interpret Proteinuria Results

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Proteinuria can be caused by pre-renal, post-renal, and primary renal disorders (Table 3). A complete urinalysis that includes urine specific gravity (USG), semi-quantitative evaluation of proteinuria on chemistry dipstrip, and urinary sediment microscopy should be performed on every dog or cat in which routine blood work is indicated as part of a minimal database. Evaluation of proteinuria with other methods such as UPC (urine protein to creatinine ratio) or MA (microalbuminuria) should be used to confirm and further characterize the presence or absence of proteinuria.

Protein in the urine is measured by qualitative, semi-quantitative, or quantitative methods. Standard urine chemistry dipstrips contain one pad that detects protein. Zoetis provides an automated reader of dipstrips, the VETSCAN® UA Urine Analyzer, with chemistry pads to measure urinary proteins that include standard urine protein, urinary creatinine as part of UPC, and MA. Urine chemistry by dipstrip provides optimal results when read by an automated strip reader instead of manual reporting by visual inspection.[1] Integration of dipstrip results for standard protein, UPC, and MA has the potential to provide more clinically useful information than from individual protein results. Examples for the interpretation of various combinations of results for proteinuria based on standard dipstrip, UPC, and MA are presented in Table 4.

Measurement of urinary protein using dipstrip, UPC, and MA may provide useful information in the following circumstances:

- Screening apparently healthy geriatric or senior dogs and cats before the patient is clinically ill
- Animals with confirmed or suspected systemic hypertension
- Sub-staging CKD by IRIS criteria
- Following CKD patients before and during treatment
- Screening dogs or cats to allow early detection of an hereditary nephropathy
- Animals with chronic inflammation, infections, neoplasia or immune-mediated disease
- Animals with endocrinopathy such as hyperadrenocorticism, diabetes mellitus, and hyperthyroidism
- Animals with critical illness

Dipstrip for Measurement of Urinary Proteins

The standard semi-quantitative reagent test strip for protein measures total proteins. It is most sensitive for the detection of albumin and less sensitive to globulins, Bence Jones protein, mucoproteins, and hemoglobin. The degree of protein is reported as 0, trace, 1+, 2+, and 3+, which corresponds to < 15 mg/dL, 15 mg/dL, 30 mg/dL, 100 mg/dL, and 300 mg/dL semi-quantitative values, respectively.[2]

False positive reactions are common, and may occur with blood contamination, highly alkaline urine (pH>8), urine contaminated with benzalkonium chloride, or highly concentrated urine samples (>1.050). False negative reactions can occur in very acidic or dilute urine. Dipstrip reagent pads that are negative for protein may show proteinuria when more sensitive methods, such as UPC or MA, are used. A dipstrip positive result ≥ trace for protein should be confirmed with another method. Trace and 1+ dipstrip reactions are the most problematic to know if they indicate pathological proteinuria without confirmatory testing (UPC or MA). Dipstrip protein ≥ 2+ are usually associated with pathologic proteinuria and albuminuria.

Urinary Protein to Creatinine Ratio (UPC)

UPC is a unitless number following the division of urinary protein in mg/dL by urinary creatinine in mg/dL. UPC is measured on benchtop analyzers as the gold standard, but can be estimated on urinary dipstrips. Dipstrips that contain a pad to measure urinary creatinine concentration allow the UPC to be calculated and reported in semi-quantitative bins, see Table 1 below. A UPC < 0.2 is normal for most dogs and cats. Borderline values are from 0.2 to < 0.4 for the cat and from 0.2 to < 0.5 for the dog. Values ≥ 0.4 for the cat and ≥ 0.5 for the dog are considered proteinuric. Values ≥ 2.0 are often associated with primary glomerular disease and albuminuria.

Table 1. VETSCAN UA Semi-quantitative UPC Results

	Dog	Cat
Normal	< 0.2	< 0.2
Borderline Proteinuric	0.2 to 0.5	0.2 to 0.4
Proteinuric	≥ 0.5 to < 2.0	≥0.4 to < 2.0
Severe Proteinuric	≥ 2.0	≥ 2.0

In general, UPC results are not affected by differences in sex, method of urine collection, fasted vs fed states, or by time of day at collection. However, UPC may increase when urinary creatinine excretion declines with loss of muscle mass, as frequently occurs with chronic kidney disease (CKD).[3] The variability in UPC may be more dramatic in those with underlying glomerular disease. Proteinuria based on UPC can be observed in apparently healthy animals that could indicate random biologic variability of protein filtration or could indicate an underlying disorder that has not yet been diagnosed.

