Associations of patient characteristics, disease stage, and biopsy technique with the diagnostic quality of core needle renal biopsy specimens from dogs with suspected kidney disease

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OBJECTIVE
To identify factors affecting the diagnostic quality of core needle renal biopsy specimens from dogs with suspected kidney disease.

DESIGN
Cross-sectional study.

ANIMALS
522 client-owned dogs with suspected kidney disease for which core needle renal biopsy specimens (n = 1,089) were submitted to the International Veterinary Renal Pathology Service for evaluation and inclusion in their database.

PROCEDURES
Data regarding dog signalment, clinical variables, biopsy method, needle brand and gauge, biopsy results, and other variables were extracted from the database. Variables were tested for association with 3 outcomes of light microscopic evaluation of core specimens: number of glomeruli per core specimen, attainment of < 10 glomeruli, and presence or absence of renal medullary tissue.

RESULTS
Number of glomeruli per core specimen was significantly associated with needle gauge, dog age, serum creatinine concentration, and degree of proteinuria, whereas biopsy method and submitting hospital were significantly associated with the presence of renal medullary tissue in specimens. Mean numbers of glomeruli per core specimen obtained with 14- or 16-gauge needles were similar, but both were significantly greater than the mean number obtained with 18-gauge needles. Needle gauge had a similar association with the likelihood of obtaining < 10 glomeruli in a core specimen. Specimens obtained via laparotomy or laparoscopic approaches more commonly contained medullary tissue than those obtained by ultrasound-guided approaches.

CONCLUSIONS AND CLINICAL RELEVANCE
Overall, findings suggested that ultrasound-guided biopsy with a 16-gauge needle should maximize the diagnostic quality of renal biopsy specimens from dogs with suspected kidney disease, while avoiding potential adverse effects caused by larger needles. (J Am Vet Med Assoc 2018;252:67–74)

Renal biopsy is the reference (gold) standard for determining the cause of proteinuric nephropathy and acute kidney injury in dogs and is used to direct treatment of these conditions. To facilitate classification of glomerular diseases, renal biopsy specimens should be evaluated by 3 techniques: LM, immunofluorescence, and TEM. Specimens for each technique must contain adequate numbers of glomeruli. In human medicine, the recommendation is that renal biopsy specimens evaluated via LM contain a minimum of 10 glomeruli to allow assessment of the distribution of nondiffuse glomerular diseases such as focal segmental glomerulosclerosis. Indeed, some nephropathologists prefer 15 to 20 glomeruli for LM evaluation. In contrast, the veterinary literature suggests that histologic evaluation of 5 to 10 glomeruli is adequate.

By logic, use of larger-gauge needles would increase the number of glomeruli available for evaluation but could concurrently increase the risk of complications, particularly hemorrhage. Similarly, clinician experience with performing biopsies, disease severity or chronicity, patient size, and various other factors are expected to impact the diagnostic yield of renal biopsy. Comparisons of human renal biopsy specimens have shown that an increase in needle diameter from 18 to 14 gauge is associated with a significant increase in the number of glomeruli obtained per core specimen. Bleeding, however, is reportedly more severe in humans undergoing

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>IVRPS</td>
<td>International Veterinary Renal Pathology Service</td>
</tr>
<tr>
<td>LM</td>
<td>Light microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
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<tr>
<td>UPC</td>
<td>Urine protein-to-creatinine concentration ratio</td>
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JAVMA • Vol 252 • No. 1 • January 1, 2018

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ultrasound-guided renal biopsy with 14-gauge rather than 18-gauge biopsy needles. A study of renal biopsy techniques involving medium-sized healthy dogs revealed that glomerular yield was higher when 14-gauge versus 18-gauge biopsy needles were used. This finding failed to be validated in a later study involving clinical biopsy specimens from dogs with naturally acquired renal disease, although incomplete recording of the types of biopsy needles used might have reduced the statistical power to detect such differences.

Owing to the lack of conclusive data regarding the effect of needle gauge on diagnostic quality of renal biopsy specimens from dogs with kidney disease, no definitive recommendations exist for the most appropriate needle gauge for this purpose, and clinicians are left to weigh the desire to obtain a diagnostic specimen against concerns regarding the risks of complications. In addition, further information on the effect of dog body size, disease severity and chronicity, biopsy technique, and operator experience would be helpful in developing evidence-based guidelines for clinicians who plan to submit renal biopsy specimens for pathological evaluation. The goal of the study reported here was to use a recently developed centralized IVRPS database, which is a collaborative effort between The Ohio State University and Texas A&M University, to investigate the effects of various dog signalment, clinical, and biopsy variables on the diagnostic quality of core needle renal biopsy specimens from dogs with naturally acquired kidney disease. We hypothesized that needle gauge, biopsy technique, operator experience, dog breed size, and stage of renal disease would significantly affect the number of glomeruli obtained per core specimen.

