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Prevalence of Immune-Complex Glomerulonephritides in Dogs Biopsied for Suspected Glomerular Disease: 501 Cases (2007–2012)

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Background: Glomerulonephropathies are common causes of kidney disease in dogs.

Objective: To determine the prevalence of immune-complex glomerulonephritis (ICGN) in North American dogs biopsied for suspected glomerular disease.

Animals: Renal biopsies (n = 733) submitted to the Texas Veterinary Renal Pathology Service between January 1, 2007 and December 31, 2012 were reviewed. Dogs were included if the biopsy was performed for suspected glomerular disease.

Methods: Specimens were evaluated by light microscopy (LM), immunofluorescence (IF), and transmission electron microscopy (TEM). Findings were retrospectively evaluated to categorize the diagnosis for each case. For the diagnosis of ICGN, TEM findings were considered conclusive when LM and IF were equivocal.

Results: Of the 501 dogs included in the study, 241 (48.1%) had ICGN; 103 (20.6%) had primary glomerulosclerosis; 76 (15.2%) had amyloidosis; 45 (9.0%) had nonimmune complex (IC) glomerulopathy; 24 (4.8%) had non-IC nephropathy; and, 12 (2.4%) had primary tubulointerstitial disease. Many (66/241; 27.4%) ICGN cases required TEM for definitive diagnosis, including 14 cases (5.8%) that were not suspected on LM. Of cases not diagnosed as ICGN, a substantial proportion (60/260; 23.1%) required TEM to rule out immune complex deposits, including 14 of 189 cases (7.4%) presumptively diagnosed as ICGN on LM.

Conclusions and Clinical Importance: Approximately half of all dogs biopsied for suspected glomerular disease had conditions other than ICGN. Renal biopsy is needed to accurately categorize the underlying disease and direct appropriate treatment. Additionally, TEM and IF evaluations by experienced nephropathologists are necessary to obtain an accurate diagnosis in many cases.

Key words: Immunofluorescence; Kidney; Proteinuria; Transmission electron microscopy.

Glomerular diseases occur frequently in dogs and are important causes of morbidity and mortality in this species. Immune-complex glomerulonephritides (ICGN) often are cited as the most common causes of glomerular disease in dogs, with other possible causes (eg, amyloidosis, glomerulosclerosis, hereditary nephropathy) acknowledged but typically given less emphasis.[1–6] Currently, however, no data are published regarding the prevalence of ICGN in a large cohort of dogs being evaluated for clinical suspicion of glomerular disease using advanced diagnostic modalities such as transmission electron microscopy (TEM) and immunofluorescence (IF).

Abbreviations:

CR	Congo red
GBM	glomerular basement membrane
GS	glomerulosclerosis
H&E	hematoxylin and eosin
ICGN	immune-complex glomerulonephritis (nephritides)
IC	immune complex(es)
IF	immunofluorescence microscopy
JMS	Jones methenamine silver
LM	light microscopy
MT	Masson's trichrome
PAS	periodic acid-Schiff
TEM	transmission electron microscopy
TVRPS	Texas Veterinary Renal Pathology Service
UPC	urine protein:creatinine

Clinical suspicion of immune-mediated disease generally prompts consideration of treatment with immunomodulatory drugs. Indeed, contemporary reviews of canine glomerular disease invariably discuss this possible therapeutic strategy, albeit with reservations because of the lack of good evidence supporting the efficacy of any particular treatment regimen in any specific clinical setting.[1–6] Nonetheless, pathophysiologic and pharmacologic rationale as well as anecdotal experience strongly suggest that at least some dogs with ICGN are likely to benefit from treatment with immunomodulatory drugs. A substantial therapeutic dilemma thus is created because the potential benefit must be weighed against the risks that accompany administration of immunomodulatory drugs.[7] Importantly, the potential risk-benefit trade-off likely would be negative if the disease were not actually immune-mediated because immunosuppression of dogs without

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biopsy-proven ICGN could expose such patients to unnecessary risks. In order to assess the magnitude of such risks, an estimate of the general prevalence of ICGN in dogs showing clinical evidence of glomerular disease (eg, persistent, clinically relevant renal proteinuria) would be useful.

