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REVIEW ARTICLE

Kidney Biopsy in Dogs and Cats

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ABSTRACT

Kidney biopsy is a diagnostic technique allowing in vivo collection of kidney tissue material for a histopathological examination, which makes it possible to diagnose disorders in the normal organ structure. The most common biopsy used for a sample collection is oligobiopsy. Renal biopsy is a safe and very useful diagnostic method allowing direct evaluation of disorders in the normal kidney structure and precise determination of the disease process nature with regard to qualitative and quantitative changes.

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INTRODUCTION

Diagnosing the reasons for the kidney insufficiency in dogs and cats on the basis of history, clinical examination, laboratory blood (hematological and biochemical) test, urinalysis and the kidney ultrasound examination frequently does not allow determination of its causes. In order to ultimately determine the causes of the renal insufficiency, a fragment of the kidney should be collected for further examinations, that is a biopsy should be performed. Kidney biopsy is a diagnostic technique which makes intravital collection of tissue material from the kidney for histopathological or cytological examinations possible. Both examinations allow determination of disorders in the normal structure of the organ. The most common kind of biopsy performed in collection of tissue samples from the kidney is oligobiopsy (Horauf, 1995; Drost *et al.,* 2000; Brovida, 2003; Jankowski, 2003; Groman *et al.,* 2004; Vaden, 2004; Rezaie *et al.,* 2008; Lees *et al.,* 2011; Silva *et al.,* 2012).

Indications for kidney biopsy: Kidney biopsy is recommended in cases when histopathological examination of the samples may change a way of treatment in the dog and cat with renal insufficiency. Consequently it is performed with the aim of making a diagnosis, evaluation of the renal disease progress (in its qualitative and quantitative aspect), determination of a treatment and prognosis. Kidney biopsy is then recommended in animals suspected of protein losing nephropathies, acute renal insufficiency and renal tumors. Indications for oligobiopsy and fine needle aspiration biopsy have been presented in Table 1. It should be stressed that in case of suspected kidney tumor the recommended method is fine needle aspiration biopsy (FNAB), because this kind of biopsy causes little tissue trauma, hence minimal bleeding, which prevents the spread of neoplastic cells by a hematogenous way. Only when this biopsy technique provides too little material and final diagnosis still cannot be made, a different kind of biopsy, e.g. oligobiopsy, should be performed (Kučera and Rychla, 1990; Osborne *et al.,* 1996; Nowicki and Depta, 2001; Borjesson, 2003; Jankowski, 2003; Nowicki *et al.,* 2005; Vaden, 2005; Venkateswaran, 2007; Lees *et al.,* 2011).

Contraindications for kidney biopsy: Contraindications for kidney biopsy include a lack of one kidney, pyonephrosis, presence of perirenal abscesses, polycystic kidney disease (PKD), hydronephrosis, big renal cysts, severe kidney insufficiency, uncontrolled hypertension, blood coagulation disorders, severe anemia, extensive pyelonephritis, terminal kidney insufficiency and severe respiratory-circulatory insufficiency (Osborne *et al.,* 1974; Kubiak *et al.,* 2003; Zatelli *et al.,* 2003; Nicpoń *et al.,* 2004; Vaden *et al.,* 2005; Haers *et al.,* 2011).

Techniques of kidney biopsy: In small animal medicine, several biopsy techniques are used to collect tissue samples from the kidney. They include blind biopsy, radiological-guided biopsy, keyhole biopsy, ultrasound-guided biopsy, laparoscopic-guided biopsy and biopsy through laparotomy. It should be emphasized that the

choice of the kidney biopsy technique depends on the animal species, its size, the operator's experience, available equipment and the clinical condition of the patient referred for biopsy (Barr, 1995; De Rycke *et al.,* 1999; Brovida, 2003; Nowicki *et al.,* 2005; Vaden, 2005; Rezaie *et al.,* 2008).

