Consensus Statement
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Pathologic Evaluation of Canine Renal Biopsies: Methods for Identifying Features that Differentiate Immune-Mediated Glomerulonephritides from Other Categories of Glomerular Diseases

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Background: Human renal biopsies are routinely evaluated with light microscopy (LM) using a panel of histologic stains, transmission electron microscopy (TEM), and immunofluorescence (IF) microscopy to obtain a diagnosis. In contrast, the pathologic evaluation of glomerular disease in veterinary medicine has relied mostly on LM and was of limited utility. To address this problem, recently established veterinary renal diagnostic centers have adopted methods used in human nephropathology for evaluation of renal biopsies. Three broad categories of disease, which have the greatest implications for clinical management of proteinuric dogs, have been established and include amyloidosis, immune complex-mediated glomerulonephritis (ICGN), and non-ICGN.

Objective: To demonstrate histopathologic, ultrastructural, and IF findings in renal biopsy specimens that experienced veterinary nephropathologists utilize to make accurate and clinically useful diagnoses in dogs with proteinuric glomerular disease and to provide guidelines for the proper evaluation of renal biopsies.

Methods: Renal biopsy specimens were routinely examined by LM, IF, and TEM. Samples were reviewed by members of the World Small Animal Veterinary Association Renal Standardization Study Group to identify lesions that were diagnostic for, or suggestive of, the presence of immune complexes (IC) or amyloidosis in all modalities. Ten guidelines for renal biopsy evaluation were formulated.

Results: Each method of investigation contributed important findings that were integrated to make an accurate final morphological diagnosis. The guidelines were validated by an independent group of veterinary pathologists.

Conclusions and Clinical Importance: Routine evaluation of renal biopsies with LM, TEM, and IF is feasible and necessary for making accurate, morphologic diagnoses that can be used to guide clinical management of dogs with glomerular disease.

Key words: Canine; Glomerulonephritis; Immune complex; Immunofluorescence; Proteinuria; Transmission electron microscopy.

Although human renal biopsies are routinely evaluated with light microscopy (LM) using a panel of special stains, transmission electron microscopy (TEM), and immunofluorescence (IF) to obtain a specific and clinically useful diagnosis, the evaluation of glomerular disease in veterinary medicine has often relied on LM alone, which has been of limited utility.

Abbreviations:

CR	Congo red
GBM	glomerular basement membrane
HE	hematoxylin and eosin
ICGN	immune complex glomerulonephritis (nephritides)
IC	immune complex(es)
IF	immunofluorescence microscopy
IVRPS	International Veterinary Renal Pathology Service
JMS	Jones methenamine silver
LM	light microscopy
MT	Masson's trichrome
Non-ICGN	nonimmune complex glomerulonephropathy (ies)
PASH	periodic acid Schiff hematoxylin
TEM	transmission electron microscopy
UVNS	Utrecht Veterinary Nephropathology Service
WSAVA-RSSG	World Small Animal Veterinary Association – Renal Standardization Study Group

Since 2008, two collaborating centers, one located in North America and the other in Europe, have routinely performed these specialized diagnostic evaluations (LM, TEM, and IF) on all adequate samples of renal tissue submitted for pathologic evaluation. The North American center was initially organized at Texas A&M University and was known as the Texas Veterinary Renal Pathology Service. Recently, it has been reorganized as a cooperative effort between the Ohio State University and Texas A&M University and is now known as the International Veterinary Renal Pathology Service (IVRPS). The European center is

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located at Utrecht University in the Netherlands and is known as the Utrecht Veterinary Nephropathology Service (UVNS). By the middle of 2013, the combined cumulative experience of both centers exceeded 960 canine cases. One goal of these centers is to evaluate a sufficient number of specimens annually to develop and maintain the technical and interpretive expertise needed to examine renal specimens with these specialized methods and to reliably make correct diagnoses. In human nephropathology, it has been suggested that the number of cases required for this purpose is 200 per year.[1]

The veterinary pathologists associated with these centers have developed expertise in the evaluation of each modality. These diagnostic centers have not only created a strong foundation for the diagnosis of canine glomerular disease but they also have revealed the deficiency of each modality when used alone. Illustrations of lesions that are definitively diagnostic for, suspicious for, or inconsistent with 3 main categories of glomerular disease (ICGN, amyloidosis, and non-ICGN) are the main goals of this publication. These 3 broad diagnostic categories were created because they have the greatest implications for prognosis and treatment.

Materials and Methods

Cases were evaluated retrospectively by the World Small Animal Veterinary Association Renal Standardization Study Group (WSAVA-RSSG) from the pool of more than 900 canine renal tissue specimens to identify representative cases that clearly demonstrate features, which are definitively diagnostic for, suggestive of, or inconsistent with ICGN, amyloidosis, or non-ICGN. Only specimens that had sufficient tissue for evaluation by all modalities (LM, TEM, and IF) were examined, except for some cases in which amyloidosis was diagnosed via LM. Tissues for LM were sectioned at 3-μm thickness and stained with hematoxylin and eosin (HE), Masson's trichrome (MT), or periodic acid Schiff hematoxylin (PASH). Most tissue sections were also stained with Jones methenamine silver (JMS) and Congo red (CR) the latter of which was performed on 8-micron-thick sections.