In human medicine, full and optimal evaluation of a renal biopsy specimen requires light microscopy (LM), IF, and TEM.[8] Identification of immune complexes (ie, immunoglobulin [Ig] heavy chains of IgG, IgM, and IgA, kappa and lambda light chains, and complement factors C3 and C1q) is facilitated by IF evaluation of unfixed, frozen tissue. Ultrastructural evaluation provides useful diagnostic information in nearly 50% of nontransplant human renal biopsy specimens,[9,10] allowing assessment of the glomerular basement membrane (GBM) and podocyte foot processes, as well as confirmation of the presence or absence of immune complex deposits. A previous preliminary study of TEM in canine and feline renal pathology concluded that TEM also is necessary to complement histological examinations in dogs.[11] Indeed, many glomerular diseases are at least partially defined by distinctive changes in cells, extracellular matrix, or both that can only be detected by TEM.[12]

There are no large-scale studies defining the prevalence of ICGN in dogs with clinical evidence of glomerular disease for which renal specimens were evaluated by LM, IF, and TEM in all cases. Prior studies have examined smaller cohorts and either drew from cases with a previous diagnosis of glomerulonephritis, did not exclude known lower urinary tract diseases, examined dogs with histories of chronic disease that were euthanized, or sampled dogs submitted for euthanasia without histories.[13–21] When modalities other than hematoxylin and eosin (H&E) were used for evaluation, not all cases were examined, only known GN cases were examined, or TEM was performed only on formalin-fixed and paraffin-embedded tissue (which may result in more artifacts).

The purpose of this study was to determine the prevalence of ICGN in a large cohort of North American dogs with clinical evidence of glomerular disease that had pathologic evaluation of their renal tissue performed using LM, IF, and TEM.

Materials and Methods

Renal Biopsies

Cases were evaluated retrospectively from 733 renal biopsy cases, including wedge and needle core biopsies of various sizes, submitted to the Texas Veterinary Renal Pathology Service (TVRPS) from January 1, 2007 through December 31, 2012. Most cases were submitted by veterinarians affiliated with specialty practices and tertiary care institutions. Specimens were processed routinely for LM, TEM, and IF. Specimens for LM were sectioned at 3 μm thickness and stained with H&E, Masson's Trichrome (MT), and Periodic acid-Schiff (PAS). After December 1, 2008 all samples also were stained with Jones' methenamine silver (JMS). Congo red (CR) staining was performed if amyloid was suspected on LM. To minimize cost to the client, TEM was not always performed in cases with amyloidosis diagnosed by LM and CR staining (ie, typical apple-green birefringence when viewed under polarized light). For TEM, tissues were fixed in chilled 3% buffered glutaraldehyde. Specimens were postfixed in 1% osmium tetroxide, dehydrated in a series of graduated alcohols, infiltrated in an acetone/epoxy plastic, and embedded in a plastic mold. Plastic blocks were cut with an ultramicrotome.[a] Thick sections were stained with toluidine blue. Sections then were evaluated, and appropriate areas identified for thin sectioning. Thin sections were cut at silver-grey interference color (55–60 nm) and placed on slotted copper grids coated with Formvar.[b] Grids were stained with uranyl acetate and lead citrate and were examined in a transmission electron microscope.[c] TEM specimens initially were evaluated by the authors (FJC and CAB) at the Texas Heart Institute and University of Georgia, respectively. Unfixed tissue samples for IF evaluation were embedded in Optimal Cutting Temperature medium[d] and snap frozen in liquid nitrogen vapor. After cryosectioning at 4 μm thickness, 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. All slides were examined by one of the authors (GEL) with an epifluorescence microscope[g] using appropriate filters.

Criteria for Case Selection

To identify all adequately studied biopsy cases that involved North American dogs being evaluated for suspected glomerular disease, cases were excluded if they were noncanids, were from outside of North America, had no clinical indication of glomerular disease or had no submitted historical information, had a previous biopsy sample from which a diagnosis had been obtained (no single dog was included more than once), or had tissue considered insufficient for diagnosis as determined by the pathologist. Because the extent and detail of the clinical history varied widely, and terminology differed among submitted histories, primary glomerular disease was considered if one or more of the following criteria were met: the clinical history specifically mentioned nephritic syndrome, nephrotic syndrome, or glomerulonephritis as the reason for submission; urine protein:creatinine (UPC) was >2; or the primary clinical sign was persistent renal proteinuria (proteinuria identified on routine urinalysis with or without UPC >0.5 on multiple occasions in the absence of prerenal or postrenal causes for proteinuria). In 3 cases, the initial biopsy specimen was inadequate, and the second diagnostic specimen was used for the purposes of this study. Animals biopsied for suspected juvenile-onset renal disease or renal dysplasia were only included if they had a UPC >2.

