A randomised controlled comparison of aspiration and non-aspiration fine-needle techniques for obtaining ultrasound-guided cytological samples from canine livers

K.L. Fleming[a,*], E.J. Howells[a,b], E.J. Villiers[c], T.W. Maddox[a]

[a] School of Veterinary Science, University of Liverpool, Leahurst Campus, Chester High Road, Neston, CH64 7TE, UK
[b] Bilton Veterinary Centre, 259 Bilton Road, Rugby, CV22 7EQ, UK
[c] Dick White Referrals, Station Farm, London Road, Six Mile Bottom, CB8 0UH, UK

ARTICLE INFO

ABSTRACT

Ultrasound-guided fine-needle sampling to obtain cytological samples is a well-established technique. However, the application of suction during sampling is controversial. Evidence from the human literature and one previous veterinary study suggest that non-aspiration may be superior for a number of organs. This prospective study compared the quality and diagnostic value of cytological samples from canine livers obtained by fine-needle aspiration (FNA) and non-aspiration (FN-NA) techniques. A total of 119 dogs that required ultrasound-guided FNA of the liver as part of their clinical investigation were recruited and randomly assigned to either FNA ($n = 54$) or FN-NA ($n = 65$) sampling groups. Specimens were reviewed by external cytopathologists masked to the technique used. Cytological reports were reviewed for their overall diagnostic value, cellularity, cell preservation and haemodilution.

Overall, 88.2% (95% confidence intervals [CI], 82.4–94.0) of samples were diagnostic. There was a significant difference, as demonstrated by Chi-squared statistical analysis, in the prevalence of diagnostic samples between the FNA (81.5%; 95% CI, 71.1–91.8) and FN-NA groups (93.9%; 95% CI, 88.0–99.7; $P = 0.037$). Non-diagnostic samples were significantly associated with lower cellularity, poorer cell preservation and more severe haemodilution ($P < 0.001$ for each). However, there were no significant differences in the frequency of these specific variables between the FNA and FN-NA groups. In this study, fine-needle non-aspiration was superior to an aspiration technique for sampling the canine liver, as it resulted in higher rates of diagnostic cytology samples, with greater cellularity, less haemodilution and better cytological preservation.

Crown Copyright © 2019 Published by Elsevier Ltd. All rights reserved.

Introduction

Cytological samples of the liver are commonly obtained in small animal veterinary medicine and are an important step in the diagnostic investigation of dogs with hepatic disease (Weiss et al., 2001; Rothuizen and Twedt, 2009). Liver cytology may be used in the investigation of elevated hepatic enzymes, ultrasonographic abnormalities such as diffuse hepatomegaly, altered echogenicity and masses, or during neoplastic disease staging (Kerwin, 1995; Sharkey et al., 2014). Sampling can obtain a definitive diagnosis or narrow the differential diagnoses list and may help guide further testing (Roth, 2001; Rothuizen and Twedt, 2009; Bahr et al., 2013). However, limitations are recognised: primarily cytology is unsuitable when

assessment of liver architecture is required, since cells are obtained without histological context (Roth, 2001; Bahr et al., 2013). Cytology for diagnosis of liver lesions has been shown to have lower accuracy than lesions in other organs (Cohen et al., 2003). In one study of 97 cases, cytological findings agreed with the histopathological diagnosis in only 30% of dogs and 51% of cats (Wang et al., 2004). Cytology has been shown to be most effective in the diagnosis of diffuse vacuolar change or neoplastic disease (Roth, 2001; Wang et al., 2004; Bahr et al., 2013), with variable sensitivity demonstrated for inflammatory liver disease (Weiss et al., 2001; Wang et al., 2004; Bahr et al., 2013).

