Sensitivity of Tru-cut and fine-needle aspiration biopsies of liver and kidney for diagnosis of feline infectious peritonitis

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Background: The detection of typical lesions and feline coronavirus (FCoV) antigen in tissues is the only conclusive method for making a diagnosis of feline infectious peritonitis (FIP). A positive result using Tru-cut biopsy (TCB) and fine-needle aspiration biopsy (FNAB) has high diagnostic specificity, but information about the capacity of these techniques to correctly identify cats with FIP lesions is not available. **Objectives:** The diagnostic sensitivity of TCB and FNAB for detecting liver and kidney histologic lesions caused by FIP was evaluated. **Methods:** TCB and FNAB specimens collected mainly at necropsy from 25 cats with FIP were analyzed. Diagnostic sensitivity was calculated on the basis of the number of false-negative and true-positive specimens, compared with the number of organs bearing histologic lesions of FIP. **Results:** Diagnostic sensitivity was higher for hepatic TCB (64%) and FNAB (82%) than for renal (39% and 42%, respectively) procedures. A high percentage of renal cytologic and TCB specimens were inadequate. Combined analysis of TCB and FNAB specimens collected from the same organ increased the diagnostic sensitivity for liver (86%) and kidney (48%). The sensitivity of immunohistochemical/cytochemical analysis was low (11–38% depending on the technique), probably due to variable distribution of feline coronavirus in the lesions. **Conclusion:** Biopsy of liver and kidney can correctly identify FIP lesions. However, false-negative results or inadequate samples occur with moderate frequency, especially for immunochemical analysis. Diagnostic sensitivity may be increased when both TCB and FNAB specimens from the same organ are examined. (*Vet Clin Pathol*. 2005;34:368–374)

Key Words: Diagnostic sensitivity, feline infectious peritonitis, fine-needle aspiration biopsy, kidney, liver, Tru-cut biopsy

Feline infectious peritonitis (FIP) is a systemic, fatal, immune-mediated disease affecting domestic and wild felids. The etiologic agent is a highly pathogenic feline coronavirus (FCoV) strain derived from a mutation of the more common enteric FCoV of low pathogenicity.[1–3] The 2 FCoV strains cannot be distinguished by either serologic or molecular approaches, and, thus, diagnosis of FIP cannot be based solely on serologic or polymerase chain reaction (PCR) test results. History, clinical findings, and laboratory data (hematologic and biochemical analyses, serum protein electrophoresis, serologic testing, PCR analysis, and analysis of effusions) can provide a presumptive diagnosis of FIP; however, the only reliable methods for definitive diagnosis of FIP are histologic examination and immunohistochemical analysis of affected organs.[4–9] Unfortunately, the poor health status of cats affected with FIP limits the possibility of surgery and laparoscopic biopsy and, thus, is rarely used in routine diagnosis of the disease.

A possible solution for collecting histologic samples without severe health risks for affected cats would be the use of minimally invasive techniques such as Tru-cut biopsy (TCB) and fine-needle aspiration biopsy (FNAB) of liver and kidney, in which pathognomonic lesions of FIP usually are evident.[4] Several reports dealing with the diagnostic accuracy of these techniques are available in literature,[10–12] although the authors conclude that only rare cases of FIP have been studied. Based on these reports and on our knowledge of the distribution of FIP lesions in liver and kidney,[4,13] TCB and FNAB have high diagnostic specificity for FIP. To the authors' knowledge, studies of the utility of these techniques for collecting adequate specimens and avoiding false-negative results (diagnostic sensitivity) are not currently available. This information would be extremely important for clinicians in deciding whether to include TCB or FNAB in diagnostic protocols for FIP. Therefore, the aim of the study reported here was to evaluate the diagnostic sensitivity of these minimally invasive biopsy techniques (and of immunohistochemical or immunocytochemical analysis of acquired specimens) performed on liver and kidney containing lesions caused by FIP.