Biopsy Classification

Transmission electron microscopy was used as the principal method for immune-complex detection, and IF findings were used to support the interpretation of electron-dense deposits when TEM findings were equivocal or difficult to interpret. Importantly, the workflow of the diagnostic service was such that LM was evaluated first, followed by IF, TEM, or both approximately 2–6 weeks later. Hence, a preliminary diagnosis based solely on LM findings was rendered initially and a final diagnosis that included TEM and IF findings was issued subsequently. For the purposes of this study, all biopsy specimens with glomerular deposits of immune complexes were placed in a single category (ICGN). Biopsy specimens without evident glomerular immune-complex (IC) deposits were further categorized as amyloidosis, glomerulosclerosis (ie, focal segmental glomerulosclerosis), other non-IC glomerulopathy, non-IC nephropathy (ie, diseases of the

entire kidney), or primary tubulointerstitial disease. Categorization of cases as ICGN and amyloidosis was consistent with diagnostic criteria developed and used by the World Small Animal Veterinary Association Renal Standardization Study Group.[22]

Statistical Analysis

Sensitivity and specificity were calculated for LM detection of ICGN as compared with EM, and 95% confidence intervals were determined using the Wilson score method. Pearson's chi-squared test was used to test for significant differences in sex distribution among categories. This evaluation was followed by a posthoc 2-sample test of proportions to determine which categories had a significantly different sex distribution as compared with the total cohort. Significance was set at $P < .05$.

Results

After exclusions were applied (Table 1), 501 cases were identified as dogs from North America that were evaluated for suspected glomerular disease. Inconsistencies in terminologies used in medical histories provided when biopsy specimens were submitted precluded accurate attribution of the clinical sub-category of suspected glomerular disease (eg, nephrotic syndrome, nephritic syndrome, persistent renal proteinuria, multiple factors) in this retrospective study. In most (479/501; 95.6%) cases, however, a UPC value >2 was reported (n = 470) or there was a history of marked or persistent proteinuria without any reported UPC value (n = 9). Only 22 (4.4%) of the 501 cases of suspected glomerular disease were included based on a history of persistent proteinuria despite a reported UPC <2. These cases were present in each of the categories as follows: ICGN (n = 6); non-IC glomerulopathy (n = 6); 3 each for glomerulosclerosis, non-IC nephropathy, and primary tubulointerstitial disease; and amyloidosis (n = 1).

Signalment

The average age of the 501 dogs that were evaluated for suspected glomerular disease was 6.8 years (range, 4 months to 14 years). Females slightly outnumbered males (Table 2), and most males and females were neutered or spayed, respectively. Dogs with suspected glomerular disease were from all geographic regions of North America, but fewer were from the Mountain or Southwest regions than other regions (Table 2). More than 100 different breeds were evaluated, but dogs of 12 different breeds each accounted for 2% or more of the cohort (ie, ≥10 dogs of that breed were included) and together accounted for nearly half (47.1%) of the entire cohort (Table 3).

Table 1. Cases excluded from the study for specified reasons.

Total number of cases available for review	733
Exclusions	
Animals other than dogs	45
Dogs not from North America	28
Dogs without suspected glomerular disease	96
Dogs without adequate history provided	4
Dogs with existing biopsy diagnosis	18
Dogs with samples inadequate for diagnosis	41
Total number of cases excluded	232
Total number of cases included	501

Table 2. Sex and geographic origins of 501 dogs biopsied for evaluation of suspected glomerular disease.

	n	%
Sex		
Intact males	41	8.2
Neutered males	177	35.3
All males	218	43.5
Intact females	27	5.4
Spayed females	256	51.1
All females	283	56.5
All dogs	501	100.0
Geographic origin		
Northeast	132	26.3
Southeast	59	11.8
Midwest	137	27.3
Mountain	17	3.4
Southwest	30	6.0
Pacific	126	25.1
All dogs	501	100.0

Northeast: ME, VT, NH, MA, RI, CT, NY, PA, DE, MD; Southeast: KY, WV, VA, TN, NC, MS, AL, GA, SC, FL; Midwest: ND, SD, NE, KS, MN, IA, MO, WI, MI, IL, IN, OH; Mountain: AB, ID, MT, WY, NV, UT, CO; Southwest: AZ, NM, TX, OK, AR, LA; Pacific: British Columbia, Alaska, WA, OR, CA.

Table 3. Breeds of dogs biopsied for evaluation of suspected glomerular disease.

	n	%
Labrador Retriever	52	10.4
Golden Retriever	38	7.6
Yorkshire Terrier	29	5.8
Beagle	15	3.0
Miniature Schnauzer	15	3.0
Shetland Sheepdog	14	2.8
Boxer	13	2.6
Cocker Spaniel	13	2.6
Doberman Pinscher	13	2.6
Soft-Coated Wheaton Terrier	13	2.6
English Bulldog	11	2.2
Standard Poodle	10	2.0
All other breeds (n > 88)[a]	265	52.9
All dogs	501	100.0

[a]Eighty-eight named breeds plus assorted mixed breeds.

Pathologic Evaluation

ICGN was identified in slightly fewer than half of the dogs biopsied for evaluation of suspected glomerular disease (Table 4). IC deposits were distributed in various different patterns within the glomerular capillary walls as demonstrated by TEM, including subepi-

Table 4. Categories of pathologic conditions found in dogs biopsied for suspected glomerular disease.

