Increasing Incidence of Inadequate Kidney Biopsy Samples Over Time: A 16-Year Retrospective Analysis From a Large National Renal Biopsy Laboratory

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Introduction: Renal biopsy remains an essential tool for the diagnosis and treatment of patients with medical kidney disease. Recently, there has been a perceived change in the number of inadequate samples. The aim of this study was to determine the native renal biopsy miss rate from 2005 to 2020 at Arkana Laboratories, a nationwide kidney biopsy service.

Methods: From 2005 to 2020, a total of 123,372 native kidney biopsies were received from >2,500 nephrologists practicing across 44 US states. The miss rate was determined by age and year. In a subset of biopsies received in 2005 and 2018, the biopsy operator was determined, nephrologist or radiologist. Furthermore, the miss rate, needle gauge, biopsy depth by operator, and biopsy core width by gauge were measured.

Results: The miss rate increased markedly from 2% in 2005 to 14% in 2020. Radiologists performed 5% of biopsies in 2005 and 95% in 2018 using smaller diameter (18g/20g) needles 92% of the time. Glomeruli per centimeter of core biopsy and mean core width were significantly lower with smaller needles. The miss rate deep was significantly lower for nephrologists and remained consistent within operator between the 2 time points. The miss rate did not correlate with the increasing age of the population who had biopsies.

Conclusion: This increase in kidney biopsy miss rate significantly affects patient care in the management of medical kidney disease. Its correlation with the complete reversal in operators suggests an urgent need for interaction with and training of radiologists in this critical technique.

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Although the first detailed study of the percutaneous renal biopsy (PRB) was published in a Cuban journal in 1950, the publications by Iversen and Brun and the modification by Kark and Muehrcke led to its widespread use.1–3 By 1960, the rapid advances in the understanding of renal pathology brought on by PRB, along with significant progress in renal dialysis, transplantation, and the substantially increased understanding of renal physiology, led to the formation of Nephrology as a separate discipline.4

Today, PRB remains a critical tool for accurate diagnosis and thus treatment of medical renal disease.5

As in all biopsy procedures, safety and yield remain the critical outcome determinants. In the last 16 years, we retrospectively observed a decline in tissue adequacy. By 2015, there were certain centers with insufficient material in as high as 50% of biopsies performed. Analysis of these centers revealed a change in operators from nephrologists to radiologists. The time frame correlates with the shift in operators nationwide.6,7

The purpose of this study was to evaluate the change in renal biopsy yield in the last 16 years in our laboratory. Our hypothesis is that there has been a significant decrease in biopsy tissue obtained and that this correlates with the change to radiologists as operators and the attendant use of smaller gauge needles.8

METHODS

Institutional Board Review

The Solutions Institutional Review Board approved this study as minimal risk research as the data collected were those typically obtained for routine clinical
practice. Thus, the requirement for informed consent was waived.

**Histology**

All samples were processed using standard techniques as in our previous studies.9,10 Biopsy samples for light microscopy were fixed and transported in neutral-buffered formalin. The tissue was dehydrated in a step-wise fashion in graded alcohol solutions. The alcohol was removed with graded xylene solutions, and the tissue was embedded in paraffin. Serial 3µm sections were cut and stained with hematoxylin and eosin, periodic acid–Schiff, Jones methenamine silver, or Mason’s trichrome using standard reagents.

**Miss Rate Over Time**

All native renal biopsies received at Arkana Laboratories from January 1, 2005 to December 31, 2020, were analyzed to determine the number of inadequate samples on light microscopy. Biopsies were considered inadequate when the diagnosis line included any of the following terms: “inadequate for diagnosis,” “insufficient for diagnosis,” “medulla only,” “no kidney tissue available,” “fat,” and/or “connective tissue” and/or “skeletal muscle” only and/or ≤7 glomeruli.11 Cases with a diagnosis including the terms “limited sample” or “limited tissue” or “limited material” were evaluated for adequacy on a case-by-case basis by one of us (PDW).

**Miss Rate by Operator**

In 2005, the operator was known in 93% of the cases. During that year, Arkana (then Nephropath) received biopsies from 338 nephrologists from 24 states. Nevertheless, the number of biopsies received in 2006 was 41% greater than in 2005 and the operator could only be determined in 42% of the cases. Analysis of 200 consecutive cases received in mid-2008 found that the operator could only be determined in 36% of the cases. Thus, an accurate analysis of misses by operator could not be performed after 2005. To address misses by operator at a later time point, native kidney biopsies received in a 20-week period during April to August of 2018 were evaluated. The operator was determined by requesting that information from the sending facility by phone. Of the 5201 biopsies received, the operator could still not be determined in 67 cases (1%) resulting in 5134 biopsies for analysis.

