European Veterinary Renal Pathology Service: a survey over a 7-year period (2008-2015)

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Abstract: BACKGROUND: The European Veterinary Renal Pathology Service (EVRPS) is the first Web-based registry for canine renal biopsy specimens in Europe. HYPOTHESIS/OBJECTIVES: The aim was to verify whether differences exist between the clinical and laboratory presentation of dogs with nephropathy according to renal pathological findings, as defined by light and electron microscopy of renal biopsy specimens submitted to EVRPS. ANIMALS: Renal biopsy specimens of dogs were collected from the archive of the service (n = 254). Cases were included if both light and electron microscopy were available (n = 162). METHODS: Renal biopsy specimens were classified based on the morphological diagnoses. Thereafter, they were grouped into 3 disease categories, including immune-complex-mediated glomerulonephritis (ICGN), non-immune-complex-mediated GN (non-ICGN), and renal lesions not otherwise specified (RL-NOS). Differences among morphological diagnoses and among disease categories were investigated for clinical and laboratory variables. RESULTS: Serum albumin concentration was lower in dogs with ICGN than in those with non-ICGN (P = 0.006) or RL-NOS (P = 0.000), and the urine protein-to-creatinine ratio (UPC) was significantly higher in ICGN than in the other 2 disease categories. Regarding morphological diagnoses, albumin was significantly lower in amyloidosis (AMY) and membranous (MGN), membranoproliferative (MPGN) or mixed glomerulonephritis (MixGN) than in minimal change disease, primary (FSGS I) or secondary (FSGS II) focal and segmental glomerulosclerosis and juvenile nephropathies (JN). The UPC was higher in MPGN than in FSGS I and FSGS II. CONCLUSIONS AND CLINICAL IMPORTANCE: Dogs with ICGN, in particular MPGN, had higher protein loss than those with non-ICGN or RL-NOS, leading to more severe hypoalbuminemia. Clinical and laboratory differentiation among dogs with the different morphological diagnoses and among dogs with different disease categories was difficult due to overlapping results.

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Keywords: Renal biopsy; Glomerulonephritis; Dog; Electron microscopy; Diagnosis

Abbreviation

AMY: amyloidosis

EVRPS: European Veterinary Renal Pathology Service

FSGS I: primary focal and segmental glomerulosclerosis

FSGS II: secondary focal and segmental glomerulosclerosis)

FWER: family-wise error rate

ICGN: immune-complex mediated glomerulonephritis

IF: immunofluorescence

JN: juvenile nephropathies

LM: light microscopy

MCD: minimal change disease

MD: miscellaneous diseases

MGN: membranous glomerulonephritis

MixGN: mixed glomerulonephritis

MPGN: membranoproliferative glomerulonephritis

Non-ICGN non immune-complex mediated glomerulonephritis

RBs: renal biopsies

RL-NOS: renal lesions not otherwise specified

RL-NOS: renal lesions not otherwise specified

TEM: transmission electron microscopy

UPC: urine protein/creatinine

UTI: urinary tract infection

UVNS: Utrecht Veterinary Nephropathology Service

WSAVA-RSSG: World Small Animal Veterinary Association-Renal Standardization Study Group

Abstract

Background: The European Veterinary Renal Pathology Service (EVRPS) is the first web-based registry for canine renal biopsies in Europe.

Hypothesis/Objectives: The aim was to verify whether there are differences between clinical and laboratory presentation of dogs with nephropathy according to renal pathological findings, as defined by light and electron microscopy of renal biopsies submitted to EVRPS.

Animals: Renal biopsies of dogs were collected from the archive of the service (n=254). Cases were included if both light and electron microscopy were available (n=162).

Methods: Renal biopsies were classified based on the morphological diagnoses. Thereafter, they were grouped into 3 disease categories, including immune-complex mediated glomerulonephritis (ICGN), non immune-complex mediated GN (non-ICGN) and renal lesions not otherwise specified (RL-NOS). Differences among morphological diagnoses and among disease categories were investigated for clinical and laboratory variables.