Fine-needle sampling is a valuable technique as is relatively non-invasive, avoids the need for general anaesthesia, is inexpensive and has a low complication rate. Two main techniques have been described to obtain cytological samples of the canine liver, either by fine-needle aspiration (FNA) or by a fine-needle non-aspiration technique (FN-NA) also known as a cutting or capillary

* Corresponding author.
  E-mail address: kathryn.fleming@liverpool.ac.uk (K.L. Fleming).

technique (Weiss and Moritz, 2002; Rothuizen and Twedt, 2009). FNA is typically performed with a 20–23 G hypodermic needle (Kerwin, 1995; Burkhard and Meyer, 1996; Weiss and Moritz, 2002; Rothuizen and Twedt, 2009), attached to an empty or air-filled 5–12 mL syringe (Guillot et al., 2009; Rothuizen and Twedt, 2009). With FNA the needle is inserted into the liver and a few millilitres of suction is applied creating a slight vacuum, this suction is maintained as the needle is rapidly passed through the parenchyma several times to different depths and angles (Burkhard and Meyer, 1996; Weiss and Moritz, 2002). With the FN-NA technique the hypodermic needle (with or without an attached syringe) redirected through the parenchyma has a cutting action, dislodging cells and driving them into the needle shaft where they are maintained by capillary tension (Santos and Leiman, 1988; Menard and Papageorges, 1995).

Ultrasound-guided sampling facilitates real-time visualisation of the needle tip permitting sampling of focal or diffuse lesions (Kerwin, 1995; Menard and Papageorges, 1995). Adjacent structures can be avoided, reducing the risk of complications (Kerwin, 1995), thus improving the success and safety of the procedure (Hager et al., 1985). Complications, including haemorrhage, following FNA are rare and typically minor if they occur (Léveillé et al., 1993; Rothuizen and Twedt, 2009); coagulation testing is not routinely indicated (Rothuizen and Twedt, 2009). However, animals with liver disease may have consequential coagulopathies, increasing the risk of haemorrhage.

In the human literature, multiple studies have evaluated FNA vs. FN-NA sampling techniques in various tissues (Dey and Ray, 1993; Ali Rizvi et al., 2005; Sajeev and Siddaraju, 2009; Misra et al., 2015). Few specifically focus on the liver (Fagelman and Chess, 1990; Jahromi et al., 2015). While differences in these studies exist, all have demonstrated that FN-NA yields samples of superior quality or confers other advantages. There is a lack of veterinary evidence regarding which technique provides optimal sample quality. A study comparing the two techniques for splenic sampling concluded FN-NA was superior to obtain high-quality cytological specimens from the canine and feline spleen (LeBlanc et al., 2009). An advantage of FN-NA when sampling highly vascular tissue such as the spleen, is reduced blood contamination (LeBlanc et al., 2009). Optimal diagnostic quality samples obtained using fine-needle sampling techniques should contain a high cellularity representative of the lesion/pathology, good cell preservation and only mild haemodilution.

Our aim was to compare cytological samples of the canine liver, obtained by FNA and FN-NA, to determine if one technique was superior. The primary aim was to ascertain the diagnostic yield of the two techniques, defined as the likelihood the sample would provide the information needed to establish a diagnosis. Secondary aims included assessment of cellularity, cell preservation and haemodilution with each technique. The null hypothesis was there would be no statistical difference in the proportion of non-diagnostic samples obtained using FNA vs. a FN-NA technique.

Materials and methods

The study was granted ethical approval by the Veterinary Research Ethics Committee, University of Liverpool (Approval number: VREC174; Approval date: 20 December 2013) Dogs were prospectively recruited from the population at the Small Animal Teaching Hospital (SATH), University of Liverpool between January and July 2014. Dogs requiring ultrasound guided fine-needle sampling of the liver as part of their clinical diagnostic work-up were included. Individuals were randomly assigned to groups for sampling by either FNA or FN-NA technique, by flipping a coin at the time of sampling. Exclusion criteria were clinical contraindications to sampling, including known coagulopathy or marked thrombocytopenia (platelet count $<50 \times 10^9$/L), or if it was decided that random selection of a sampling technique was inappropriate for ethical reasons. Signalment data including breed, age, sex and weight was recorded for each dog sampled.

Fine-needle samples were obtained by a senior radiologist or diagnostic imaging resident supervised by the former, using a 22 G 40 mm needle, redirected a minimum of 3–4 times. Either FNA using a 5 mL syringe and up to 3 mL of negative pressure, or FN-NA using a needle alone and capillary action was employed, the later only attaching the syringe after sampling. With both techniques the obtained sample was expressed onto glass slides using a partially air-filled 5 mL syringe, then smeared with another slide before air drying.

