ABSTRACT

Key words: paraclinical diagnosis, kidney, disease, cat

Renal disease can be induced by many processes kidneys having a limited ability to respond to them. The result is prolonged renal disease in most cases, chronic renal failure, which is the most common disorder among patients with such disease, clinical manifestations, progression and prognosis is variable depending almost every case.

Early identification of kidney diseases is important to limit secondary disorders and to identify the exact etiology in order to establish a correct diagnosis and treatment. If the agent is identified early, treatment can provide a chance for kidney function recovery, However, in patients with advanced chronic renal failure, the treatment will not only lead to re-stabilizing the patient until a new rebound effect of the inflammatory process. Irreversible changes in the kidney may mask the true cause of the inflammatory process, thus making it difficult to determine the exact diagnosis. Besides early diagnosis establishment it is necessary to determine the stage of the nephropathy after the Guide made available by the International Society of Renal Interest (IRIS) (depending on the value of serum creatinine), to establish a protocol for monitoring patients with kidney failure in chronic phase.

The risk factors involved in certain diagnostic procedures such as renal biopsy or imaging techniques, must be well understood, for correct application of methods in kidney disease diagnosis.

So far in the literature are numerous studies related to the diagnosis of renal disease in both human medicine and veterinary medicine, studies detailing the updated methods of clinical and laboratory diagnosis; there are continuous changes of disease, so new methods require the use of and necessary to identify early markers of renal disease.

The aim of the present study was to describe the applicability of laboratory diagnostic methods in the diagnosis of renal diseases in cats, including border diagnostic methods, such as imaging methods and laboratory methods as well as the establishment of a correlation between these methods in order to improve diagnosis.

The research for my PhD thesis with the title "*Researches regarding paraclinic diagnosis in cat nephropathies*" were performed at the University of Agricultural Sciences and Veterinary Medicine of Iaşi and the Faculty of Veterinary Medicine Frederico II in Naples, Italy, during four years of study in the period of October 1, 2010-September 1, 2014. The thesis is structured in two parts: first part entitled "*The present state of knowledge,*" and the second entitled "*Personal Contribution*".

First part it is structured in three chapters, in which they are synthesized data from the literature on the topic of the thesis.

To fully understand the pathological processes affecting the kidney is necessary to know basic morphological data about this organ, which is why the first chapter of the thesis is titled "*Bibliographical data regarding the morphophysiology of kidney in cat "*, reveals the normal morphological aspects of the kidney and also the physiological processes that take place at the level of each segment, emphasizing the importance of each segment.

Chapter II of the thesis entitled *" Etiopathogeny of renal diseases in cats "* shows the main aspects of kidney disease pathogenesis broken down by type. The last chapter of the first part titled "*Updated bibliography regarding paraclinical diagnosis of cat nephropathies"* , makes a review of the diagnostic methods used in determination of renal diseases in cats, also underlining the current state of knowledge in this area.

Second part „P*ersonal Contribution"* comprises six chapters (IV-IX). Chapter IV presents the purpose and objectives of the thesis and the following chapters describe the materials and working methods, the results obtained and their interpretation, discussion and partial conclusions. The last chapter includes the final conclusions of the thesis, which summarizes the results.

Chapter IV with the title" *Aim and objectives "* describes the aim and main objectives of the thesis with the activities necessary to achieve them. The main goal of this thesis was to enrich the knowledge related to the laboratory diagnosis of nephrophathies in cats and to establish a diagnostic protocol for early discovery of these diseases in order to improve the effectiveness of treatment and reduce mortality. It was intended to establish a correlation between pathological processes found in the kidney and the data obtained after the applying paraclinical diagnostic methods. For this a series of hematological, biochemical blood products parameters, urine analysis and also imaging methods, and last but not least the anatopathological examination were monitored; in the case of the last type of exams, correlations were done for

each type of affection, between the parameter values and macroscopic and microscopic lesions of the kidney. One of the major objectives of this thesis was represented by the use of imaging diagnosis in cat renal diseases, objective achieved by identifying the archives through clinical examination and through direct Radiology techniques with, and without contrast substance, the ultrasound examination, including Doppler technique and also computer tomography examination in these conditions, where it was possible. Another aim of the thesis was to apply the laboratory methods in the diagnosis of renal diseases in cats, including: blood analyses to establish specific hematological and biochemical markers in these disorders, urine analyses, as well as conducting histopathological examinations of kidney biopsy specimens, necropsy and histopathological examination of samples.

Researches in chapter V, entitled "*Epidemiological study on cat nephropaties*" followed the determination of the prevalence of renal failure in cats in three veterinary centers in Europe, emphasizing the influence of age or sex on the evolution of this disease. The study included cats presented from November 2010 until June 2014 at three veterinary centers; from the total number of cases only those with renal pathology were selected. The results obtained established a prevalence of renal insufficiency (IR) of 10.83% in the period November 2010-June 2014 for the three veterinary centers.