Materials and Methods

Animals and study design

The study included 25 client-owned cats (19 domestic shorthair, 3 Siamese, 1 Chartreux, 1 Persian, 1 Maine Coon), 13 females and 12 males, presented with a clinical suspicion of

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FIP, a differential diagnosis supported by the results of a CBC, serum protein electrophoresis, and serum α_1-acid glycoprotein (AGP) concentration and analysis of the effusion (when present).[9] Five cats were older than 1 year, and the remaining cats were younger than 1 year. Samples were obtained from 22 cats at necropsy, which was performed immediately after death to minimize postmortem artifacts. In contrast, 3 cats (nos. 13, 14, and 15) were biopsied while under general anesthesia (pretreatment with acepromazine [0.5 mg/kg IM], followed by anesthesia induced with diazepam [0.5 mg/kg IV] and ketamine [5 mg/kg IV] and maintained with halothane in oxygen) just prior to euthanasia.

Tru-cut biopsy

The TCB specimens were taken using a Tru-cut biopsy needle (16-gauge, 10-cm long for the liver or 18-gauge, 10-cm long for the kidney; Temno model, Gallini Medical Devices, Mantova, Italy). In live cats, ultrasound-guided percutaneous biopsy, using a 7.5–10-MHz mechanical sector transducer (Pandion 300S, Pie Medical, Maastricht, Holland), was performed. The TCB specimens were obtained using standard specimen collection sites and procedures, with cats positioned in lateral or dorsal recumbency.[10,11] Hepatic biopsy was performed by puncturing the left lateral or left medial liver lobe through the skin[12]; renal biopsy was performed by inserting the needle into the left lumbar region to tangentially access the caudal pole of the left kidney. In cats from which specimens were obtained at necropsy, the abdomen was opened to keep the organs to be biopsied firmly in place, although the needle was randomly inserted into the organs independent of the presence of macroscopic lesions. The stylet was advanced into the parenchyma, and a tissue core specimen (approximately 1-cm long and 0.2-cm thick) was collected by triggering the outer cannula, then retracting the needle. Two to 7 biopsy specimens were taken from each cat.

Fine-needle aspiration biopsy

The FNAB specimens were obtained before the TCB specimens were acquired from 22 cats, using a standard 10-mL syringe with a 21-gauge needle. The same sites indicated for TCB were sampled following the procedures suggested by Cowell et al.[14] Drops of the material collected were smeared on glass slides. For specimens from 16 cats, an aliquot of the material contained in the needle was placed in 200 μL of a phosphate buffer solution. After resuspension, this material was spun in a cytocentrifuge. Some smears and cytocentrifuged specimens were stained with May Grünwald-Giemsa stain (Merck, Darmstadt, Germany). The remaining smears and cytocentrifuged specimens were used for immunocytochemical analysis.

Necropsy and tissue processing

All 25 cats included in the study (including the 3 cats biopsied antemortem) underwent complete necropsy. Specimens (approximately 1 cm^3) for histologic examination were collected from each affected organ. Liver and kidney specimens were obtained even in the absence of gross lesions. Specimens collected during necropsy and TCB were fixed in 10% formalin and embedded in paraffin. Five-micron-thick sections were stained with H&E.

Immunochemical analysis

Immunohistochemical and cytochemical analyses were performed using the avidin-biotin complex (ABC) technique with a commercially available kit (Vectastain Elite, Vector Laboratories Inc, Burlingame, CA, USA). After deparaffination and rehydration of the biopsy specimens, endogenous peroxidases were inhibited by addition of H_2O_2 (1%) in 0.1% sodium azide for cytologic examination and in methanol for histologic examination. Antigen-unmasking was carried out only for histologic sections using a microwave pretreatment (2 cycles of 5 minutes in citrate-buffered solution, 0.01*M*, pH 6). Blocking serum (30 minutes at room temperature) was obtained from horses. The primary antibody (a monoclonal antibody against the FCoV was kindly provided by Dr NC Pedersen, Davis, CA, USA) was applied overnight at 4°C for histologic sections and for 1 hour at 37°C for cytologic specimens. After 3 washes in Tris-buffered solution (pH 7.6), biotinylated secondary anti-mouse antibody (30 minutes at room temperature) and the ABC complex (30 minutes at room temperature) were applied to the samples. Diaminobenzidine tetrahydrochloride (DAB, Histo-line Laboratories, Milano, Italy) was used as the chromogen for the reaction, following the manufacturer's instructions. After blocking the reaction by washing in running tap water, the slides were counterstained with Mayer's hematoxylin and coverslipped. Negative controls consisted of histologic and cytologic specimens incubated with normal mouse serum (DAKO A/S, Glostrup, Denmark).

