

DOI 10.2478/pjvs-2013-0118

Review

Fine needle biopsy of abdominal organs in dogs – indications, contraindications and performance technique

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Abstract

Recent years have seen in both human and veterinary medicine the development of numerous techniques allowing for evaluation and classification of changes in individual organs and tissues. Despite introduction of such techniques into diagnostics as among others, CT, MRI, CEUS or elastography, biopsy is still considered a “golden standard” and it is a procedure performed in order to obtain a final diagnosis. There are many biopsy techniques, such as fine needle aspiration biopsy, core biopsy as well as methods of performing a procedure, e.g. blind biopsy, biopsy under USG control and biopsy during laparotomy. In the article usefulness of biopsy techniques in relation to diagnostics of individual abdominal organ, as well as the procedure technique, contraindication and complications are discussed.

Key words: fine needle biopsy, dog, abdominal organs

Introduction

In recent years veterinary medicine has seen increasing importance of fine needle biopsy in diagnostics of lesions developing in abdominal and thoracic organs. Fine needle aspiration biopsy (FNAB) is a more and more frequently used diagnostic technique allowing the collection of cell material for cytological and microbiological examinations (Barr 1995, Britton 1999, Gani at al. 2011, Wypij 2011). It is especially useful in the diagnosis of neoplastic processes and infectious diseases (Bonfanti at al. 2004).

Fine needle aspiration biopsy is a safe technique possible to perform even in out-patient clinic conditions. In many cases it is not necessary to anaesthize a patient; only in over-reactive animals sedation is recommended. Additionally, FNAB is characterized by a low complications rate and is cheap in comparison with other biopsies (Livraghi at al. 1983, Roussel at al. 1989, Smith 1991, Gazelle at al. 1992, Léveilé at al. 1993, Britton 1999, Nyland at al. 2002, Vignoli and Saunders 2011, Klopffleisch at al. 2011, Wypij 2011). The time from biopsy to its result is relatively short, thanks to which it is possible to quickly diagnose and

apply a proper treatment. The combination of high diagnostic effectiveness and minimum invasiveness leads to a conclusion that fine needle aspiration biopsy should be routinely carried out in patients with a suspicion of neoplastic diseases, despite the fact that in some cases the ultimate diagnosis requires collection of material from several sites (Itoh 1984, Hager et al. 1985, Beaty et al. 1997, Britton 1999).

Fine needle aspiration biopsy technique

Fine needle aspiration biopsy is most frequently performed using injection needles (4 cm long) and lumbar puncture needles (9 cm long) of 0.6-0.9 mm (20-23G) in diameter attached to 5-20 ml syringes (Wypij 2011). Prior to biopsy an ultrasound examination must be performed in order to precisely evaluate location of lesions, their size and depth of situation as well as to determine the best biopsy needle insertion site (Wang et al. 2004, Gani et al. 2011). One of the best biopsy techniques is ultrasound guided biopsy. After determining the site for collection of bioplate under ultrasound guidance, the biopsy needle is inserted (Wypij 2011). When it reaches the area of the affected tissue, the piston of a syringe is pulled out so as to create a vacuum in order to aspirate the material. In cases of small skin tumors, lymphatic nodes and spleen, the biopsy needle is inserted without a syringe (fine needle fenestration – FNF) and turned around its axis several times (Watson et al. 2011). After removing the needle the cytological material is blown out on to a slide similarly as in FNAB.

In case of small lesions (e.g. small neoplastic tumors) it is recommended to collect aspirate from their central part. This is due to the fact that in the course of many neoplastic processes a lesion is very frequently surrounded by the secondary inflammatory process (Bonfanti et al. 2004, Wypij 2011). In this case collection of cytological material from the margin may distort the result. However, in large neoplastic changes bioplate should rather be collected from peripheral areas because due to ischemia the central part may be affected by necrosis which will also distort the result (Blue et al. 1999).

In recent years a new method of imaging has been introduced – dynamic elastography. This method allows for a more precise evaluation of changes and determination of a site for collecting the most diagnostic material.

Fine needle aspiration biopsy effectiveness is estimated at 70-80% (Wypij 2011). The most common reasons for obtaining non- or poorly diagnostic material include: contamination of the sample with blood, collection of too few cells (which is related to

excessive firmness of the affected tissue or little experience of an operator), damage to cells while making a smear and a too thick smear – difficult to evaluate. Similarly, contamination of a sample with gel used in an ultra-sound examination may lead to origination of artifacts, which substantially impedes cytological evaluation of smear due to gel turquoise colour and amorphic appearance (Wypij 2011).