Results: Serum albumin concentration was lower in dogs with ICGN than in those with non-ICGN ($p=0.006$) or RL-NOS ($p=0.000$) and the urine protein-to-creatinine ratio (UPC) was significantly higher in ICGN than in the two other disease categories. Regarding morphological diagnoses, albumin was significantly lower in amyloidosis and membranous, membranoproliferative or mixed glomerulonephritis than in minimal change disease, primary or secondary focal and segmental glomerulosclerosis and juvenile nephropathies. UPC was higher in membranoproliferative glomerulonephritis than in primary focal and segmental glomerulosclerosis.

Conclusions and clinical importance: Dogs with ICGN, in particular membranoproliferative glomerulonephritis, had higher protein loss than those with non-ICGN or RL-NOS, leading to more severe hypoalbuminemia. Clinical and laboratory differentiation among dogs with the different morphological diagnoses and among dogs with different disease categories was difficult due to overlapping results.

Keywords: Renal biopsy; Glomerulonephritis; Dog; Electron microscopy; Diagnosis

In collaboration with the World Small Animal Veterinary Association-Renal Standardization Study Group (WSAVA-RSSG), the Utrecht Veterinary Nephropathology Service (UVNS) has been created in 2008, providing the first web-based and prospective registry system for canine and feline renal biopsies (RBs) in Europe. In 2014, the service was reorganized and included into the European Veterinary Renal Pathology Service (EVRPS) and located within the Department of Comparative Biomedicine and Food Science, University of Padova, Italy.

To date, the EVRPS serves European veterinarians providing renal pathology consultations (LA, AVD) and detailed diagnoses including light microscopy (LM), immunofluorescence (IF) and transmission electron microscopy (TEM), in a short turnaround time. A diagnosis is obtained by the consensus of three pathologists with renal pathology experience (LA, SB, JVL). The EVRPS represents also the first continental registry to record pathological diagnosis, as well as clinical and laboratory data of dogs undergoing RBs. Registries represent important tools, providing epidemiological data or characteristic findings of the various diseases and are widely used in human medicine. In 2013, Scheneider and colleagues collected the largest series of canine RBs, which were obtained from 501 dogs living in North America, and provided thorough description of pathological findings.[1] They reported a wide array of renal diseases in dogs but clinical and laboratory findings were not provided. However, it is worth mentioning that collection of clinical, laboratory and pathological data from dogs with renal disease is difficult for many reasons. First, RBs are not usually included in the diagnostic workup in clinical practice. Furthermore, it is now recognized that the complete

evaluation of a RB requires LM and TEM examination combined with IF.[2-3] Unfortunately, TEM facilities are not always available in private and academic veterinary diagnostic centres and costs may represent a limiting factor. Therefore, comprehensive surveys are always difficult to be performed and require excessively long time periods or multicentre investigations.

The aims of the present report from EVRPS were to characterize the clinical and laboratory presentation of dogs with suspected renal disease that underwent RB submitted to the EVRPS, to classify the RB according to LM and TEM examination, and to identify possible differences between clinical and laboratory findings based on renal morphological diagnoses and based on renal disease categories. The survey included dogs living in Europe.

Materials and methods

Case selection

RBs submitted between 2008 and 2015 were collected from the archive of the UVNS/EVRPS. Although standard RB evaluation consists in LM, IF, and TEM, the contemporary presence of LM and TEM report was the sole inclusion criterion for the selection. The IF results were considered to implement the diagnosis but not included in the data analysis. For all cases, clinical and laboratory variables were retrieved from signalment, history, haematology and biochemical profile, urinalysis. All data were provided by the referring veterinarian.