Diagnostic criteria

The FNAB, TCB, and histologic specimens collected at necropsy were submitted for "blind" microscopic examination by the authors (TCB were examined by SP, EM, MP and FNAB by AG, WB). Liver and kidney specimens collected by TCB or at necropsy were considered diagnostic for FIP when typical lesions were observed. In particular, fibrinous perihepatitis, intraparenchymal pyogranulomatous foci, and perivascular lymphoplasmacytic infiltrates in liver specimens were considered diagnostic for FIP. Pyogranulomatous cortical foci with central necrosis in kidney specimens also were considered diagnostic for FIP.[15]

The TCB specimens were classified as follows: (1) nondiagnostic, specimens that were too small[12] or composed only of tissues other than liver or kidney (eg, muscle or fat); (2) consistent with FIP, specimens containing typical FIP lesions, as described previously; (3) dubious, specimens showing only lesions consistent with, but not specific for FIP (eg, lymphohistiocytic perivasculitis in liver, peritubular mononuclear cell infiltrates in kidney); and (4) not consistent with FIP, specimens representative of the collected tissue, but without

Table 1. Results of macroscopic and histologic analyses of liver and kidney and of corresponding Tru-cut biopsy (TCB) and fine-needle aspiration biopsy (FNAB) specimens (immunochemical results for feline coronovirus antigen in parentheses).*

Cat No.	Liver					Kidney				
	Gross	Histology	TCB	FNAB-S	FNAB-C	Gross	Histology	TCB	FNAB-S	FNAB-C
1	NC	fp (–)	fp (–)	ND	ND	NC	NC	ND	NC (–)	NC (–)
2	NC	fp, pg (–)	fp (–)	ND	ND	NC	NC	NC (–)	NC (–)	NC (–)
3	NC	pg (–)	D (–)	C (–)	C (–)	C	pg (+)	D (–)	C (–)	C (–)
4	NC	pg (–)	D (–)	C (–)	C (–)	C	pg (–)	pg (–)	C (–)	ND
5	C	fp, pg (+)	fp, pg (–)	ND	ND	NC	NC	ND	ND	ND
6	C	fp, pg (+)	NC (–)	C (–)	C (–)	NC	NC	ND	NC (–)	ND
7	C	fp (+)	pg (–)	C (–)	ND	NC	NC	NC (–)	NC (–)	ND
8	NC	pg (+)	D (–)	ND	ND	NC	pg (+)	pg (+)	C (–)	ND
9	C	pg (+)	fp, pg (+)	C (–)	ND	C	pg (–)	NC (–)	C (+)	C (+)
10	C	fp (–)	D (–)	C (+)	C (+)	NC	NC	NC (–)	ND	ND
11	C	fp (+)	fp (+)	NC (–)	ND	NC	NC	D (–)	ND	ND
12	NC	fp, pg (+)	pg (–)	ND	ND	C	pg (–)	pg (–)	C (–)	NC (–)
13	NC	fp (–)	fp (–)	C (–)	C (–)	NC	NC	ND	NC (–)	ND
14	NC	fp (–)	D (–)	NA	NA	C	pg (–)	NC (–)	NA	NA
15	NC	fp (+)	pg (+)	C (+)	ND	NC	NC	NC (–)	NC (–)	ND
16	C	fp (–)	pg (–)	C (–)	C (–)	C	pg (–)	ND	C (–)	ND
17	C	fp, pg (+)	pg (–)	C (+)	ND	NC	pg (–)	pg (–)	C (–)	ND
18	NC	pg (–)	pg (–)	NA	NA	C	pg (–)	ND	NA	NA
19	NC	pg (+)	pg (+)	NA	NA	C	pg (+)	ND	NA	NA
20	C	fp, pg (+)	pg (+)	C (+)	NA	C	pg (+)	pg (+)	C (+)	NA
21	C	pg (–)	NC (–)	NC (–)	NA	C	pg (+)	pg (+)	NC (–)	NA
22	C	fp (–)	NC (–)	C (NA)	NA	NC	NC	NC (–)	NC (–)	NA
23	C	fp (+)	D (+)	NC (–)	NA	NC	NC	NC (–)	NC (–)	NA
24	C	fp (+)	pg (–)	C (–)	NA	NC	NC	NC (–)	NC (–)	NA
25	C	fp (+)	pg (–)	C (+)	NA	NC	pg (+)	pg (–)	NC (–)	NA