Transmission electron microscopy specimens were fixed in chilled 3% buffered glutaraldehyde and then postfixed in 1% osmium tetroxide, serially dehydrated, infiltrated in an acetone/epoxy plastic, and then embedded in a plastic mold. Plastic blocks were cut with an ultramicrotome[a] and thick sections were stained with toluidine blue to identify optimal areas for thin sectioning. Thin sections were cut at 55–60 nm and placed on copper grids[b] and stained with uranyl acetate and lead citrate. They were examined in a transmission electron microscope[c] and representative digital images were captured by the authors (FJC, CAB, and LA) at the Texas Heart Institute, University of Georgia, and University of Padova, respectively.

For IF, unfixed tissue samples were embedded in Optimal Cutting Temperature compound[d] and snap-frozen in liquid nitrogen vapor. Tissues were cryosectioned at 4-μm thickness, and sections were labeled with an appropriate dilution of fluorescein isothiocyanate-labeled anti-IgG,[e] anti-IgM,[e] anti-IgA,[e] anti-C1q,[f] anti-C3,[e] anti-Lambda light chains,[f] and anti-Kappa light chains[f] antibodies. Labeling patterns and fluorescent intensity were evaluated by the authors (GEL and JvdL) with an epifluorescence microscope[g] using appropriate filters and digital imaging.

High resolution, whole-section digital images of all LM slides were obtained with a slide scanning instrument.[h] The scanned LM sections and the digital images of the TEM and IF evaluations were stored on a server where the digital images were managed by digital pathology software[i] that delivers the images via the internet to participating pathologists for viewing[i-k] either independently or jointly during online[l] case-review conferences. In each modality, features diagnostic for, suggestive of, or inconsistent with the presence of IC deposits or amyloid were identified, discussed, and annotated as such. These features were used for the categorization of ICGN, amyloidosis, or non-ICGN.

In addition, the WSAVA-RSSG formulated guidelines for the pathologic evaluation of renal tissue. These guidelines were then distributed among the RSSG and also sent to 5 additional non-RSSG members for external validation. This procedure involved voting (strongly agree, agree, neutral, disagree, and strongly disagree) on each guideline using an anonymous ballot and allowed validators to provide written comments. After ballot submission, the guidelines with greater than 75% agreement were considered to be validated.

Results

ICGN

Definitive diagnosis of ICGN depends on ultrastructural findings of electron-dense immune deposits in subepithelial, subendothelial, intramembranous, or mesangial locations, with many cases also demonstrating IC in multiple locations within the glomerular capillary tuft (Fig 1A,B). Other electron-dense material that is not IC can also be identified with TEM (eg, hyalinosis), but the appearance of these substances differs from IC (Fig 3E,F). Care must be taken by the pathologist to correctly interpret these findings. In our experience to date, glomerular IC deposition is diffuse (ie, affecting every glomerulus) in dogs. Specifically, in cases of canine ICGN where multiple glomeruli were available for evaluation by EM and IF, IC were present in all patent glomerular tufts.

Immune deposits are further characterized by immunofluorescent labeling with antibodies against immunoglobulins and complement components (Fig 1H). A granular, global staining pattern of glomeruli clearly associated with capillary walls, the mesangium, or both with antibodies against C3 and at least 1 class of immunoglobulin is diagnostic of IC, whereas splotchy, segmental staining is considered nonspecific (Fig 3H). Inexperience in the evaluation of IF is cited as the main cause of diagnostic dilemmas in the evaluation of renal biopsies by physician nephropathologists and, therefore, staining patterns should be interpreted with care.[2]

Light microscopy can reveal a wide variety of lesions in ICGN; some of which provide a presumptive diagnosis before TEM or IF results become available. The presence of uniform, prominent remodeling (spikes, holes, or double and irregular contours) of the glomerular basement membrane (GBM) in most glomeruli is highly suggestive of ICGN. Jones Methenamine Silver is the preferred technique for the evaluation of these features (Fig 1E,F), although they can also be observed with PASH (Fig 1C,D). Notably,