Processing of RBs

All renal biopsies were routinely processed for LM and TEM examination.[4] Specimens for LM were sectioned at 3 μm thickness and stained with Haematoxylin and Eosin, Periodic acid-Schiff, Masson's Trichrome, and periodic acid methenamine silver. Congo red staining was performed on 8-10 μm sections if amyloid was suspected, to confirm the diagnosis. For TEM, tissues were fixed in chilled 3% buffered glutaraldehyde and processed according to standard procedures.[4]

Evaluation of RBs

The evaluation and classification of renal lesions were based on the diagnostic criteria developed by WSAVA-RSSG.[4] TEM was used as the gold standard method for immune-complex detection. RBs were analysed singularly and then grouped in 3 disease categories: a) immune-complex mediated glomerulonephritis (ICGN) when glomerular immune-complex deposits were identified by TEM, including membranous GN (MGN), membranoproliferative GN (MPGN), mixed GN (MixGN) and focal and segmental glomerulosclerosis pattern secondary to immune-complex deposits (FSGS II); b) non immune-complex mediated GN (non-ICGN) when glomerular immune-complex deposits were not identified by TEM, including minimal change disease (MCD), amyloidosis (AMY), focal and segmental glomerulosclerosis pattern not associated with other glomerular diseases (FSGS I); c) renal lesions not otherwise specified (RL-NOS), including juvenile nephropathies (JN) and miscellaneous diseases (MD). Dogs without evident lesions at TEM were arbitrarily included in the latter group.

Statistical analysis

Analyses were performed with a commercial software (SPSS v20.0 for Windows) to detect possible associations between clinical or laboratory findings and pathological results. At first, the analysis was conducted comparing all morphological diagnoses and subsequently was re-conducted considering the 3 disease categories. The following variables collected from signalment and history were used in the analysis: age at diagnosis, sex (male, female), disease onset (acute, chronic), body weight and appetite (decreased, increased), anorexia, vomiting, polyuria and polydipsia, lethargy, previous episodes of urinary tract infection (UTI), subcutaneous edema, ascites, hypoalbuminemia, gross haematuria and proteinuria (present, absent). Furthermore, laboratory values were considered in the analysis if recorded within one month prior to biopsy, including: haematocrit, leukocyte count, serum albumin, total proteins, creatinine, urea nitrogen, sodium, potassium, total calcium, phosphorus and antithrombin III, urinalysis including colour, specific gravity, pH, glycosuria, erythrocytes, leukocytes and UPC.

In particular, contingency tables were prepared for each of the aforementioned variables, and the Pearson's χ^2 test was performed to assess their possible association with morphological diagnoses and disease categories. When appropriate, the Fisher's exact test was used for 2x2 contingency tables.

For continuous variables, a Shapiro-Wilk test was performed to assess whether the data were normally distributed.

Kruskal-Wallis or ANOVA tests were performed to compare means among the different morphological diagnoses and disease categories. When a significant variation among groups

occurred, post-hoc analysis was performed with Mann-Whitney, Bonferroni or Dunnett tests, based on data distribution and homoscedasticity assessment. Significance was set at $p \leq 0.05$ for all tests except for Mann-Whitney test whereby based on the number of possible paired contrasts the significance threshold was set at $p \leq 0.016$ for diseases categories and at $p \leq 0.001$ for morphological diagnoses to reduce the family-wise error rate (FWER) in multiple comparisons.

When only two groups were compared, the independent-samples t-test or the Mann-Whitney test was performed according to data distribution. Significance was set at $p \leq 0.05$ for both tests.

Finally, Cohen's kappa was calculated to assess the level of agreement between LM and TEM in the disease categories and morphological diagnoses assignment. Results were evaluated according to Landis and Koch (1977).[5]

Results

Cases

Over a period of 7 years, 254 RBs were sent to the UVNS/EVRPS. Ninety-two samples were excluded because LM or TEM were missing. Veterinarians from different European countries took advantage of the service; RBs coming from Sweden were the most frequent (n=43), followed by United Kingdom (n=41), Netherlands (n=33), Belgium (n=8), Norway (n=7), Finland (n=6), France (n=6), Germany (n=6), Italy (n=4), Ireland (n=3), Hungary (n=2), Switzerland (n=2), and Spain (n=1). The proportion of cases for which the different data were

available varied widely within the study population, ranging from 6.8% (for antithrombin III activity) to 100% (for vomiting). More details are provided in Tables 2 to 5 and S1 to S4.