Prepared slides were sent to an external cytopathology service (Dick White Referrals Diagnostics) and evaluated by one of the authors (EJV, DipECVCP; 53% samples) or a second cytopathologist (DipACVP, Clinical Pathology; remaining 47% samples). Both were masked to sampling technique and unaware their findings were to be appraised in this study. Normal laboratory guidelines were used for sample evaluation. Cellularity was graded as low (occasional nucleated cells), moderate (nucleated cells in small groups or individually with wide spaces between them, with or without blood contamination) or high (large clusters or sheets of cells covering at least 20% of the smear area). Cell preservation was graded as poor when a high proportion of the cells were disrupted; moderate when some cells were disrupted but most were intact, and good when the vast majority of the cells were well preserved. Haemodilution was categorised as mild when a few red blood cells were observed, severe when the smear had a blood-like appearance, or moderate when many fields of view contained predominantly nucleated cells with smaller numbers of red blood cells. Cytological reports were reviewed by one author (TWM) masked to collection technique. Samples without specific comments pertaining to cellularity, cell preservation and haemodilution were excluded. Cytological diagnosis was recorded for each sample, based upon the cytology report alone. If no conclusion was reached due to sample quality and/or if repeat sampling was advised the cytological diagnosis was recorded as 'non-diagnostic'. Only once all information was collected were cases matched with the sampling technique used.

All dogs were monitored for post-sampling complications. Ultrasonographic assessment for the development of peritoneal fluid suggesting haemorrhage, was performed immediately after sampling and at the end of the ultrasound examination. Dogs were monitored during the remainder of their hospital stay for any clinical deterioration (e.g. development of tachycardia, hypotension, altered capillary refill time or mucous membrane pallor) suggestive of significant internal haemorrhage. If any suspicion of complications led to repeat abdominal ultrasonographic assessment this was recorded. Following discharge, hospital records were retrospectively reviewed for evidence of complications.

All statistical analyses were performed using dedicated statistical software (SPSS 20.0, SPSS). Independent variables were derived from the signalment data, cytological reports and follow-up. Descriptive statistics were generated for all variables; continuous data were summarised as median values with interquartile ranges (IQR), and categorical data were amalgamated into appropriate groups if required (due to small group sizes) and expressed as frequencies with 95% confidence intervals (CI). Normality of distribution for continuous variables was also assessed via the Kolmogorov–Smirnov test. The primary outcome considered was whether samples were diagnostic or non-diagnostic. Chi-squared statistical analysis was performed to determine if there was a significant difference in the prevalence of diagnostic samples between the two groups. The secondary outcomes considered were cellularity, cell preservation and haemodilution; each categorised into one of three groups based on the cytological report. The independent variables examined were breed, sex, age, weight, diagnosis and sampling technique; association was also assessed using Chi-squared analysis for categorical variables and the Mann–Whitney U test for continuous variables. $P < 0.05$ was considered significant.

A previous clinical audit at the SATH showed that approximately 80% of liver FNA's conducted at the hospital were considered diagnostic (unpublished data). Assuming a difference of approximately 20% in the proportion of cytological reports considered diagnostic between the two groups would be relevant, a sample size of approximately 54 animals per group was required with a significance level of 0.05 and study power of 0.8. This study therefore aimed to recruit a minimum of 110 dogs in total.

Results

A total of 120 dogs were recruited, one was excluded since the cytology report did not specifically comment on all cytological features under evaluation. Of the remaining 119, 65 (55%) were sampled by FN-NA and 54 (45%) by FNA. No clinical complications occurred in any dogs sampled. The median age of the study population was 9.9 years (IQR 7.2–11.3 years), the youngest dog was 1 year 5 months and the eldest 14 years 6 months. The median age in the FN-NA group was 10.1 years (IQR 7.7–11.2 years) and in the FNA group was 9 years (IQR 7.0–11.5 years). The most commonly represented breeds were: cross breeds ($n = 20$, 17%), Labrador retriever ($n = 18$, 15%), English springer spaniel ($n = 6$, 5%), German shepherd dog ($n = 6$, 5%), miniature schnauzer ($n = 6$, 5%), West Highland white terrier ($n = 6$, 5%), Border collie ($n = 5$, 4%)