Fine needle aspiration biopsy of the internal organs may provide valuable information, yet it is not commonly used in veterinary medicine. It may result from fears of veterinarians concerning possibility of complications or lack of biopsy know-how. Prior to fine needle aspiration biopsy, blood coagulation parameters should be determined (Vignoli and Saunders 2011). It should be noted that the risk of internal bleeding in FNAB is considerably lower than in other biopsy techniques. Based on research conducted in human medicine the complication rate in FNAB was estimated at 0.05%; in veterinary medicine it is estimated at 2% (Livraghi et al. 1983, Wypij 2011). Complications after FNAB occur more frequently in cats than in dogs and more often due to inflammatory rather than neoplastic processes (Livraghi et al. 1983, Smith 1991, Gazelle et al. 1992, Leveille 1993, Nyland et al. 2002). While collecting bioplates from neoplastic changes one should remember about the risk of dissemination of neoplastic cells along the biopsy needle path or a hematogenic way. It most frequently happens in biopsy of the pancreas and prostate neoplastic tumors (Bergenfeldt et al. 1988, Nyland et al. 2002, Herman et al. 2005). However, many authors think that the risk of neoplastic cells implantation is extremely rare and should not affect the decision of biopsy (Livraghi et al. 1983, Vignoli et al. 2007). Other authors think that data concerning neoplastic metastases caused by biopsy are understated due to high mortality in animals with a neoplastic disease. They proved that 20% of animals in which percutaneous fine needle biopsy of neoplastic tumors had been performed were affected by metastases only 4 years after the procedure (Klopfleisch et al. 2011). However, in humans the rate of neoplastic metastases occurring along the biopsy needle path is estimated at 0.007% (Klopfleisch et al. 2011).

Biopsy of abdominal organs

Liver

In clinical practice of dogs and cats diseases fine needle aspiration biopsy of the liver is most commonly performed (Wypij 2011). It should be stressed that in many liver diseases the analysis of data obtained from

the history, clinical examination, laboratory blood and urine tests, X-ray examination and/or ultrasound examination are not sufficient to make a final diagnosis, and hence to administer the right treatment. The cytological examination of material collected from the liver allows for a diagnosis of the liver neoplastic changes and many disorders of non-neoplastic origin, e.g. cholestasis, the liver lipidosis, glycogen storage, nodular regeneration, different kinds of inflammation, copper storage (Kristensen et al. 1990, Kedar et al. 1991, Willard et al. 1999, Roth 2001, Wang et al. 2004). The most common liver neoplastic changes include metastases, mainly of the mammary gland carcinoma, mastocytoma (MCT), spleen hemangiosarcoma and lymphoma (Fig. 1). The primary liver neoplasms [e.g. hemangiosarcoma, the liver carcinoma (Fig. 2), the primary liver lymphoma] are less frequently diagnosed (Willard et al. 1999, Roth 2001, Wang et al. 2004).

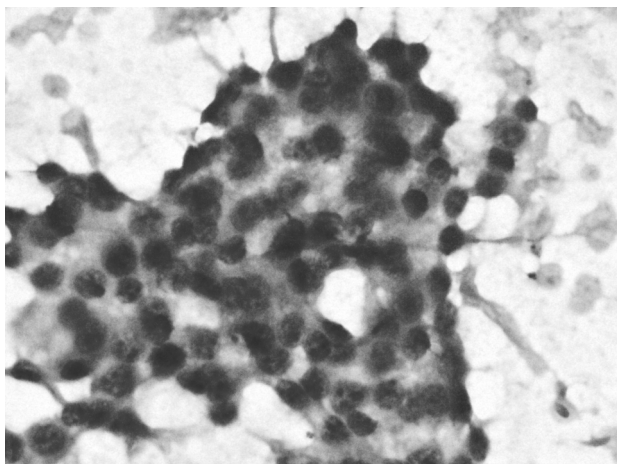


Fig. 1 Liver. Dog. Tissue aspirate. Numerous atypical lymphocytes. Liver lymphoma. HE staining, 400x.