Table 1: Indications for oligobiopsy and fine needle aspiration biopsy in dogs and cats

Oligobiopsy	Fine needle aspiration biopsy
- proteinuria of unknown origin - hematuria of unknown etiology - nephrotic syndrom - diagnosis and differentiation of glomerulonephritis - acute kidney insufficiency of unknown origin - disorders in the normal kidney shape (a larger or smaller size, irregular shape) - tubulointestinal nephritis of unknown etiology - renal amyloidosis - renal dysplasia	- neoplastic changes - confirmation of perirenal cysts - confirmation of infectious nephropathy (microbiological or cytological examination)

Blind biopsy is a technique in which insertion of the biopsy needle in direction of the kidney is consistent with its topographic location and is performed with a simultaneous immobilization of the organ through the abdominal wall. It is a relatively simple procedure in cats due to the phenomenon of migrating kidneys (freely translocating in the abdominal cavity), which allows quite easy seizing and immobilizing of the organ through the abdominal wall. However, it is problematic in dogs, because the right kidney is entirely hidden under the costal arch and only the caudal end of the left kidney can be reached. The advantage of blind biopsy is its low cost as only a biopsy needle is needed to perform a procedure. However, the great disadvantage is the operator's inability to control location of the biopsy needle in the abdominal cavity and to determine the place for the kidney sample collection, which consequently may lead to serious complications after biopsy (Osborne *et al.,* 1971; Osborne *et al.,* 1996; Vaden *et al.,* 2005; Silva *et al.,* 2012).

Radiological-guided biopsy (syn. fluoroscopic biopsy) – is at present a rarely used kidney biopsy technique. It consists of intravenous infusion of contrast medium in order to visualize the kidneys (nephrogram), and insertion of a biopsy needle is done under roentgenotelevision control. The advantage of this technique is quite precise collection of the kidney sample. However, the disadvantages are: firstly – contrast medium may not always be safely administered (e.g. the contraindication is severe kidney insufficiency), secondly – exposure of the animal and the operator to harmful activity of X-rays (Hoppe *et al.,* 1986; Jankowski, 2003; Uppot *et al.,* 2010).

Keyhole biopsy means incision of the abdominal wall behind the costal arch on the right side. Next, the kidney is immobilized with the index finger inserted through the opening into the abdominal cavity. Through the neighboring, significantly smaller incision of the abdominal wall the biopsy needle is inserted into the abdominal cavity and led to the kidney surface under the finger guidance. The advantages of this biopsy technique include the low examination cost and increased precision of the kidney sample collection as compared with blind biopsy. However, the disadvantages of keyhole biopsy include the use of this technique only in dogs and, moreover, only in the right kidney, as well as considerable trauma due to incision of the abdominal wall (Wise *et al.,* 1989; Osborne *et al.,* 1996; Nowicki and Depta, 2001; Nicpoń *et al.,* 2004; Vaden, 2005).

Ultrasound-guided biopsy is at present the most commonly used technique of kidney biopsy in dogs and cats. It consists of collection of the kidney sample under ultrasound control. This technique allows visualization of the kidney, evaluation of its size and internal structure and depth of its location in the abdominal cavity, which enables the operator to precisely perform the kidney biopsy and minimize the risk of possible complications. It should be emphasized that application of an ultrasound probe with a special track for a biopsy needle allows very precise determination of the sample collection place (Hager *et al.,* 1985; Yamamoto *et al.,* 1991; Bigge *et al.,* 2001; Rawlings *et al.,* 2003; Zatelli *et al.,* 2005; Jankowski *et al.,* 2008; Lees *et al.,* 2011; Manashirova *et al.,* 2011).

Laparoscopy-guided biopsy allows for collection of a tissue sample from the kidney under control of laparoscopy. This biopsy technique allows a very precise biopsy collection from the kidney (effectiveness comparable with ultrasound-guided biopsy), which minimizes of possible complications. However, the laparoscopy set is very expensive (Grauer *et al.,* 1983; Wise *et al.,* 1989; Nowicki and Lew, 2001; Lew *et al.,* 2003; Vaden, 2005; Nowicki *et al.,* 2010).

Biopsy through laparotomy (syn. surgical biopsy) is surgical opening of the abdominal cavity and collection of a wedge-shaped renal cortex sample by means of a scalpel. It should be emphasized that this technique is used only when there is no possibility to use any of the above mentioned biopsy techniques or when they provide too little tissue material for the final diagnosis (Kučera and Rychla, 1990; Osborne *et al.,* 1996; Nowicki and Depta, 2001; Vaden *et al.,* 2005).

Kidney biopsy needles: In the past the needles used in kidney biopsy were Vim-Silverman needles modified by Franklin. Those are cutting needles, consisting of an external cannula and internal bifurcating mandarin with grooves (Jeraj *et al.* 1982; Nash, 1983; Brovida, 2003; Donnelly *et al.,* 2008).

