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ORIGINAL RESEARCH

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Diagnostic quality of ultrasound-guided fine-needle aspirates samples from the canine liver and spleen is not significantly affected by using 22-, 23-, and 25-gauge needles

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Abstract

Ultrasound-guided fine-needle aspirates (FNA) of the liver and spleen for cytological analysis are a commonly performed procedure in canine veterinary practice. Based on our review of the literature, this is the first published study investigating whether needle size affects the diagnostic quality of hepatic and splenic samples. The aim of this prospective analytical study was to compare the diagnostic quality of ultrasound-guided FNA cytological samples of canine liver and spleen based on cellularity, blood contamination, and overall cell preservation between three different needle sizes (22-, 23-, and 25-gauge). A total of 282 splenic aspirates from 94 dogs and 348 hepatic aspirates from 116 dogs were enrolled in the study and examined by two board-certified veterinary clinical pathologists. In this study, no significant differences in diagnostic quality were identified between different needle gauge sizes when sampling canine liver and spleen. Blood contamination was higher using 22-gauge needles compared with 25-gauge needles ($P = 0.024$) when sampling the liver.

KEYWORDS
cytology, dog, fine needle aspiration, sampling

1 | INTRODUCTION

Transabdominal ultrasound-guided fine-needle aspirates (FNA) of the liver and spleen are a well-established and utilized procedure in veterinary medicine. Canine and feline liver and spleen are commonly sampled for cytological diagnosis of focal or diffuse parenchymal changes, screening of inflammatory and infectious conditions, and for oncological staging. These organs are generally superficial and easily accessible to FNA with few reported complications including minimal peritoneal bleeding, small hematomas, or pain.[1] Severe complications leading to death due to hemorrhage have been reported in 2 of 307 cats[2] and 2 of 600 dogs[3] undergoing hepatic FNA.

There is some controversy regarding which FNA technique yields the most diagnostic samples. Multiple veterinary and human studies have compared the effect of needle gauges on the cytological quality in multiple body regions.[4,5] A human systematic review and meta-analysis comparing different needle sizes when sampling pancreatic masses reported no differences in diagnostic quality.[6] A recent veterinary study comparing 22-gauge and 25-gauge FNA of cutaneous, subcutaneous, and intracavitary masses also reported no significant difference in diagnostic quality but reported less blood contamination using the smaller needles.[4] Other studies suggest that a specific

Abbreviation: FNA, fine-needle aspirates.

needle size yields better results.[5,7,8] In addition, several human and veterinary studies have compared the diagnostic quality of aspiration versus nonaspiration techniques. For hepatic and splenic canine samples, a nonaspiration technique has been reported to be superior.[9,10] In comparison, an aspiration technique has been reported superior in five different canine tumor.[8] Similar results between both techniques have been reported in multiple organs in humans[11,12] and conflicting results between aspiration and non-aspiration technique have been found in canine lymph nodes.[13,14]

In our institution, there is a large discrepancy in the preferred needle gauge size between different board-certified radiologists and diagnostic imaging residents. Based on our review of the literature, there are no veterinary studies objectively investigating the effect of needle gauge in the diagnostic quality of ultrasound-guided FNA of the liver and spleen in dogs. The aim of this study was to compare the diagnostic quality of ultrasound-guided fine-needle aspirate cytological samples based on cellularity, blood contamination, and overall cell preservation between three different needle sizes (22-, 23-, and 25-gauge). We hypothesized that diagnostic quality would not be affected by needle size.