Materials and Methods

Dogs and data collection

The IVRPS database was searched to identify dogs that had a core needle renal biopsy performed, with samples submitted to the IVRPS for analysis, from 2005 through 2013 and for which the gauge of the needle used had been recorded. Data were then collected and categorized regarding dog breed size (small, medium, large, or giant), sex and reproductive status, age and age group (puppy [0 to 6 months], juvenile [7 months to 2 years], adult [3 to 8 years], or geriatric [≥ 9 years]), body weight, and body condition score at the time of biopsy; duration of clinical signs of kidney disease (chronic [≥ 3 months] or acute [< 3 months]); systolic arterial blood pressure (< 150 mm Hg, 150 to 159 mm Hg, 160 to 179 mm Hg, or ≥ 180 mm Hg); serum creatinine concentration category (no azotemia [< 1.4 mg/dL], mild azotemia [1.4 to 2.0 mg/dL], moderate azotemia [2.1 to 5.0 mg/dL], or severe azotemia [≥ 5.0 mg/dL]); serum albumin concentration (< 2.6 mg/dL or ≥ 2.6 mg/dL); UPC (nonproteinuric [< 0.2], borderline [0.2 to 0.5], proteinuric [≥ 0.5 to 3.5], or nephrotic-range proteinuric [≥ 3.5]); method of core specimen collection (laparotomy, ultrasound-guided approach, or laparoscopic approach); biopsy device type (automated, semiautomated, or manual); biopsy needle gauge (18, 16, or 14) and brand; total number of glomeruli identified via LM, immunofluorescence, and TEM; percentage of renal cortical tissue identified via LM; and pathological diagnosis.

When biopsies were performed on multiple dates for the same patient, data from only the first submission were considered.

Categories used for serum creatinine concentration, systolic arterial blood pressure, and UPC were created in accordance with the International Renal Interest Society scale for kidney disease. Biopsy needles were grouped on the basis of the manufacturer into 4 categories for the most commonly used needle types: brand 1, brand 2, brand 3, and brand 4. On the basis of the histologic and ultrastructural findings of the IVRPS, dogs were assigned to 1 of 8 biopsy interpretations: nondiagnostic, immune-mediated glomerulonephritis, amyloidosis, focal segmental to global glomerulosclerosis, glomerulopathy not otherwise specified, non–immune-mediated nephropathy, primary tubulointerstitial disease, and normal kidney via all modalities. The category of glomerulopathy not otherwise specified was comprised of specimens in which important primary lesions of the glomeruli were identified but did not fall into other categories.

Examples included lesions of the glomerular basement membrane, podocyte lesions without sclerosis, glomerular lipidosis, and glomerulocystic disease. Specimens assigned a diagnosis of non–immune-mediated nephropathy had non–immune-complex-mediated lesions in both the glomeruli and tubulointerstitial regions. In these situations, both compartments were similarly affected and the primary disease process could not be discerned.

Examples of such diseases included juvenile nephropathy or renal dysplasia and nephrosclerosis. For statistical analysis, the nondiagnostic group was excluded.

Statistical analysis

Descriptive statistics were generated for all data by use of statistical software. Independent variables were tested for their association with the 3 assessed outcomes of LM evaluation of specimens (number of glomeruli detected, obtainment of < 10 glomeruli, and presence of renal medullary tissue). Because the number of core specimens available for LM evaluation varied widely between 1 and 7/dog, data were standardized as the mean number of glomeruli per core specimen submitted.