The International Renal Interest Society (IRIS) uses UPC to substage CKD in dogs and cats as:

Table 2. IRIS Substaging by Proteinuria

	Dog	Cat
Non-proteinuric	< 0.2	< 0.2
Borderline Proteinuric	0.2 to 0.5	0.2 to 0.4
Overtly Proteinuric	> 0.5	> 0.4

The degree of proteinuria as assessed by UPC at the time of diagnosis affects the survival of cats[3] and dogs[4] with CKD.

Microalbuminuria (MA)

Urine from normal dogs and cats contains < 1.0 mg/dL of albumin. Microalbuminuria is defined as 1 to 29 mg/dL of urinary albumin; ≥ 30 mg/dL is considered overt proteinuria/albuminuria.[5] Measurement of MA is useful to determine loss of protein into urine below the detection of urine on dipstrips and to confirm results of positive dipstrip reactions for protein.[6,7]

The VETSCAN UA reports semi-quantitative MA results as either < 2.5 mg/dL (negative) or ≥ 2.5 mg/dL (positive) to be used as a screening test.

MA measured at a referral lab may be needed in order to determine a specific value for MA between 2.5 and 29 mg/dL and to determine a baseline to document trends. It is more appropriate to follow UPC when MA values exceed 29 mg/dL or UPC >2.[5]

MA positive status is found in 25% of apparently healthy dogs[8] and 9 to 14%[9,10] of apparently healthy cats[10]; this increases with age and when a dog or cat is ill. MA positive status was found in 31% of dogs and 26% of cats with various clinical conditions.[6,7] MA occurs in greater than 50% of critically ill dogs, a finding that is more frequent than an increased UPC.[11]

MA positive status develops in dogs and cats early on in many disease processes (Table 3), often before UPC is overtly increased. The same diseases shown to be associated with increased UPC usually have MA positive status at the same time. **It appears likely that MA becomes positive first, followed by UPC, and then dipstrip protein positive in diseases with progressive glomerular injury.**[5]

Detection of MA can indicate the presence of early glomerular damage not detectable by other methods.[12] Albuminuria that is persistent or of increasing magnitude may be the earliest clinical indicator of glomerular disease.[13] Systemic inflammation can be associated with endothelial leakage of protein and MA. Dogs with severe inflammatory response syndrome (SIRS) frequently have increased UPC and MA, associated with glomerular dysfunction. Deposition of immune complexes within the glomerulus, as well as decreased blood flow to the kidneys and injurious molecules synthesized by tumors, are thought to contribute to glomerular injury and proteinuria in patients with neoplasia.[14]

Renal Disease and Proteinuria

Systemic hypertension from any cause can be associated with glomerular proteinuria, especially when increases in systemic blood pressure are severe. Systemic blood pressure should be measured in all patients with glomerular proteinuria since successful lowering of blood pressure can lessen this type of proteinuria.

For a general approach to renal proteinuria, refer to Figure 1. **The hallmark of glomerular-origin proteinuria (regardless of specific disease) is the documentation of excess urinary protein in association with a non-inflammatory urinary sediment (≤ 10 RBC/HPF, ≤ 5 WBC/HPF, < 2 non-squamous epithelial cells/HPF).** However, it should be noted that not all patients with excess WBC or RBC in urinary sediment are associated with increased UPC or MA status. If the UPC or MA is increased in patients with an active sediment, these measurements should be repeated when urinary sediment is inactive.[13] The presence of

glomerular proteinuria (urine reagent strip, UPC, or MA) allows the identification of patients with renal disease in which glomerular permeability to primarily albumin has increased. Longstanding proteinuria of glomerular origin can lead to further glomerular injury, tubular damage, tubulointerstitial inflammation, and progressive nephron loss.[13] Despite less proteinuria for cats with CKD compared to dogs with CKD, survival for cats was significantly related to the degree of proteinuria based on UPC in two studies. Cats with UPC < 0.2 lived the longest and cats with > 1.0 or > 0.4 UPC lived the shortest times.[3,15] Dogs with UPC ≥ 1.0 at the time of CKD diagnosis did not live as long as dogs with UPC < 1.0 and were at greater risk for a uremic crisis.[4] The risk for persistent MA positive status to result in progressive CKD requires more research.