The miss rate by operator and age of patient (by decade) were also determined.

**Needle Gauge by Operator**

For the first time point (2005), 100 consecutive native biopsies were examined for gauge and operator. In 3 cases, one or the other criterion could not be determined leaving 97 biopsies reported (87 performed by nephrologists and 10 by radiologists). In 2018, needle gauge data were available for 20 of 660 biopsies (3%) performed by nephrologists and 374 of 12,819 biopsies (3%) done by radiologists.

**Glomeruli by Needle Gauge**

Glomeruli, percent cortex, and total core length were determined in 250 consecutive biopsies from 2018 using 16g or 18g needles. Of the 250 cases, 25 had <40% cortex and were discarded leaving 225 for inclusion. Because 14g and 20g needles were much less often used, 25 consecutive biopsies using 14g needles and 20 consecutive biopsies using 20g needles with ≥60% cortex were evaluated.

**Needle Gauge Width**

The biopsy width of the tissue was calculated using the serially sectioned, periodic acid–Schiff-stained slide in the middle of the slide set. There were 102 consecutive biopsies that were evaluated for gauge, width, and glomerular number for 14g, 16g, or 18g needle. Because 20g needle biopsies were less frequent, 15 consecutive cases using a 20g needle were evaluated. Mean biopsy width was determined using 5 measurements per sample with an Olympus UC90 digital camera and Olympus cellSens image analysis software on an Olympus BX51 microscope with 100× objective (Olympus Corporation, Tokyo, Japan).

**Miss Depth**

The microscopic description in the report was used to determine the nature of the tissue received (reviewed by PDW). A deep miss was defined as medulla. A shallow miss was defined as perirenal tissue, such as muscle, fat, and/or connective tissue. In both settings, scant renal cortex was occasionally present.

**Statistical Analysis**

Descriptive statistics are presented with counts, percentages, and a bar chart for categorical variables, means, and SEM and box plots for continuous variables. A one-way analysis of variance test was used to evaluate the number of glomeruli per centimeter obtained from different needle gauges, controlling for all pairwise comparisons using the Tukey-Kramer adjustment for multiplicity. The Cochran-Armitage statistic was used to test for a trend over time in the biopsy miss rate. A Pearson correlation with 95% CI was used to describe the relationship between the width of the renal biopsy core and the needle gauge used to obtain it. The Cochran-Mantel-Haenszel test was used to test for differences in needle gauge choice and deep misses between operators, controlling for year of biopsy.
RESULTS

Biopsy Demographics
Arkana Laboratories received 1749 PRBs from 338 referring nephrologists across 24 US States in 2005 (Figure 1). By 2012, there were 5528 PRBs from 1252 nephrologists in 32 US States. In 2020, the last year of the study, there were 14,210 native PRBs from 2466 nephrologists in 44 US states. Taken together, 123,372 biopsies were analyzed from 2005 to 2020.

Miss Rate Over Time
The miss rate in 2005 was 2% of biopsies received, whereas in 2020, it was 14%, with a notable increase in the 2009 miss rate of 9% compared with the 3% miss rate in 2008 (Figure 2). The overall miss rate for the 16-year time frame was 11%, lowest in 2005 to 2007 at 2% and highest in 2013, 2014, and 2020 at 14%. The trend test is highly significant ($Z = -26.20$, $P < 0.001$), indicating the miss rate has generally been increasing in the last 20 years.

Change in Operator Over Time
Nephrologists performed almost all medical renal biopsies received by Arkana Laboratories in the early 2000s accounting for 95% in 2005 with a miss rate of 1%. Radiologists did only 82 PRBs in 2005 but had 14 misses (17%). In 2018, radiologists performed 95% of PRBs with a miss rate of 13% whereas nephrologists, performing 5% of PRBs, missed 8% of the time (Table 1). The miss rate was lower in each group proportional to the number of biopsies performed. Nevertheless, comparing the miss rate by nephrologists when they were the primary operators (2005, 1%) with radiologists as the primary operators (2018, 13%), the overall miss rate has increased by $>800\%$ (Table 1).