Multivariable models were developed to assess the association of each independent variable with each of the 3 outcome variables. A multivariable linear model was developed to identify factors associated with the mean number of glomeruli per core specimen, which was transformed by calculation of
the natural logarithm to fulfill the assumption of normal data distribution. Multivariable logistic regression models were also developed to identify the factors associated with obtaining < 10 glomeruli and the presence of renal medullary tissue in core specimens. Adjusted ORs and their associated 95% confidence intervals were calculated from the model coefficients and their SEs. Variables screened for independent associations with each outcome included submitting hospital; dog breed size, age category, sex and reproductive status, body weight, and body condition score; serum creatinine concentration; UPC; presence or absence of nephrotic-range proteinuria (UPC > 3.5); experience level of the attending clinician (number of renal biopsies previously performed); categorized duration of clinical signs; and biopsy method, needle brand and gauge, and complications. Variables identified as independently associated with each outcome were tested for inclusion in multivariable models by use of a forward selection procedure. Differences were considered significant at values of \( P < 0.05 \).

Results

Dogs

A total of 522 dogs, each with 1 submission to the IVRPS (n = 1,089 core needle renal biopsy specimens in total), met the selection criteria and were included in the study; 276 (52.9%) dogs were female (241 spayed) and 246 (47.1%) were male (203 neutered). For the 500 dogs for which age was recorded, ages ranged from 3 months to 14 years, with 8 (1.6%) classified as puppy, 52 (10.4%) classified as juvenile, 305 (61.0%) classified as adult, and 135 (27.0%) classified as geriatric.

Ninety-four pure breeds were included, representing 441 of the 514 (85.8%) dogs for which breed was recorded. Breeds represented by \( \geq 10 \) dogs included Labrador Retriever (n = 48), Golden Retriever (31), Yorkshire Terrier (19), Boxer (18), Shetland Sheepdog (16), Soft Coated Wheaten Terrier (14), Beagle (14), Miniature Schnauzer (13), Doberman Pinscher (12), German Shorthaired Pointer (11), and Australian Shepherd (11). Because approximate size of the dog was considered more pertinent than breed, dogs were classified by breed size as small (146 [28.0%]), medium (185 [35.4%]), large (164 [31.4%]), and giant (19 [3.6%]).

Historical and clinical variables

Serum creatinine concentration was high (\( > 1.4 \text{ mg/dL} \)) for 284 of the 510 (55.7%) dogs for which it was recorded, which were classified as having stage 2 (n = 85 [16.7%]), stage 3 (132 [25.9%]), or stage 4 (67 [13.1%]) kidney disease. Of the 442 dogs with a recorded blood pressure, 78 (17.6%) had hypertension, defined as a systolic arterial blood pressure > 180 mm Hg. Of the 481 dogs with a recorded UPC, 459 (95.4%) had proteinuria (UPC > 0.5) and 349 (73.0%) had nephrotic-range proteinuria (UPC > 3.5). Duration of clinical signs of kidney disease was available for 447 (85.6%) dogs and was categorized as acute for 157 (35.1%) and chronic for 290 (64.9%) dogs.

Biopsy variables

Core needle renal biopsy specimens had been collected by use of 30 types of needles from 14 manufacturers; however, to simplify the analyses, only the 4 most commonly used brands were assessed. Although mean numbers of glomeruli per core specimen collected with brands 1 and 2 were greater than those collected with brands 3 and 4, the differences were not significant.

No biopsy device type was specified for 244 of the 522 (46.7%) submissions. Of the 278 dogs with a recorded device type, automated devices were used for 149 (53.6%), semiautomated for 123 (44.2%), and manual for 6 (2.2%). The type of biopsy device had no significant effect on the number of glomeruli identified via LM or on the prevalence of inadvertent inclusion of renal medullary tissue. Needle diameter was specified for 501 (96.0%) dogs. An 18-gauge needle had been used for 256 (47.1%) of these dogs, a 16-gauge needle for 177 (35.3%), and a 14-gauge needle for 88 (17.6%).

Institution and operator experience

Overall, 25 hospitals submitted specimens to the IVRPS for > 5 dogs during the study period, representing 341 (65.3%) dogs. Only 3 hospitals submitted specimens from > 20 dogs (representing 94 [18.0%] dogs in total). No institution was specified for submissions regarding 2 dogs.

Only 128 (24.5%) dogs had core specimens submitted by clinicians who had supervised or performed > 5 renal biopsy procedures. Most biopsies (for 394 [75.5%] dogs) had been performed by clinicians with less experience. Only 5 clinicians submitted > 10 core specimens to the IVRPS during the study period.