Golden retriever ($n = 4$, 3%), Staffordshire bull terrier ($n = 4$, 3%) and Cocker spaniel ($n=4$, 3%); other breeds comprised the remainder ($n=39$; 33%). Overall, sex and neuter status comprised: eight entire females, 59 neutered females, 13 entire males and 39 neutered males. There was no statistical difference between the two sampling groups in age ($P = 0.536$), weight ($P = 0.184$), breed ($P = 0.734$) or sex ($P = 0.927$).

Overall, 88.2% of the samples were diagnostic (95% CI 82.4–94.0). Samples obtained using the FN-NA technique (61/65; 93.9%; 95% CI 88.0–99.7) were more likely to be diagnostic ($P = 0.037$) than samples obtained using the FNA technique (44/54; 81.5%; 95% CI 71.1–91.8). The cytological diagnoses were classified into 13 different groups, shown in Table 1, the most common of which were normal/no significant findings (24.3%), vacuolar hepatopathy (17.6%), or hepatitis (13.4%).

For the secondary outcomes: cellularity was high, moderate or low in 45.4% (54/119), 37.8% (45/119) and 16.8% (20/119) of samples, respectively; cellular preservation was good, moderate or poor in 79.8% (95/119), 16.8% (20/119) and 3.4% (4/119) of samples, respectively; haemodilution was mild, moderate or severe in 30.3% (36/119), 43.7% (52/119) and 26.0% (31/119) of samples, respectively. Results for each feature, categorised by sampling technique, are provided in Table 2 (and Fig. 1). No significant difference in cellularity ($P = 0.15$), cell preservation ($P = 0.62$) or haemodilution ($P = 0.70$), was found between samples from the FN-NA and FNA groups. Chi-squared analysis showed non-diagnostic samples, in both groups, were significantly associated with lower cellularity, poorer cell preservation and more severe haemodilution ($P < 0.001$ for all).

Discussion

In this study, the overall proportion of diagnostic liver samples achieved was 88.2%. Contrary to our null hypothesis we demonstrated a significant difference in the prevalence of diagnostic samples between the FN-NA group (93.9%) and the FNA group (81.5%; $P = 0.037$).

The use of a non-aspiration sampling technique was first described over 30 years ago (Briffod et al., 1982). Since then many studies comparing FN-NA and FNA techniques, in various tissues, have been published in the human literature (Fagelman and Chess, 1990; Dey and Ray, 1993; Ali Rizvi et al., 2005; Jahromi et al., 2015). The use of FN-NA to acquire ultrasound-guided fine-needle tissue samples was first reported in veterinary patients in 1995 (Menard and Papageorges, 1995), with authors advocating FN-NA to obtain samples with relatively improved cellularity because of reduced haemodilution. A comparison of FNA and FN-NA techniques for sampling the canine and feline spleen concluded that FN-NA was a superior method to obtain high-quality samples (LeBlanc et al., 2009). In that study, splenic samples obtained by FN-NA had significantly higher cellularity, less blood, and similar cell morphology compared with samples obtained by FNA.

The liver, like the spleen, is also highly vascular and as such inherently bloody samples are likely (Sharkey et al., 2007). Two studies specifically evaluated liver sampling using FN-NA and FNA have been described in the human and the pig. One, conducted using porcine livers, showed the technique equivalent to our FN-NA technique provided the greatest cellularity (Jahromi et al., 2015). Another concluded that FN-NA of the human liver was equivalently good to FNA, but that FN-NA smears were easier to interpret (Fagelman and Chess, 1990). In our study, despite showing improved likelihood of obtaining a diagnostic sample with an FN-NA technique, no statistical difference was identified between each group for cellularity, cellular preservation or haemodilution.