Miss Rate by Operator and Age of Patient Over Time
There was a significant increase in biopsies performed on patients aged $\geq 60$ years over time (Figure 3). The Cochran-Armitage trend test for 2005 to 2020 comparing $>60$ versus $<60$ was significant ($Z=35.49$, $P < 0.001$). Similarly, a direct comparison of the age distributions in 2005 and 2018 also revealed an older population in 2018 (Cochran-Mantel-Haenszel $\chi^2$ (1) = 82.50, $P < 0.001$), primarily driven by approximately a 10% increase in the native biopsies of 60- to 79-year-olds. Comparing miss rate by operator and age of patient in 2005, there is no difference in miss rate for either group in the older age group compared with their mean miss rate. In 2018, both nephrologists and radiologists miss more frequently in the older age group than their mean miss rate (Table 2). Nevertheless, when the miss rates by the predominate operator are compared (nephrologists in 2005 and radiologists in 2018), the miss rate for patients aged $\geq 60$ years old is comparable with the overall miss rate.

Conversely, we observed a decrease in pediatric (ages 0–19) biopsies over time (Figure 3). In 2008, 14% of biopsies were from this group, whereas in 2020,
pediatric biopsies accounted for only 4% of all biopsies. The Cochran-Armitage trend test comparing age groups <20 versus 20+ was significant ($Z = 28.50$, $P < 0.001$) for the years 2005 to 2020. In 2005, the miss rate was 1% for pediatric patients (1 of 145) and 2% for adult patients (38 of 1603). This rate increased for both sets of patients by 2018: 7% for pediatric patients (18 of 244) and 13% for adult patients (630 of 4890). The miss rate for adult patients was twice that of the pediatric patients for both years.

### Needle Gauge by Operator

In general, nephrologists use larger needles (smaller gauge numbers) than radiologists (Table 3, $\chi^2 (1) = 195$, $P < 0.001$). In 2005, nephrologists were the primary operators and used mainly 14g (22%) and 16g needles (76%). In 2018, radiologists were the primary operators and mostly preferred the 18g (86%) and 20g needles (7%).

### Glomeruli per Centimeter by Gauge

A total of $N = 270$ samples were analyzed to evaluate needle gauge impact on the number of glomeruli observed per centimeter of core biopsy. Box-and-whisker plots are presented revealing the mean (denoted by $X$), median (50%), interquartile range (25% and 75%), and minimum/maximum; there were no outliers detected in these data (Figure 4).

The mean number of glomeruli per centimeter is inversely related to needle gauge size, dropping from $25 \pm 0.9$ cm to $2 \pm 1.0$ cm (mean $\pm$ SEM) as the gauge size increases from 14g to 20g. The pairwise comparisons between each set of gauges are significantly different based on a one-way analysis of variance with the Tukey-Kramer correction applied for multiple comparisons (all $P < 0.01$).

### Needle Gauge Width

The width of the renal biopsy core changes dramatically as the needle bore size decreases (Figure 5a, Pearson’s $r = -0.91$, 95% CI $[-0.94$ to $-0.87]$). The mean width of the renal tissue obtained with differing needle gauges was determined (Table 3): 14g—$894 \pm 20.0$, 16g—$563 \pm 10.1$, 18g—$303 \pm 29.0$, and 20g—$155 \pm 22.5$ (mean $\pm$ SEM in $\mu$m). A 14g biopsy sample fills the photographic field at 100×, and the available tissue rapidly declines with smaller bore needles (Figure 5b). Given that the mean width of a glomerulus from a healthy adult is approximately 250 $\mu$m, the bore of the most often used 18g needle is only approximately 1.2× larger than a glomerulus.

### Biopsy Depth

In 2005, nephrologists performed 1667 biopsies (95%) missing 25 times (1%), 14 (56%) of which were deep. Radiologists did 82 biopsies (5%) and missed 14 times (17%). Of these 14, 11 (79%) were deep. In 2018, radiologists performed 12,819 (95%) and had 1648 (13%)...
Table 2. PRB miss rate by operator and patient age, 2005 vs. 2018

| Age        | Nephrologists | Radiologists |
|------------|--------------|--------------|
| 0–19       | 0.7          | 7            |
| 20–39      | 1.9          | 13           |
| 40–59      | 1.8          | 7            |
| ≥60        | 1.2          | 10           |
| Total      | 1.5          | 8            |

PRB, percutaneous renal biopsy of native kidneys. Red font highlights the miss rate in patients aged ≥60 years; blue font highlights miss rate across all ages.

misses, 1340 (81%) deep. Nephrologists did 660 biopsies (5%) with 50 (8%) misses, 29 (58%) deep in that same year. The 23% higher rate of deep misses for radiologists compared with nephrologists, controlling for year, is highly significant ($\chi^2$ (1) = 18.33, $P < 0.001$).