Golden Retrievers (n=11), Labrador Retrievers (n=9) and Schapendoes (n=8) were the most represented breeds. Female were slightly predominant (52%) over males (48%). The median age at presentation was 74 months (6.2 years), with 54.3% of dogs being <7 years.

Morphological diagnosis

By LM, 53 (32.7%) renal biopsies were diagnosed as FSGS I, 28 (17.3%) as MGN, 26 (16%) as MPGN, 11 (6.8%) as AMY, 4 (2.5%) as JN, 2 (1.2%) as MixGN, and 1 (0.6%) as MCD; 21 (13%) dogs had MD and 16 (9.9%) showed no abnormalities. By TEM, the definitive diagnosis changed in 71 dogs (Table 1). The final diagnosis did not change for dogs identified by LM as AMY, MCD, MixGN and JN. Interestingly, none of the dogs had FSGS II based on LM, whereas at TEM it was diagnosed in 19 cases. Cohen's Kappa was 0.494.

Age significantly differed among morphological diagnoses (p=0.002); in particular, dogs with JN were younger than dogs with MPGN (p=0.001) and FSGS I (p=0.000) (Table 2).

Considering historical data, significant differences in proportions among groups were found for the detection of UTI (p=0.040), and ascites (p=0.014) (Table 3). Indeed, UTI was less frequent in dogs with MPGN and MixGN; ascites and abdominal distension were less frequent in dogs with MPGN and FSGS I.

Concerning haematology and biochemical profiles, significant differences in proportions among groups were found for alterations of serum concentration of albumin and total proteins

(p=0.000 and p=0.001) (Table 3). Hypoalbuminemia and hypoproteinemia were more common in dogs with AMY, MGN, MPGN and MixGN. Significant differences among groups were present for serum concentrations of albumin (p=0.000), total proteins (p=0.001), creatinine (p=0.013), urea nitrogen (p=0.021) and phosphorus (p=0.012) (Table 2). In particular, albumin was lower in dogs with AMY, MPGN and MixGN than in dogs with FSGS I, FSGS II and JN (p-values of paired contrasts from 0.000 to 0.001), and in dogs with MGN than in dogs with MCD, MixGN and JN (p=0.000 for all contrasts).Total proteins were lower in dogs with AMY or MGN than in dogs with FSGS II (p=0.040 and p=0.007, respectively). Creatinine was lower in dogs with MCD than in dogs with MPGN (p=0.000). Concerning urea nitrogen, no significant result was obtained for paired contrasts. Phosphorus was higher in dogs with MD than in dogs with FSGS I (p=0.001) .

Regarding urinalysis, significant differences were found among groups for the presence of erythrocytes (p=0.009) and proteinuria (p=0.000) (Table 3). Erythrocytes were less commonly observed in the urine of dogs with FSGS I and more commonly in that of dogs with MPGN and MixGN. Proteinuria was slightly less common in dogs with FSGS II and frequently absent in those with JN; in the latter, approximately half had no proteinuria. Mean UPC and urine specific gravity were significantly different among groups (p=0.017and p=0.000, respectively) (Table 2). In particular, UPC was higher in dogs with MPGN than in dogs with FSGS I (p=0.035) and urine specific gravity was higher in dogs with MixGN than in dogs with AMY, FSGS I and FSGS II (p=0.034, p=0.008 and p=0.043, respectively).

Significant differences among morphological diagnoses were not detected for the remaining variables (Tables S1-S2).

Disease categories

By LM, 64 (39.5%) RBs were diagnosed as non-ICGN, 57 (35.2%) as ICGN and 41 (25.3%) as RL-NOS. By TEM, the final diagnosis changed in 46 dogs; overall, 82 (50.6%) dogs had ICGN, 59 (36.4%) non-ICGN and 21 (13%) RL-NOS. In particular, the final diagnosis changed for 21 (32.8%) dogs diagnosed as non-ICGN, 2 (3.5%) diagnosed as ICGN and 23 (56.1) diagnosed as RL-NOS based on the LM results, respectively. Cohen's Kappa between LM and TEM was 0.560.