While limited literature pertaining to comparison of the two sampling techniques in the liver is available, multiple human studies have evaluated the use of FN-NA vs. FNA to sample the thyroid. Obviously, the afferent blood supply to the liver is very different to that of the thyroid gland (approximately 80% is via the portal venous system); however, the liver is a similarly highly vascular organ that typically yields bloody aspirates (Fagelman and Chess, 1990). Two studies appraising thyroid sampling, and a third involving various tissues (including the liver and thyroid) found diagnostically superior specimens were obtained significantly more frequently with a FN-NA technique (Santos and Leiman, 1988; Dey and Ray, 1993; Ali Rizvi et al., 2005). Not all identified a statistically significant difference however, with two of these studies concluding the techniques were comparable (Tublin et al., 2007; Song et al., 2015). Pothier and Narula's (2006) review and metanalysis found the odds ratio favoured the FN-NA technique despite the lack of statistical significance and concluded that FN-NA may produce superior samples. Increased aspiration of blood can compromise cellular preservation and haemodilution may hamper interpretation; reducing it therefore improves smear quality (Ali Rizvi et al., 2005). This supports our findings that non-diagnostic samples, obtained by either technique, were significantly associated with more severe haemodilution, lower cellularity and poorer cell preservation ($P < 0.001$ for all) consistent with our initial assumptions that diagnostic quality would be affected by haemodilution, preservation and cellularity. FN-NA is reportedly likely to produce less haemorrhage, as the cellular yield depends on spontaneous capillary action rather than negative suction (Dey and Ray, 1993). With an FN-NA technique resultant smears had more concentrated cellular material which was less obscured by blood (Ali Rizvi et al., 2005).

Table 1
Cytological diagnosis of samples obtained by fine-needle non-aspiration (FN-NA) or fine-needle aspiration (FNA) techniques, for all dogs in the study ($n = 119$).

Diagnosis	Total number of samples	Percentage of total (%)	Breakdown by technique	
			FN-NA	FNA
Normal/no significant findings	29	24.3	19	10
Vacuolar hepatopathy	21	17.6	12	9
Hepatitis	16	13.4	12	4
Nodular hyperplasia	11	9.2	6	5
Lymphoma	6	5.0	3	3
Hepatocellular carcinoma	5	4.2	3	2
Metastatic neoplasia	4	3.4	2	2
Histiocytic sarcoma	3	2.5	1	2
Cholestasis	2	1.7	0	2
Unclassified sarcoma	1	0.8	0	1
Extramedullary haematopoiesis	1	0.8	1	0
Other	6	5.0	2	4
Non-diagnostic	14	11.8	4	10
Total	119	100	65	45

Table 2
The prevalence of samples obtained by fine-needle non-aspiration (FN-NA) or fine-needle aspiration (FNA) exhibiting: high, moderate or low cellularity; good, moderate or poor cell preservation and mild, moderate or severe haemodilution. Results classified by sampling technique FN-NA ($n = 65$) and FNA ($n = 54$). The two sampling groups were compared using the Chi-squared test.

	FN-NA ($n = 65$)			FNA ($n = 54$)			P
	High/Good/Mild n (%)	Moderate n (%)	Low/Poor/Severe n (%)	High/Good/Mild n (%)	Moderate n (%)	Low/Poor/Severe n (%)	
Cellularity	32 (49.2)	26 (40.0)	7 (10.8)	22 (40.7)	19 (35.2)	13 (24.1)	0.15
Cellular preservation	54 (83.1)	9 (13.8)	2 (3.1)	41 (75.9)	11 (20.4)	2 (3.7)	0.62
Haemodilution	21 (32.3)	29 (44.6)	15 (23.1)	15 (27.8)	23 (42.6)	16 (29.6)	0.7
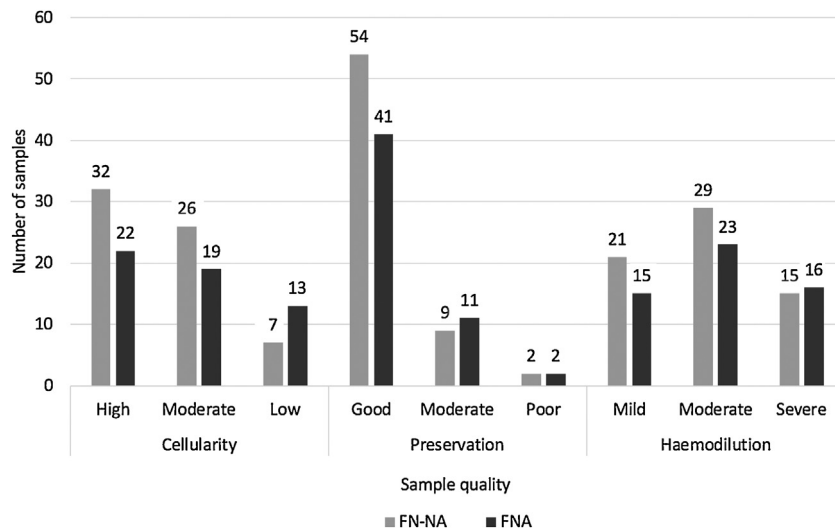
Fig. 1. Sample quality in relation to sampling technique. The distribution of cytology samples exhibiting: high, moderate or low cellularity; good, moderate or poor cell preservation and mild, moderate or severe haemodilution, grouped by either fine-needle aspiration (FNA) or fine-needle non-aspiration (FN-NA) sampling technique.