The age of males with renal failure (7.9 ± 4.4) was significantly lower than the age of female with renal failure (9.3 ± 4.7) ($p = 0.0001$). The mean age of the control group was 7.3 ± 4.9 years, being significantly lower than that of cats in the IR group ($p = 0.0001$). The increased number of females with renal impairment was recorded for the age group 9-11years while for males for the age group 4-5 years. Significant statistical differences were observed between the mean age of cats with the IR between Centre 1 (10,47 ± 4,98) and 2 (8,69 ± 4,47).

In chapter VI, entitled" *Imaging methods used in the diagnosis of renal diseases in cats"* the imaging diagnosis by ultrasound, x-ray with and without contrast substance, as well as computer-tomography in cat renal disease were described, highlighting specific aspects of diagnostic techniques and lesional aspects. Ultrasound diagnosis has been applied in 194 cases, the x-ray in 22 cases, and computer-assisted tomography was applied for a single case. The study material was represented by cats for a medical examination of the kidneys in the Medical Clinic and the laboratory of Roentgendiagnostic of the Faculty of Veterinary Medicine of Iaşi, Romania, in the Interdepartmental Radiology Centre within the Faculty of Veterinary Medicine in Naples, Italy, as well as within the Veterinary Clinic Laterzza from the same town. After

ultrasound examination the following conditions were diagnosed (number of cases): chronic nephritis 72, acute nephritis 30, pyelonephritis 1, renal abscesses 1, renal infarction 2, polycystic kidney 16, hypoplasia1, tumors14, nefrocalcinoza 1, hydronephrosis 24, renal lithiasis 24, renal cysts 6, perirenal pseudocysts 4 , adding up to a total of 194 cases. Plain radiography revealed changes in volume, position and density of the organ; contrast radiography revealed nephrogram changes changes in pyelographic phase and drainage phase. A total of 22 cats with renal disease were diagnosed using X-ray with or without contrast enhancement; these methods are thus complementary in addition to ultrasound examination, a diagnosis assisting in particular in the case of obstructive nephropathies, or renal lithiasis. Computer-assisted tomography is a technique that can be utilized in the case of obstructive nephropathies disease, or in the case of neoplastic processes, recommendations for the use of this method in the diagnosis of renal diseases are few, beacause of its high costs and existence of alternative imaging methods.

Chapter VII entitled" *Researches regarding the lboratory diagnistic methods of renal diseases in cats* " includes data on blood analysis, the determination of hematological, biochemical parameters and blood gases. Combined with hematology and urine analysis, biochemical profiles, can form a basis of diagnosis for most kidney diseases. Interpretation of the results requires an understanding of pathological biochemistry of each abnormal result. Hematological examination carried out has highlighted that the number of RBC, hemoglobin values and hematocrit along with progression of renal failure from one stage to another. Anemia in chronic kidney disease is a normochromic, normocitar nonregenerative anaemia, arising mainly due to production shortages of eritropoetin by the kidneys, and in particular the lack of trophic effect of eritropoetin on hematogene bone marrow. A moderate limfopoenia was observed with neutrophylia , the last being due to glucocorticoid effect in renal failure.

Biochemical examination was carried out for 198 cases of the 384 taken into study, a significant difference was observed for BUN between stage I and stage III and IV of renal failure. For creatinine a significant statistical difference was noted between stage I and all other stages, proving that creatinine has a greater specificity in the diagnosis of renal diseases than BUN. Elevated phosphorus values were observed for stage IV, while for stages II and III showed no statistically significant difference in relation to the values registered for the stage I. There were no significant differences for cholesterol, calcium, sodium, serum amylase. As a result of the statistical analysis of the data obtained through the examination of blood gases we observed a decrease in Tc CO2 and HCO3 values for stages III and IV compared to stage I. Low HCO2

values for stages III and IV indicate metabolic acidosis of renal origin due to the inability of the kidneys to excrete metabolic acid material.

Another chapter deals with aspects of urine analysis in patients with kidney failure. Regarding the methods of urine sampling, cistocentesys has been very well tolerated by cats, and urine samples were sterile in the majority of cases. From the parameters examined, there were no significant statistical differences for specific gravity and Ph between the stages of kidney failure. However urine specific gravity may be a marker of tubular dysfunction, characterized by a decrease in urine reabsorption and tubular secretion. At the examination of urinary sediment, crystals were identified for any of kidney failure stages. The struvite, calcium oxalate and urate were found most often.

The last section provides categorized data about anatomopathological examinations, represented by necropsy, biopsy and histopathological examination. The biopsy technique is also, described in this section. The study material was composed of necropsied cats from November 2010 to June 2014 at the discipline of Pathology and Legal Medicine at the Faculty of Veterinary Medicine Iassy. Gross examinations of the kidneys were made at the surface of the organ, after decapsulation, and sectioning on the great curvature for examining basinetal, cortical and medular areas. Renal Biopsy was performed either by means of guidance concerning or through blind technique. Renal Vascularization was identified with the help of color Doppler to avoid high caliber vessels penetrating and considerable bleeding. Histological samples were processed in the Laboratory of Cell Biology, Histology and Embryology, at the Faculty of Veterinary Medicine of Iassy.