*FNAB-S indicates FNAB smears; FNAC-C, FNAB cytocentrifuged specimen; C, consistent with FIP; NC, not consistent with FIP; D, dubious; ND, nondiagnostic; fp, fibrinous perihepatitis; pg, pyogranulomatous (lesion); NA, not available.

lesions consistent with FIP. In the case of discrepancies between the results obtained from repeated biopsies on the same animal, biopsy specimens were classified on the basis of the most frequent finding.

The FNAB specimens were classified as: (1) nondiagnostic, specimens with poor cellularity, or with excessive blood contamination or large numbers of ''naked nuclei'' and fragmented cells or both; (2) consistent with FIP, highly cellular samples containing the normal cell population of the sampled organs (hepatocytes, renal tubular epithelial cells), but also neutrophils, macrophages, plasma cells, and lymphocytes, supporting a diagnosis of pyogranulomatous inflammation[16,17]; and (3) not consistent with FIP, highly cellular samples containing only the normal cell population of the sampled organs, without any sign of inflammation.

Statistical analysis

Because the aim of this study was to evaluate the ability of TCB and FNAB to detect FIP lesions in liver and kidney, the numbers of true-positive (TP) and false-negative (FN) TCB or FNAB results were calculated for specimens collected from organs at necropsy that had histologic FIP lesions. Inadequate specimens were not included in this calculation. Diagnostic sensitivity was then calculated as follows: sensitivity = TP/(TP+FN).[18]

Results

Necropsy and routine histologic/immunohistochemical analysis

All cats included in the study were affected with FIP. Specifically, 22 of the 25 cats had effusive FIP, whereas cats 18, 21, and 25 had the noneffusive form. Gross findings were consistent with a diagnosis of FIP, and microscopic lesions and/or viral antigen were detectable in at least 1 affected tissue.

Gross hepatic lesions were detected in 14 cats (Table 1). Nonetheless, all cats had histologically detectable lesions, including fibrinous hepatitis (n = 12), intraparenchymatous pyogranulomatous foci (n = 7), or mixed perihepatitis and

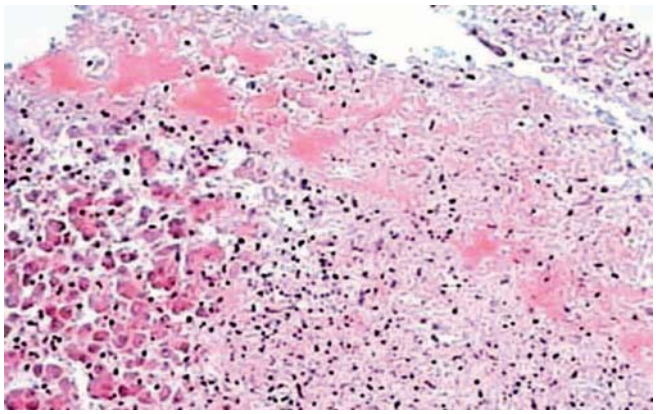

Figure 1. Histologic section of a Tru-cut biopsy specimen from the liver. Note the fibrinous perihepatitis within a subserosal pyogranulomatous lesion. H&E, ×25 objective.

pyogranulomatous lesions (n = 6). FCoV antigen was detected by immunohistochemical analysis in 14 specimens, mainly those with pyogranulomatous foci, whereas specimens with poorly cellular fibrinous perihepatitis were negative for FCoV antigen in 6 of 12 cats. Gross renal lesions were found in 10 cats, and FIP lesions were observed in histologic specimens from 13 cats, 6 of which also were FCoV-positive by immunohistochemical analysis.