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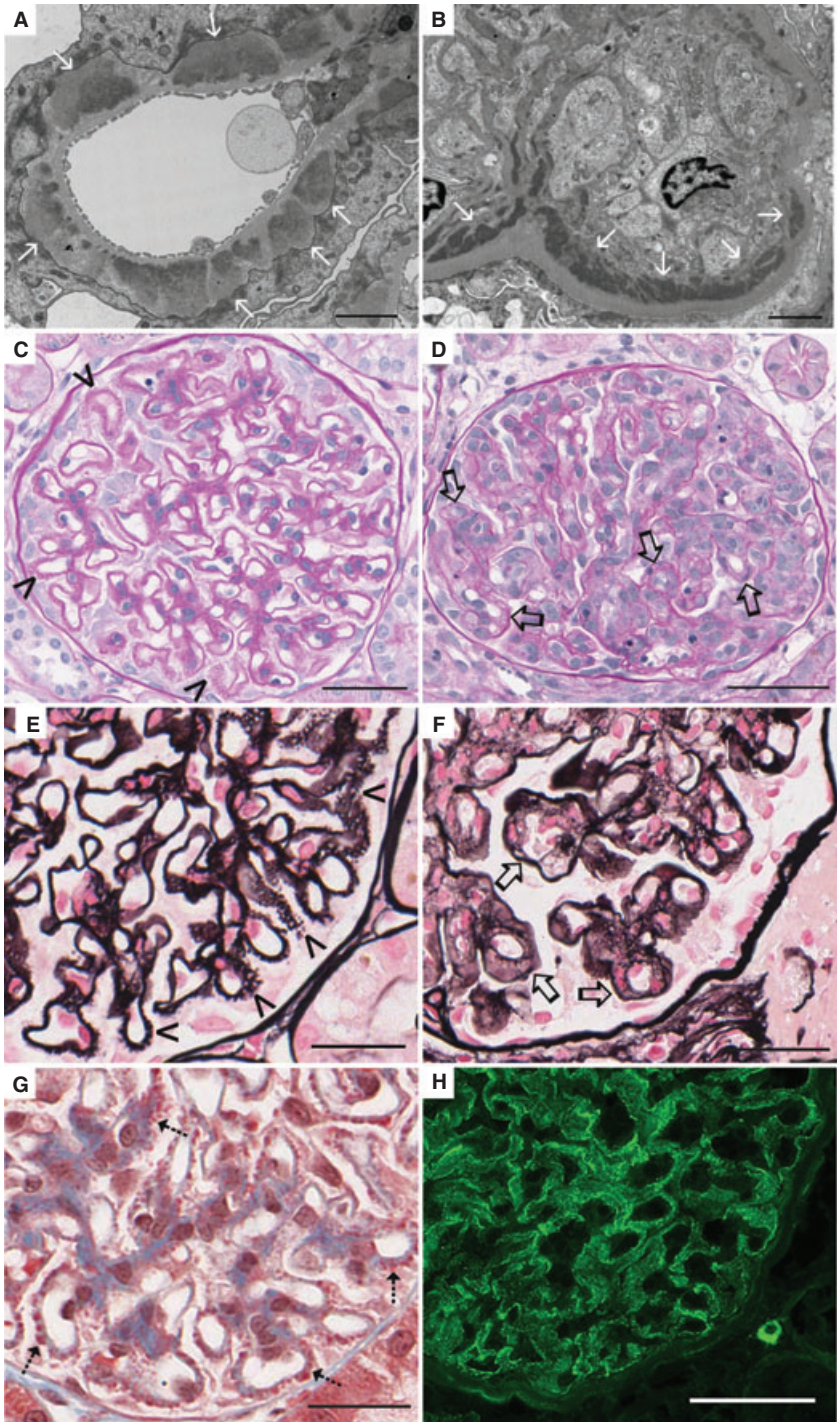

Fig 1. Histologic, ultrastructural, and immunofluorescent findings in cases of ICGN. **(A, C, E, G, H)** Renal biopsy from a 6-year-old castrated male English Setter dog with proteinuria (UPC = 6.6), but not azotemia. (A) Electron microscopy reveals immune complexes (IC) (white arrows) in the abluminal side (subepithelial) of the capillary wall. Scale bar = 2 μm. (C, E) Periodic acid Schiff hematoxylin (PASH) and Jones methenamine silver (JMS) stains of a glomerulus, respectively, reveals remodeling of the glomerular basement membrane (GBM) characterized by spike-like projections (arrowheads) on the subepithelial surface. Scale bar = 50 μm. (G) Masson's trichrome stain demonstrates numerous IC on the subepithelial surface (dotted arrows). Scale bar = 50 μm. (H) Immunofluorescence staining with an antibody against Lambda light chain demonstrates the presence of immunoglobulins along capillary walls. Scale bar = 50 μm. Staining for C3 and IgG revealed a similar pattern (not shown). **(B, D, F)** Renal biopsy from a 3-year-old castrated male Boxer dog with proteinuria and azotemia (UPC = 12.4, serum creatinine 4.4 mg/dL). (B) Electron microscopy reveals IC (white arrows) along the luminal side (subendothelial) of the capillary wall. Scale bar = 2 μm. (D, F) PASH and JMS stains of a glomerulus, respectively, reveals remodeling of the GBM characterized by double contours of the GBM (open arrows). Glomeruli also have global endocapillary and mesangial hypercellularity. Scale bar = 50 μm.

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uniform thickening of the GBM alone without evidence of spikes, holes, or double or irregular contours is not sufficient for a presumptive diagnosis of ICGN, because it can be seen in cases of non-ICGN. Likewise, the lack of GBM remodeling viewed by LM does not rule out ICGN, especially in cases of relatively recent onset of proteinuria, as those cases have had less time for modification in the GBM. In those cases, TEM and IF are required for the correct diagnosis. Masson's trichrome may reveal the presence of large immune complexes, especially those in a subepithelial location (Fig 1G). Occasionally, IC can also be observed on the luminal side of the GBM with this stain.