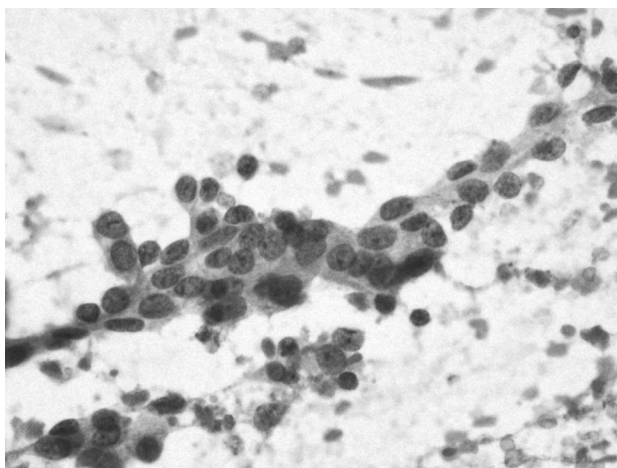


Fig. 2 Liver. Dog. Tissue aspirate. – Atypical epithelial cells with a large nucleus, dense chromatin and scant amounts of basophilic cytoplasm. Carcinoma cell. HE staining, 400x.

In the years 2005-2008, Bahr et al. (2013) performed the analysis of the research results obtained on the basis of FNAB and a core biopsy. They have concluded that the cytological examination of aspirates obtained during the FNAB procedure is characterized by high sensitivity in the diagnostics of the liver neoplastic changes (86.7%) and the vacuolar change diagnostics (51.6%). On the other hand, it shows low sensitivity in the diagnostics of inflammation, necrosis, and hyperplasia (Bahr et al. 2013).

In turn, the research carried out by other authors has proven that in case of the liver lipidosis sensitivity of the liver cytological examination is 25-95% (Willard et al. 1999).

At present the fine needle aspiration biopsy of the liver is most frequently performed under USG guidance (Fig. 2). The biopsy needle is most commonly inserted from the side of the abdominal cavity or through an intercostal space into the left medial or lateral liver lobe at a safe distance from the diaphragm, large blood vessels and the gall bladder (Itoh 1984, Hager et al. 1985, Kristensen et al. 1990, Blue et al. 1999, Roth 2001, Wang et al. 2004).

In case of the performance of FNA procedure, there is a very low risk of occurrence of minor complications (0.5%) and serious complications (0.05%). It is thought that in case of the liver FNAB, a risk of mortality amounts to less than 0.01%. A frequency of occurrence of the complications is conditioned by vascularization and location of changes in the liver, a thickness of a needle, an applied biopsy technique, and experience of a person who carries out the biopsy. Dissemination of neoplastic cells along the needle path constitutes a separate issue. In case of the liver neoplastic tumour FNAB, dissemination of neoplastic cells along the needle path constitutes a separate issue, however, there are only a few descriptions of this type of complications in the world literature (Chhieng 2004).

Gall bladder

The gall bladder biopsy is a relatively safe procedure. It is performed using needles 3-10 cm long and 0.7-0.9 mm in diameter (22-20G) attached to 10 ml syringes. The gall bladder FNAB is most commonly carried out in cats with suspicion of hepatitis and biliary ducts inflammation with an aim of performing bile microbiological and cytological examinations. Biopsy should be carried out under USG control (Fig. 4). An indication for the gall bladder FNAB is also its decompensation in animals with extra liver biliary ducts obstruction (Iton 1984). In this case the

gall bladder should be maximally emptied during a puncture, which reduces the risk of bile leak to the peritoneal cavity and development of bile derivative hepatitis and peritonitis (Itoh 1984, Dodd et al. 1996, Das et al. 1997, Vörös et al. 2002, Savary-Bataille et al. 2003, Herman et al. 2005).

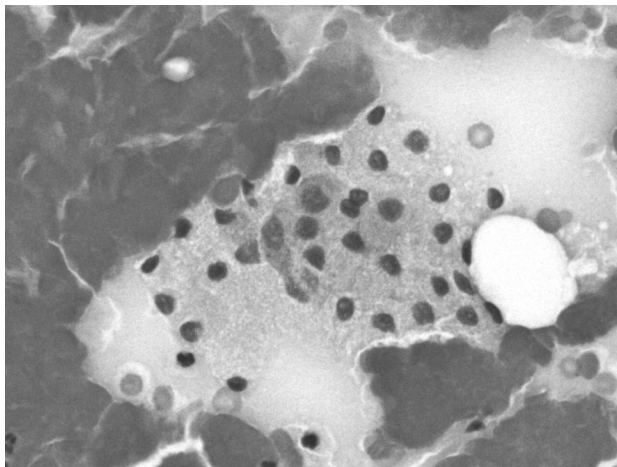


Fig. 3. Ultrasound – guided FNAB of the liver.