Table 3. Biopsy core diameter and needle gauge use by operator and year

| Needle gauge | Biopsy core diameter (µm, mean ± SEM) | Nephrologist | Radiologist |
|--------------|--------------------------------------|--------------|-------------|
| 14g          | 894 ± 20.0                           | 19 (22)      | 4 (20)      |
| 16g          | 563 ± 10.1                           | 66 (76)      | 16 (80)     |
| 18g          | 303 ± 29.0                           | 2 (2)        | 0 (0)       |
| 20g          | 155 ± 22.5                           | 0 (0)        | 0 (0)       |

Cochran-Mantel-Haenszel test for differences in operator’s choice of needle gauge, controlling for year: $\chi^2$ (1) = 195, $P < 0.001$. Red font denotes needle gauge usage for the primary operators that year.

DISCUSSION

The greatest strength of this study is its size and breadth of data. More than 120,000 native renal biopsies referred by >2500 nephrologists from 44 US States were evaluated in the 16-year study period. On this basis, these data are likely the best indicator of PRB miss rate in the United States. Though this breadth markedly increases the likelihood that this is representative of the overall renal biopsy miss rate, it does conceal the individual operator and operator group outcomes. Looking at these subsets, the miss rate varies from <1% to >50% (data not shown). It also conceals improvement over time owing to training and consistent review of outcomes. Several groups have improved from >40% miss rate to <2% miss rate, 18g and 20g needles to all 16g needles, and a mean of <10 glomeruli/cm to >30 glom/cm with a marked decrease in significant complications (data not shown). The cause(s) of the variability in miss rate after the initial 3-fold increase in 2009 could not be determined. Still, the overall increase from a 2% miss rate (2005) to a 14% miss rate (2020) is a 7-fold increase and represents thousands more patients with insufficient tissue when, ideally, one miss is too many.

This increased miss rate correlates with the change from nephrologists (95%, 2005) to radiologists (95%, 2018) as primary operators. The lack of operator data in the entire study is a weakness. Nevertheless, this is significantly mitigated by the data that were gathered on a case-by-case basis from 2005 to 2018, the published national trends regarding the switch to radiologists as operators and the extensive anecdotal analysis of our database regarding miss rates in centers that have switched from primarily nephrologists to entirely radiologists as operators (data not shown). The introduction of the automated biopsy gun and improved visualization techniques led to a radical change in ease and safety of the PRB. With radiologists controlling access to ultrasound and scanning instruments and because the biopsy gun was so much faster, easier to use, and safer, more and more biopsies were done by radiologists. Still, nephrologists have practically been forced to hand off this technique owing to increased regulations, liability costs, and the time constraints imposed by using radiology’s imaging facilities.

The change from nephrologists to radiologists as the primary operators in PRBs is associated not only with an overall increased miss rate but an increased miss rate by nephrologists in our study. This may reflect a decrease in the number of biopsies performed by an individual practicing nephrologist and/or inadequate training as this transition has taken place in training centers. The latter has been disputed by Korbet et al. in their review of the change from nephrologists to radiologists as operators doing PRBs in their center.
in both years. Further confirmation that a decrease in cortical thickness owing to age and/or chronic kidney disease is not related to the increased miss rate is the 10-fold increase in the miss rate among pediatric patients between nephrologists as primary operators in 2005 and radiologists as primary operators in 2018.

In our study, the needle biopsy gauge changed significantly between 2005 and 2018. The 18g needle is now the most common size by far and even 20g needles are used. The increased use of smaller 18g needles correlates with the change from nephrologists to radiologists as the primary operators both in our study and in other reports.6,12,16 The rationale for using a smaller gauge is likely based on an intuitive but incorrect assumption that smaller means safer. In a recent systematic review and meta-analysis of 87 manuscripts describing >118,000 PRBs, Poggio et al.17 found a numerical trend toward more hematomas and transfusions with 18g needles and a significant increase in pain with the 18g needles when compared with 16g needles. Nevertheless, there are also multiple studies revealing that the safety of the 14g needle is not less than either 16g or 18g needles.18–20 The 20g needle, while useful in endoscopic biopsies of the liver and pancreas, has a core diameter less than the diameter of a glomerulus and produces significantly less volume of tissue that is much more fragile and fragmented. Therefore, the 20g needle should not be used for a PRB.5,21–23

Confirming previous studies, our data reveal that the 18g needle produces fewer glomeruli/cm than the larger core needles.16,20,24 Although significantly different, the number of glomeruli/cm obtained is comparable (mean ± SEM: 18g—9.8 ± 0.32; 16g—12.3 ± 0.69). This similarity may be related to the wide variability in the bevel of needles collectively referred to only by gauge number.22,23 Nevertheless, there are 3 other important factors that greatly reduce the use of the smaller 18g needle of any bevel. The smaller needle produces greater fragmentation of the sample impairing an accurate evaluation of the tubulointerstitial compartment, the most important area for patient prognosis.26 It has also been found that as many as 50% of glomeruli are lost or floating in 18g biopsies.27