2 | MATERIALS AND METHODS

2.1 | Selection and description of subjects

This was a prospective, analytical design. Client-owned canine patients were included in the study if they required hepatic and/or splenic aspirates as part of their diagnostic workup for suspected diffuse hepatic and/or splenic pathology, oncologic staging, or screening for inflammatory/infectious diseases. Patients were recruited from December 2018 to June 2022 at the Queen Mother Hospital for Animals. Ethical approval was granted by the Clinical Research Ethical Review Board at the Royal Veterinary College (URN: M2018 0149). Inclusion criteria included the following: (1) a clinical justifiable reason for hepatic and/or splenic FNA and (2) an ultrasonographically unremarkable liver/spleen or diffuse changes in the organ's echogenicity, echotexture or size. Samples for which FNA were targeted toward a specific focal hepatic or splenic lesion or defined mass were excluded from the study. Hepatic and splenic masses were excluded as necrotic intralesional areas[15,16] could potentially interfere with the results. Additionally, significant differences in cell content have been found in different canine tumors.[8] CBC analysis, including a manual platelet count, was performed in each patient prior to sampling. Additionally, a coagulation panel was performed in animals with clinically suspected coagulopathy or increased risk of bleeding. Dogs with evidence of bleeding or a confirmed bleeding disorder (including thrombocytopenic dogs) were also excluded. Dogs were sedated at the discretion of the clinician or anesthetists in charge. Decisions for the inclusion or exclusion of dogs were made by a board-certified veterinary radiologist (E.F., European College Veterinary Radiology) and a second-year diagnostic imaging resident (C.L.).

2.2 | Data recording and analysis

2.2.1 | Samples

Ultrasound-guided FNA were obtained by a board-certified veterinary radiologist or a resident under supervision. All the samples were performed using the same ultrasound unit (RS80A system, Samsung Medison), using a microconvex probe and frequencies ranging from 4–9 MHz. Patients were positioned in lateral or dorsal recumbency for scanning. The fur was clipped over the region of interest and any residual coupling gel was removed along with any gross debris. Chlorhexidine and/or surgical spirit was applied to the skin if needed.

Each patient had three ultrasound-guided FNA taken from the liver, spleen, or both organs. Each sample was performed using one of the following needle gauge diameters (22-, 23-, and 25-gauge). The order in which they were used was determined by a predefined sheet with a randomized numbering system (https://www.random.org/). The needle was held with no syringe attached and four needle passes were performed in each organ using a nonaspiration technique. This technique has been reported superior in splenic and hepatic aspirates due to greater cellularity and less blood contamination.[9,10] Once the needle was removed from the patient a 5 mL syringe with a partially air-loaded plunger was attached to the needle hub and used to expel the aspirated material onto glass microscope slides (Colourslides, Solmedia). This process was done immediately to avoid blood clotting within the hub. If excessive sample material was evacuated onto the slide, the sample was split with another slide to make a duplicate. The "squash prep" technique was used to smear the aspirated material.[1] The aspirates were smeared using a consistent technique and subsequently air-dried. The slides, both the original and duplicate if present, were labeled with the case number and numbered (1, 2, 3) with the first, second, and third needles used. In our institution, we routinely aim for three diagnostically optimal samples for each patient. If any of these samples were considered diagnostically suboptimal (e.g., no or minimal sample acquired or excessively blood contaminated), further FNAs were performed as needed; these additional samples were not included in the study. All pertinent case and sampling information were recorded in a file including the operator name and person preparing the slides. The board-certified clinical pathologists evaluating the cytological samples were blinded to this information.

2.2.2 | Cytological evaluation

Slides were stained with modified Wright stain (Hematek 3000®, Siemens) and stored for study purposes after the initial diagnosis. The slides were then individually examined by two board-certified veterinary clinical pathologists (A.J. European College of Veterinary Clinical Pathology and E.H. American College of Veterinary Pathology). Between 3 and 6 slides per organ were examined; in cases of duplicate slides, the best-scoring slide was used in each case. Any case missing a slide was excluded from the examination. A four-tier

TABLE 1 Four-tier cytological grading system adapted from LeBlanc et al.[9] Diagnostic quality was based on cellularity, blood contamination, and cellular preservation.