The application of suction can traumatise fragile cells, producing artefacts (Fagelman and Chess, 1990; Rajasekhar et al., 1991); these may lead to diagnostic error (Fagelman and Chess, 1990). In particular, malignant cells are more fragile and therefore more prone to degeneration and the trauma of suction can compromise their diagnostic value (Misra et al., 2015). FN-NA has been reported to be less traumatic than FNA (Zajdela et al., 1987; Rajasekhar et al., 1991; Dey and Ray, 1993; Chowhan et al., 2014). FN-NA is therefore said to be superior for sampling malignant lesions, producing smears with superior diagnostic quality (Rajasekhar et al., 1991; Raghuveer et al., 2002; Misra et al., 2015). Tissues sampled with aspiration are often fragmented with distorted histologic architecture (Suo et al., 2018). Insufficient numbers of samples from each group represented a neoplastic diagnosis to be able to identify whether one technique (FN-NA vs. FNA) was superior in their diagnosis. Further as histological characterisation of non-diagnostic samples was not performed in this study diagnostic accuracy of identifying neoplasia could not be compared between groups; this remains an area for future study.

Multiple sources consider FN-NA technically easier to perform than FNA (Fagelman and Chess, 1990; Pothier and Narula, 2006; Sajeev and Siddaraju, 2009; Misra et al., 2015). With FN-NA the needle is said to be easier to control within the lesion (Misra et al., 2015). Ease of sample collection was not evaluated as an outcome in our study.

Coagulation testing was not routinely performed in this study, but platelet count prior to sampling was available for all dogs. No significant complications occurred with either sampling technique, based on ultrasonographic assessment of the dog during sampling and at the conclusion of the ultrasound scan and subsequent monitoring of demeanour and clinical parameters until discharge.

Study limitations include the involvement of multiple clinicians in sample collection. Despite establishing sampling protocols and use of the same equipment, specific sampling methodology may have varied slightly between operators. Similarly, bias may have been introduced by use of two cytopathologists, although this should be limited as they work closely together, apply agreed criteria to reach a diagnosis and consult with each other when cases are challenging. These limitations are inherent to this type of randomised controlled study. The variety of underlying disease processes affecting the liver parenchyma may have influenced the results (e.g. friability, vascularisation). Concurrent pathology not detected by cytology (e.g. fibrosis), cellular fragility or poorly exfoliative lesions may reduce diagnostic yield. However, randomised group allocation (supported by comparable age, weight, breed and sex distribution) allowed a fairly even spread of diagnoses between the two sampling methods (Table 1). This variation is therefore expected, with no particular bias to one method; consequently, the results should be relevant for sampling of a range of hepatic conditions.

