

USEFULNESS OF SERUM CYSTATIN C MEASUREMENT FOR ASSESSING RENAL FUNCTION IN CATS

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Abstract

The aim of the work was to determine the concentration of serum cystatin C and usability of its measurement in diagnostics of renal failure in cats. The reference point was the value of glomerular filtration rate (GFR), calculated on the basis of plasma clearance of iohexol (Pcio). Seventy cats were divided into two groups: group I – 24 healthy cats, group II – 46 cats affected with renal dysfunction. The measurement of serum cystatin C was done according to immunonefelometric method. In order to estimate Pcio, the following blood samples were collected: pre-sample – 0, and samples collected 120, 180, 240, and 300 min after intravenous administration of iohexol. Its concentration was determined using high performance liquid chromatography. Pcio was obtained using a one-compartment model, according to the method developed by Krutzen Back and Nilsson-Ehl. Serum concentration of cystatin C in cats with renal dysfunction was significantly statistically higher (1.3 ± 0.6 mg/L) as compared to the group of healthy animals (0.7 ± 0.2 mg/L). Statistical analysis of the data confirmed strong correlation between GFR and serum concentration of cystatin C ($r = -0.51$). Data analysis on the basis of the receiver operating characteristic curve proved that cystatin C seems to be more reliable parameter than creatinine in the assessment of kidneys function in cats and the measurement of cystatin C in blood serum can be of a considerable meaning as far as early diagnosis of renal failure in cats is concerned.

Key words: cats, renal failure, cystatin C, iohexol, glomerular filtration rate.

Aiming at the assessment of renal function in cats, the measurement of serum concentrations of urea and creatinine is the most commonly used in practice. Unfortunately, the usefulness of both of these parameters in early diagnosis of renal diseases is far from being satisfactory. Significant increase in concentration of both parameters in blood is observed but at the moment when 70% of active nephrons have already been damaged (22, 24). Moreover, a number of factors of non-renal origin (body weight, age, sex, diet, other diseases) additionally affect concentration of both parameters in blood (10, 18, 22, 24). It has been generally assumed that the most reliable single parameter for the assessment of renal function is glomerular filtration rate (GFR). Numerous authors believe that plasma clearance of iohexol (Pcio), apart from inulin, is a “gold standard” in determination of GFR in cats and dogs (5, 7-9, 19, 20). The mean GFR in healthy cast measured by Pcio is 1.83 ± 0.64 (mL/min/kg) (11). The dosage of iohexol given intravenously is different: 450 mg/kg b.w. (9) or 90 mg/kg b.w. in healthy cats and 45 mg/kg b.w. in cats with reduced kidney tissue (19). In human medicine, cystatin C has been considered as a new marker of renal failure, a promising alternative to creatinine concentration in blood serum measurement. It is a

protein belonging to the family of cysteine protease inhibitors, produced, at constant speed by all nucleated cells of an organism (24, 16, 25). Considering its physical and chemical properties, it seems to be an ideal endogenous indicator, whose concentration in blood is nearly totally dependent on GFR. The mentioned protein undergoes free filtration and, after its absorption in proximal tubules, it becomes decomposed, without returning to blood (12, 24, 16). Practically, it does not enter urine unless proximal tubules get injured (26, 6). In nephrology, this endogenous substance is viewed as a marker of renal excretory function, at least as sensitive, or even more sensitive than creatinine (21, 22). In veterinary medicine, positive trials involving the use of cystatin C concentration measurements have been conducted in rats (3) and dogs (1, 28). Unfortunately, except the article by Martin (17), no reports regarding the attempts to use the serum concentration of cystatin C in diagnostics of renal failures in cats can be found in the literature.

The purpose of this research was determination of reference range for serum concentration of cystatin C in healthy cats, the assessment of the effect of non-renal originating factors on cystatin C concentration, and comparison of concentration of cystatin C with concentration of creatinine. The comparison of these two parameters was possible using relation with

measured plasma clearance of iothexol (P_{cio}), and an analysis based on receiver operating characteristic (ROC). The last purpose of this research was to assess the possibility of using cystatin C concentration measurements in early diagnosis of renal failure in cats.