Characteristic	Score	Interpretation	Description
Cellularity	3	High	High numbers of nucleated cells
	2	Moderate	Moderate numbers of nucleated cells
	1	Low	Low numbers of nucleated cells
	0	Acellular	No to rare nucleated cells
Blood	3	Marked	High numbers of RBCs, often densely packed
	2	Moderate	Moderate numbers of RBCs
	1	Mild	Low numbers of RBCs
	0	None	No to rare RBCs
Preservation	3	Excellent	Majority (>90%) of cells intact
	2	Good	Low numbers of lysed cells
	1	Fair	Moderate numbers of lysed cells
	0	Poor	Majority (>90%) of cells lysed

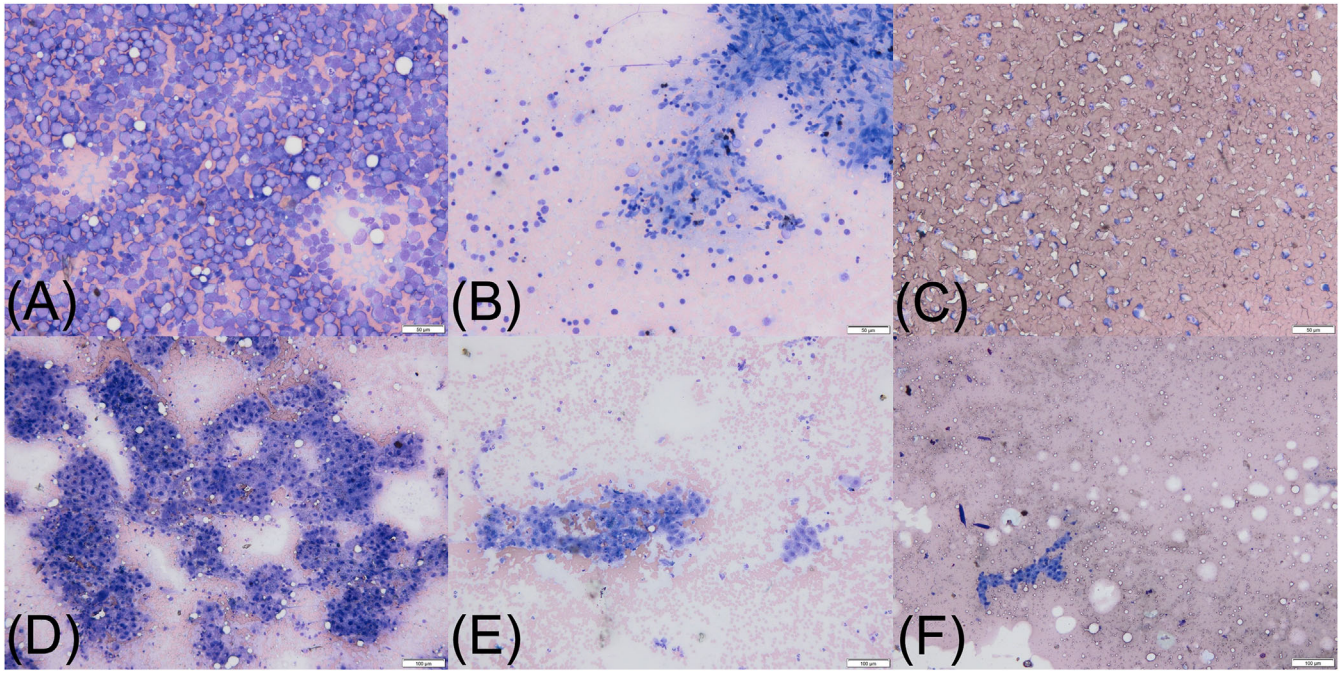

FIGURE 1 Photomicrographs of different degrees of cellularity, blood contamination and cell preservation in splenic (left, A–C) and hepatic (right, D–F) aspirates. Spleen: (A) high cellularity (3), low blood contamination (1), excellent cell morphology preservation (3); (B) moderate cellularity (2), moderate blood contamination (2) and good cell morphology preservation (2); (C) low cellularity (1), marked blood contamination (3) and fair cell morphology preservation (1). Liver: (D) high cellularity (3), mild blood contamination (1), excellent cell morphology preservation (3); (E) moderate cellularity (2), moderate blood contamination (2) and good cell morphology preservation (2); (F) low cellularity (1), marked blood contamination (3) and fair cell morphology preservation (1). Modified Wright stain in 200× (A–C) and 100× (D–F) magnification. [Color figure can be viewed at wileyonlinelibrary.com]

grading system (Table 1 and Figure 1), adapted from LeBlanc et al., was used for the classification of slide criteria which included cellularity, blood contamination, cell preservation, and whether slides were considered clinically diagnostic.[9] Scores (0-3) were entered into individual Excel sheets for each examiner and organ before being merged after completion of all slides. Each criterion was examined for agreement and any disagreement between pathologists resulted in review of the slide and a consensus score determined for discrepant criteria. After completion of slide examination, original results prior to consensus agreement from both examiners were compared for interobserver agreement. Complete agreement was reached when both examiners gave the same score for either cellularity, blood contamination, or