Glomerular endocapillary hypercellularity is suggestive of, but not diagnostic for, ICGN. A diffuse and global increase in numbers of nucleated cells, either circulating polymorphonuclear leukocytes or native cell types (endothelial and interposed mesangial cells) within capillaries (ie, endocapillary hypercellularity), is often caused by the presence of subendothelial or intramembranous IC (Fig 1D). Focal or segmental endocapillary hypercellularity and mesangial hypercellularity can have multiple etiologies that may or may not be associated with IC and are, therefore, nonspecific (Fig 3G). Importantly, ICGN with predominantly subepithelial deposits is usually not associated with endocapillary hypercellularity.

Findings inconsistent with a diagnosis of ICGN are the absence of IC on TEM, IF, or both. There are no LM findings that definitively rule out ICGN. Furthermore, LM is required for assessment of the distribution and severity of the disease, as well as for evaluation of the degree of glomerular and tubulointerstitial scarring.

Amyloidosis

Definitive diagnosis of amyloidosis requires the presence of green birefringent material in CR-stained sections (cut at 8- to 10-μm thickness) when viewed with polarized light (Fig 2C). The CR-stained material is peach to orange when viewed with regular nonpolarized LM (Fig 2B). Although TEM is usually not required for definitive diagnosis, ultrastructural evaluation reveals distinct fibrillar material (9–11 nm in diameter) in the mesangium and GBM (Fig 2A). The degree of effacement of glomerular capillary lumens is the basis for determining the severity of the lesion. In addition, similar Congophilic material may be present in the interstitium (often medullary) or in arterial/arteriolar walls.

Light microscopy lesions suggestive of amyloidosis include expansion of mesangial zones with or without thickening of the walls of capillary loops by material that is eosinophilic on HE, pale pink on PASH (Fig 2D), a mix of pale blue to pale orange on MT (Fig 2F), and does not take up the silver on the JMS (Fig 2E). The HE stain can be unreliable for this diagnosis because there is significant overlap in the eosinophilia of amyloid and sclerotic collagen; however, foci of sclerosis will take up silver on the JMS enabling differentiation between these two processes. Furthermore, nonamyloid, noncongophilic fibrillary deposits, with similar tinctorial qualities on PASH, MT, HE, and JMS stains, have been identified in canine glomeruli,[3,4] and therefore a CR should always be performed for a definitive diagnosis.

There are no LM features, which rule out a diagnosis of amyloid. Importantly, cases of early or mild amyloidosis may have very small or subtle amyloid deposits, which are only visible by TEM.

Non-ICGN

Non-ICGN is a broad category of glomerular lesions in which neither IC nor amyloid can be identified and is therefore often a diagnosis of exclusion. The non-ICGN group includes, but is not limited to, cases of focal segmental to global glomerulosclerosis, abnormalities of the GBMs without associated sclerosis, glomerular lipidosis, and congenital or developmental nephropathies that involve glomeruli. Segmental glomerulosclerosis is solidification of a portion of the peripheral capillary loops by extracellular matrix, which is positively stained by MT, PASH, and JMS (Fig 3A–D,G).

Definitive diagnosis of non-ICGN requires ultrastructural and IF evaluation of the tissue to rule out the presence of IC. As mentioned previously, the pathologist's experience in these advanced diagnostic modalities is essential to making the correct diagnosis. The presence of electron-dense material without the ultrastructural characteristics of ICs in the mesangium or GBM may be confusing. For example, lipid can be electron-dense and is present in various types of glomerular injury (Fig 3E).[5] Likewise, hyalinosis, which is the result of plasma insudation into the GBM, is also electron-dense, but usually well circumscribed and finely granular or glassy (Fig 3F). Furthermore, hyalinosis may stain nonspecifically with IF (so-called trapping) in areas of glomerulosclerosis (Fig 3H).

As this is a broad category, there are a wide variety of LM lesions in these patients. Importantly, smooth outer contours of the GBM and lack of double contours with the JMS method (Fig 3C) suggests the absence of IC deposition in the GBM, but this is only valid in cases of chronic, sustained proteinuria where one would expect some degree of GBM remodeling. However, there are few studies examining serial biopsies from dogs with ICGN,[6,7] so the duration of time required for GBM remodeling to occur is currently unknown. Glomerular hypercellularity and expansion of the mesangial zones are highly variable in dogs with non-ICGN (Fig 3G) and their presence or absence does not assist in the categorization of these cases.

The presence of IC precludes a diagnosis of non-ICGN. In addition, glomeruli that are normal via LM, EM, and IF should not be classified as non-ICGN. In those rare cases, an alternate etiology or explanation of the proteinuria should be investigated.

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Validation of Guidelines for the Evaluation of Renal Biopsies

Based on our experience evaluating canine renal biopsies, the WSAVA-RSSG created 10 guidelines for the processing and evaluation of such biopsies (Box 1). All 10 guidelines surpassed the 75% cut-off for validation by the RSSG and 5 external (non-RSSG) veterinary pathologists. Specifically, 4 guidelines received 100% agreement and 6 received 90% agreement.