At present the most commonly used kidney biopsy needles are Tru-cut needles. Similarly to Vim-Silverman needles modified by Franklin, the Tru-cut needles represent the cutting type and the collected sample is in shape of an incomplete cylinder. Tru-cut needles are made up of an external cannula of a sharpened end and an inner, also sharpened, solid stylet with a special incision for the tissue sample, a so-called specimen notch. The needle is inserted into the kidney at the depth of 1-2 mm under the kidney capsule with the stylet specimen notch inside the cannula. Next, the stylet is inserted into the kidney parenchyma, the sharpened cannula is slid down, the kidney fragment is cut off with it and placed in the stylet specimen notch. There are three biopsy systems: manual – collection of the sample is carried out by hand (Fig. 1); semi-automatic – insertion of the stylet in the kidney is

performed manually and the needle automatically slides the cannula (Fig. 2) and automatic – so-called biopsy guns are used, insertion of the stylet and sliding of the cannula are fully automated (Fig. 3). In veterinary medicine the semi-automatic needles, most commonly used in kidney biopsy in dogs and cats, have the diameter of 14G or 16G (Hoppe *et al.,* 1986; Yamamoto *et al.,* 1991; De Rycke *et al.,* 1999; Brovida, 2003; Groman *et al.* 2004; Vaden, 2005; Zatelli *et al.,* 2005; Manashirova *et al.,* 2011; Silva *et al.,* 2012).

Qualification of dogs and cats for kidney biopsy: Qualification of dogs and cats for kidney biopsy should be based on the history, the clinical examination, laboratory blood and urine tests and the ultrasound examination results.

When obtaining the history and during the clinical examination special attention should be paid to the clinical signs which suggest the kidney disease (e.g. all types of miction disorders, tenderness in the kidney area, edema in the kidney area, smell of ammonia from the oral cavity, polydipsia). The hematological examination should include such parameters as erythrocyte count (RBC), hemoglobin concentration (HGB), hematocrit value (HCT), erythrocytes indicators (MCV, MCH, MCHC), leukocytes count (WBC) and leukogram. The examination should also include parameters determining normal activity of the coagulation system, e.g. blood platelets count (PLT), coagulation time, bleeding time, prothrombin time, which will prevent uncontrolled post-biopsy bleeding. The biochemical examination should include evaluation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine, total protein, albumins, Ca++, Pi, K^+, Na^+, Cl^-. These examinations are expected to show the function of the kidneys and other organs as well, e.g. the liver, which is related to safety of anesthesia performed before the kidney biopsy. Urinalysis should include such parameters as protein, glucose, acetone, blood, urobilinogen and bile pigments, and presence of erythrocytes, leukocytes, epithelial cells, mineral components, bacteria and atypical cells, e.g. neoplastic cells in the sediment. Particular attention should be drawn to occurrence of proteinuria and/or hematuria, which may indicate permeability of glomeruli related to disturbances in their structure. Ultrasound examination of the urinary system plays an important role in qualification of animals for kidney biopsy. This examination is considered as a basic imaging technique used in diagnostic procedures concerning renal diseases in dogs and cats, because it allows for determining a size, shape and location of the kidneys and evaluating their parenchyma and renal pelvis structure. In some cases the final diagnosis of the disease is already possible only after ultrasound kidney examination. Such disorders include, e.g. polycystic kidney disease, hydronephrosis, renal pelvis lithiasis. However, in the case of e.g. glomeruli disease or hypertrophic changes ultrasound examination does not give an ultimate diagnosis but orients further diagnostic procedure. A valuable complement to standard ultrasound examination is Doppler blood flow measurement which is of great significance in diagnosing hypertension. Additionally, ultrasound examination frequently allows for diagnosing diseases of the lower urinary tract or other organs, which may secondarily cause renal function disorders (Barr, 1995; Bigge *et al.,* 2001; Zatelli *et al.,* 2003; Jankowski *et al.,* 2008; Rezaie *et al.,* 2008; Haers *et al.,* 2011).