The possibility of obtaining samples using both FN-NA and FNA from each dog was considered during study design. However, the increased potential for complications, most notably haemorrhage, resulting from doubling the number of samples obtained per dog, was not granted ethical approval. Additionally, the masking process for the clinical pathologist would have been compromised, as sampling technique used would have been recorded on the slides. Diagnostic accuracy of the fine-needle sampling (i.e. the ability of the sample to correctly detect or exclude disease) was not assessed since

histopathological comparison was not performed for the majority of cases. Pathologists may add qualifiers (e.g. suggestive for) or modifiers to the diagnosis, to indicate slight hesitation when sample evaluation is challenging due to suboptimal sample quality or complex sample interpretation. For these reasons and the inherent limitations of FNA cytology mentioned earlier it cannot be assumed that the diagnostic accuracy is equivalent to the diagnostic yield. Finally, there will be bias in the referral population.

In view of the limitations described and since the differences identified between the two sampling techniques were relatively modest (with overlap of the 95% CI), further studies are indicated to corroborate these findings and provide a stronger evidence base for our clinical practice.

Conclusions

For fine-needle sampling of the canine liver, a non-aspiration technique was marginally superior to aspiration, resulting in a greater proportion of diagnostic samples. Extrapolation of data to support an ultrasound-guided fine-needle sampling technique for other organs is limited, due to differences in accessibility, vascular supply, ease of cell exfoliation and specific pathologies.

Conflict of interest statement

None of the authors of this paper has any financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

References

Ali Rizvi, S.A., Husain, M., Khan, S., Mohsin, M., 2005. A comparative study of fine needle aspiration cytology versus non-aspiration technique in thyroid lesions. Surgeon 3, 273–276.

Bahr, K.L., Sharkey, L.C., Murakami, T., Feeney, D.A., 2013. Accuracy of us-guided FNA of focal liver lesions in dogs: 140 cases (2005–2008). Journal of the American Animal Hospital Association 49, 190–196.

Briffod, M., Gentile, A., Hebert, H., 1982. Cytopuncture in the follow-up of breast carcinoma. Acta Cytologica 26, 195–200.

Burkhard, M.J., Meyer, D.J., 1996. Invasive cytology of internal organs: cytology of the thorax and abdomen. Veterinary Clinics of North America: Small Animal Practice 26, 1203–1222.

Chowhan, A.K., Sreedhar Babu, K.V., Sachan, A., Rukmangdha, N., Patnayak, R., Radhika, K., Phaneendra, B.V., Kumaraswamy Reddy, M., 2014. Should we apply suction during fine needle cytology of thyroid lesions? A prospective study of 200 cases. Journal of Clinical and Diagnostic Research 8, FC19–FC22.

Cohen, M., Bohling, M.W., Wright, J.C., Welles, E.A., Spano, J.S., 2003. Evaluation of sensitivity and specificity of cytologic examination: 269 cases (1999–2000). Journal of American Veterinary Medical Association 222, 964–967.

Dey, P., Ray, R., 1993. Comparison of fine needle sampling by capillary action and fine needle aspiration. Cytopathology 4, 299–303.

Fagelman, D., Chess, Q., 1990. Nonaspiration fine-needle cytology of the liver: a new technique for obtaining diagnostic samples. American Journal of Roentgenology 155, 1217–1219.

Guillot, M., D'Anjou, M.A., Alexander, K., Bédard, C., Desnoyers, M., Beauregard, G., del Castillo, J.R.E., 2009. Can sonographic findings predict the results of liver aspirates in dogs with suspected liver disease? Veterinary Radiology & Ultrasound 50, 513–518.

Hager, D.A., Nyland, T.G., Fisher, P., 1985. Ultrasound-guided biopsy of the canine liver, kidney and prostate. Veterinary Radiology 26, 82–88.

Jahromi, A.H., Ballard, D.H., Bahrami, R., D'Agostino, H.R.V., 2015. Comparison of different techniques of ultrasound-guided fine needle biopsy of liver in a swine model. Hepatitis Monthly 15, 1–4.

Kerwin, S.C., 1995. Hepatic aspiration and biopsy techniques. Veterinary Clinics of North America: Small Animal Practice 25, 275–291.

LeBlanc, C.J., Head, L.L., Fry, M.M., 2009. Comparison of aspiration and nonaspiration techniques for obtaining cytologic samples from the canine and feline spleen. Veterinary Clinical Pathology 38, 242–246.