Category	n (%)	Male (%)	Female (%)	Age (years) Median (Range)	UPC Median (Range)
ICGN	241 (48.1%)	109 (45.2%)	132 (54.8%)	6.2 (0.3–14.0)	8.3 (0.6–42.7)
Glomerulosclerosis	103 (20.6%)	29 (28.2%)	74 (71.8%)	8.0 (2.0–13.0)	6.0 (1.6–27.0)
Amyloid	76 (15.2%)	43 (56.6%)	33 (43.4%)	7.2 (2.0–14.0)	10.3 (1.5–40.1)
Other non-IC glomerulopathy	45 (9.0%)	18 (40.0%)	27 (60.0%)	6.5 (0.4–13.0)	5.4 (1.4–20.8)
Non-IC nephropathy	24 (4.8%)	14 (58.3%)	10 (41.7%)	8.0 (0.3–13.5)	3.4 (0.5–25.9)
Primary TI disease	12 (2.4%)	5 (41.7%)	7 (58.3%)	6.0 (2.5–10.0)	3.9 (0.6–8.6)
All cases	501 (100.0%)	218 (43.5%)	283 (56.5%)	7.0 (0.3–14.0)	7.6 (0.6–42.7)

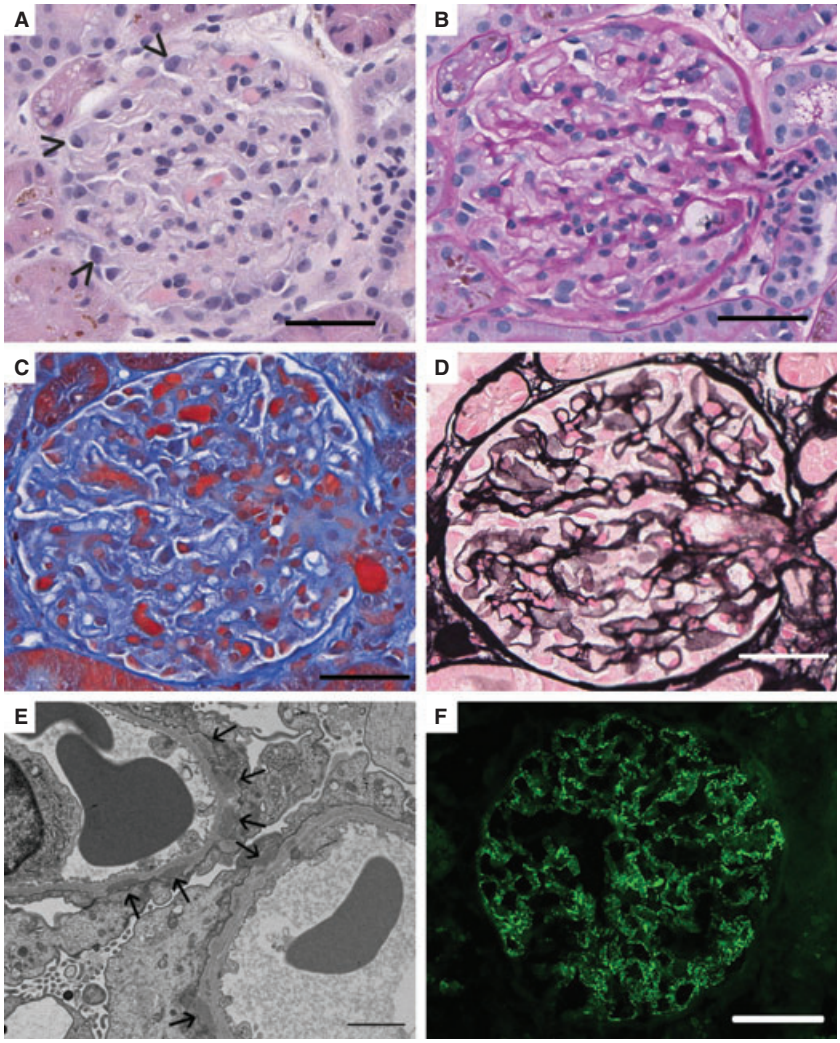

Fig 1. Histologic, ultrastructural and immunofluorescent findings in a renal biopsy specimen from a large mixed-breed castrated male young adult dog with proteinuria. **(A–D)** H&E, PAS, MT, and JMS stains of a glomerulus, respectively reveal podocyte hypertrophy (arrowheads) and lack of endocapillary or mesangial hypercellularity. Scale bar = 50 μm. **(E)** TEM demonstrates numerous ICs on the abluminal surface of the GBM. Scale bar = 2 μm. **(F)** IF staining with an antibody against lambda light chains identifies the presence of immunoglobulins. IgG and C3 were also present in a similar pattern (data not shown). Scale bar = 50 μm.