In patients with CKD, therapeutic plans designed to normalize or at least reduce the degree of proteinuria are often undertaken with the goal to reduce the risk for progressive nephron loss.[13] It has not yet been determined if treatments for IRIS CKD Stage 1 that are designed to lower MA (diet or drugs) should be prescribed to improve outcome in survival or stability of renal function. The long-term effects of stable MA or borderline UPC levels on renal function or survival during primary glomerular disease or systemic disease are not known.[10]

The magnitude of proteinuria and trends for increasing or decreasing proteinuria over time are important considerations in determining how aggressive the diagnostic approach and treatment should be (Figure 2.) **Persistence of protein positive status based on UPC or MA should be confirmed 3 times within 6 weeks.** If MA is positive and UPC is overtly positive, UPC is monitored in the future. If MA is positive and UPC is normal, MA should be followed to see if the magnitude of MA is increasing over time. A low level of MA that is not increasing may reflect previous renal damage or a disease process that is no longer active. Treatment details regarding dietary or pharmacologic therapies designed to reduce the magnitude of renal proteinuria are beyond the scope of this white paper.

Table 3. Classification of Proteinuria Based on Site or Mechanism[6,7,10,12,16-21]

Category	Subcategory	Mechanism	Causes/Examples	Comments
PRE-RENAL		• Glomerular barrier function is normal – selectivity maintained for molecular size and charge • Overload of small MW molecules in plasma that can cross the normal glomerulus into urine	• Myoglobinuria • Hemoglobinuria • Bence Jones Proteinuria (light chains) – Multiple Myeloma or Paraproteinemia (lymphoma)	• Myoglobin has no plasma carrier – clear plasma • Hemoglobin has a carrier protein that must first become saturated before free hemoglobin can enter urine – pigmented plasma
POST-RENAL	Urinary	• Glomerular barrier is normal • Blood loss into urine – capillary bleeding or inflammation • Ureter, Bladder, Urethra	• Trauma, Cystocentesis, Urolithiasis, Urothelial Neoplasia, Infection • Cystitis (including interstitial) Urethritis, Urethral Obstruction, Ureteral Obstruction	• History – LUT urgency or trauma; physical exam, imaging, urine culture, urine cytology, histopathology
	Genital	• Glomerular barrier is normal • Blood loss into urine • Prostate, penis, prepuce, uterus, vagina vestibule, vulva	• Prostatitis, Prostatic Neoplasia, Benign Prostatic Hypertrophy (BPH) • Estrus, pyometra, metritis, balanoposthitis, TVT	• History, physical exam, imaging, urine cytology, histopathology
PRIMARY RENAL	Functional	• No structural renal lesions. Transient dysfunction of the glomerular barrier results in mostly albuminuria. Renal vascular congestion and adrenergic system activation result in proteinuria	• Fever, seizures, exposure to extreme heat or cold, strenuous exercise (non-conditioned dogs), swimming dogs, severe stress, congestive heart failure, large tumors obstructing blood return from vena cava	• UPC usually < 1.0 • History, physical exam, thoracic and abdominal imaging, echocardiogram • Transient nature of proteinuria
	Pathological	• Result of functional or structural lesions within any region of the kidney		• Proteinuria is persistent