Diagnoses

Overall, 30 (5.7%) dogs had core needle renal biopsy specimens submitted to the IVRPS that were classified as nondiagnostic. Pathological diagnoses for the remaining 492 dogs included immune-mediated glomerulonephritis (n = 212 [40.6%]), focal segmental to global glomerulosclerosis (96 [18.4%]), amyloidosis (64 [12.3%]), tubulointerstitial disease (43 [8.2%]), non–immune-mediated nephropathy (38 [7.3%]), glomerulopathy not otherwise specified (34 [6.5%]), and normal kidney (5 [1%]). A total of 11,616 glomeruli were identified via LM as available for evaluation, representing a mean of 22.3 glomeruli/submission. Total number of core specimens per submission was variable, with 158 submissions that included 1 core specimen, 220 that included 2 core specimens, 103 that included 3 core specimens, 27 that included 4 core specimens, 6 that included 5 core specimens, 5 that included 6 core specimens, and 2 that included 7 core specimens for LM evaluation. For dogs with fo-
cal segmental to global glomerulosclerosis (n = 96), a condition that requires examination of multiple glomeruli, a mean of 25.6 glomeruli were available for LM evaluation.

For the 30 dogs with core specimens considered nondiagnostic, the total number of glomeruli available for LM evaluation ranged from 0 to 15. Specifically, specimens from 11 dogs contained no glomeruli, from 15 dogs contained 1 to 5 glomeruli, from 1 dog contained 6 to 10 glomeruli, and from 3 dogs contained > 10 glomeruli. Regarding the 11 dogs with no glomeruli visible via LM, 4 had no glomeruli visible via immunofluorescence or TEM evaluation, whereas 7 with glomeruli visible via these 2 techniques contained nonspecific lesions such as mild podocyte foot process effacement or splotchy immunostaining. For the 15 dogs with 1 to 5 glomeruli visible via LM, 8 had no glomeruli visible via other techniques, 4 had glomeruli visible via other modalities with nonspecific lesions, and 3 had glomeruli that were globally sclerotic and inadequate for evaluation. For the 1 dog with 6 to 10 glomeruli visible via LM, immunofluorescence preparations had nonspecific, splotchy staining, but no glomeruli were visible via TEM to definitively rule out an underlying lesion of the glomerular basement membrane. For the 3 dogs with 11 to 15 glomeruli visible via LM, glomerulosclerosis was identified, but no glomeruli were visible in immunofluorescence or TEM preparations to rule out the presence of underlying immune-complex deposition.

Of the 492 dogs with a diagnostic core specimen, 43 (8.7%) had ≤ 5 glomeruli visible via LM, 49 (10.0%) had 6 to 10 glomeruli visible, and 400 (81.3%) had > 10 glomeruli visible.

Complications
Complications following renal biopsy were recorded for 18 (3.4%) dogs. The most common complication was hematuria (n = 14), but hydronephrosis (1), retroperitoneal effusion (1), perirenal edema and related abdominal pain (1), and other unspecified events (3 [1 of which required nephrectomy]) were also recorded. Two of the dogs had 2 complications (both hematuria and hemorrhage). Insufficient data were available to allow analysis of the effect of needle gauge on the risk of complications; however, hematuria was recorded for 3 dogs in the 14-gauge group, 4 dogs in the 16-gauge group, and 7 dogs in the 18-gauge group, suggesting that needle gauge had no strong association with this particular complication. All of the clinically relevant recorded complications (unspecified injury requiring nephrectomy, hydronephrosis, and perirenal edema and related abdominal pain) were associated with biopsies involving 14-gauge needles.

Factors associated with < 10 glomeruli/core specimen
In analyses of the relationship between needle gauge and LM findings of > 10 visible glomeruli, which is the number of glomeruli deemed to be sufficient for evaluation of human biopsy specimens, 113 (21.6%) dogs had < 10 total glomeruli visible via LM. Needle gauge was specified for 501 (96.0%) dogs’ submissions. Obtainment of > 10 glomeruli was achieved for 148 (83.6%) submissions via 16-gauge needles, 172 (72.9%) submissions via 18-gauge needles, and 68 (77.3%) submissions via 14-gauge needles. Additionally, when the total number of glomeruli per core specimen was analyzed (ie, total number of glomeruli in the whole submission divided by the number of core specimens evaluated), then only 85 (36.0%), 96 (54.2%), and 46 (52.3%) dogs in which 18-, 16-, and 14-gauge needles were used, respectively, had > 10 glomeruli. Needle gauge significantly affected the diagnostic quality of core specimens, with 18-gauge needles more often yielding specimens with < 10 glomeruli than 16- and 14-gauge needles (18 gauge vs 16 gauge, P < 0.001; 18 gauge vs 14 gauge, P = 0.04). Sixteen-gauge needles also yielded a slightly higher proportion of specimens with > 10 glomeruli than did 14-gauge needles (P = 0.0445).
Several other factors were associated with outcome regarding diagnostic quality of specimens through multivariable analysis (Table 1). Although the mean number of glomeruli obtained decreased in association with azotemia (Table 2), the likelihood of obtaining an insufficient specimen with < 10 glomeruli was significantly greater in dogs with severe azotemia (> 5 mg/dL) than in nonazotemic dogs (< 1.4 mg/dL). Dog age at the time of biopsy was also associated with outcome, whereby the odds of having < 10 glomeruli/submission decreased with increasing age. Hypoalbuminemia (< 2.6 mg/dL) was not associated with < 10 glomeruli. Instead, dogs with normoalbuminemia were at higher risk for having < 10 glomeruli per submission.