thelial, subendothelial, both subepithelial (Fig 1) and subendothelial, and intramembranous locations. Deposits also were often present in the mesangium. In affected dogs with ICGN, the proportions of males and females were not statistically different from that of the entire cohort (P = .23), and the distribution of ages also was similar. Among the cases of ICGN, 175/241 (72.6%) were diagnosed as such by LM with confirmation of deposits on TEM; 52/241 (21.6%) had LM changes suggestive of IC disease but required confirmation of deposits on TEM; and 14/241 (5.8%) did not have LM changes suggestive of IC disease, but

had immune deposits on TEM. An additional 46/260 (17.7%) of the non-ICGN cases had LM lesions consistent with (but not definitively diagnostic for) IC deposition. In these cases, evaluation by TEM did not identify IC deposits. Lastly, in 14/260 (5.4%) of non-ICGN cases the LM findings were sufficient to render a presumptive LM diagnosis of ICGN, requiring a reversal of the diagnosis once TEM findings became available. The frequency of this misdiagnosis decreased over time, with 11 cases occurring between 2007 and 2009, 3 between 2010 and 2011, and none in 2012. Based on these findings, LM had a sensitivity of 94% (95% confidence interval, 91–97%) and a specificity of 77% (95% confidence interval, 72–82%), with LM-positive cases including all of those in which ICGN was either diagnosed or suspected by a nephropathologist based on LM evaluation of PAS, H&E, JMS, and MT stains. Of note, the overall frequency that TEM findings either reversed the initial LM diagnosis or clarified an initial LM diagnosis that was recognized to be uncertain from the outset was 126/501 (25.1%) in this cohort of cases.

Glomerular lesions consisting primarily of sclerotic changes without evidence of underlying ICGN were identified in one-fifth of the cases (Table 4). Absence of immune complexes was verified by TEM and IF evaluations in these cases of glomerulosclerosis (GS) (Fig 2). There was a significant ($P < .001$) preponderance of females among dogs with this category of glomerular lesions; 74 (71.8%) of the dogs with GS were female (69 spayed and 5 intact). Within this category, a range of focal segmental to global GS was observed. Five (4.9%) of the 103 cases in this category originally were diagnosed as IC disease based on LM, but they