Box 1
Consensus Recommendations for the Pathologic Evaluation of Renal Biopsies from Dogs with Suspected Glomerular Disease

1 For optimal evaluation, renal biopsy specimens should be sent to a diagnostic center capable of evaluating the tissue by the 3 modalities of light microscopy (LM), transmission electron microscopy (TEM), and immunofluorescence (IF) microscopy with interpretation by veterinary pathologists experienced in nephropathology.
2 All specimens must be sectioned at 3 μm or less, except for the Congo Red section, which should be 8–10 μm thick. Special stains must be performed to highlight the glomerular basement membrane (Periodic acid schiff hematoxylin [PASH] and Jones methenamine silver or Periodic acid-methenamine silver) and to identify immune deposits and matrix collagen deposition (Masson's trichrome).
3 Tissue should be sectioned serially (2–3 sections per slide), so that focal, segmental lesions are not missed. All glomeruli should be evaluated in a core biopsy and at least 50 glomeruli should be evaluated in wedge specimens.
4 The glomerular basement membrane (GBM) is best visualized in the peripheral capillary loops of the glomerular tufts and should be carefully examined for a normal smooth outer contour. Signs of GBM remodeling include spike-like projections, holes, double contours, irregular outer contours, or generalized thickening without the previously mentioned features. Thickened capillary loops alone are not pathognomonic for immune complex glomerulonephritis (ICGN). If there is no LM evidence of GBM remodeling or immune complex deposition, a comment regarding the insensitivity of this method should be included in the preliminary interpretation, especially for cases with a recent onset of proteinuria.
5 Glomerular hypercellularity should be specified as to location (mesangial, endocapillary, or both), cell type, and severity.
6 Synechiae (adhesions between the tuft and Bowman's capsule) and GBM hyalinosis (insudation of plasma into the capillary wall) should be evaluated.
7 Severity of interstitial fibrosis, inflammation, and tubular atrophy must be evaluated. Arterio- and arteriolosclerosis, as well as arterial and arteriolar hyalinosis (changes indicative of hypertension) should be assessed.
8 Immunofluorescence staining is preferable to immunohistochemistry because of its greater sensitivity, and IF should be performed for immunoglobulin heavy chains (IgG, IgM, IgA) as well as the complement component, C3. Additional IF stains that can support a diagnosis of ICGN include lambda light chains and C1q. Evidence of ICGN is provided by finding granular staining with C3 and at least 1 immunoglobulin along peripheral capillary loops, mesangium, or both. The staining pattern (granular, linear, splotchy), staining intensity, and location for each immuno-reactant should be assessed. IF findings are sometimes equivocal and must be verified using TEM. Segmental, splotchy staining indicates trapping of plasma constituents in areas of segmental sclerosis or hyalinosis and therefore is not diagnostic of ICGN even if it appears to be strongly positive. As there may be nonspecific staining patterns, experience in the evaluation of IF is essential for proper interpretation of the findings.
9 Transmission electron microscopy should be performed on either formalin- or glutaraldehyde-fixed tissue. If patent glomeruli cannot be identified in samples processed for TEM, tissue with glomeruli can be removed from the paraffin block for reprocessing for TEM, although there will be mild artifacts associated with this procedure. Ultrastructural evaluation should be performed on glomeruli that are not undergoing atrophy or global glomerulosclerosis. The peripheral capillary loops and mesangium should be examined for the presence or absence of IC, amyloid, or other fibrils. The GBM should be assessed for remodeling or splitting. Depending on the stage of the disease, ICs range from electron dense to electron lucent. Location of deposits (subendothelial, subepithelial, intramembranous, mesangial) should be determined. Podocyte injury and foot process effacement should also be evaluated. As with IF evaluation, experience in the interpretation of TEM findings is crucial to the diagnostic workup of canine renal biopsies.
10 The written report of the findings should include the number of glomeruli evaluated, the number (or percent) of globally sclerotic glomeruli, whether the distribution of glomeruli with lesions is less than 50% (focal) or greater than 50% (diffuse), and the degree of lesion involvement in the glomerulus as either occupying a portion of the glomerular tuft (segmental) or all of the tuft (global). A description of salient glomerular features as evidenced by all 3 diagnostic modalities (as specified in the previously stated guidelines) should be included in the report. Finally, an assessment of pathologic changes in the tubulointerstitial and vascular compartments should also be included as the condition of these compartments is important in formulating an overall prognosis.

Discussion

The accumulation of prognostic information and the development of optimal treatment strategies in humans with glomerular disease has relied upon the integration of clinical history, clinicopathologic data, and accurate, detailed morphologic diagnoses achieved through the routine use of LM, IF, and TEM. To facilitate treatment and monitoring of canine and feline patients, veterinary nephrologists currently use the International Renal Interest Society staging system for chronic kidney disease. This system utilizes serum creatinine, urine-specific gravity, and renal palpation or diagnostic imaging, and patients are then substaged based on the degree of proteinuria or hypertension. Notably, the definitive pathologic diagnosis is not factored into this system. Furthermore, these guidelines only apply to patients with chronic, stable renal disease.[8] Therefore, four sets of recommendations for treatment of dogs with proteinuric kidney disease have been recently formulated and validated by veterinary nephrologists.[9] One of these sets of recommendations is for dogs that have undergone renal biopsy, and determination of the presence or absence of IC is vital to the algorithm. Separation of ICGN from