Table 1—Results of multivariable logistic regression analysis of factors associated with < 10 glomeruli in LM evaluations of core needle renal biopsy specimens from dogs with suspected kidney disease (n = 522).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Adjusted OR</th>
<th>95% confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine concentration (mg/dL)</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&lt; 1.4</td>
<td>1.0</td>
<td>Reference</td>
<td>—</td>
</tr>
<tr>
<td>1.4–2.0</td>
<td>2.7</td>
<td>1.4–5.4</td>
<td>—</td>
</tr>
<tr>
<td>2.1–5.0</td>
<td>2.6</td>
<td>1.4–4.8</td>
<td>—</td>
</tr>
<tr>
<td>&gt; 5.0</td>
<td>4.1</td>
<td>2.1–8.4</td>
<td>—</td>
</tr>
<tr>
<td>Serum albumin concentration (mg/dL)</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&lt; 2.6</td>
<td>1.0</td>
<td>Reference</td>
<td>—</td>
</tr>
<tr>
<td>≥ 2.6</td>
<td>2.3</td>
<td>1.4–3.7</td>
<td>—</td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.87</td>
<td>0.80–0.97</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Needle gauge</td>
<td>0.005</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>2.2</td>
<td>1.2–3.8</td>
<td>—</td>
</tr>
<tr>
<td>16</td>
<td>1.0</td>
<td>Reference</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>1.2</td>
<td>0.5–2.5</td>
<td>—</td>
</tr>
</tbody>
</table>

— = Not applicable.
Number of glomeruli per core specimen

Differences in glomerular yield between the 18-gauge and other needle sizes were significant (18 gauge vs 16 gauge, \( P < 0.001; 18 \text{ gauge vs 14 gauge, } P = 0.002 \)), with the mean glomerular yield from 18-gauge needles being the lowest. However, no difference was identified between 14- versus 16-gauge needles in the mean number of glomeruli per core specimen (Table 2).

Similar to findings for the other outcome variables, serum creatinine concentration, dog age, and needle gauge were all significant (Table 2). Of note, dogs with nephrotic-range proteinuria (UPC > 3.5) had more glomeruli per core specimen than dogs with a UPC \( \leq 3.5 \). Presence of hypoalbuminemia was not significantly associated with number of glomeruli per core specimen.

Factors associated with inclusion of renal medullary tissue

The multivariable logistic regression model of factors associated with inadvertent inclusion of renal medullary tissue in core specimens included biopsy method and number of submissions received from the submitting hospital (presumed to reflect biopsy experience; Table 3). Specimens obtained via laparotomy or laparoscopic approaches more commonly contained medullary tissue than those obtained by ultrasound-guided approaches. Hospitals that had submitted specimens for \( \leq 5 \) dogs during the study period were more likely to submit specimens containing renal medullary tissue than those with more submissions.

Discussion

The primary goal of the study reported here was to provide evidence-based recommendations to clinicians as they consider their approach to renal biopsy in dogs with kidney disease. To do this, we compared the diagnostic quality of core needle renal biopsy specimens from a large number of dogs with naturally acquired kidney disease, focusing specifically on factors that can be controlled by a clinician (eg, needle gauge and brand) or are influenced by clinical aspects of the patient (eg, dog signalment, size, and severity of renal disease). Findings suggested that ultrasound-guided percutaneous biopsy by use of a 16-gauge needle should maximize the diagnostic quality of core specimens. Other factors associated