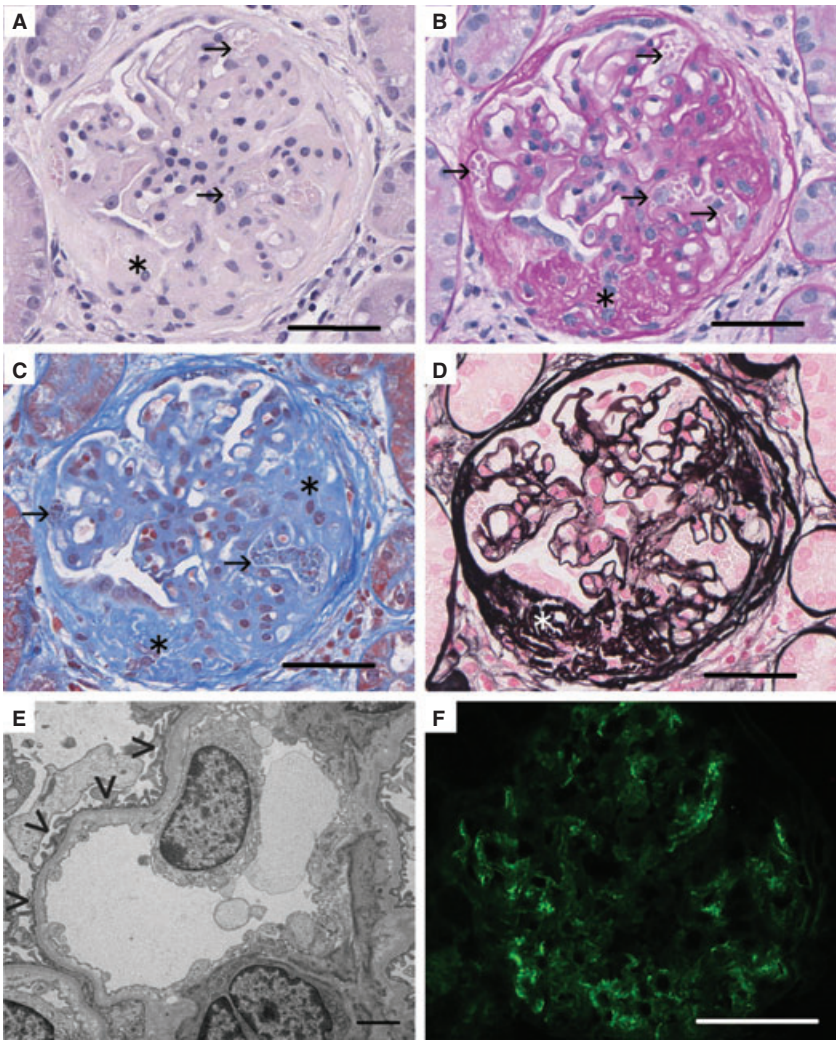

Fig 2. Histologic, ultrastructural, and immunofluorescent findings in a renal biopsy specimen from an adult spayed female Rhodesian Ridgeback dog with proteinuria. **(A–D)** H&E, PAS, MT, and JMS stains of a glomerulus, respectively reveal segmental sclerosis and synechiae (*). Podocytes are hypertrophied and contain large protein droplets in their cytoplasm (arrows). Scale bar = 50 μm. **(E)** TEM demonstrates widespread effacement of podocyte foot processes (arrowheads) and absence of ICs. Scale bar = 2 μm. **(F)** IF staining with an antibody against IgM does not demonstrate granular staining along capillary walls or in GBM (compare with positive staining pattern on Fig 1F). Scale bar = 50 μm.

did not have electron-dense deposits on TEM or positive IF staining for immunoreactants. Because GS is a diagnosis of exclusion (wherein underlying ICGN with secondary glomerular scarring has been ruled out), an additional 32 cases (31%) required TEM to definitively document the absence of immune complexes.

Amyloidosis was identified in nearly one-sixth of the dogs evaluated (Table 4). In contrast to the entire cohort, the sex distribution among dogs with amyloidosis was characterized by slightly more males than females but was not significantly different from the entire cohort ($P = .27$). The youngest dog was 2 years old, but the age distribution was otherwise similar to that of the entire cohort. Amyloidosis, however, appeared to be over-represented in 2 breeds; 8 of 9 Chinese Shar Peis and 9 of 11 English Bulldogs biopsied for suspected glomerular disease were found to have amyloidosis. In 3 of 76 cases, amyloid deposits were small and nodular and distributed along the glomerular capillary walls with associated GBM remodeling observed with JMS, mimicking the LM features that can occur in ICGN. However, CR staining and TEM confirmed the presence of amyloid deposits in these cases.

Dogs with an assortment of primary glomerular lesions that were not ICGN, amyloidosis, or glomerulosclerosis were grouped in a single category that accounted for nearly one-tenth of the cases (Table 4). The ages and sex distribution of dogs in this category were similar to those of the entire cohort, with no significant difference observed for sex ($P = .20$). Five (11.1%) of the 45 cases in this category originally were diagnosed as IC disease based on LM, but did not have electron-dense deposits on TEM or positive IF staining for immunoreactants. TEM was required to definitively determine that IC were not present in the glomeruli in 17/45 (37.8%) of the non-IC glomerulopathy cases. Most cases had nonspecific evidence of podocyte injury with or without synechiae (25 cases), but other cases had nonamyloidotic fibrillary deposits (6 cases), were suspected to have fibronectin glomerulopathy (1 case) or collagenofibrotic glomerulopathy (1 case), or had primary GBM defects (4 cases). Seven other cases had nonspecific primary glomerular changes without IC deposits.

Dogs with chronic changes in both the glomerular and tubulointerstitial compartments (for which it was unclear whether the initial insult to the kidney was primarily glomerular or tubulointerstitial) were included in the non-IC nephropathy category. These accounted for <5% of the cases (Table 4). Electron-dense deposits were not seen on TEM, but it is possible that immune-complex deposits initiated the damage and later resolved during disease progression. A single case in this category was diagnosed as IC disease based on LM, but did not have electron-dense deposits on TEM or positive IF staining for immunoreactants.

Dogs with primary tubulointerstitial changes were the least frequent category, which accounted for <3% of the cases (Table 4). Conditions included in this category were a case of lymphoma (UPC, 1.7), a possible toxin exposure (UPC, 3), 2 cases of leptospirosis (median UPC, 2.2), 4 cases of severe interstitial nephritis of unknown etiology (median UPC, 5.9), 2 cases of tubular degeneration and necrosis (median UPC, 4.0), and 2 cases of advanced tubulointerstitial scarring (median UPC, 3.6). Only 2 dogs in this category (1 dog with leptospirosis and a UPC of 2.2 and 1 dog with tubular necrosis and a UPC of 4.1) had normal glomeruli on LM, EM, and IF and in fact were the only 2 dogs in the cohort with normal glomeruli. All other dogs in this category had ultrastructural evidence of moderate to marked remodeling of the glomerular basement membrane and podocyte foot process effacement, but were placed into this group because the tubulointerstitial changes were the predominant findings in the specimen.