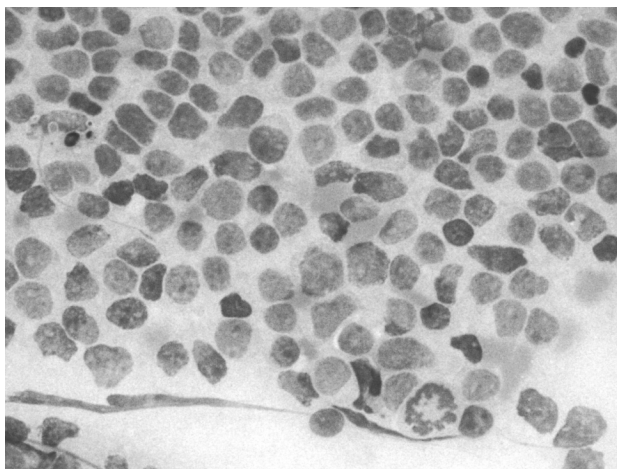


Fig. 4. Ultrasound – guided FNAB of the gallbladder.

An indication for the gall bladder FNAB can also be suspicion of a neoplastic process. The gall bladder neoplasms are relatively rarely observed in the veterinary practice; it is considered that they constitute approximately 4-5% of neoplasms that derive from the biliary duct epithelium. They are usually described for older animals. Adenoma, lipoma, hemangioma, and leiomyoma belong to benign neoplasms of the gall bladder. However, adenocarcinomas are the most frequent neoplasms of the gall bladder (Glińska et al. 2008). The gall bladder FNAB and the cytological examination of bile may provide a lot of important information enabling determination of a type and

character of changes and establishing the best therapeutic option. There are few reports on the complications related to the gall bladder biopsy in the literature. It is most likely to be associated with the fact that the biopsy is relatively rarely performed. There can be the following complications after the gall bladder FNAB: bleeding, choleperitonitis, and a possible dissemination of the neoplastic process along the needle path. It ought to be remembered that a big limitation of the cytological evaluation of bile is a degenerative impact of bile acid salts on cells, which may limit a possibility of a correct diagnosis (Dood 1996).

Spleen

There are many reasons for splenomegaly, including extra bone marrow hematopoiesis, lymphoma or splenitis. The cause of the spleen shape disturbances may be e.g. hematomas or neoplastic changes. The spleen biopsy, although performed rarely, may be useful in making a diagnosis and establishing further treatment (Wypij 2011). The most common spleen neoplasms diagnosed on the basis of cytological evaluation of the material collected in biopsy include: lymphoma and mastocytoma (the primary form – more common in cats, the secondary form – in dogs) (Kinney et al. 1993). At present splenectomy is still a golden standard in treatment of the spleen lesions in veterinary medicine. However, it is an invasive procedure, relatively expensive, requiring general anaesthesia and not always necessary. That is why when detecting the organ enlargement, as well as changes in its shape, a fine needle biopsy should be always taken into consideration in the diagnostic procedure (Watson et al. 2010). The exception is a risk of heavy bleeding which may follow the biopsy in case of multilocular tumors e.g. hemangiosarcoma. In that situation laparotomy and excision of the whole organ is recommended. Due to the spleen vascularity a fine needle fenestration biopsy is recommended, not FNAB. Based on the research conducted on 42 dogs in which spleen biopsy was performed under USG control complications were noted only in one individual (Kinney et al. 1993). Numerous studies carried out in human medicine also confirmed that FNF is safety and represents the effectiveness of biopsy (Gazelle et al. 1992, Vignoli and Saunders 2009, Gani et al. 2011). Needles 0.7-0.9 mm (22-20G) in diameter are recommended for use in a fine needle fenestration biopsy. The needle is inserted into the affected organ area under USG control and next cytological material is collected by repeated needle turning. It is worth noting that in spleen biopsy tissue aspiration must be avoided as it increases the risk of contaminating

a sample with blood, which makes cytological interpretation difficult (Savage et al. 1995, Watson et al. 2010). The most frequently described complications of the spleen biopsy are bleeding and accidental biopsy of the neighbouring organs e.g. kidneys, colon. It is assumed that a risk of bleeding after the spleen biopsy amounts to 0%-2% (Quinn 1986). The examination, performed by Solbiati et al. (1983), of people in whom splenectomy was performed a short time after the spleen biopsy, showed that there were neither haematomas nor other changes at a place where the biopsy was performed. In case of tumorous lesions, if possible, it is recommended to perform a biopsy from circumferentially located changes as this reduces damage of the spleen parenchyma and thus a risk of bleeding.