The median age in dogs diagnosed with ICGN was 70 months, 90 months for dogs with non-ICGN, and 27 months for dogs with RL-NOS. A significant difference was found among the 3 disease categories (p=0.000); in particular, dogs with RL-NOS were younger than dogs with ICGN (p=0.001) and non-ICGN (p=0.000), whereas dogs with ICGN and non-ICGN had comparable age (Table 4).

Considering the historical information, significant differences among groups were found for the detection of previous UTI (p=0.035), presence of ascites (p=0.040), and hypoalbuminemia (p=0.006) (Table 5). Overall, previous UTI was less frequent in dogs with ICGN. Ascites was more common in dogs with ICGN whereas hypoalbuminemia was scarcely identified in dogs with RL-NOS.

With regard to recent biochemical data, significant differences among groups were found for alterations of serum concentration of albumin, total proteins and calcium (p=0.003, p=0.019 and p=0.050, respectively) (Table 5). Hypoalbuminemia and hypoproteinemia were more common in dogs with ICGN. Hypocalcemia was more common in dogs with ICGN and hypercalcemia in dogs with RL-NOS. Significant differences in serum concentrations of albumin (p=0.000), urea nitrogen (p=0.022) and phosphorus (p=0.003) were present among the 3 disease categories (Table 4). In particular, albumin was lower in dogs with ICGN compared to those with non-ICGN (p=0.006) or RL-NOS (p=0.000), urea was significantly higher in dogs with ICGN than in those with non-ICGN (p=0.008), and phosphorus was significantly higher in dogs with RL-NOS than in those with non-ICGN (p=0.001).

Considering urinalysis, significant differences in proportions among groups were found for the presence of erythrocytes (p=0.015) and proteinuria (p=0.035) (Table 5). Above 10 erythrocytes/high power field were more common in the urine of dogs with ICGN compared with the other two disease categories. Proteinuria was slightly less frequent in RL-NOS dogs than in the other two disease categories. The mean UPC was higher in ICGN dogs than in non-ICGN (p=0.002) and RL-NOS (p=0.000) dogs (Table 4).

No significant differences among disease categories were detected for the remaining variables (Tables S3-S4).

Because it is perceived by pathologists that AMY and FSGS I are relatively easy to differentiate from any ICGN, further analysis was performed to compare the two morphological diagnoses against the whole group of ICGN. In particular, it was found that dogs with AMY

were less likely to be anemic than those with ICGN (p=0.038; Table S1 and S3, respectively), had higher mean HCT values (p=0.049; Table S2 and S4, respectively), and lower mean urinary specific gravity (p=0.047; Table 2 and 4, respectively). Dogs with FSGS I were more commonly reported to have previous UTI than those with ICGN (p=0.018; Table 3 and 5, respectively), less commonly decreased serum concentration of albumin (p=0.002; Table 3 and 5, respectively) and total proteins (p=0.006; Table 3 and 5, respectively). In addition, dogs with FSGS I, compared to ICGN, had higher concentrations of albumin (p=0.000; Table 2 and 4, respectively) and total proteins (p=0.039; Table 2 and S4, respectively), and lower UPC (p=0.002; Table 2 and 4, respectively). Finally, dogs with FSGS I, compared to ICGN, less frequently had erythrocyturia (p=0.000; Table 3 and 5, respectively).

Discussion

In the present study we analysed the clinical, laboratory and pathological data of 162 canine RBs examined at the EVRPS between 2008 and 2015. The number of submitted RBs has risen constantly over the 7 years (9% per year), likely due to increasing awareness of the service by European veterinarians and the recent publication on RB indications combined with the new criteria to diagnose renal diseases in dogs.