TABLE 2 Percentage of diagnostic samples using different needle sizes (22-, 23-, and 25-gauge) for the spleen and liver.

	Spleen ($n = 94$)			Liver ($n = 116$)		
	22G	23G	25G	22G	23G	25G
Diagnostic samples	81 (86.2%)	76 (80.9%)	85 (90.4%)	74 (63.8%)	74 (63.8%)	71 (61.2%)

TABLE 3 Grading of the cytological factors evaluated for the splenic samples.

	Liver											
	22G				23G				25G			
	Grade 0%	Grade 1%	Grade 2%	Grade 3%	Grade 0%	Grade 1%	Grade 2%	Grade 3%	Grade 0%	Grade 1%	Grade 2%	Grade 3%
Cellularity	4.3%	38.3%	39.4%	18.1%	3.2%	40.4%	40.4%	16%	0%	40.4%	41.5%	18.1%
Blood contamination	2.1%	5.3%	25.5%	67%	1.1%	3.2%	30.9%	64.9%	0%	4.3%	31.9%	63.8%
Cellular preservation	4.3%	11.7%	57.4%	26.6%	5.3%	10.6%	59.6%	24.5%	1.1%	9.6%	58.5%	30.9%

preservation. Partial agreement was marked when scores differed by 1 score (e.g. examiner 1 scored blood contamination as 2 whereas examiner 2 scored blood contamination as 1 or 3). Cases where the discrepancy was >1 were classified as a disagreement.

2.3 | Statistics

Statistical analysis was carried out by two observers (E.F. and C.L.) with training in biostatistics as part of their Masters degree (MVetMed), using a commercially available statistics software (SPSS Statistics for Macintosh, Version28.0.; IBMCorp.). The Kolmogorov–Smirnov test was used to evaluate the normality of the data. Continuous variables with non-normal distribution are presented as median values and ranges. Normally distributed data are presented as mean ± standard deviation. The McNemar test was used to assess any differences between the number of diagnostic and non-diagnostic samples between the different needle gauge sizes. The Wilcoxon signed-rank test was used to assess any differences between the grades of cellularity, blood contamination, and preservation between the different needle gauge sizes. The sample size for this study was calculated using a power analysis of 90% with a global significance level of 5% (http://www.rad.jhmi.edu/jeng/javarad/samplesize/). A minimum of 84 patients were included in each of the liver and spleen sampling groups. *P*-values of less than 0.05 were considered significant (with a 95% confidence interval).