Material and Methods

Research was conducted on 70 cats of different sex and breed (67 European cats, one Persian, two Devon Rex), various age (9 months to 17.5 years of age), and body weight (2-8 kg). All cats were patients of the ambulatory of our Department. On the basis of the data obtained from interview, clinical examination, blood and urine analysis, and ultrasonography of abdominal cavity, the cats were divided into two groups: 24 healthy cats and 46 affected cats. Additionally, the group of affected cats was subdivided into four subgroups, according to the classification by the International Renal Interest Society (IRIS) (7, 23). In the past, chronic renal failure (CRF) was classified as mild, moderate, or severe, based on laboratory findings and clinical signs. In IRIS system, IV stages of CRF are based on the degree of kidney function. Prior to blood test, the cats underwent 12 h starvation with free access to water. Blood analysis (morphology and chemistry) was done with the use of the following devices: apparatus ABC Vet (Horiba ABX) for blood morphology examination in animals, and apparatus Max Mat PI (Alfa Diagnostics) for chemistry examination. The reference data elaborated by Winnicka (29) were accepted as standard values. Ultrasonographic examination of abdominal cavity, especially the urinary tract, was done using apparatus Envisor C (Philips) with 3/12 MHz linear probe.

Blood samples were collected from all cats at 0 min and, subsequently, after 120, 180, 240, and 300 min from intravenous administration of iothexol. The dose of this preparation was 90 mg/kg b.w. for non-azotaemic cats and 45 mg/kg b.w. for azotaemic cats (19). Serum

cystatin C concentration and plasma clearance of iothexol were measured in the Department of Laboratory Medicine, Medical University in Wrocław. Cystatin C concentration was determined with immunonefelometric method – Penia, using the Dade Behring apparatus, according to tests N Latex Cystatin C OQNM11. Iothexol concentration in blood serum was determined by high performance liquid chromatography (HCLP), with the use of the HP1100 device (Hewlett Packard). To calculate plasma clearance of iothexol, the method modified by Miyamoto (19) was applied. Serum creatinine was determined by enzymatic colorimetric assay. Serum urea was measured using method comprising urease and glutamate dehydrogenase (GLDH). GFR was estimated from plasma clearance of iothexol by the method of Brochner–Mortensen (4). In brief, a plasma tracer elimination curve was generated and clearance was calculated by dividing the injected dose by the area under the curve (AUC), estimated using a one-compartment pharmacological model. The obtained values of P_{cio} were standardised according to body weight. GFR, estimated basing on P_{cio} , was applied to compare cystatin C, creatinine, and urea data as exponents of renal function in cats.

The obtained results were expressed as mean values and standard deviation values (SD). The results were analysed statistically using Tukey and Mann-Whitney tests. Mutual correlation between data was investigated using Spearman correlation coefficient. Permissible error of statistical analysis was assumed at the level of 5%, assigning it as the level of statistical significance at $P < 0.05$.

Results

The results obtained in the group of healthy cats and cats with kidney diseases, are presented in Table 1. Data shown in Table 2, represent the results obtained in the group of affected cats additionally divided into four subgroups according to IRIS classification.

Table 1

Mean values of selected parameters in the groups of healthy and affected cats. Classification of animals on the basis of creatinine concentration in their blood serum according to standards accepted by the laboratory at Department of Internal Diseases with Clinic for Horses, Dogs and Cats of Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences

Cats	Urea (mmol/L)	Creatinine (μ mol/L)	P_{cio} (mL/min/kg b.w.)	Cystatin C (mg/L)	Number of cats
Healthy	8.9 ± 1.8	131.0 ± 34.3	2.4 ± 0.8	0.7 ± 0.2	24
Affected	20.9 ± 22.0	340.2 ± 437.7	1.2 ± 0.7	1.3 ± 0.6	46

P_{cio} - plasma clearance of iothexol (GRF); $\pm$ SD.