Liver biopsy

All of the TCB specimens contained sufficient tissue to be considered diagnostic. Histologic findings in most specimens (16/25) were consistent with FIP, based on intraparenchymatous pyogranulomatous lesions (n = 10), fibrinous perihepatitis (n = 4), or both (n = 2, Figure 1, Table 1). Results of the remaining 9 TCBs were dubious (n = 6), or the specimen did not have any lesions (n = 3). All TCB specimens obtained from the same cat had similar results, with rare exceptions. FCoV antigen was detected in TCB specimens from 6 cats.

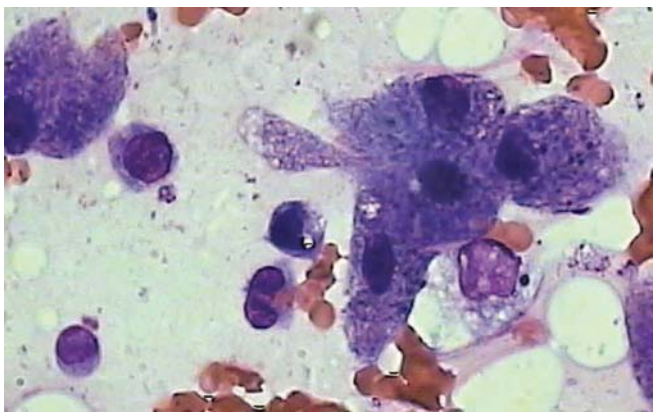

Figure 2. Fine-needle aspiration biopsy specimen from the liver. Notice the mixed inflammatory cell infiltrate, with lymphocytes, a neutrophil, and a macrophage. May-Grünwald-Giemsa, ×100 objective.

Table 2. Summary of results obtained for Tru-cut biopsy (TCB) and fine-needle aspiration biopsy (FNAB) specimens from liver in which FIP lesions were detected in routine histologic examination.*

	TCB	FNAB-S	FNAB-C	TCB or FNAB
Histology/cytology	(n = 25)	(n = 22)	(n = 16)	(n = 22)
Nondiagnostic	0	5	10	0
False negative (FN, not consistent with FIP or dubious)	9	3	0	3
True positive (TP, consistent with FIP)	16	14	6	19
Diagnostic sensitivity [TP/(FN+TP)]	0.64	0.82	1.0	0.86
Immunochemistry	(n = 25)	(n = 16)	(n = 6)	(n = 21)
IHC or ICC negative (FN)	19	11	5	13
IHC or ICC positive (TP)	6	5	1	8
Diagnostic sensitivity [TP/(FN+TP)]	0.24	0.31	0.17	0.38

*FNAB-S indicates FNAB smears; FNAB-C, FNAB cytocentrifuged specimens; IHC, immunohistochemistry; ICC, immunocytochemistry.

Of the 22 FNAB specimens, cytologic findings were nondiagnostic (n = 5), not consistent with FIP (n = 3), or consistent with FIP (n = 14) (Figure 2, Table 1). Most of the latter were taken from a liver for which routine histologic examination revealed highly cellular pyogranulomatous lesions. FCoV antigen was detected in 5 FNAB specimens. Most of the cytocentrifuged FNAB specimens (10/16) were nondiagnostic, mainly because of the presence of artifacts. Results for the remaining 6 specimens were consistent with FIP, but only in 1 specimen were FCoV-positive cells found.

In 5 cats, cytologic findings in FNAB specimens were consistent with FIP, but histologic findings in TCB specimens were either not consistent or dubious. As a consequence, only 3 of the 22 cats from which TCB and FNAB specimens were available had results that were not consistent with FIP for both specimens.