Category	Subcategory	Mechanism	Causes/Examples	Comments
PRIMARY RENAL (cont'd)	Glomerular	• Abnormal glomerular barrier • Loss of glomerular permselectivity	• Systemic hypertension (any cause) • Primary glomerulonephritis, hereditary nephropathy or glomerulopathy, glomerulosclerosis (CKD), amyloidosis • Secondary glomerulonephritis: chronic infectious or parasitic disease, acute and chronic inflammation, neoplasia, immune-mediated disease, drug reactions, leptospirosis (rarely), any disease with circulating chronic antigen excess (immune complexes) • Exogenous glucocorticosteroids (D), Cushing's disease (D), hyperthyroidism (C), diabetes mellitus, critical illness	• UPC often > 0.5 – 1.0 • UPC > 1.0 and often > 2.0 at time of diagnosis
	Tubular	• Glomerular barrier is normal • Decreased tubular reabsorption (tubulopathy): less reabsorption of small MW molecules and some moderate MW molecules (albumin) • Increased tubular secretion • Leakage of tubular cell proteins	• AKI, ATN, ARF Leptospirosis • Fanconi syndrome – congenital (D) – Basenji • Fanconi syndrome – acquired: jerky treats (D), drugs; sometimes part of AKI; Copper associated hepatitis (D) • CKD – tubules fail to reabsorb small amounts of albumin that are normally reabsorbed by proximal tubules	• UPC usually < 2.0 UPC often 0.5 – 1.0 • Glucosuria & aminoaciduria if generalized proximal tubulopathy
	Interstitial	• Glomerular barrier is normal • Exudate or plasma enters tubular lumens from peritubular capillaries	• Allergic drug reactions (acute interstitial nephritis) • Leptospirosis Pyelonephritis (bacterial)	• Tubules and interstitium are often involved with the same disease process at the same time • Tubulo-interstitial disease is the term often used

MW – molecular weight, MA – microalbuminuria, LUT – lower urinary tract, UPC – urinary protein to urinary creatinine ratio, TVT – transmissible venereal tumor, AKI – acute kidney injury, ATN – acute tubular necrosis, ARF – acute renal failure

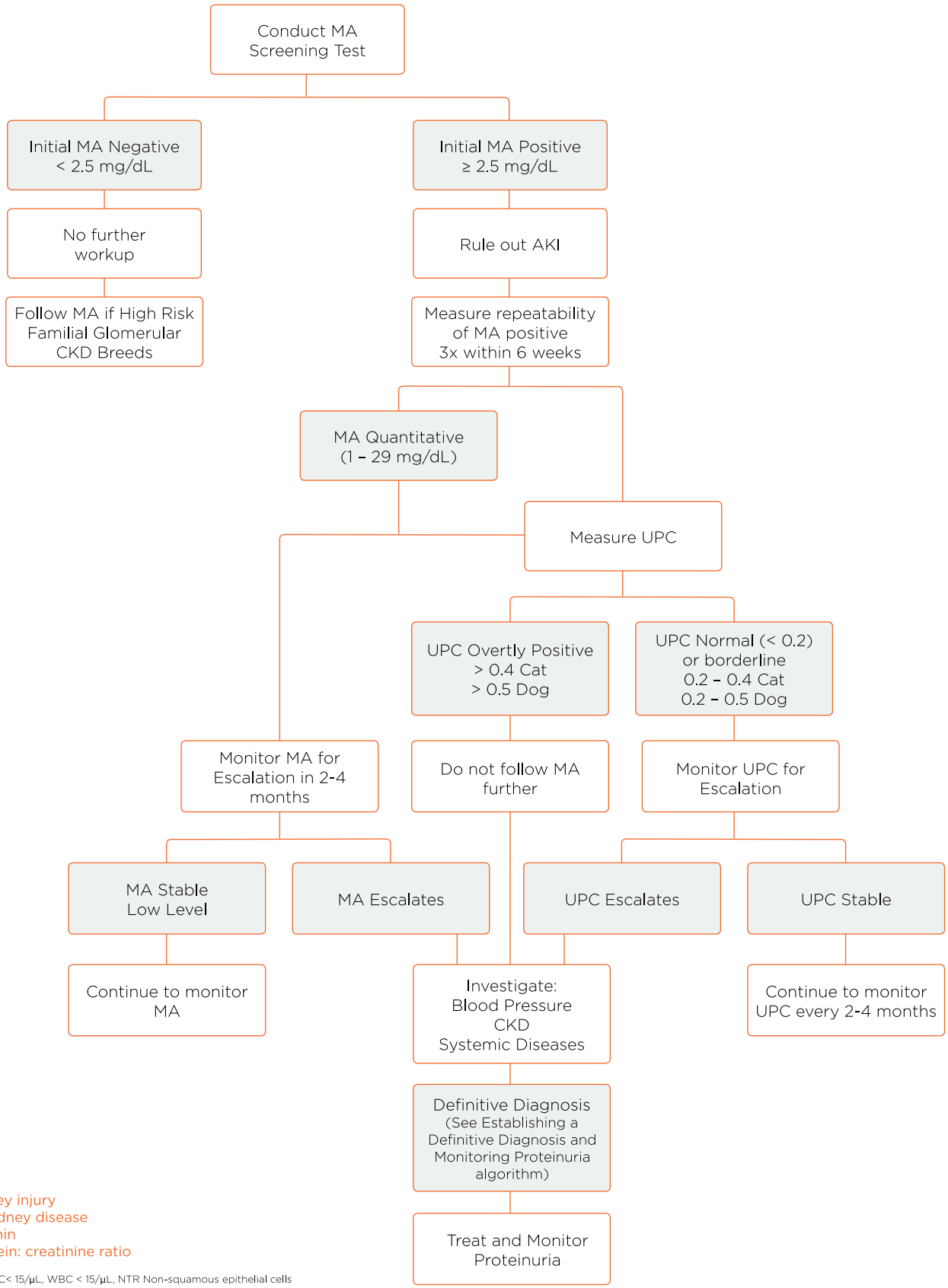
Table 4. Hypothetical Interpretation of Various Combinations of Proteinuria Results by Dipstrip, UPC,* and MA**