Interestingly, when examining the geographical origin of the veterinarians using the service, RBs from the North European countries were overrepresented, possibly causing a bias in the distribution of renal diseases. Indeed, northern countries are not endemic for Leishmaniasis, which is a major cause of ICGN in dogs from southern Europe.[6-7] However, the

rate of dogs with ICGN in our case series (50.6%) was in line with a recent investigation of Scheneider and colleagues (i.e. 48.1%), confirming that approximately half of the renal diseases in dogs have an immune-mediated origin.[1]

In this series, the agreement between LM and TEM diagnosis for either morphological diagnoses or disease categories was only moderate. Hence, these results emphasize the importance of TEM during the diagnostic evaluation of RBs as this tool can identify lesions that would be undetected by LM. Identification of immune-complexes is necessary for proper classification of renal diseases and the combined use of LM and TEM allowed us to localize them within the glomerulus and to achieve the final morphological diagnosis. Furthermore, this study confirms the necessity to evaluate RBs both with LM and TEM in FSGS cases, since LM alone cannot differentiate between a primary podocyte injury (possibly genetic) and the deposition of immune complexes.[8-9]

When considering cases divided according to disease categories, dogs with RL-NOS were significantly younger compared to dogs affected by ICGN and non-ICGN. This was mainly related to the high frequency of JN in this disease category, which is a major cause of chronic kidney disease in young pure breed dogs.[10-11] In contrast to age, gender was similarly distributed among disease categories or morphological diagnoses.

Concerning history, differences were documented for UTI, being less frequent in dogs with ICGN than in those with non-ICGN or RL-NOS. Dogs with UTI may develop renal disease due to bacterial invasion of the kidneys. In this setting, the pathogenesis of kidney damage is not expected to be associated with deposition of immune-complexes but, depending

on the distribution of bacteria or binding affinity of their toxins, with direct tubular or glomerular lesions, possibly leading to non-ICGN.[12] Indeed, the majority of dogs with a morphological diagnosis of MCD and FSGS I (i.e. non-ICGN) had a history of UTI; conversely, UTI was reported in less than 10% of dogs with MPGN and MixGN (i.e. ICGN). Whether bacteria triggered the development of MCD and FSGS I or were the consequence, cannot be answered. The reason why dogs with RL-NOS had higher chances of UTI as compared to ICGN is likely due to the fact that some of the cases within the former disease category had JN, which has been shown to predispose towards UTI.[13]

With regard to urinalysis, proteinuria was more frequent in dogs with ICGN or non-ICGN than RL-NOS, although significance was small. However, the overall magnitude of proteinuria in dogs with ICGN was 2-fold and 3-fold higher than in those with non-ICGN and RL-NOS, respectively. The more severe proteinuria observed in dogs with ICGN explained the fact that in the biochemical profile hypoalbuminemia was more common and with lower concentrations, in comparison to the other groups. The marked hypoalbuminemia, in turn, accounted for the more frequently documented hypoproteinemia and hypocalcemia as well as the more common ascites reported in dogs with ICGN. Hence, protein-losing nephropathies due to immune-complexes deposition may cause larger alterations in the permselectivity of the glomerular capillary wall than those caused either by non-ICGN or RL-NOS in dogs. Although dogs with non-ICGN had proteinuria slightly more commonly than those with RL-NOS, the degree of severity did not differ. As expected, the biochemical profile showed no differences in albumin concentrations between the 2 groups and the frequency of hypoproteinemia,

hypocalcemia and ascites were also similar. The suspected larger permselectivity of glomeruli in dogs with ICGN might also explain the more frequent erythrocyturia observed in this disease category.