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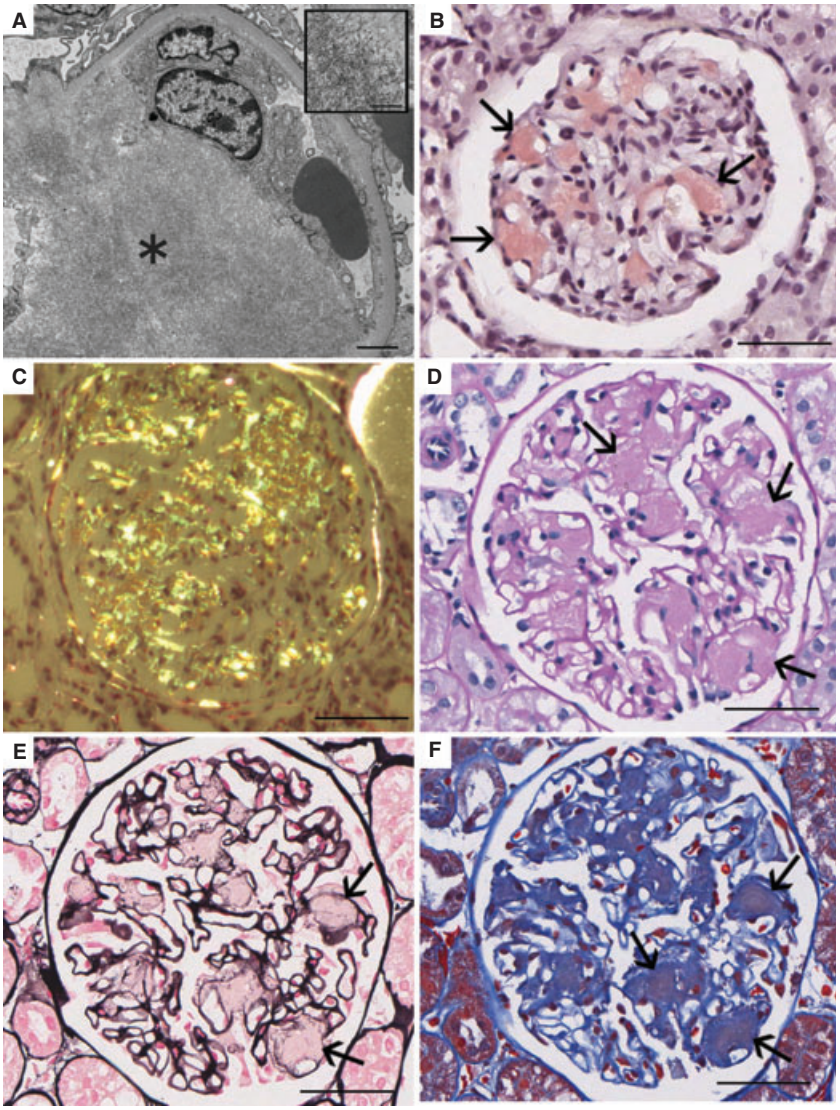

Fig 2. Histologic, ultrastructural, and immunofluorescent findings in amyloidosis. A renal biopsy from a 13-year-old castrated male small mixed breed dog with proteinuria (UPC = 9.8), but not azotemia. **(A)** Electron microscopy reveals expansion of the mesangial zone by haphazardly arranged fibrils. Scale bar = 2 μm. Inset: Higher magnification of fibrils. Scale bar = 250 nm. **(B–F)** Histologic appearance of a glomerulus. Mesangial zones are expanded by Congophilic peach material (B) that demonstrates apple green birefringence when viewed with polarized light (C) and is consistent with amyloid (arrows). This material (arrows) is pale pink on periodic acid Schiff hematoxylin (D), does not take up silver with the Jones methenamine silver method (E), and is a mix of blue-orange on Masson's trichrome (F). Scale bar = 50 μm.

non-ICGN by LM can be difficult, as demonstrated above, and both are common causes of proteinuria in dogs.[10] Even so, the pathologist must provide an accurate diagnosis, so that future evaluations of treatment efficacy are possible.

Before the establishment of the IVRPS and UVNS, the advanced modalities of renal biopsy evaluation were difficult to coordinate for a feasible diagnostic workflow. Because of this, proteinuric animals were generally diagnosed via LM alone by veterinary pathologists without advanced training in EM or IF. In many instances, clinicians felt that the expense and the potential risks of the renal biopsy procedure outweighed the benefits of having a definitive pathologic diagnosis, especially when LM alone can be misleading. Our adoption of—and familiarity with—methods used by our physician nephropathology colleagues has enabled us to establish 3 broad categories of canine proteinuric kidney disease: ICGN, amyloidosis, and non-ICGN. As stated previously, these categories were created because they have the greatest implications for treatment and prognosis.