Finally, the smaller volume of an 18g sample is such that fewer total sections can be obtained. This is revealed by the significantly lower mean width of the renal core obtained by 18g and 20g needles compared with 14g and 16g needles. This lack of tissue can be critical in focal segmental lesions (focal segmental glomerulosclerosis, lupus nephritis, vasculitis, crescentic glomerulonephritis, etc.) that will more often be missed and because deeper sections for additional special stains are more often unavailable.28

Figure 4. Number of glomeruli/cm biopsy core by needle gauge. The data are expressed as the median (middle line) in a box bounded by the IQR 25%–75%, the mean (x), and the min and max lines. 14g—med 25.0, mean 25.1, IQR 14.8–33.2, min/max 11.0–52.5, n—25; 16g—med 10.6, mean 12.3, IQR 7.6–17.5, min/max 6.2–23.3, n—40; 18g—med 9.4, mean 9.8, IQR 7.7–11.7, min/max 5.9–14.4, n—185; 20g—med 1.8, mean 2.1, IQR 1.3–2.7, min/max 0.1–4.6, n—20. In pairwise comparisons from a one-way analysis of variance, the mean number of glomeruli/cm from each needle gauge is significantly different from all other means; all P < 0.01 controlling for multiple comparisons. #, number; IQR, interquartile range; max, maximum; min, minimum.

and the review of the Walter Reed training program outcomes regarding competency in the performance of the PRB by Yuan et al.12

Another explanation for the marked increase in biopsy miss rate is the possibility that biopsies were done in older patients with more chronic diseases. These patients would have a thinner cortex increasing the likelihood of a miss unrelated to operator. We document that there has been a significant increase in biopsies performed on patients aged ≥60 years. As previously discussed, in 2018, nephrologists miss significantly more often when the patient is 60 years or older compared with their overall miss rate. Nevertheless, whether comparing the nephrologists, the radiologists, or most operators (nephrologists in 2005 and radiologists in 2018), the miss rate for patients aged ≥60 years is very similar to the overall miss rate.
Biopsy depth plays a role in the avoidance of significant bleeding complications. A cutting needle of any gauge that passes through a medium or large artery is likely to cause serious bleeding.\(^{20,29}\) Given that the medulla contains larger vessels and is very rarely the location of the diagnostic material, it is considered inadequate for diagnosis and potentially leads to serious bleeding. In our study, the miss rate deep is markedly higher when a radiologist is the operator (>80% of misses). Nevertheless, nephrologists miss deep almost 60% of the time. As a result of Arkana being an independent laboratory, we are unable to obtain information regarding complications from the PRB; this is a major weakness that cannot be remedied.

The ability to make a diagnosis on limited tissue was not evaluated as this does not relieve the operator of the requirement to provide an adequate sample. Rarely, the diagnosis can be made on 1 glomerulus (e.g., membranous glomerulopathy). Nevertheless, even when this occurs, the biopsy remains an inadequate sample in that a possible second diagnosis, the degree of global sclerosis, tubulointerstitial fibrosis, and arterial disease all remain indeterminate.

This is the largest ever study of renal biopsy adequacy for diagnosis from a single laboratory receiving samples from a highly representative sample of nephrologists and radiologists across the United States. In it, we document the markedly increased miss rate over time correlated with the change in operator from almost entirely nephrologists to almost entirely radiologists. This change also correlates with an increased miss rate deep and the use of the smaller 18g needle that has been found to result in less glomeruli/cm kidney core and less overall renal volume.

Given that it is unlikely that this change in operator will be reversed, one solution lies in outreach to radiologists to inform them of this marked increase in the miss rate of cortical material required for the diagnosis of medical renal disease. Then, in combination with the nephrologists, radiologists, and renal pathologists, to offer intensive short course training in correct technique. That such training can improve outcome has been documented anecdotally among radiology groups that have received hands-on training by one of us (PDW) or by departmental intensive internal training and follow-up of biopsy quality.\(^{30}\) A regional workshop format has already proven successful (http://kidneycon.org/). Though this event focuses on nephrologists and nephrology trainees, radiologists and radiology residents would benefit equally from such a short, hands-on session devoted to the performance of kidney biopsy. Regardless of how it is done, a rapid intervention is required to reverse the significantly increased miss rate of the medical renal biopsy.
All the authors declared no competing interests.

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