Diagnostic sensitivity was 64% for TCB, but increased to 86% when TCB and FNAB specimens from the same cat were considered together (Table 2). Conversely, smeared and cytocentrifuged FNAB specimens had high diagnostic sensitivity (82% and 100%, respectively). The diagnostic sensitivity of immunochemical analysis ranged from 17% (cytocentrifuged FNAB specimens) to 38% (simultaneous analysis of TCB and FNAB specimens) and was generally lower than the sensitivity of the corresponding FNAB or TCB specimen.

Kidney biopsy

Histologic findings in the 25 renal TCB specimens were nondiagnostic (n = 7), consistent with FIP (n = 7), or classified as false negative because the results were dubious (n = 2) or because the specimen did not contain any lesions (n = 9) (Table 1). The TCB specimens from the same cat often

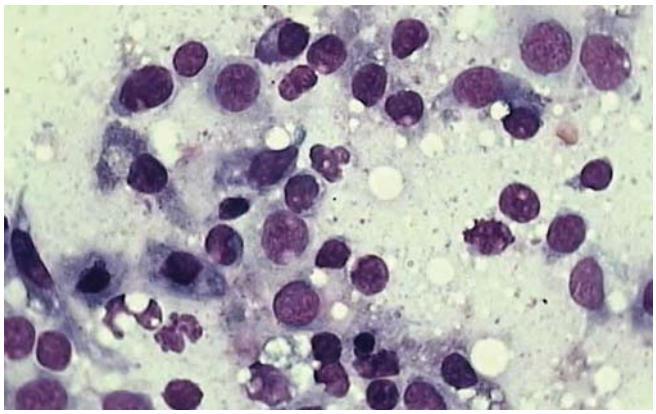

Figure 3. Fine-needle aspiration biopsy specimen from the kidney. Despite some postmortem artifacts, a mixed inflammatory cell population composed of plasma cells, neutrophils, rare lymphocytes, and a macrophage is seen. May-Grünwald-Giemsa, ×40 objective.

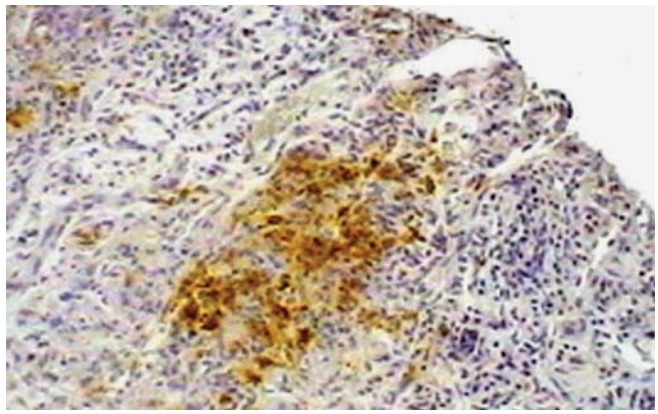

Figure 4. Histologic section of a Tru-cut biopsy specimen from the kidney. Feline coronavirus (FCoV) antigen is detected within a pyogranulomatous lesion. Anti-FCoV immunohistochemistry, avidin-biotin complex technique developed with diaminobenzidine, hematoxylin counterstain, ×25 objective.

provided a different result and were classified according to the most frequent finding.

Results of smears for 3 of the 22 FNAB specimens were nondiagnostic (Table 1). Cytologic findings in the remaining 19 FNAB specimens were not consistent with FIP (n = 11) or consistent with FIP (n = 8) (Figure 3). Results for most of the cytocentrifuged FNAB specimens (11/16) were nondiagnostic. A cell population consistent with FIP was detected in 2 of the remaining 5 smears from cytocentrifuged FNAB specimens. FCoV antigen was detected in 3 renal TCB specimens (Figure 4), 2 FNAB smears, and 1 cytocentrifuged FNAB specimen.

Renal TCB and FNAB specimens occasionally provided different results. In particular, 10 of the 22 cats from which TCB and FNAB specimens were available had results that were consistent for FIP in at least 1 biopsy specimen, and FCoV antigens were detected in 3 of these cats. In only 1 cat were results of FNAB and TCB specimens nondiagnostic.