A diagnosis of amyloidosis can often be made solely by LM; however, the distinction between ICGN and non-ICGN is difficult because their LM lesions overlap or can be subtle depending on the stage of the disease. Specifically, cases of ICGN with recent onset proteinuria might not have had enough time for the GBM to

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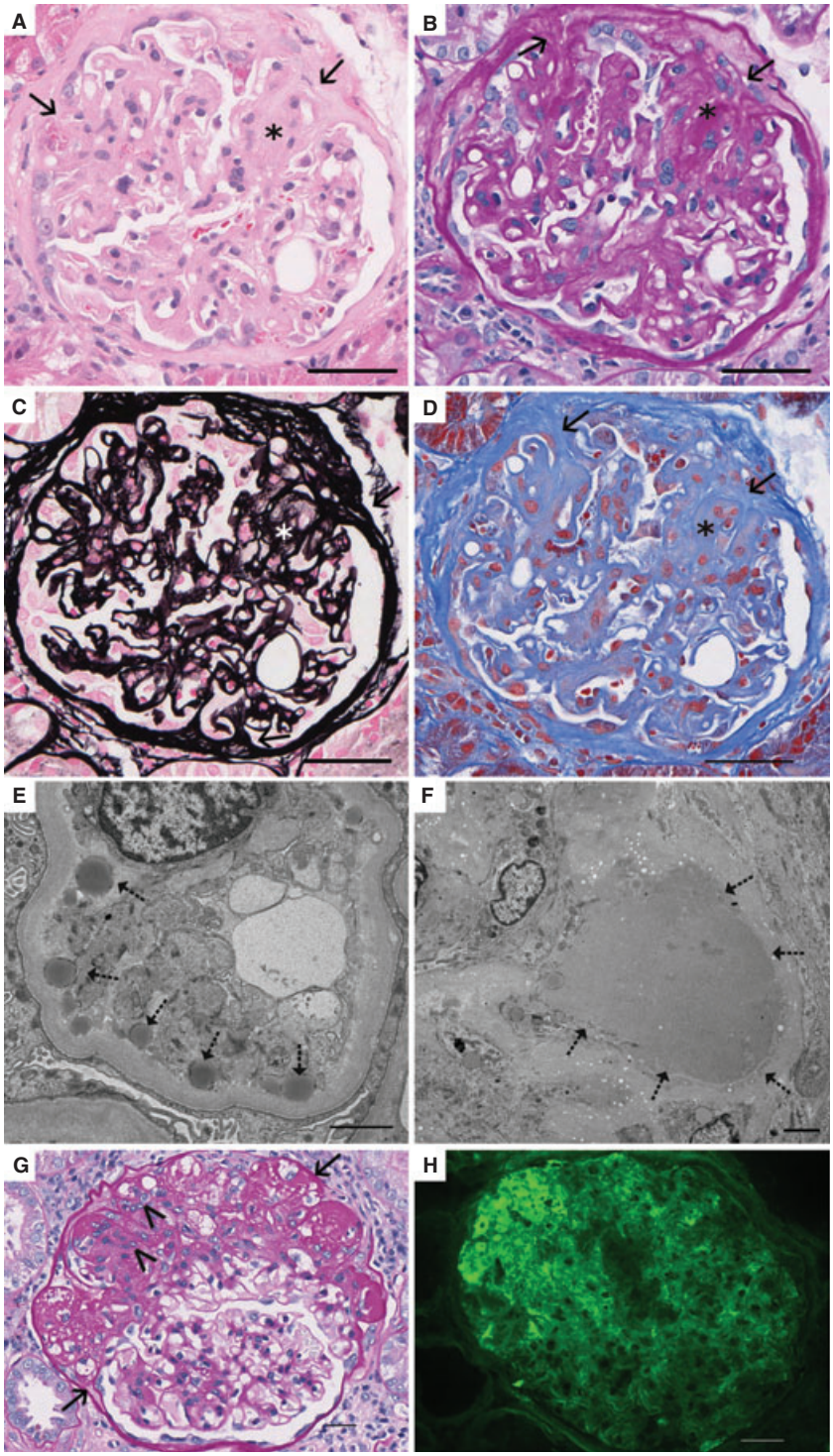

Fig 3. Histopathologic, ultrastructural, and immunofluorescence findings in non-ICGN. **(A–D)** hematoxylin and eosin, periodic acid Schiff (PAS), Jones methenamine silver (JMS), and Masson's trichrome stained serial sections of a glomerulus from a 12-year-old Yorkshire Terrier with severe proteinuria (UPC: 12.7). Segments of the tuft are effaced by extracellular matrix (segmental sclerosis) (*) and are adhered to Bowman's capsule (synechiae) (arrows). Glomerular basement membrane is thickened, but has a smooth outer contour. Scale bar = 50 μm. Electron microscopy (E-F) of the same dog reveals thickened GBM with multifocal electron-dense material (dotted arrows) that is consistent with lipid (E) and hyalinosis (F). Scale bar = 2 μm. Immune deposits were not identified via electron microscopy (EM) or immunofluorescence (IF). (G) PAS-stained glomerulus from a 4-year-old Rhodesian Ridgeback dog with proteinuria (UPC: 2.0) associated with a congenital anomaly of the kidney reveals moderate mesangial hypercellularity (arrowhead), segmental sclerosis, and a broad synechia (arrows). EM and IF ruled out the presence of immune complexes in this case. Scale bar = 50 μm. (H) Immunofluorescence staining for IgG in a glomerulus from a 14-year-old standard poodle with proteinuria (UPC: 3.2) reveals strong, splotchy (as opposed to granular) staining in a portion of the tuft, indicative of nonspecific trapping within a sclerotic segment. EM did not reveal immune complex deposition. Scale bar = 50μm.