Dipstrip	UPC*	MA**	Interpretation
0	< 0.2	Neg	• No proteinuria
0 or trace	< 0.2	Positive	• Albuminuria • Mild or early proteinuria
Trace to 1+	< 0.2	Neg	• No proteinuria
Trace to 1+	> 0.4(c) > 0.5(d)	Neg	• False negative MA (uncommon) • Non-Albumin Proteinuria (Tubular most common)
1+	< 0.2	Positive	• Albuminuria
Trace to 2+	< 0.2	Negative	• False positive dipstrip
2+	≥ 0.2 – < 0.4 (c) ≥ 0.2 to < 0.5 (d)	Positive	• Albuminuria • Mild to moderate proteinuria
3+	≥ 0.4 – < 2.0 (c) ≥ 0.5 – < 2.0 (d)	Positive	• Albuminuria • Moderate to severe proteinuria
3+	≥ 2.0	Positive	• Severe proteinuria • Primary glomerular disease • Protein-losing nephropathy • Rule out nephrotic syndrome – Evaluate serum albumin. cholesterol, and clotting cascade

* The UPC on the VETSCAN UA is reported as < 0.2, ≥ 0.2 to < 0.5, ≥0.5 to < 2.0, ≥ 2.0 for the dog and as < 0.2, ≥ 0.2 to < 0.4, ≥0.4 to < 2.0, ≥ 2.0 for the cat

** MA < 2.5 mg/dL is negative; MA ≥ 2.5 mg/dL is positive

Figure 1. Algorithim General Approach to Renal Proteinuria Using MA and UPC in Dogs and Cats With an Inactive Sediment**