Diagnostic sensitivity was similar for TCB specimens (39%), smears from FNAB specimens (42%), and cytocentrifuged FNAB specimens (40%), and was slightly higher (48%) for specimens from combined FNAB and TCB (Table 3). Similar to the liver, the diagnostic sensitivity of immunochemical analysis of the kidney was low, ranging from 11% (smears of FNAB specimens) to 20% (cytocentrifuged FNAB specimens).

Discussion

The detection of typical lesions and FCoV antigens in tissue is the only conclusive method for diagnosing FIP.[4,9] Histologic and cytologic examinations and immunocytochemical or histochemical analysis also can be performed on TCB and FNAB specimens, especially those from cats that will not likely survive the anesthesia required for collecting specimens via laparotomy. Due to high specificity,[7] the presence of FIP lesions or FCoV antigens will confirm a clinical suspicion of FIP, but the lack of lesions or FCoV antigens in biopsy specimens is difficult to interpret. Generally speaking, the possibility of accessing a lesion by using Tru-cut or syringe needles depends not only on technical factors (eg, size of the needle and skill of the operator), but also on the tissue distribution and the cellular composition of inflammatory foci. The multifocal nature and the inflammatory composition of FIP lesions[4,13] decrease the possibility of accessing the lesions and detecting specific patterns in biopsy specimens.

From a clinician's perspective, it would be valuable to know which organ must be sampled to have the highest probability of detecting FIP lesions. Theoretically, any organ that has obvious changes, such as an abdominal mass,[13] is the best sampling site. However, liver and kidney frequently are affected with FIP,[4,13] and techniques to biopsy them already have been standardized.[10–12] Our necropsy results confirmed

Table 3. Summary of results obtained for Tru-cut biopsy (TCB) or fine-needle aspiration biopsy (FNAB) specimens from kidney in which FIP lesions were detected in routine histologic examination.*

	TCB	FNAB-S	FNAB-C	TCB or FNAB
Histology/cytology	(n = 25)	(n = 22)	(n = 16)	(n = 22)
Nondiagnostic	7	3	11	1
False negative (FN, not consistent with FIP or dubious)	11	11	3	11
True positive (TP, consistent with FIP)	7	8	2	10
Diagnostic sensitivity [TP/(FN+TP)]	0.39	0.42	0.40	0.48
Immunochemistry	(n = 18)	(n = 19)	(n = 5)	(n = 21)
IHC or ICC negative (FN)	15	17	4	18
IHC or ICC positive (TP)	3	2	1	3
Diagnostic sensitivity [TP/(FN+TP)]	0.17	0.11	0.20	0.14

*FNAB-S indicates FNAB smears; FNAB-C, FNAB cytocentrifuged specimens; IHC, immunohistochemistry; ICC, immuncytochemistry.

the frequent presence of histologic FIP lesions in these organs, even in the absence of macroscopic changes.[15] Based on our sampling results, liver was affected more frequently than kidney, probably due to the high number of cats with the effusive form of FIP that were included in the study. A higher rate of renal lesions might have been found by examining more cats with the noneffusive form of FIP, in which the kidney is the main organ affected.[13] Both organs are good biopsy sites for sampling, and clinical biochemical changes or diagnostic imaging might drive the clinician's decision about which organ should preferentially be sampled.

A high percentage (about 25%) of inadequate renal TCB specimens was found, whereas hepatic specimens always contained a sufficient amount of tissue for interpretation. It is well known that FIP mainly affects young cats,[13] which have smaller kidneys; this would help to explain the high number of nondiagnostic TCB specimens. This number was similar to that reported in previous studies of the diagnostic quality of percutaneous renal biopsy specimens in dogs and might be a consequence of the small size of the needle, which, however, is required to minimize the risk of bleeding.[19] Moreover, multiple TCBs of the same kidney often provide different results. Thus, it would be advisable to perform multiple renal biopsies, although this again would increase the risk of severe bleeding in living animals; this risk can be reduced to about 25% by obtaining complete coagulation profiles[20] and performing the biopsy carefully.[19]