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**Table 2**—Results of multivariable linear regression analysis of factors associated with the number of glomeruli obtained per core needle renal biopsy specimen for the dogs (n = 522) in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>( \beta ) coefficient</th>
<th>SE of ( \beta ) coefficient</th>
<th>Least squares mean*</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine concentration (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&lt; 1.4</td>
<td>1.25</td>
<td>1.11</td>
<td>10.80(^a)</td>
<td></td>
</tr>
<tr>
<td>1.4–2.0</td>
<td>0.96</td>
<td>1.09</td>
<td>8.33(^{a,b})</td>
<td></td>
</tr>
<tr>
<td>2.1–5.0</td>
<td>0.92</td>
<td>1.07</td>
<td>8.00(^b)</td>
<td></td>
</tr>
<tr>
<td>&gt; 5.0</td>
<td>Reference</td>
<td>—</td>
<td>7.85(^{a,b})</td>
<td></td>
</tr>
<tr>
<td>UPC</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>( \leq 3.5 ) (nephrotic-range proteinuria)</td>
<td>0.84</td>
<td>1.05</td>
<td>7.24(^a)</td>
<td></td>
</tr>
<tr>
<td>&gt; 3.5</td>
<td>Reference</td>
<td>—</td>
<td>10.38(^b)</td>
<td></td>
</tr>
<tr>
<td>Needle gauge(^\dagger)</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>18</td>
<td>0.80</td>
<td>1.06</td>
<td>6.96(^a)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1.15</td>
<td>1.06</td>
<td>9.97(^b)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Reference</td>
<td>—</td>
<td>9.39(^b)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>1.03(^\dagger)</td>
<td>1.01</td>
<td>0.023</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)The least squares mean is the expected number of glomeruli per core specimen, adjusted for other independent variables in the model. \(^\dagger\)Results are based on the 501 dogs for which needle gauge was specified. \(^\dagger\)Coefficient represents the expected change in the natural logarithm of the number of glomeruli per core specimen for each 1-year increase in dog age.

**Table 3**—Results of multivariable logistic regression analysis of factors associated with the presence of renal medullary tissue in core needle renal biopsy specimens from the dogs (n = 522) in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted OR</th>
<th>95% confidence interval</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy method</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ultrasound-guided approach</td>
<td>1.0</td>
<td>Reference</td>
<td>—</td>
</tr>
<tr>
<td>Laparotomy</td>
<td>2.7</td>
<td>1.7–4.3</td>
<td>—</td>
</tr>
<tr>
<td>Laparoscopic approach</td>
<td>4.0</td>
<td>1.8–9.8</td>
<td>—</td>
</tr>
<tr>
<td>No. of submissions/submitting hospital</td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>( &gt; 5 )</td>
<td>1.0</td>
<td>Reference</td>
<td>—</td>
</tr>
<tr>
<td>( \leq 5 )</td>
<td>1.6</td>
<td>1.1–2.4</td>
<td>—</td>
</tr>
</tbody>
</table>

See Table 1 for key.
with biopsy quality in our study included the experience of the submitting hospital as well as the patient’s age, serum creatinine concentration, and UPC.

In establishing the diagnostic quality of renal biopsy specimens, it is important to discuss how the cutoffs used in the present study were chosen. Diagnosis of certain diseases, such as amyloidosis, can be reached by identification of amyloid in a single glomerulus; however, other glomerular diseases such as focal segmental glomerulosclerosis are generally unevenly distributed and require examination of multiple glomeruli before a pathologist can be comfortable ruling out this condition. Some veterinary references have suggested that 5 intact glomeruli are required for accurate interpretation of a renal biopsy specimen, whereas in human medicine the cutoff for nephropathological assessment is at least 10 glomeruli and some nephropathologists prefer 20 glomeruli to improve the likelihood of detecting focal lesions. Twenty-six of the 30 (87%) nondiagnostic specimens in the present study contained 0 to 5 glomeruli, and only 3 (10%) contained >10 glomeruli. The number of glomeruli in core specimens was also clearly related to the likelihood of obtaining a diagnosis. However, there were several instances in which the pathologist was able to make a definitive diagnosis with <10 glomeruli visible via LM. Therefore, we recommend that renal biopsy specimens contain a minimum of 10 glomeruli to facilitate accurate diagnosis in all dogs, including those with focal glomerular lesions, specimens containing lower numbers may be sufficient for diagnosis of diffuse diseases in many situations.