Pancreas

Pancreas biopsy was not performed for many years because it was thought to be a reason for this organ inflammation. Numerous studies conducted in human and veterinary medicine have proven that intraoperative collection of the organ sample may increase an activity level of the pancreas inflammation markers (such as, e.g. amylase, lipase, TLI) (Itoh 1984, Bergenfeldt et al. 1988, Wypij 2011). However, such changes were not observed in FNAB. The main indication for pancreas biopsy is suspicion of the organ neoplastic changes, in particular pancreas carcinoma, which in the clinical picture, laboratory tests as well as in an ultra-sound picture may imitate pancreatitis. Pancreas biopsy is also performed in differential diagnosis of inflammation, abscesses and pseudocysts. The fine needle aspiration biopsy should always be carried out under USG control. However, as the pancreas is difficult to visualize in the USG examination, experience of a person who determines a place from where an aspirate is taken, plays an important role (Mix and Jones 2006). In order to decrease the risk of complications an operator should avoid passing a biopsy needle through the healthy pancreatic tissue (Itoh 1984, Hager et al. 1985, Bergenfeldt et al. 1988). The research carried out by Hovdenak et al. (1982) has proven that sensitivity of the pancreas FNA in the oncological diagnostics amounts to 94%. However, the studies of other authors have revealed that in case of the pancreas biopsy as recommended by an oncologist, a possibility of receiving false negative results amounts from 3% to 20%. False positive results were not found (Hancke et al. 1975).

Kidney

The fine needle aspiration biopsy is performed in cases when only cytological material is sufficient to diagnose a disease. Hence, main indications for the kidney FNAB in dogs and cats include: confirmation of perirenal cysts, confirmation of infectious nephropathy (microbiological or cytological examination of the collected material) and suspicion of the kidney neoplastic changes (Hager et al. 1985, Cajulis et al. 1993, Jankowski et al. 2013). However, when suspecting such diseases as e.g. glomerulonephritis, renal amyloidosis, renal dysplasia oligobiopsy should be performed instead of a fine needle aspiration biopsy (Vaden et al. 2005). Most frequently a fine needle aspiration biopsy of the kidney is used for determining the kind and malignancy of neoplastic changes, which substantially affects the treatment (pharmacological treatment – chemotherapy and/or surgery – nephrectomy) and prognosis. It should be remembered that due to the renal vascularization biopsy may be followed by such complications as microscopic haematuria (most common), macroscopic haematuria, bleeding to the abdominal cavity, subcapsular hematoma, hydronephrosis (late complication), as well as the animal's death (very seldom) (Wypij 2011). Kidney biopsy should be performed under USG control (Fig. 5) or laparoscopy control because it allows for



Fig. 5. Ultrasound – guided FNAB of the kidney.

a precise bioplate collection and minimizes possible complications (Moon 2002). In cats the biopsy performance may be considerably facilitated by immobilizing the kidney with a hand. Unfortunately, it is very difficult to carry out in dogs. Bioplates should be collected from the renal cortex avoiding insertion of the biopsy needle into the renal medulla and pelvis. The ideal place for insertion of the needle into the kidney is then cortex in its caudal end. A good alternative is also insertion of the needle parallel to the

organ long axis. As for neoplastic changes, the primary renal tumors such as carcinoma (Fig. 6) or sarcoma are rarely diagnosed (Murphy et al. 1993). However, the most common renal neoplasm diagnosed on the basis of the cytological examination is renal lymphoma (Hager et al. 1985, Cajulis et al. 1993, Murphy et al. 1993, Beaty et al. 1997, Truong et al. 1999, Vaden et al. 2005, Moon 2009).