The collected samples were fixed in formalin for 24 hours, dehydrated, cleared, impregnated with paraffin and embedded in paraffin. For histological examination 5 µm sections were obtained, which have been then colored HEA, PAS, MASSON, Van GIESSON, Von KOSSA and NOVELLI. Quantification of renal pathology using digital image analysis was performed using conventional Image software J.

Also to achieve subsequent biochemical data correlation with histological results, we applied a system of histological scoring. Glomerular activity indices (GAI), tubular activity index (TAI) and chronicity index were calculated. In order to achieve this, every lesion received a score from 0 to 3, given by two different morphologists.

During gross pathological examination 40 cases were necropsied and in one case echo guided biopsy was applied. Necropsied cases had the following distribution: 7 cats with

diabetes, 11 with renal failure, 2 poisoning, 4 kidney tumors, 2 hydronephrosis, 2 renal lithiasis, 4 renal cysts, 1 hypoplasia, 1 pyelonephritis, 6 that died from other causes. Macroscopic and microscopic aspects were described extesively for each condition. Following the glomeruluar tuf area measurement mean values of 5708 ± 2348µm^2 for cats euthanized due to other causes than the renal disease 6760 ± 1208µm^2 for diabetic cats and 4768 ±960,8 µm^2 for cats with chronic renal failure were obtained. Lesions encountered for 7 cases of cats with diabetes were represented by Glomerular sclerosis with thickening of the basal membrane Glomerulonephritis, mebranoproliferative, proteinuria, glycogen or hyaline cylinders and seeping lymfohistiocitar proliferation. Renal cysts in polycystic kidney disease have a familiar character, affecting usually Persian breed cats, or crossbreeds of Persian cats. Renal cysts are either due to polycystic kidney disease, either can be acquired due to the presence of lymphohistiocitar compresion or the presence of urinary stones.

Glomerular activity index was lower than in the case of chronic renal failure for cats with diabetes or acute renal failure. Chronicity index showed elevated values for chronic renal failure in relation to the other two groups of disorders for. It is useful to obtain renal tissue samples for histological examination and cytological examination in some patients with kidney disease. However, kidney biopsy is rarely used, being cosidered an invasive method which involves sedation of the animal , involving a large enough risk in patients with this type of disorder. Histopathological examination as a result of renal biopsy and its correlation with other laboratory methods can be the path to a diagnosis, prognosis and therapy of clinical cases ofkidney disease in cats.

Chapter VIII entitled *" Correlation between the results of laboratory tests and changes in renal diseases diagnosed in the cat "* established correlations between macroscopic examinations and direct exams, such as blood tests, biopsy or renal abnormalities, this correlation being a real help in establishing a correct diagnosis and therapy of a protocol in patients with renal disease. To emphasize the importance of these correlations, two studies were carried out, which are widely discussed in this chapter, studies regarding the evaluation of renal pathology using Doppler resistivity index (RI) and expression of nerve growth factor, its specific receptor Tropomiosin kinase A and immunoglobulin G in the kidneys of cats with diabetes and chronic renal failure.

For the first study cats presented at Interdepartmental Veterinary Radiology Center of Naples, in the period from January 2003 to April 2014, subject to an ultrasound examination of

the urinary tract were considered; a total of 116 cats were included in the study. Healthy cats group was represented by 24 subjects, 15 males and 9 females, with mean age of 6.5 years, ranging from 5 months to 15 years; the kidney disease group was represented by 92 of cats, 53 male and 39 female, 20 of which with chronic nephritis, 14 with acute nephritis, polycystic kidneys, 13 with 12 end stage renal failure, renal lithiasis, with 12 11 with hydronephrosis, 10 with kidney tumors (carcinomas focal, 7 3 lymphoma diffuse). The mean value of the RI obtained for normal subjects was significantly different between the two kidneys, a unique situation for studies in veterinary medicine, until now. Our results also showed a weak influence of age for the left kidney. However, by grouping the sample of the normal subjects according to the age groups, no influence of age was demonstrated. The lack of correlation with age in cats permits us to consider RI as an independent index useful in subjects of any age.

For the second study 7 cats with diabetes and chronic renal failure were taken into account, and tissue samples were collected for histopathology and immunohistochemistry. Histological results highlighted glomerular lesions of nodular glomerulosclerosis (Kimmelstiel-Wilson nodules), glomerular hyalinization , interstitial fibrosis and glomerular proteins in the urinary space of glomeruls. Also tubular atrophy, glomerular sclerosis and mild mesangial expansion were observed .

Intraepithelial localization of IgG was observed after the immunochemical reactions in the proximal tubes, with intensely colored nuclei. No Ig G deposits were observed in glomeruli Intense positivity for NGF (nerve growth factor) and its receptor tropomyosin receptor kinase A (trkA) in the tubular cells was also observed, the proximal and distal convoluted tubes being especially targeted.

These studies come to supplement the existing data in the literature and to bring new information for diagnosis improvement, and also for prognosis and eventually treatment of renal diseases at the cat.

The last chapter of the thesis, chapter IX, includes general findings of research regarding diagnosis of nephropathies in the cat, both for practice and for future studies on this topic.