Data from the IVRPS indicated that 18-gauge needles were inferior to 14- and 16-gauge needles with respect to obtainment of core specimens of sufficient diagnostic quality. The narrower caliber explains the increased risk for unsuccessful biopsies with 18-gauge needles, a condition that also pertains to human nephropathological assessments. Despite being slightly smaller in diameter, 16-gauge needles were no different from 14-gauge needles in the total number of glomeruli obtained per core specimen in the present study. Data from this and previous research indicate that 14-gauge needles are more likely to harvest renal medullary tissue, and by default this might decrease the amount of cortical tissue in the specimen while simultaneously increasing the likelihood of hemorrhage from arcuate arteries at the corticomedullary junction. Therefore, we recommend the use of the 16-gauge needles for renal biopsies of dogs with kidney disease.

Proper patient selection also affects the ability to obtain an adequate renal biopsy specimen. Multivariable analysis of data from the IVRPS database revealed that specimens with <10 glomeruli were more commonly obtained from dogs with severe azotemia (serum creatinine concentration, >5 mg/dL) than from other dogs. Severely azotemic patients might be less stable under anesthesia, and clinicians might be reluctant to attempt multiple biopsies. Azotemia is reportedly an important risk factor for hemorrhage in dogs undergoing renal biopsy, although an association with that complication was not detected in the present study. In scenarios involving chronic azotemia, there is parenchymal scarring with associated global glomerulosclerosis, whereas acute severe azotemia often leads to interstitial edema or inflammation, both of which might reduce glomerular density (ie, number per unit of tissue volume) in the renal cortex and result in fewer glomeruli per core specimen.

Although disease chronicity was evaluated as a factor in the present study, no association was identified between this variable and outcomes and so it was not included in the multivariable modeling process. Disease chronicity is often estimated on the basis of subjective assessment of clinical signs and is likely inaccurate. Patients with chronic renal disease can decompensate abruptly, obscuring the clinical assessment. Although our data indicated increased odds of obtaining an inadequate biopsy tissue from severely azotemic dogs, this did not suggest that clinicians should forego the procedure in all such patients. Rather, these data suggested that additional specimens might be required from dogs with severe azotemia to increase the likelihood of obtaining an adequate number of glomeruli per core specimen and that renal biopsy should be pursued early after the detection of proteinuria, rather than waiting until the disease has progressed and is associated with more severe azotemia. Certainly, the indication for the biopsy, the ultrasonographic appearance and size of the kidneys, and the stability of the patient under anesthesia should all remain part of the clinical assessment.

Interestingly, hypoalbuminemic dogs were more likely to have adequate renal specimens than dogs with a serum albumin concentration within reference limits. Similarly, nephrotic-range proteinuria (UPC >3.5) was associated with the harvest of more glomeruli per core specimen than a UPC ≤3.5. It is interesting that hypoalbuminemia was identified as having a significant association with the likelihood of obtaining an adequate specimen (>10 glomeruli) in the multivariable model, whereas magnitude of proteinuria (ie, having nephrotic-range proteinuria) was significantly associated with the number of glomeruli per core in that multivariable model. We did not expect that protein-losing nephropathies would increase the density of glomeruli in the renal cortical specimens obtained, and we cannot explain the increased likelihood of obtaining a diagnostic specimen from affected dogs. One possibility is that clinicians performing biopsy for the clinical indication of proteinuria would attempt multiple biopsies to ensure adequate harvest of renal cortical tissue.

Dog age also influenced specimen quality, with younger dogs having fewer glomeruli in core specimens than older dogs. We suspect that selection bias affected the findings for young dogs in that renal biopsy involving young animals is often prompted by juvenile nephropathy, a condition that can be due to a paucity of functional nephrons. Additionally, the small
body size of young dogs may have increased the difficulty of obtaining adequate specimens (ie, small size of the biopsy target relative to adjacent large vessels). Several aspects of biopsy technique had significant associations with inadvertently including the renal medulla in the obtained specimens. Although seemingly counterintuitive, biopsy performed via laparotomy was associated with 2.7 times the odds of obtaining renal medullary tissue, compared with an ultrasound-guided approach. One would expect that direct visibility of and access to kidneys during an open surgical technique would improve the diagnostic quality of the specimens. However, without the benefit of ultrasound guidance to identify the exact location of the needle tip, clinicians might embed the needle deep in the renal cortex before triggering the cutting cannula. This apparent adverse effect of an open surgical approach on the quality of biopsy specimens has been noted by other investigators as well.