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remodel in response to the deposits. In those cases, the glomerulus would look completely normal by LM. Likewise, some cases of non-ICGN demonstrate glomerular hypercellularity or thickened GBMs. Therefore, a definitive diagnosis necessitates verification of IC deposition by TEM, IF, or both. As illustrated above, these advanced modalities require specialized training for correct interpretation. For example, electron-dense IC have a specific ultrastructural appearance, which differs from that of hyalinosis, and circulating immunoglobulins can be nonspecifically trapped in areas of glomerulosclerosis. It is essential that the final morphologic diagnosis is an integration of all 3 modalities.

We recognize that some veterinary pathologists may argue against our decision to establish the category of non-ICGN because it includes such a wide range of lesions, ranging from segmental glomerulosclerosis to ultrastructurally abnormal GBM to developmental renal anomalies that affect glomeruli. Notably, the significance of glomerulosclerosis, specifically focal segmental glomerulosclerosis, as a cause of proteinuria is under-recognized in veterinary medicine, even though it is considered to be a common cause of proteinuria in humans.[11] Multiple lines of evidence point to podocyte injury as an inciting factor in the development of glomerulosclerosis, and mutated podocyte genes, toxic insults, viral infections, and cytoskeletal stress from cell hypertrophy have all been demonstrated to injure this cell lineage.[12] This diagnostic entity is mentioned rarely in veterinary literature, although recent research has identified candidate genes associated with this disease in certain breeds of dogs.[13] Furthermore, IC deposition can also lead to podocyte damage and secondary sclerosis, further confounding the pathologist's ability to categorize a case by LM alone. The significance of many of the other lesions in the category of non-ICGN is currently unknown. For instance, multifocal glomerular lipidosis has been viewed as an incidental lesion, because it has been identified rarely in sections of kidneys from "normal" dogs at necropsy. The lack of recent urinalysis results from these "normal" dogs and the presence of this lesion as the only diagnostic abnormality in renal biopsy specimens from proteinuric dogs suggest that it should be investigated further.

The validated guidelines presented herein provide a starting point for the proper examination of renal biopsy tissue. Guidelines 4 through 9 mention lesions that, in our experience, are often encountered in canine renal biopsy specimens. Many of the listed lesions provide the basis for the classification of the case as ICGN, amyloidosis, or non-ICGN, whereas others (eg, synechiae) are less specific, but still carry prognostic weight. For aspiring veterinary nephropathologists and nephrologists, these features are merely the beginning of what can be gleaned from a renal biopsy specimen and studies to examine and categorize lesions in finer detail are currently under way (see below).

Importantly, the final interpretation of the lesions must be placed into the context of the patient's clinical presentation. The severity of interstitial fibrosis and tubular atrophy must be assessed in every case, so that the clinician can determine the likelihood that renal function will deteriorate or improve. In azotemic, proteinuric dogs without histologic evidence of tubular injury, the clinician should be alerted to evaluate the areas not captured by the kidney biopsy (eg, renal medulla) using diagnostic imaging or to search for pre- and postrenal causes of azotemia. In that same vein, ICGN cases with significant scarring of the glomeruli need to be documented as such, as they may not respond to the same therapies as cases of recent onset ICGN. Lesions in arteries and arterioles can prompt a clinician to consider the role of hypertension in their patient.

Additional studies are currently under way to subdivide the 3 broad categories to aid in the identification of possible etiologies as well as to improve prognostication. In its ongoing studies, the WSAVA-RSSG examines and annotates more than 100 LM, EM, and IF features of each renal biopsy specimen. Subcategories of canine glomerular diseases exhibiting particular combinations of features are identified and associated with their respective clinical characteristics (ie, clinical findings at diagnosis, response to treatment, and outcome). Needless to say, these studies entail extensive, long-term clinical follow-up. In the meantime, dogs undergoing a renal biopsy need to be categorized correctly, so that therapeutic regimens are based on treatment of IC-mediated disease, amyloidosis, or the lack thereof. Incorrect categorization will skew efficacy and prognostic data. Furthermore, it will always be important for pathologists and nephrologists to have a systematic approach to harvest, submit, prepare, and evaluate kidney tissue. Therefore, the validated guidelines provided herein will help optimize the diagnostic workflow in veterinary nephropathology.

Footnotes

[a] UltraCut S, Reichert Technologies, Depew, NY
[b] Electron Microscopy Sciences, Hatfield, PA
[c] JEM-1240; JEOL USA, Peabody, MA
[d] Tissue-Tek OCT Compound, Sakura Finetek USA, Torrance, CA
[e] Bethyl Labs, Montgomery, TX
[f] Dako North America, Carpinteria, CA
[g] Olympus, Center Valley, PA
[h] ScanScope CS, Aperio, Vista, CA
[i] Spectrum, Aperio, Vista, CA
[j] ImageScope, Aperio, Vista, CA
[k] WebScope, Aperio, Vista, CA
[l] GoToMeeting, Citrix Systems, Inc, Santa Barbara, CA

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