Discussion

This retrospective study determined the prevalence of various categories of renal diseases that were identified in dogs from North America that had renal biopsy performed because of suspected glomerular disease during a 6-year period. The most common reason for obtaining a renal biopsy specimen from dogs was presence of proteinuria thought to be of glomerular origin, and the most important finding is that approximately half (51.9%) of all such dogs did not have ICGN. Moreover, even after excluding all dogs with amyloidosis, a large proportion (43.3%) of the remaining dogs with suspected glomerular disease did not have ICGN. These findings indicate that it is unwise to make a presumptive (ie, not biopsy-proven) diagnosis of ICGN in a dog with glomerular proteinuria because this assumption will often be incorrect.

Ours is the largest and most comprehensive study to date of renal biopsy specimens from dogs with suspected glomerular disease. Studies of glomerular disease in dogs using LM, IF, and TEM have not previously been published on such a large scale. Historically, ICGN has been considered the most common cause of glomerular disease in dogs,[1–6] and it often has been diagnosed based solely on LM findings. Overall, ICGN was the most common cause of glomerular disease in this study, but fewer than half of the dogs (48.1%) had evidence of glomerular IC deposition. The prevalence of non-ICGN glomerular diseases was higher in this study than found in some previous studies.[14,15] However, it is difficult to compare this study with previous studies because earlier studies have examined subjects that differed widely including dogs randomly selected for euthanasia,[16] dogs with a previous histologic diagnosis of glomerulonephritis,[17–19] dogs with and without clinical evidence of glomerular disease,[15,20] dogs with clinical evidence of chronic renal disease,[21] and dogs with any urinary tract lesions, including postrenal disease.[14]

In cases of ICGN, the IC deposits were distributed in various different patterns, usually within the glomerular capillary walls (eg, subepithelial, subendothelial) but also often in the mesangium. Although the patho-

logic relevance and prognostic implications of different IC distributions are well defined in human medicine, the clinical relevance of various patterns has not yet been established in dogs. Localization of IC deposits in individual cases can be difficult, and defining pathologic subtypes of ICGN in dogs was beyond the scope of this study.

Transmission electron microscopy was used as the gold standard for IC detection in this study, and it was needed to diagnose ICGN in a large proportion (27.4%) of cases with that diagnosis. Of these, 5.8% of cases with ICGN confirmed by TEM were not suspected based on LM alone. Of the dogs with diagnostic samples that did not have ICGN (260 dogs), 46 (17.7%) had LM changes consistent with (but not definitive for) ICGN and required TEM to exclude the presence of IC deposits. In 14 (5.4%) of the non-ICGN cases, a misdiagnosis of ICGN was made based on LM evaluation alone. These findings demonstrate that TEM often is needed to verify the presence or absence of glomerular immune deposits when LM findings are suggestive of the possible presence of underlying ICGN. Importantly, there are no large-scale studies to determine the sensitivity and specificity of EM for the diagnosis of IC in dogs. Therefore, the number of glomeruli sampled could be problematic in the diagnosis of focal ICGN. In humans, ICGN with IgG-dominant complexes almost always involves all glomeruli.[23] Furthermore, in this study, ICGN cases with multiple glomeruli available for evaluation with EM and IF did not demonstrate focal IC deposition.

Although sensitivity was excellent and specificity was relatively high when ICGN was suspected based on LM, it is important to note that several LM sections were examined by specialists with extensive training in nephropathology. Nevertheless, and not surprisingly because of the cumulative nature of growing expertise, the occurrence of incorrect preliminary diagnoses of ICGN decreased appreciably during the 6-year period spanned by this study. The frequency of misdiagnoses or inconclusive diagnoses rendered by nonspecialist veterinary pathologists is not known, but it is unlikely that the high sensitivity and specificity observed in this study would be achieved by pathologists lacking nephropathology experience or those evaluating only H&E-stained sections. For example, in a study of renal tumors in humans evaluated by physician surgical pathologists, clinically relevant lesions in the nontumor tissue were missed in more than half of the samples.[24] Although the lack of recognition of glomerular lesions by non-nephropathologists in that study in part may represent failure to critically evaluate the entire sample once a diagnosis of neoplasia was made, the glomerular lesions subsequently were identified correctly by nephropathologists, highlighting the need for evaluation of samples using all modalities and by pathologists with expertise in nephropathology.