Notably, from a clinical standpoint, differentiation of dogs with one or the other disease category based on the degree of proteinuria was not possible due to the fact that there was large overlap between the 3 disease categories. However, in this series none with non-ICGN and RL-NOS had UPC >12.5 while 21.1% of those with ICGN had UPC >12.5 (data not shown). Although dogs with renal proteinuria above this threshold might be more likely to have a nephropathy with an immune-complex pathogenesis, in the authors experience dogs with amyloidosis can also have higher UPC. Among morphological diagnoses of ICGN, dogs with MPGN had the most severe proteinuria. However, differentiating dogs with the different forms of ICGN based on the degree of proteinuria was not achievable. Unexpectedly, approximately 10% of dogs with FSGS II had no proteinuria. The absence of proteinuria in a disease characterized by glomerular deposition of immune-complexes might suggest that in some affected dogs there was an increased tubular reabsorption capacity that compensated for the protein loss. Alternatively the sclerosis might indicate that an improvement of glomerular lesions has occurred.[14-15] Unfortunately, it was unknown whether dogs were receiving any medication at the time of RB. Similarly to ICGN, it was not feasible to differentiate morphological diagnoses among dogs with non-ICGN or RL-NOS. Also, dogs with amyloidosis (i.e. non-ICGN), which is frequently associated with severe proteinuria, had protein loss that overlapped with dogs affected by other non-ICGN.[16]

The biochemical profile of dogs with ICGN showed higher serum urea concentrations, but not creatinine. This difference was observed relative to dogs with non-ICGN and not to those with RL-NOS. Increased protein catabolism to counteract the more severe degree of hypoalbuminemia in dogs with ICGN might have partly explained this observation. With regard to serum creatinine concentration, dogs with MCD had lower levels than those with MPGN. In several studies, the elevation of serum creatinine concentration correlated more with tubulo-interstitial than glomerular damage.[17-18] MCD is by definition characterized by normal glomeruli at LM and lesions are detected only at TEM. In contrast, MPGN can have a variable involvement of the tubulo-interstitium and histological changes. The absence of tubulo-interstitial damage in MCD in this particular case can, at least partially, explain why serum creatinine concentration was lower compared to MPGN.

Furthermore, the biochemical profile showed that dogs with RL-NOS were more likely to have hypercalcemia and hyperphosphatemia. Increased concentrations of phosphorus and, less often, of calcium are observed if glomerular filtration rate is severely impaired. However, serum creatinine did not differ between the 3 disease categories, suggesting that the estimated degree of renal dysfunction was probably not responsible for these results. Because dogs with RL-NOS included cases of JN, which might be diagnosed at a young age, it is possible that hypercalcemia and hyperphosphatemia was associated with physiologic growth; indeed, among dogs with RL-NOS, 25% were below 1-year old (data not shown).

Because amyloidosis is relatively easy to diagnose with LM and Congo Red staining and FSGS I still represents a diagnostic conundrum, we decided to compare the two morphological

diagnoses with the whole group of ICGN. Anemia was less frequent in dogs with amyloidosis and their urine was less concentrated; a reason to justify the former is elusive, whereas the latter might be explained by the fact that amyloid deposits may be often observed also in the medulla, possibly reducing the hypertonicity of this compartment and, in turn, water reabsorption.[19] In dogs with FSGS I hypoalbuminemia and hypoproteinemia as well as proteinuria and erythrocyturia were less marked than in the whole group of ICGN; these results are in line with the above differences observed between ICGN and the whole group of non-ICGN.

One limit of this study is the lack of IF data. Indeed, IF integrated with LM and TEM may help characterizing glomerulonephritis in dogs, further refining the morphological diagnosis. Another relevant limit is represented by the fact that different laboratories performed blood and urinalysis, with reference ranges that might have differed. However, the potential bias was probably evenly distributed among disease categories and morphological diagnoses, reducing its confounding effect.

In conclusion, this survey provides for the first time information about the frequency of canine renal diseases across Europe, with ICGN and non-ICGN representing almost 90% of the RBs. From a clinical perspective, dogs with ICGN, in particular membranoproliferative glomerulonephritis, had higher protein loss than those with non-ICGN or RL-NOS, leading to more severe hypoalbuminemia. Clinical and laboratory differentiation among dogs with the different morphological diagnoses and among dogs with different disease categories was difficult due to overlapping results. Based on our results RBs examined by LM and TEM are

recommended to allocate dogs with renal lesions to a certain morphological diagnosis and disease category.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

References

1. Scheneider SM, Cianciolo RE, Nabity MB, Clubbet al. Prevalence of immune-complex glomerulonephritides in dogs biopsied for suspected glomerular disease: 501 cases (2007-2012). J Vet Intern Med 2013; 27 Suppl 1:S67-75.