3 | RESULTS

3.1 | Spleen

A total of 282 splenic aspirates from 94 dogs were enrolled in the study. The mean age was 7.7 ± 3.3 years with a median weight of 16.7 kg (range 2.2–57 kg). There were 10 intact males, 40 neutered males, 11 intact females, and 33 spayed females. Thirty-seven different breeds were included, the most common being cross breeds (11), Labrador Retriever (8), French Bulldog (7), Staffordshire Bull Terrier (6), German Shepherd (4), Cocker Spaniel (4), and Miniature Schnauzer (4). The most common reasons for sampling included oncologic staging, inflammatory/infectious screening, or abnormal imaging findings. The original cytological diagnosis of the smears comprised a total of 11 different conditions with multiple samples containing more than one condition. The most common cytological diagnoses were extramedullary hematopoiesis (68), reactive lymphoid hyperplasia (55), lymphoma (4), and normal (4).

A total of 15 board-certified, board-eligible radiologists and radiology residents were involved in performing the FNAs. Seven of fifteen (46.6%) of these people performed three or less FNAs. A variety of people were involved in preparing the slides including senior clinicians and residents from other disciplines.

Overall, 81 of 94 (86.2%) samples were considered diagnostic using a 22-gauge needle, 76 of 94 (80.9%) using a 23-gauge needle, and 85 of 94 (90.4%) using a 25-gauge needle (Table 2). No significant difference was observed between different needle gauge sizes.

Cellularity, blood contamination, and cellular preservation were graded from 0 to 3 for each needle-gauge size. A summary of these results is outlined in Table 3. No significant differences were observed between different needle gauge sizes in any of the categories. Examples of the variable cytological pictures are given in Figure 1(A–C).

A total of 345 slides were evaluated cytologically by each individual examiner. The results for the interobserver agreement can be found in Table 4. The highest agreement was reached for the cellularity (78% complete agreement) and blood contamination (77% complete agreement) whereas preservation showed 56% complete agreement. Partial agreement ranged from 22% for cellularity to 44% for preservation. Disagreement was only observed in a single slide for blood contamination (0%).

TABLE 4 Interobserver agreement for the splenic samples between the two observers.

Agreement	Cellularity	Blood	Preservation
Complete agreement	268 (78%)	264 (77%)	192 (56%)
Partial agreement	77 (22%)	80 (23%)	153 (44%)
Disagreement	0 (0%)	1 (0%)	0 (0%)

TABLE 6 Interobserver agreement for the hepatic samples between the two observers.

Agreement	Cellularity	Blood	Preservation
Complete agreement	282 (68%)	305 (74%)	212 (51%)
Partial agreement	132 (32%)	109 (26%)	198 (48%)
Disagreement	0 (0%)	0 (0%)	4 (1%)

3.2 | Liver

A total of 348 hepatic aspirates from 116 dogs were enrolled in the study. The mean age was 8.5 ± 3.4 years with a median weight of 14.9 kg (range 2.2–57 kg). There were 17 intact males, 52 neutered males, 6 intact females, and 41 spayed females. Forty-seven different breeds were included, the most common being cross breeds (9), Labrador Retriever (9), Cocker Spaniel (9), French Bulldog (8), Staffordshire Bull Terrier (8), Miniature Schnauzer (5), Bichon Frise (4), and German Shepherd (4). The most common reasons for sampling included oncologic staging, inflammatory/infectious screening, increased liver enzymes, and abnormal imaging findings. The original cytological diagnosis of the smears comprised a variety of different conditions. The most common diagnoses were vacuolar hepatopathy (78), neutrophilic, lymphoplasmacytic, macrophagic or mixed inflammation (21), cholestasis (16), normal (12), extramedullary hematopoiesis (11), lymphoma (9), and necrosis (6).

A total of 13 board-certified, board-eligible radiologists and radiology residents were involved in performing the FNAs. Six of thirteen (46.1%) of these people performed three or less FNAs. A variety of people were involved in preparing the slides including senior clinicians and residents from other disciplines.

