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Review

Advances in the evaluation of canine renal disease

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ABSTRACT

Many recent advances in the evaluation of dogs with kidney disease have improved our diagnostic algorithms and have impacted our therapeutic strategies. Non-invasive techniques, such as urinary and serologic biomarker evaluation, can help a clinician diagnose and treat a patient that cannot undergo a renal biopsy for clinical or financial reasons. Some biomarkers might help localize the affected structure (glomerulus vs. tubule) and indicate the type or severity of injury present. Although more research is needed, studies indicate that some biomarkers (e.g. urine protein to creatinine ratio and urinary immunoglobulins) can be useful in predicting adverse outcomes. Importantly, the sensitivity and specificity of biomarkers for renal injury should be established and clinicians need to understand the limitations of these assays.

If a renal biopsy is performed, then it should be evaluated by a specialty diagnostic service with expertise in nephropathology. A panel of special stains, immunofluorescence for the detection of immunoglobulins and complement factors, and transmission electron microscopy can be routinely employed in cases of glomerular disease. These advanced diagnostics can be used to detect immune deposits in order to definitively diagnose immune complex mediated glomerular disease. Integrating the results of biomarker assays and comprehensive renal biopsy evaluation, the clinician can make informed therapeutic decisions, such as whether or not to immunosuppress a patient.

Introduction

Diseases of the kidney are common in small animals, and guidelines for the clinical staging and grading of renal disease have been created and adopted by the International Renal Interest Society (IRIS).[1] Criteria for both acute kidney injury (AKI) and chronic kidney disease (CKD) have helped identify cases in which the injury is reversible or can be mitigated by therapeutic intervention; however, the veterinarian must first determine the nature of injury. Recent advances in urinary and serum biomarker analysis and in the evaluation of renal tissue have markedly improved our diagnostic algorithms. They have enhanced our ability to categorize diseases based on the structure affected (glomerulus vs. tubule) and pathogenesis (e.g. immune complex-mediated disease). These diagnostic techniques are especially useful in the current era of hemodialysis, plasmapheresis and immunosuppression for immune-mediated diseases.

Because great strides have been made in the evaluation of proteinuric kidney disease, the discussion here is focused on urinary biomarkers in protein losing nephropathy. Where appropriate, the diagnostic relevance of the various urinary biomarkers in AKI will be mentioned. This will be followed by discussion of serum biomarkers of kidney disease. Finally, the techniques used for comprehensive evaluation of renal tissue (especially glomeruli) will be presented. Much of the knowledge regarding urinary biomarkers and comprehensive evaluation of renal biopsy specimens has been gained by the authors' experience in directing the International Veterinary Renal Pathology Service (IVRPS), which is a collaborative effort between the Ohio State University and Texas A&M University that routinely evaluates urine by gel electrophoresis and renal tissue with transmission electron microscopy (TEM), immunofluorescence (IF) and histopathology using a panel of special stains. For simplicity's sake, the term 'renal biopsy' will imply comprehensive analysis using these modalities.

Proteinuric kidney disease

Renal proteinuria is commonly observed in dogs with kidney disease. While proteinuria can result from either glomerular or tubular disease, a urine protein to creatinine ratio (UPC) >2.0 is strongly indicative of glomerular disease. Moreover, the role of proteinuria in the development of CKD is likely under-appreciated because affected dogs are identified late in their disease course. Renal biopsy (discussed below) is the gold standard for evaluating canine glomerular disease; however, many dogs are not candidates for the

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[1] See: http://www.iris-kidney.com/guidelines/index.html (accessed 2 May 2016).

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1090-0233/

procedure due to health or financial reasons. Using less invasive and inexpensive diagnostic methods not only provides evidence for the presence or absence of glomerular disease, but the results might also influence the decision to perform a renal biopsy. Several extensive reviews discuss the pathophysiologic mechanisms and appropriate interpretation of proteinuria in small animals (Lees et al, 2005; Grauer, 2007, 2011; Harley and Langston, 2012). Despite consensus statements and reviews about the importance of evaluating, monitoring, and treating renal proteinuria, proteinuria is often still overlooked in small animals as an early marker of kidney disease. In some cases, this may lead to clinicians not detecting renal disease until development of azotemia, missing the opportunity for timely therapeutic intervention.

Proteinuria is defined by the detection of an excessive amount of protein in the urine by means of semiquantitative tests on urinalysis (dipstick) or quantitative measurements of UPC or urinary albumin concentration (Lees et al, 2005). The origin of proteinuria, its persistence and its magnitude must be established (Lees et al, 2005). A step by step guide to localize the source of proteinuria is discussed in detail in the 2005 ACVIM consensus statement (Lees et al, 2005). Persistent renal proteinuria (UPC ≥0.5 in at least three samples two or more weeks apart without a contributing prerenal or postrenal cause) could be glomerular, tubular, or both. In the discussion below, 'proteinuria' refers to 'renal proteinuria', assuming pre- and post-renal causes of proteinuria have been ruled out.