In the present study, a laparoscopic approach to renal biopsy was associated with even greater odds of inadvertent biopsy of renal medullary tissue. These results are in contrast to those in a previous prospective study, in which investigators compared laparoscopically acquired specimens to specimens obtained with ultrasound guidance. These discordant results can be explained by the fact that laparoscopic procedures in the previous study were performed at a single institution in clinically normal dogs, whereas the present study included actual case material submitted by clinicians from multiple institutions with various levels of experience performing renal biopsy. Likewise, the importance of institutional experience was clearly evident, wherein hospitals from which ≤ 5 submission were received during the study period had 1.6 times the odds of submitting specimens containing renal medullary tissue than hospitals from which more submissions were received.

Several variables in the present study had no significant association with diagnostic quality of renal biopsy specimens. We were unable to detect an effect of device type (manual versus automated needle biopsy), although this might have been attributable to the lower number of biopsies that were performed with a manual device. Spring-loaded needles (automated and semiautomated) are reportedly easier to use, decrease the risk of renal laceration, and cause less tissue distortion. Needle gauge had no significant association with complication rates in the study reported here; however, the reliability of this finding is unknown given the retrospective nature of the study and the possibility that complications may not have been consistently recognized or reported. Although findings of previous experimental research suggest an increased risk of hemorrhage with larger needle gauge, this finding could not be supported or refuted by our data.

Although biopsy technique and patient factors are known to affect the diagnostic quality of specimens, it is important to remember that postbiopsy specimen processing can also affect a pathologist’s ability to diagnose glomerular disease in dogs. Optimal methods for evaluation of glomerular disease include the assessment of biopsy specimens by use of histologic, immunofluorescence, and TEM techniques to identify and elucidate pathogenic mechanisms in humans and dogs. Separation of biopsy material for each technique occurs optimally at the time of the biopsy, and a protocol was described in a previous report.

Immunofluorescence and TEM evaluations can be performed on 1 or 2 intact glomeruli because these techniques are used to detect immune-complex deposition, which almost always affects all glomeruli. Ideally, division of core specimens is performed after examination of glomeruli under a dissecting microscope or an ocular loupe to ensure that adequate numbers of glomeruli are included in each specimen. In the authors’ experience, the number of required glomeruli varies by modality, and our preference is to see 2 to 3 glomeruli for TEM, 4 to 8 for immunofluorescence evaluation, and > 10 for LM.

Although retrospective analysis of a large, standardized database such as the one used in the present study has its strengths, some limitations also exist to the information that can be obtained. For example, it was noted that completion of the complications section of specimen submission forms was uncommon. On the basis of this situation and the temporal nature of form submission at the time of biopsy, it was likely that some complications occurred that were not documented or not recognized at the time of submission. Similarly, although the statistical methods used in the present study accounted for anticipated potential confounding factors (eg, breed size, degree of azotemia, and needle gauge), other factors not accounted for may also be associated with diagnostic quality of core needle renal biopsy specimens. Finally, in performing these analyses, we assumed that 10 glomeruli would have been required to obtain a specimen of adequate diagnostic quality. However, in reality, nephropathologists would typically attempt to make a diagnosis with the available tissue and some conditions can be accurately diagnosed even with specimens that fail to meet the stated criteria.

In the study reported here, analysis of patient data from the largest renal biopsy database in veterinary medicine suggested that patient factors, disease severity, and biopsy method all affect the diagnostic quality of core needle renal biopsy specimens from dogs with suspected kidney disease. Findings suggested that ultrasound-guided renal biopsy with 16-gauge needles should maximize the diagnostic quality of obtained specimens while avoiding potential adverse effects caused by larger needles. Given that only 54.2% of core specimens obtained with 16-gauge needles included > 10 glomeruli, we would recommend that at least 2 good-quality core specimens be submitted for evaluation.
Acknowledgments

Dr. Crivellenti was supported by a scholarship from the Fundação de Amparo à Pesquisa do Estado de São Paulo (2012/25515-0).

Footnotes

b. Biopty, Magnum, Max core, and Monopty needles, CR Bard Inc, Murray Hill, NJ.
c. EZ core, Surgivet, and Vetcare needles, Cook Medical, Bloomington, Ind.
d. Temno, Tru-cut, and Vim Tru-cut needles, CareFusion, San Diego, Calif.
e. JMP, version 10, SAS Institute Inc, Cary, NC.

References