Overall, 74 of 116 (63.8%) samples were considered diagnostic using a 22-gauge needle, 74 of 116 (63.8%) using a 23-gauge needle, and 71 of 116 (61.2%) using a 25-gauge needle (Table 2). No significant difference was observed between different needle gauge sizes.

Cellularity, blood contamination, and cellular preservation were graded from 0 to 3 for each needle-gauge size. A summary of these results is outlined in Table 5. No significant differences were observed between different needle gauge sizes in cellularity or cellular preservation. There was a significant difference ($P = 0.024$) in blood contamination between the 22-gauge and 25-gauge needles. Examples of the variable cytological pictures are given in Figure 1(D–F).

A total of 414 slides were evaluated cytologically by each examiner. The results for the interobserver agreement can be found in Table 6. The highest agreement was reached for blood contamination (74% complete agreement) and cellularity (68% complete agreement). Disagreement was seen for the preservation in four slides (1%) and preservation showed the highest degree of variability in the agreement (51% complete agreement and 48% partial agreement).

4 | DISCUSSION

In this study and in agreement with our hypothesis, the diagnostic quality of canine hepatic and splenic FNA was not significantly affected by needle gauge when using 22-, 23-, and 25-gauge needles. These results are in accordance with multiple veterinary and human papers.[4,6] Nevertheless, splenic FNA using 25-gauge needles resulted in more diagnostic samples and were associated with slightly better cellular preservation when compared to 22- and 23-gauge needles. In comparison, hepatic FNAs using 22- and 23-gauge needles resulted in more diagnostic samples when compared with 25-gauge needles. In the liver, the use of 25-gauge needles resulted in significantly less blood contamination when compared to 22-gauge needles ($P = 0.024$). The fact that no significant difference in blood contamination was identified in the spleen is not surprising due to its inherent high vascularity.[1]

This prospective study was conducted over a 3.5-year period. This is partly due to the strict inclusion criteria and the relatively high number of patients required based on our power calculations. However, department closures and the COVID pandemic also contributed to the delay. Only ultrasonographically unremarkable organs or organs with diffuse changes in echogenicity, echotexture, or size were included in the study. A nonaspiration technique is the preferred method in our department and was chosen for this research as it has been

TABLE 5 Grading of the cytological factors evaluated for the hepatic samples.

	Liver											
	22G				23G				25G			
	Grade 0%	Grade 1%	Grade 2%	Grade 3%	Grade 0%	Grade 1%	Grade 2%	Grade 3%	Grade 0%	Grade 1%	Grade 2%	Grade 3%
Cellularity	17.2%	46.6%	27.6%	8.6%	22.4%	41.4%	25.9%	10.3%	13.8%	41.4%	35.3%	9.5%
Blood contamination	6.9%	26.7%	46.6%	19.8%	7.8%	30.2%	50.9%	11.2%	6.9%	31.9%	57.8%	3.4%
Cellular preservation	15.5%	11.2%	47.4%	25.9%	21.6%	12.1%	41.4%	25%	12.1%	23.3%	37.9%	26.7%

reported superior in splenic and hepatic aspirates due to greater cellularity and less blood contamination.[9,10] We decided to exclude thrombocytopenic patients and patients with confirmed or suspected bleeding disorders as this would have probably increased blood contamination and potentially also interfered with the results. Although FNA of patients with severe bleeding disorders or taking antithrombotic/anticoagulant medications appear to still be safe in humans,[17,18] a greater risk of nondiagnostic samples has been identified in human patients under antithrombotic drugs.[19] To standardize the sampling, the procedure was performed with no syringe attached to the needle. There is a discrepancy among the members of the team in this regard, with some people preferring sampling with the syringe attached with and without a partially air-loaded plunger. Anecdotally, it is believed that FNA with the syringe attached provides a better grip. In our opinion, it is unlikely that sampling with a syringe attached without an air-loaded plunger would have changed the results. However, sampling with a partially air-loaded plunger could potentially change the pressures within the needle lumen and yield different results. To our knowledge, there are no human or veterinary studies comparing all these different techniques and further studies are necessary to ascertain if they are comparable.