Fig. 6. Kidney. Dog. Tissue aspirate. Atypical, polymorphic epithelial cells, with prominent nucleoli. Renal carcinoma. HE staining, 400 x

Urinary bladder

Urinary bladder puncture (cystocentesis) is a safe and easy diagnostic technique. The most common indications for cystocentesis in dogs and cats include collection of urine for laboratory tests and urine letting in the urethra obstruction. It should be noted that in case of urine culture, cystocentesis is a technique of choice for collecting a urine specimen because it prevents contamination from the urethra (Wypij 2011). The urinary bladder puncture is carried out through the abdominal wall in a “blind way” (most commonly in cats, additionally immobilizing the urinary bladder with a hand) or under USG control. In some situations insertion of the needle into the urinary bladder lumen may be hindered by elasticity of its wall. Cystocentesis is performed using needles of 0.7-0.9 mm in diameter (22-20G) and 3-4 cm in length. The needle should be inserted into the urinary bladder wall at an angle of 45°, because it prevents leakage of urine into the peritoneal cavity. Complications following the urinary bladder puncture are very rare. They include: bleeding from the puncture site, the puncture site infection, leakage of urine into the peritoneal cavity, chemical peritonitis and the urinary bladder rupture (Murphy et al. 1993, Moon 2009, Vignoli and Saunders 2009). In case of neoplastic changes situated in the urinary bladder FNAB is not recommended due to the risk of disseminating neoplasm along the biopsy

needle pathway (particularly transitional carcinoma), which was confirmed by Nyland et al. (2002). In such cases it is best to perform cystoscopy and collect samples using biopsy forceps inserted through the endoscope working channel.

Prostate

The most commonly diagnosed diseases of the prostate based on a cytological examination of the material collected by FNAB include mild organ hypertrophy (Fig. 7), chronic prostate inflammation and its neoplasms (Wypij 2011). The research carried out by Nickel and Teske (1992) has revealed that credibility of FNAB in the diagnostics of the prostate neoplasms amounts from 79% to 80%, and the credibility of a core-needle biopsy – approximately to 89%. The research of other authors has proven that conformity of a cytological examination with a histopathological examination of the prostate is very high and amounts to 75% (Vignoli and Saunders 2009). It indicates high FNAB sensitivity as the prostate diseases diagnostic method. The prostate biopsy is relatively difficult to perform due to its location and close proximity of such organs as the aorta, the urinary bladder and the rectum. Due to this fact it should be performed under USG control. Prior to the performance of the biopsy, it is recommended to introduce a catheter into the urethra, which facilitates visualization of the prostate. The alternative prostate biopsy technique is a biopsy performed through the rectum wall (more commonly used at present in humans than in animals). The most frequent complications following prostate biopsy include damage of the urethra or large vessels, rectal puncture and mild hematuria lasting a few days (Nyland et al. 2002).



Fig. 7. Prostate gland. Dog. Tissue aspirate. A cluster of normal-appearing prostatic epithelial cells with moderate amounts of slightly basophilic cytoplasm and round nuclei. HE staining, 400x.

Contraindication for the performance of the biopsy is also the presence of abscesses in the prostate as this can lead to a septic inflammation of the peritoneum.

The cytological examination of a collected aspirate consists in a quality and quantity evaluation of the cells (e.g. cells with neoplastic atypia properties). It should be remembered that in case of a biopsy of particular organs and tissues, one should take into account a risk of receiving false positive or negative results. The reasons for an occurrence of the false positive and false negative FNAB results are a technical error and a histopathological evaluation error but the results are not limited to them. The former results from an incorrect biopsy performance technique, an incorrect determination of a place from which the material will be taken or an incorrect execution of a cytological preparation. The histopathological evaluation error may be caused by an incorrect performance of the cytological examination or a lack of experience of a histopathologist. Therefore, if the results obtained are not unequivocal and diagnostic imaging suggests possible neoplastic infiltrations, it is recommended to perform a biopsy again in order to verify the results obtained.

Summing up, it should be noted that fine needle aspiration biopsy of abdominal organs in dogs and cats is a useful, safe and cheap diagnostic method. First of all, it allows for distinguishing neoplastic changes from non-neoplastic changes in the organs, which in turn allows for precise initial diagnosis, orienting treatment and determination of prognosis. Due to a low rise of complications FNAB should be more frequently used in clinical practice of dogs and cats; diseases. Moreover, when suspecting neoplastic changes, fine needle aspiration biopsy should be considered as a basic diagnostic method.

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