Preparation of dogs and cats for kidney biopsy: The dietetic preparation of dogs and cats qualified for the kidney biopsy is similar to that of any other procedure performed under general anesthesia. It should comprise 24 h fasting and at least a 6 h break in administration of fluids before the procedure. The kidney biopsy in dogs may be carried out in sternal position or in the right or left lateral position depending on the kidney from which the biopsy is to be collected. However, in cats the lateral position is preferable. The kidney biopsy is a painful procedure and hence it should be performed under general anesthesia or in premedication combined with local anesthesia of the biopsy needle insertion canal. General anesthesia is preferable in most cases. In dogs and cats with renal insufficiency no anesthesia protocol has proved to be really safe. What is important is to maintain adequate blood pressure and renal perfusion during anesthesia. At present, anesthesia with the use of propofol is considered to be the safest anesthesia protocol in animals with renal insufficiency (Osborne *et al.,* 1971; Jeraj *et al.,* 1982; De Rycke *et al.,* 1999; Brovida, 2003; Jankowski *et al.,* 2008; Haers *et al.,* 2011; Silva *et al.,* 2012). The authors of the present article performed kidney biopsies in dogs and cats using the following anaesthesia protocols:

1. Premedication: xylazine at a dosage of 1-2 mg/kg b.w. with atropine at a dose of 0.05 mg/kg b.w. in one intramuscular injection; general anaesthesia-5 mg/kg b.w. thiopental intravenously as initial dose and next according to the effect;
2. Premedication: xylazine at a dosage of 1-2 mg/kg b.w. with 0.05 mg/kg b.w. atropine in one intramuscular injection; general anaesthesia-1- 4 mg/kg b.w. di-hydroxypropylphenol intravenously;
3. Premedication: xylazine 1-2 mg/kg b.w. with 0.05 mg/kg b.w. atropine in one intramuscular injection; general anaesthesia- 4-10 mg/kg b.w. ketamine intramuscularly.

In the authors' opinion, the above anesthesia protocols are safe and make it possible to perform the procedure in an appropriate time. However, in animals that hale not developer renal insufficiency yet, the authors recommend application of anesthesia protocols no 1 and 3, whereas in animals with renal insufficiency they recommend the protocol no 2.

Complications after kidney biopsy: It should be noted that the kidney bioptates should be collected only from the renal cortex (Fig. 4). One must avoid puncturing the deeper kidney layers as there is a danger of serious complications due to rich vascularization in this area (Fig. 5). Penetration of the renal medulla with a biopsy needle may lead to formation of large infarctions or fibroses. The frequency and type of complications occurring after the kidney biopsy is related to the biopsy technique, the condition of the animal referred for biopsy and the operator's experience; their rate is estimated at 1-20%.

Fig. 1: Tru-cut needle – manual system.

Fig. 2: Tru-cut needle – semi-automatic system.

Fig. 3: Tru-cut needle – automatic system (biopsy gun).

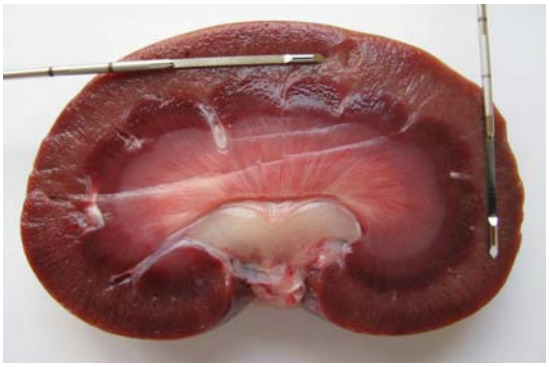

Fig. 4: Correct insertion of the biopsy needle into the kidney.

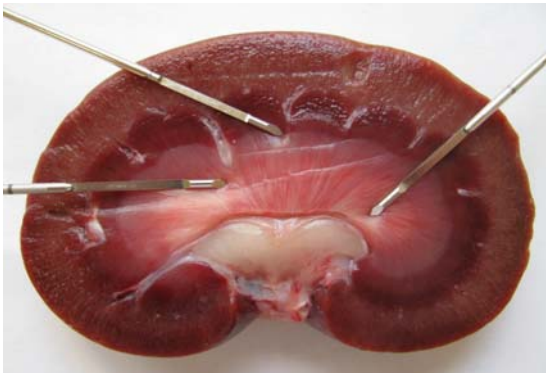

Fig. 5: Incorrect insertion of the biopsy needle into the kidney.