Léveillé, R., Partington, B.P., Biller, D.S., Miyabayashi, T., 1993. Complications after ultrasound-guided biopsy of abdominal structures in dogs and cats: 246 cases (1984–1991). Journal of the American Veterinary Medical Association 203, 413–415.

Menard, M., Papageorges, M., 1995. Technique for ultrasound-guided fine needle biopsies. Veterinary Radiology and Ultrasound 36, 137–138.

Misra, R.K., Mitra, S., Jain, R.K., Vahikar, S., Bundela, A., Misra, P., 2015. Image-guided fine needle cytology with aspiration versus non-aspiration in retroperitoneal masses: is aspiration necessary? Journal of Pathology and Translational Medicine 49, 129–135.

Pothier, D.D., Narula, A.A., 2006. Should we apply suction during fine needle cytology of thyroid lesions? A systematic review and meta-analysis. Annals of the Royal College of Surgeons of England 88, 643–645.

Raghuveer, C.V., Leekha, I., Pai, M.R., Adhikari, P., 2002. Fine needle aspiration cytology versus fine needle sampling without aspiration. A prospective study of 200 cases. Indian Journal of Medical Sciences 56, 431–439.

Rajasekhar, A., Sundaram, C., Chowdhary, T., Charanpal, M., Ratnakar, K.S., 1991. Diagnostic utility of fine-needle sampling without aspiration: a prospective study. Diagnostic Cytopathology 7, 473–476.

Roth, L., 2001. Comparison of liver cytology and biopsy diagnoses in dogs and cats: 56 cases. American Society for Veterinary Clinical Pathology 30, 35–38.

Rothuizen, J., Twedt, D.C., 2009. Liver biopsy techniques. Veterinary Clinics of North America: Small Animal Practice 39, 469–480.

Sajeev, S., Siddaraju, N., 2009. A comparative analysis of fine-needle capillary cytology vs. fine-needle aspiration cytology in superficial lymph node lesions. Diagnostic Cytopathology 37, 787–791.

Santos, J.E., Leiman, G., 1988. Nonaspiration fine needle cytology. Application of a new technique to nodular thyroid disease. Acta Cytologica 32, 353–356.

Sharkey, L.C., Dial, S.M., Matz, M.E., 2007. Maximizing the diagnostic value of cytology in small animal practice. Veterinary Clinics of North America: Small Animal Practice 37, 351–372.

Sharkey, L.C., Seelig, D.M., Overmann, J., 2014. All lesions great and small, part 2. Diagnostic cytology in veterinary medicine. Diagnostic Cytopathology 42, 544–552.

Song, H., Wei, C., Li, D., Hua, K., Song, J., Maskey, N., Fang, L., 2015. Comparison of fine needle aspiration and fine needle nonaspiration cytology of thyroid nodules: a meta-analysis. BioMed Research International 2015, 19–21.

Suo, L., Chang, R., Padmanabhan, V., Jain, S., 2018. For diagnosis of liver masses, fine-needle aspiration versus needle core biopsy: which is better? Journal of the American Society of Cytopathology 7, 46–49.

Tublin, M.E., Martin, J.A., Rollin, L.J., Pealer, K., Kurs-Lasky, M., Ohori, N.P., 2007. Ultrasound-guided fine-needle aspiration versus fine-needle capillary sampling biopsy of thyroid nodules: does technique matter? Journal of Ultrasound in Medicine 26, 1697–1701.

Wang, K.Y., Panciera, D.L., Al-Rukibat, R.K., Radi, Z.A., 2004. Accuracy of ultrasound-guided fine-needle aspiration of the liver and cytologic findings in dogs and cats: 97 cases (1990–2000). Journal of the American Veterinary Medical Association 224, 75–78.

Weiss, D.J., Moritz, A., 2002. Liver cytology. Veterinary Clinics of North America – Small Animal Practice 32, 1267–1291.

Weiss, D.J., Blauvelt, M., Aird, B., 2001. Cytological evaluation of inflammation in canine liver aspirates. Veterinary Clinical Pathology 30, 193–196.

Zajdela, A., Zillhardt, P., Voillemot, N., 1987. Cytological diagnosis by fine needle sampling without aspiration. Cancer 59, 1201–1205.