In contrast with biopsy specimens, the percentage of nondiagnostic FNAB specimens was similar for liver (22.7%) and kidney (13.6%). Some of the material collected by FNAB was cytocentrifuged in an attempt to concentrate cells and facilitate a diagnosis. However, this procedure damaged many cells and most of the cytocentrifuged FNAB specimens were nondiagnostic, suggesting that this procedure is not applicable for routine use in making a diagnosis. These artifacts might result from the fact that most of the specimens were collected at necropsy and that cytocentrifugation of FNAB specimens from solid organs is particularly stressful for the cells. Nevertheless, we preferred to standardize the techniques on samples obtained at postmortem before applying them to living animals.

When only biopsy specimens collected from organs that had FIP lesions were considered, variable proportions of FN (specimens without cells or lesions consistent with FIP) and TP (specimens with cells or lesions consistent with FIP) results were obtained for liver and kidney. The analysis of diagnostic sensitivity, calculated as the percentage of TP biopsy results for the total number of adequate biopsy specimens collected from organs bearing FIP lesions, indicated that in liver and kidney the sensitivity was higher for cytologic specimens than for TCB specimens; nevertheless, it should be remembered that most of the cytologic specimens were inadequate. Despite the high sensitivity of adequate specimens, the diagnostic value of cytologic examination alone is limited by the high number of inadequate specimens. Cytocentrifuged FNAB specimens of liver, for example, had very high sensitivity (100%), but most of the specimens were inadequate, and ultimately, only about a third of the cats with hepatic FIP lesions were correctly identified by examination of FNAB.

In some instances, FNAB supported the diagnosis of FIP better than did the corresponding TCB, and vice versa. Thus, it is advisable to use these 2 techniques in combination in routine practice. In clinical practice, FNAB is more frequently performed than is TCB, mostly due to the high risk of bleeding associated with the latter when performed in live animals. For our specimens, however, the combined analysis of both types of samples increased the diagnostic sensitivity to 86%. Interestingly, results of renal TCB were consistent with FIP in the 3 cases with the noneffusive form, suggesting that this technique can be helpful in diagnosing the dry form of FIP, which is difficult to diagnose conclusively based on other clinicopathologic changes. Analysis of TCB results for a larger number of cases with the noneffusive form of FIP would be required to confirm this supposition. Moreover, although we considered the TCB results that were dubious as ''false-negatives'', an uncertain result might still support pertinent clinical, hematologic, or electrophoretic findings consistent with FIP.[6,9,13]

Compared with the corresponding histologic and cytologic results, the diagnostic sensitivity of immunochemical staining always was very low. The analysis of specimens collected during necropsy allowed us to confirm what has been reported in the literature about the variable distribution of viral antigens within FIP lesions. The amount of viral antigen is not correlated to the size of the foci, specifically in renal lesions, which often have large, poorly cellular areas of necrosis, and viral antigen often is not uniformly distributed throughout the lesion in liver or kidney.[21–23] Thus, it is unlikely that FIP-positive cells can be detected by biopsy. In parallel tests in which TCB specimens were fixed for various lengths of time, specimens kept in formalin for 48 hours still stained positively (data not shown), thus confirming that the negative samples were due to variable distribution of viral antigen, rather than to discrepancies in specimen processing. The low diagnostic sensitivity and high cost make immunochemical techniques inadvisable for routine use.

In conclusion, the results of this study provide evidence that the liver and kidneys from cats with both forms of FIP are good sampling sites for diagnostic biopsies, even in the absence of gross changes. Nevertheless, FN results can be obtained, especially from TCB of the kidney, even in the case of multiple samplings from the same cat. The probability of FN results decreases when TCB and FNAB specimens from the same cat are examined, but increases when FNAB specimens are cytocentrifuged. In contrast, the diagnostic sensitivity of immunchemical testing was always very low, likely due to the variable distribution of viral antigens within FIP lesions.

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