The most common kidney biopsy complications include: microscopic hematuria (20-70%), macroscopic hematuria (1-4%), tenderness in the biopsy needle insertion place, perirenal hematomas (about 9%), hydronephrosis, arterio - venous fistula, damage of the other organs, urinary system infections and death (about 3%) (Grauer *et al.,* 1983; Nash, 1983; Hager *et al.,* 1985; Wise *et al.,* 1989; Groman *et al.,* 2004; Jankowski *et al.,* 2008; Haers *et al.,* 2011).

The kidney sample management: The complex nephro-pathological biopsy diagnostics includes evaluation in the light, fluorescence and electron microscope (Wright *et al.,* 1981; Aresu *et al.,* 2008; Kamiie *et al.,* 2009; Lees *et al.,* 2011).

The tissue material collected for the histopathological examination must be fixed in 4- 10% buffered formalin solution. Next the tissue material is embedded in paraffin and cut into slices not exceeding 4-6 μm in thickness. The next stage is staining by means of four basic methods: hematoxylin and eosin (H-E), Masson's, Jones' and periodic acid-Schiff (p.a.S.) methods. In case of suspected kidney amyloidosis Congo red staining should be performed, as well. The above mentioned stain allows visualizing disorders in the normal tissue structure under the light microscope. Staining with hematoxylin and eosin is a routine stain showing cell nuclei (blue), cytoplasm (pink) and other structural elements of the renal tissue. It is used for general evaluation of the biopsy collected from the kidney. Periodic acid-Schiff and Jones' methods stain basement membranes of the glomeruli capillaries, mesangium, tubules and Bowman's capsules. Those methods are useful in evaluation of basement membranes thickness and possible disorders of their structure. Staining with Masson's method makes the connective tissue green, the cell nuclei brown-red and cytoplasm orange-red. The advantage of that method is the possibility to stain immunoglobulin and fiber deposits bright red, which distinctly contrasts with staining of the examined tissue of other structures (Center *et al.,* 1987; Brovida, 2003; Zatelli *et al.,* 2003; Walker *et al.,* 2004; Vaden, 2005; Jankowski *et al.,* 2008; Scaglione *et al.,* 2008; Yhee *et al.,* 2010; Newman, 2012).

The material for examination under a fluorescent microscope should be placed on the filter paper moistened with physiological solution in the closed container, cooled down to a temperature of +4ºC and immediately sent to the immunofluorescent laboratory. Next, the biopsies are frozen to a temperature of -70ºC and sliced. Then, cryostatic slices are subjected to an immunohistochemical reaction using direct immunofluorescence with antibodies against IgG, IgA, and IgM. That method visualizes immunoglobulins deposited in the glomerulus structures (Kulig *et al.,* 1999; Aresu *et al.,* 2008; Yhee *et al.,* 2010; Lees *et al.,* 2011).

The material for examination under an electron microscope should be fixed in 2-3% glutaraldehyde solution at a temperature of +4ºC and quickly sent to the electron microscopy laboratory for further analysis. That method visualizes all types of renal tissue structure disorders which are not visible under a light microscope (Wright *et al.,* 1981; Brovida, 2003; Walker *et al.,* 2004; Scaglione *et al.,* 2008; Kamiie *et al.,* 2009; Newman, 2012).

In most cases only a histopathological examination of the collected bioptates from the kidney under a light microscope is routinely performed in veterinary practice, due to the high costs of the examinations under fluorescent and electron microscopes.

The presence of five glomeruli in the renal bioptate is considered as a criterion for a valid histopathological evaluation. Sometimes the presence of only one glomerulus in the bioptate may allow a diagnosis. However, the general rule is: the more glomeruli in the biopsy, the more definite the diagnosis (Kulig *et al.*, 1999; Vaden, 2005; Manashirova *et al.*, 2011). The authors of this article obtained 4 to 24 glomeruli (mean 14 glomeruli) in biopsies collected from dogs with glomerulonephritis (Jankowski *et al.*, 2008).

Conclusion: Kidney biopsy is a safe and very useful method to diagnose renal diseases because it makes direct evaluation of disorders and precise qualitative and quantitative determination of the disease process possible. This method allows for appropriate treatment and prognosis of the primary kidney disease. Moreover, the kidney biopsy should be considered as a basic diagnostic method in glomerulonephritis, kidney amyloidosis and kidney dysplasia and kidney neoplastic changes.

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