Table 2

Mean values of selected parameters in the groups of affected cats, formed according to IRIS classification

Subgroup	Urea (mmol/L)	Creatinine (μ mol/L)	Pcio (mL/min/kg b.w.)	Cystatin C (mg/L)	Number of cats
IRIS score					
1	9.1 $\pm$ 1.5	117.8 $\pm$ 13.1	1.5 $\pm$ 0.7	1.1 $\pm$ 0.3	16
2	10.1 $\pm$ 3.8	178.9 $\pm$ 29.0	1.4 $\pm$ 0.5	1.0 $\pm$ 0.5	16
3	30.1 $\pm$ 7.8	351.3 $\pm$ 53.4	0.7 $\pm$ 0.3	1.4 $\pm$ 0.3	6
4	59.4 $\pm$ 26.3	1,099.3 $\pm$ 629.3	0.5 $\pm$ 0.4	2.0 $\pm$ 0.7	8
Total	20.9 $\pm$ 22.1	340.2 $\pm$ 437.7	1.2 $\pm$ 0.65	1.25 $\pm$ 0.6	46

Symbols as in Table 1.

Cystatin C serum concentration in healthy individuals amounted 0.7 ± 0.2 mg/L, while in cats with kidneys' failure it was 1.3 ± 0.6 mg/L. Statistical analysis of collected data proved a strong correlation between GFR and cystatin C in blood serum ($r=-0.51$). Slightly weaker correlation was recorded between GRF and creatinine ($r=-0.46$). When analysing GFR dependence on age, sex, and body weight, the correlation coefficient was statistically equal to zero.

In data analysis by ROC method (Fig. 1), the area below the curve (AUC) for particular parameters amounted: 0.854 for plasma clearance of iohexol (Pcio) within 95% credibility interval (0.753-0.954); 0.819 for cystatin C in 95% credibility interval (0.723-0.915); 0.744 for creatinine in 95% credibility interval (0.63-0.858); 0.689 for urea in 95% credibility interval (0.567-0.811).

and serum concentrations of urea, creatinine, and cystatin C.

Discussion

The results of Pcio in healthy cats were similar to reference values published by other authors (9, 15, 27). They differ only slightly from those reported by Miyamoto (19, 20), which can probably be connected with the other procedure of examination – animals underwent sedation in the course of the experiment. As it has been proven in the present research, Pcio in cats suffering from renal disease is significantly statistically lower than in healthy cats. The results obtained in this group of animals are comparable to the ones published by Miyamoto (20), referring to the cats with experimentally removed 75% - 83% of kidney tissue. It is relatively difficult to compare the results that involve plasma clearance of iohexol in ill cats divided according to IRIS classification, since in international literature there is no information about any earlier conducted research. Based on the data presented in the Table 2, it can be seen that the serum concentrations of urea and creatinine in cats from the fourth group (division according to IRIS) deviates significantly from the values of these parameters in individuals classified to the remaining groups (I, II, and III). Such high concentrations of urea and creatinine may suggest a developing end-stage renal insufficiency. It is interesting that most of the animals classified to this group did not show clinical symptoms other than polyuria, increased thirst, loss of appetite, and a little more sleepiness. Perhaps the described behaviour of animals could be attributed to the fact that cats were mostly outgoing, middle-aged individuals. The owners, not having full control over the animals, considered the changes in cats' behaviour to be attributed to age of animals. In this group, there was only one young cat, after a traffic accident, which has developed acute renal failure due to extra renal uraemia. Unfortunately, in the author's opinion the classification of IRIS is ambiguous. According to various authors, a noticeable increase in serum creatinine results from the damage of approximately 70% of active nephrons. This fact may explain why in cats with chronic kidney disease assigned to group I (division according to IRIS)

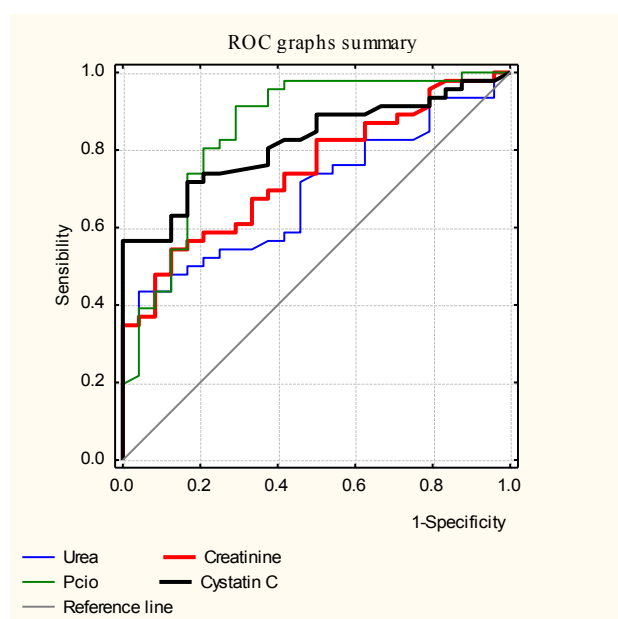


Fig. 1. Graphical expression of sensibility and specificity of four examined methods. Diagram presents the measurement of plasma clearance of iohexol (Pcio)