2. Aresu L, Pregel P, Bollo E, et al. Immunofluorescence staining for the detection of immunoglobulins and complement (C3) in dogs with renal disease. Vet Rec 2008; 163:679-82.

3. Cianciolo RE, Brown CA, Mohr FC, et al. Pathologic evaluation of canine renal biopsies: methods for identifying features that differentiate immune-mediated glomerulonephritides from other categories of glomerular diseases. J Vet Intern Med 2013; 27 Suppl 1:S10-8.

4. Cianciolo RE, Mohr FC, Aresu L, et al. World Small Animal Veterinary Association Renal Pathology Initiative: Classification of Glomerular Diseases in Dogs. Vet Pathol 2016:53:113-35.

5. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977; 33:159-174.

6. Aresu L, Valenza F, Ferroglio E, et al. Membranoproliferative glomerulonephritis type III in a simultaneous infection of Leishmania infantum and Dirofilaria immitis in a dog. J Vet Diagn Inves 2007;19:569-72.

7. Dujardin JC, Campino L, Cañavate C, et al.Spread of vector-borne diseases and neglect of Leishmaniasis, Europe. Emerg Infect Dis 2008;14:1013-8.

8. Angioi A, Pani A. FSGS: from pathogenesis to the histological lesion. J Nephrol 2016; 29:517-23.

9. Aresu L, Zanatta R, Luciani L, et al. Severe renal failure in a dog resembling human focal segmental glomerulosclerosis. J Comp Pathol 2010;143:190-4.

10. Chandler ML, Elwood C, Murphy KF, et al. Juvenile nephropathy in 37 boxer dogs. J Small Animal Pract 2007; 48:690-4.

11. Kolbjørnsen O, Heggelund M, Jansen JH. End-stage kidney disease probably due to reflux nephropathy with segmental hypoplasia (Ask-Upmark kidney) in young Boxer dogs in Norway. A retrospective study. Vet Pathol 2008; 45:467-74.

12. Roberts JA. Etiology and pathophysiology of pyelonephritis. Am J Kidney Dis 1991; 17:1-9.

13. De Morais HS, Di Bartola SP, Chew DJ. Juvenile renal disease in golden retrievers: 12 cases (1984-1994). J Am Vet Med Assoc 1996; 209:792-7.

14. Gillmore JD, Hutchinson WL, Herbert J, et al. Autoimmunity and glomerulonephritis in mice with targeted deletion of the serum amyloid P component gene: SAP deficiency or strain combination? Immunology 2004; 112:255-64.

15. Mc Causland FR, Niedermaier S, Bijol V, et al. Lyme disease-associated glomerulonephritis. Nephrol Dial Transplant 2011; 26:3054-6.

16. Cook AK, Cowgill LD. Clinical and pathological features of protein-losing glomerular disease in the dog: a review of 137 cases (1985-1992). JJ Am Anim Hosp Assoc 1996; 32:313-22.

17. Finco DR, Brown SA, Brown CA, et al. Progression of chronic renal disease in the dog. J Vet Intern Med 1999; 13:516-28.

18. Brown MR, Cianciolo RE, Nabity MB, et al. Masitinib-associated minimal change disease with acute tubular necrosis resulting in acute kidney injury in a dog. J Vet Intern Med 2013; 27:1622-6.

19. Segev G, Cowgill LD, Jessen S, et al. Renal amyloidosis in dogs: a retrospective study of 91 cases with comparison of the disease between Shar-Pei and non-Shar-Pei dogs. J Vet Intern Med 2012; 26:259-68.

Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Table S1: Distribution of 162 dogs with kidney disease according to disease category

Table S2: Mean values of different variables in 162 dogs with kidney disease, according to disease category

Table S3: Distribution of 162 dogs with kidney disease according to morphological diagnoses

Table S4: Mean values of different variables in 162 dogs with kidney disease, according to morphological diagnoses