Pathophysiology of renal proteinuria

In the healthy kidney there are several mechanisms that prevent protein loss into the urine. The glomerular filtration barrier, composed of the fenestrated endothelium and glycocalyx, trilaminar glomerular basement membrane (GBM), and podocytes with slit diaphragms, is the main mechanism for preventing proteinuria (D'Amico and Bazzi, 2003). The ultrastructural morphology of the glomerular filtration barrier will be described below. This barrier allows proteins <40 kDa (low molecular weight [LMW] proteins) to filter freely; however, intermediate molecular weight (IMW) proteins are largely restricted, and high molecular weight (HMW) proteins (>100 kDa) are almost completely restricted from glomerular filtration (D'Amico and Bazzi, 2003). A second mechanism preventing proteinuria is the ability of healthy proximal tubular epithelial cells to reabsorb proteins normally present in the urinary filtrate (D'Amico and Bazzi, 2003).

When renal damage occurs, the mechanisms that prevent proteinuria are compromised. Glomerular damage increases the permeability of the filtration barrier, allowing increased filtration of IMW and HMW proteins (D'Amico and Bazzi, 2003). Tubular damage can result in decreased protein reabsorption, leakage of proteins from tubular epithelial cells, and increased production of proteins involved in injury and repair (D'Amico and Bazzi, 2003). Glomerular damage often results in massive proteinuria whereas tubular damage is thought to result in mild proteinuria. However, even today, the magnitude of proteinuria expected in purely tubulointerstitial disease is under debate. Conflicting experimental data, predominantly in rodents, have been used to support arguments both for and against the development of mild to moderate proteinuria when proximal tubular epithelial cells are injured or lost (Comper et al., 2008; Navar, 2009; Tanner, 2009). Treatment to reduce proteinuria has been shown to mitigate progression of renal disease in dogs (Brown et al., 2003; Lees et al, 2005). The reader is referred to additional sources with detailed discussions of treatment protocols for reducing proteinuria in dogs (Lees et al, 2005; Harley and Langston, 2012).

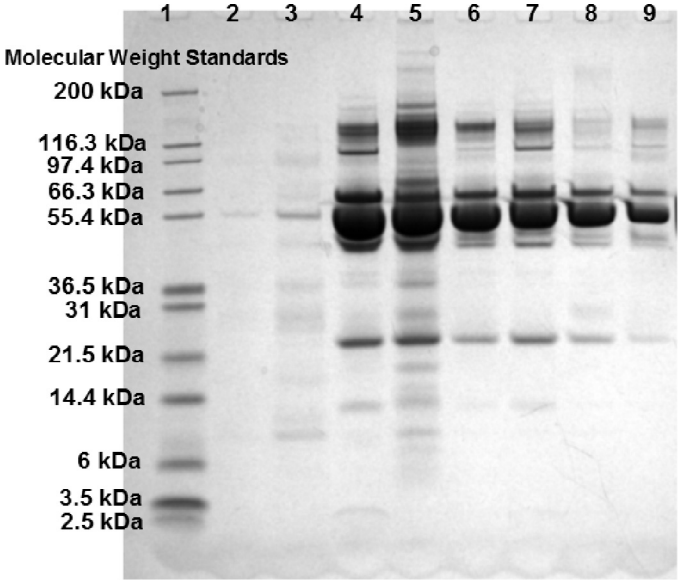

Fig. 1. Image from Bis-Tris gel electrophoresis demonstrating urine protein banding patterns obtained from dogs with primary tubular damage (lanes 2 and 3: low magnitude of proteinuria with predominantly low molecular weight bands), primary glomerular damage (lanes 6, 7, 8 and 9: relatively large magnitude of proteinuria with predominantly intermediate and high molecular weight bands and few low molecular weight bands), and mixed glomerular and tubular damage (lanes 4 and 5: mixture of prominent bands ranging from low to high molecular weight).

Patterns of proteinuria

When urine from dogs with renal injury is analyzed with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) or similar methods, the pattern of protein banding can help determine if glomerular damage, tubular damage, or both are contributing to the proteinuria (D'Amico and Bazzi, 2003; Zini et al., 2004). Primary tubular damage with limited or no glomerular damage will generate predominantly LMW proteins, while primary glomerular damage with minimal or no tubular damage generates a pattern of IMW and HMW protein bands (Schultze and Jensen, 1998; D'Amico and Bazzi, 2003; Zini et al., 2004; Giori et al., 2011). Mixed patterns with protein bands in low, intermediate, and high molecular weight ranges, however, are the predominant patterns seen in proteinuric dogs, as concurrent damage to both glomerular and tubular components commonly occurs (Schultze and Jensen, 1998; Zini et al., 2004; Giori et al., 2011). Fig. 1 demonstrates different urine protein banding patterns from proteinuric dogs evaluated by the IVRPS.

Urine protein: Creatinine ratio

Once proteinuria is deemed to be persistent and renal in origin, evaluation and monitoring of UPC are the important steps in determining the presence and severity of glomerular damage. A persistent UPC between 0.2 and 0.5 is classified as borderline proteinuria while UPC ≥0.5 is considered proteinuric.[2] UPC values ≥2 are generally considered to be indicative of glomerular proteinuria, while values <2 are thought to occur more often with tubular proteinuria (Center et al., 1985; Lees et al., 2005). Certainly, in the authors' experience, a UPC ≥2 is typically associated with at least some injury to the glomerular filtration barrier, with significant glomerular lesions identified in 97.6% of 501 renal specimens from dogs

[2] See: http://www.iris-kidney.com/guidelines/staging.html (accessed 2 May 2016).