creatinine does not exceed the limit of 220 $\mu\text{g/L}$. Moreover, often there are no clinical signs of the disease, even in individuals in the second stage of renal insufficiency (group II according to IRIS score). Own observation agrees with the observations of Plotnick (23). According to this author, the first symptoms of kidney disease in the form of polyuria/polydipsia, are noted in the cat in the late second or early third stage (division according to IRIS), when the damage includes about 66% of active nephrons. What is more, clinical signs of organ dysfunction fully reveal only in the third and fourth-stage of renal disease, at the time when the damage occurs in at least 75% of active nephrons. Plotnick, like other authors, points out that some cats with primary glomerular disease, despite azotaemia, can produce urine with a specific gravity above the 1,035.

Very interesting results can be obtained when the classification into healthy and ill animals is based not on the values of serum concentration of creatinine but on the value of plasma clearance of iohexol. Providing that in healthy cats, P_{cio} threshold value amounts 2.04 mL/min/kg, all cats showing P_{cio} value below 2.04 mL/min/kg are considered as the ill ones. The mentioned assumption results in shifting threshold values of reference range for urea and creatinine concentrations in blood serum. Threshold value for urea concentration in healthy cats should have to be diminished to 7.41 mmol/L, while individuals characterising creatinine concentration above 122 $\mu\text{mol/L}$ should have to be classified as ill, or at least suspected to be ill. As far as healthy individuals are concerned, the threshold value for cystatin C serum concentration should be set at the level of 0.89 mg/L. This seems to be a very intriguing result; especially considering the fact that the analysis of areas (AUC) under ROC curve apparently prefers P_{cio} as the method of assessing glomerular filtration rate. ROC evidently shows that measurement of plasma clearance of iohexol is the best method, out of four types of measurements, to reliably estimate glomerular filtration rate. This method is the most sensitive and the most reliable, being at the same time, very safe. The main advantage of using iohexol is the time of its elimination, which is about 1/3 quicker compared to creatinine. Thus, it makes this method safer, and it is recommended for animals displaying different degree of dehydration or weakened renal function (11). The results respecting ROC analysis obtained by Wehner (28) were nearly identical and confirmed higher diagnostics value of cystatin C in relation to creatinine. Moreover, for creatinine statistical null hypothesis concerning the equality of areas (AUC) under ROC curve for a pair of variables - P_{cio} and creatinine is true only at $P < 0.05$, while it stops being true at $P < 0.1$, contrary to the pair of variables P_{cio} and cystatin C. The latter option confirms the fact that cystatin C is a better GFR indicator than creatinine. Concerning cystatin C itself, it has been proved in this work that contrary to Martin (17), the measurement of cystatin C serum concentration in cats is possible when using commercial tests for cystatin C determination in humans, and that immunonefelometric method (Penia) is

more reliable than turbidimetric method (Petia), which was used by Martin. The problem regarding advantage of Penia method over the elder Petia method and the difference in the results obtained according to the chosen method was described by Jonkisz *et al.* (13). Factors such as age, sex, or body weight did not affect cystatin C serum concentration in cats, which remains in agreement with the results reported by other authors (2, 10, 21, 24)

In conclusion, the data obtained for the examined population of cats indicate that reference values for serum concentration of cystatin C in healthy cats do not exceed the range of 0.3 to 1.1 mg/L. The factors such as age, sex, and body weight do not affect the serum concentration of cystatin C. Analysis of the obtained results, based on ROC curve, proved that cystatin C seems to be a more advantageous parameter than creatinine in the assessment of renal function diagnosis in cats. Determination of cystatin C concentration can be a significant indicator regarding early diagnosis of kidney diseases in cats.

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