Glomerulosclerosis was the most common non-IC glomerulopathy found in this cohort of dogs (20.6%). It was even more common than amyloidosis (15.2%), which has been reported to be the second most common cause of glomerular disease in some studies of dogs.[13,25,26] Interestingly, GS is the only category in which a substantial sex bias was identified, as females were significantly over-represented (74/103, 71.8%) by a margin of nearly 3 to 1. Because the histories typically only included data on recent clinical findings, possible causes of this sex bias could not be ascertained. Most of the females (69/74; 93.2%) were spayed, but information about age at neutering and previous reproductive status generally was not available for this retrospective study. Importantly, cases in this category included both focal segmental and global GS. In human medicine, primary focal segmental glomerulosclerosis (FSGS) is a specific disease category caused by primary podocyte abnormalities, whereas adaptive forms (eg, secondary podocyte injury) develop after glomerular insults, such as hypertension, obesity, diabetes mellitus, and decreased nephron endowment.[27] Because its pathogenesis is not as well understood in dogs, all cases in which glomerulosclerosis was present without underlying IC disease were put into a single category. Glomerulosclerosis is under-recognized as a cause of clinically relevant proteinuria in dogs. It often is characterized by mesangial cell proliferation and GBM thickening in the scarred segments. Because these changes mimic the histologic lesions of ICGN (Fig 2), it may be misdiagnosed as ICGN in proteinuric dogs in which renal biopsy specimens are evaluated only by LM (as was the case in 5% of the samples in this category in this study). Because immunosuppressive therapies have not been studied in glomerulosclerosis in dogs, and in as much as they might not be effective in a disease process without immune complexes, it is important that the clinician receive a diagnosis based on a comprehensive pathologic evaluation when deciding on a treatment plan.

The cases with non-IC glomerulopathy included dogs with primary glomerular lesions that did not fit in any of the other categories, as defined. Specifically, these cases had ultrastructural evidence of abnormalities in the GBM, podocytes, or mesangium but did not have evidence of IC, amyloidosis, or sclerosis. Similar to the situation in other categories, a large portion (37.8%) of the non-IC glomerulopathy cases required TEM to definitively determine that immune deposits were not present in the glomeruli. This finding further highlights the importance of TEM in correctly diagnosing suspected glomerular disease in dogs.

Animal age did not appear to be a useful indicator of the likely diagnostic category in these dogs with suspected glomerular disease. The median age of affected dogs in every category was between 6 and 8 years, which was similar to the median age (7 years) of all dogs that were included in the study. Although renal disease generally is known to increase in prevalence as dog's age, it is important to note that in this study a dog, 4 months of age, had TEM evidence of ICGN.

Therefore, young age alone cannot be used to rule out ICGN. Indeed, the only disease categories in this study that did not include some dogs younger than 2 years of age were amyloidosis, glomerulosclerosis, and primary tubulointerstitial disease.

In dogs, UPC >0.5 is accepted as indicating clinically relevant proteinuria,[1,4,28] and UPC >2.0 or >3.0 is considered to be suggestive of glomerular disease.[28,29] A UPC ≥2.0 was used in this study as the criterion to include cases evaluated for suspected glomerular disease when the available history did not include other evidence of glomerulopathy (eg, nephritic or nephrotic syndrome, chronic proteinuria). Although the study was designed to select for cases with probable glomerular disease, 12 dogs (1 with a UPC of 8.6) were found to have primary tubulointerstitial disease. The higher than expected UPC results in most of these tubulointerstitial disease cases was at least partly explained by secondary damage to glomeruli, but it is important to note that a UPC >2 does not necessarily preclude primary tubulointerstitial disease.

Most biopsy specimens in this study were submitted by clinicians working in specialty or tertiary care facilities, and most were from high population centers in the Northeast, Midwest, and Pacific regions of North America. This distribution might cause a selection bias for owners willing to spend more money on their animals (or able to incur the expense of a complete renal diagnostic evaluation), patient environment, and severity or chronicity of the clinical histories, because many animals initially may have been seen and managed for some time by their primary care veterinarians. Additionally, TEM was not performed on most cases with amyloid deposits confirmed by CR staining, and thus concurrent ICGN could not be excluded. No instances of concurrent disease, however, were found in cases in which both modalities were used. Furthermore, given that amyloidosis is considered irreversible, diagnosis of concurrent ICGN likely would not impact prognosis or treatment.

This study focused on dogs with suspected glomerular disease, which is the most common indication for renal biopsy.[12] Proteinuria is a hallmark of glomerular disease, and in humans, proteinuric patients are most likely to benefit from therapy altered in response to biopsy findings.[30] Dogs with suspected glomerular disease are at risk for being inappropriately treated with immunosuppressive drugs, and evaluation of a renal biopsy specimen is needed to minimize such risks. The findings of this study confirm that TEM and IF should be included routinely in the evaluation of renal biopsy specimens obtained from dogs with suspected glomerular disease; otherwise, incorrect diagnoses often will be made. Veterinary nephropathology is still a developing subspecialty, and future studies are needed to formulate a useful subclassification scheme for ICGN based on IC location and other relevant pathologic features, as well as to correlate pathologic findings with clinical data (including response to treatment and long-term outcome) in order to refine the therapeutic and prognostic utility of renal biopsy findings for dogs with suspected glomerular disease.

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

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