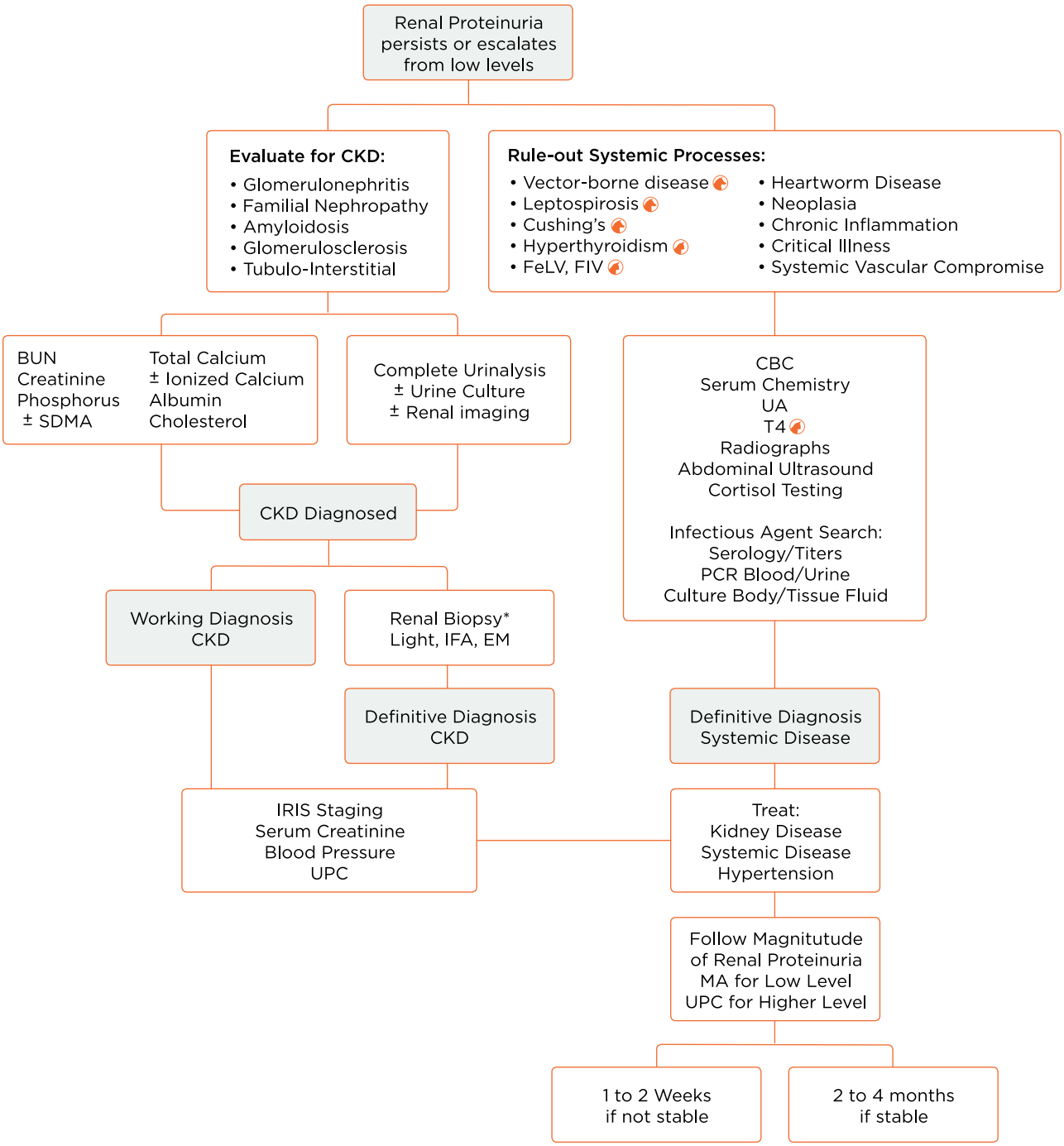


AKI = acute kidney injury
CKD = chronic kidney disease
MA = microalbumin
UPC = urine protein: creatinine ratio

**Clear Supernatant, RBC< 15/µL, WBC < 15/µL, NTR Non-squamous epithelial cells

Figure 2. Approach to Establishing a Definitive Diagnosis and Monitoring Proteinuria

(If AKI ruled out)



AKI = acute kidney injury
EM = electron microscopy
IFA = Immunofluorescent antibody
Light = light microscopy
MA = microalbumin
UPC = urine protein: creatinine ratio

*Renal biopsy is not advocated for all patients with CKD, especially if the kidneys are small and there is advanced disease. Renal biopsy is helpful to disclose immunological and non-immunological causes for those with renal proteinuria before there is obvious azotemia and extensive renal fibrosis. Pathological findings from the combination of light microscopy using special stains, immunofluorescent microscopy, and electron microscopy are needed in order to properly assess underlying renal pathology causing renal proteinuria (most commonly glomerular causes). The prognosis for patients with renal proteinuria varies by the specific pathological diagnosis. Aggressive treatment protocols using immunosuppressive drugs are best directed in patients that have undergone renal biopsy providing evidence for an immune process (e.g. immune complex deposition within the glomeruli). The International Veterinary Renal Pathology Service (IVRPS) processes renal tissues and examines them with the special techniques mentioned above, and then a group of veterinary nephropathologists consult to deliver a final diagnosis. Further information about how to handle and submit renal tissue and fees can be accessed on line from the IVRPS at https://vet.osu.edu/vmc/international-veterinary-renal-pathology-service-ivrps

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