Duplication of slides to reduce the volume of sample material on an individual slide was performed at the discretion of the operator making the slides. This technique may have artifactually reduced the severity of blood contamination or improved the diagnostic quality of samples. This is a technique routinely used in the clinic for sampling and was therefore not omitted during these cases.

Fewer diagnostic samples were achieved in liver aspirates (62.9%) compared with previous veterinary[20] (78%) and human[21–23] (85%—100%) studies. The diagnostic yield of splenic FNA in this study was 85.3% which is similar compared to humans (85%—90.9%)[5,24] and slightly less than previously published veterinary[25] (100%) literature. The cause of disparity between these results is unclear but could reflect the underlying hepatic and splenic structure/pathology.

When comparing results for interobserver agreement, only rare disagreements were found, whereas partial and complete agreements were variably distributed between the organs and different categories. Preservation seemed to be the most affected by subjective assessment with 44% partial agreement in the spleen and 48% partial agreement in the liver. This may in part be the result of different areas of the slide selected to assess for the preservation, focus of cells used to assess cellularity (e.g. organ-specific cells vs. blood cells), time attributed to each slide but also personal factors which vary between and within observers on a day-to-day basis. The intra- and interobserver variability for different areas of cytological examination has been evaluated in various studies in veterinary medicine ranging from to fair to good.[26,27]

There are several limitations to this study. The first limitation reflects the inter-operator variability. Multiple operators were involved in performing the FNA and preparing the smears. To mitigate this, a sampling protocol was created, and every member of the team was instructed on how to perform the FNA and prepare the slides, in an attempt to minimize variability. Additionally, this variability reflects the normal clinical setting, and the results of this study are therefore applicable for veterinarians taking FNA of these organs. The second limitation includes that only three needle sizes were investigated. Some veterinary and human papers additionally include 19-, 21-, and 27-gauge needles.[1,6,28,29] It is possible that selecting those sizes could have resulted in significant differences. In our department, we rarely use needles outside the 22- and 25-gauge range. In our opinion, taking additional samples with larger gauge needles would not be clinically justifiable. Finally, the lack of final histopathological diagnosis and variety of cytological diagnoses may have affected the results. Different underlying conditions could have increased or decreased the diagnostic yield of the sample.

In conclusion, our study did not reveal significant differences in diagnostic quality between 22-, 23-, and 25-gauge needles. Significant differences were however identified in blood contamination between the 22-gauge and 25-gauge needles when sampling the liver. Although no significant differences were found, the slight increase in diagnostic quality and cell preservation with 25-gauge needles in splenic aspirates and less blood contamination in hepatic aspirates suggests that this needle size may produce slightly better-quality samples.

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Category 1

(a) Conception and design: Llanos, Holmes, White, Jasensky, Fitzgerald
(b) Acquisition of data: Llanos, Holmes, White, Jasensky, Fitzgerald
(c) Analysis and interpretation of data: Llanos, Fitzgerald

Category 2

(a) Drafting the article: Llanos, Holmes, White, Jasensky, Fitzgerald
(b) Revising article for intellectual content: Llanos, Holmes, White, Jasensky, Fitzgerald

Category 3

(a) Final approval of the completed article: Llanos, Holmes, White, Jasensky, Fitzgerald

Category 4

(a) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: Cesar Llanos, Emma Holmes, Crystal White, Anne-Katherine Jasensky, Ella Fitzgerald

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PREVIOUS PRESENTATION OR PUBLICATION DISCLOSURE

No previous presentation or publication of this work.

REPORTING CHECKLIST DISCLOSURE

No reporting checklist was used for this study.

DATA AVAILABILITY STATEMENT

For consulting data supporting the results in this paper please contact the corresponding author.

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