University of Veterinary Medicine Budapest

Department and Clinic of Internal Medicine

Comparison of urinary albumin-to-creatinine ratio in urine samples taken by cystocentesis or free catch method

A vizelet albumin/kreatinin arányának összehasonlítása cisztocentézissel vett és spontán ürített vizeletekben

Alisha Schnitzer

Supervisor: Dr. Fruzsina Falus,
Department and Clinic of Internal Medicine

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1 Introduction

In the veterinary field, the gold standard urine sampling method is cystocentesis, but there are other valid solutions to get good urinalysis results. Finding the perfect fit for every patient is essential with the minimum stress for the animal and a maximum of reliable information from the urine.

Cystocentesis is the only sterile method, especially regarding bacterial contamination. Where on the other hand, free catch method has a much higher risk of bacterial contamination. Cystocentesis sampling is more expensive than free catch sample and can also cause more stress for the animal.

There are already studies that have dealt with similar topics. The study of Beatrice et al., 2010 compared urine protein-to-creatinine ratio (UPC) in samples collected via cystocentesis versus free catch in dogs [1]. Mortier et al., 2023 did a study of cats about UPC and urine specific gravity compared between cystocentesis and home sampling [2]. Both studies showed that home sampling is valid and shows no significant value change.

After these two studies, it is very interesting to figure out if that applies to other variables in the urinalysis. This thesis aims to determine if it makes any difference in the urine albumin-to-creatinine ratio (UAC) value in dogs, whether the urine sample was taken by cystocentesis or free catch.

It is a fundamental question to determine whether the sampling method significantly affects the laboratory values. If not, it could mean the owner and doctor can decide together which is the best fitting sampling method according to the best for the animal.

2 Abbreviations

ACEIs, Angiotensin-converting enzyme inhibitors

ARBs, Angiotensin receptor blockers

CKD, Chronic kidney disease

IMGN, Immune-mediated glomerulonephritis

IRIS, International Renal Interest Society

PLNs, Protein-losing nephropathies

PU/PD, Polyuria and polydipsia

RAAS, Renin-angiotensin-aldosterone system

SDMA, Symmetric dimethylarginine

SSA, Sulfosalicylic acid test

UAC, Urinary albumin-to-creatinine ratio

UPC, Urinary protein-to-creatine ratio

USG, Urine specific gravity

3 Literature review

3.1 Urinalysis

3.1.1 Importance and indications of urinalysis

Urinalysis is considered as part of the minimum database. In general, for a urinalysis, not much equipment is needed, it is easily performed with trained staff and is inexpensive. There are different indications for urinalysis, for example, changes in urine colour, clarity, volume, and odour. Other indications for urinalysis could also be dysuria (painful urination), stranguria (painful but frequent urination of small volumes), pollakiuria (frequent abnormal urination), and polyuria (increased amount of urine production). Urinanalysis helps us to diagnose urinary tract diseases but also gives information about other systemic diseases like liver failure or hemolysis. Furthermore, it is a useful method when diagnosing proteinuria, diabetes mellitus, Cushing's disease, hyperthyroidism, and other illnesses causing polyuria and polydipsia (PU/PD) [3, 4].

3.1.2 Urine sampling methods

Urine can be collected by various kinds of methods:

- cystocentesis
- free catch
- catheterisation.

Cystocentesis is done mostly by ultrasound guided with a needle of the size 22G or 23G directly introduced through the abdominal wall into the bladder. It is a very useful sampling method in veterinary medicine, as it is the only sterile way to collect urine, but on the other hand, cystocentesis sampling can also cause stress for the animal, so it can even come to the point that the patient gets aggressive. The procedure of cystocentesis has a very low risk of bladder wall tears and can cause microscopic haematuria as a side effect in the animal. Cystocentesis is also difficult to perform in low bladder filling [5].

Another method to collect urine is the free catch method. Here, you take a sterile container, open the container shortly before the animal pees and put it under the dog when urinating. The best is to catch a mid-stream sample, as this minimises bacteria contamination from the

distal urinary tract or genital tract. Free catch samples are also more easily contaminated by the owner, as the owner could touch the container on the inside.

Cystocentesis is more expensive than just sending a free catch sample to the laboratory. Cystocentesis only takes a few seconds to a minute, so it is not time-consuming, especially with a cooperative, calm, not stressed dog. For some owners, the free catch method is more challenging and takes more than just one attempt to get the urine sample, but for many animals, free catch is less stressful. Consequently, free catch may take more time than a cystocentesis, but if done correctly, it should not take much longer than a cystocentesis.

Urine sampling via catheterisation is done in a clinic by a veterinarian with a catheter placed into the urethra of the animal up to the bladder to get urine out. Catheterisation can cause trauma on the urethra and haemorrhages as a side effect. Also, there is a risk of potential iatrogenic infection of the urinary tract [5].

3.1.3 Urinalysis in general

Often, urinalysis is done in-house, as it does not require many laboratory supplies, but it can also be sent to an outside labaratory, depending on the veterinary clinic and practice. The best is to use a fresh sample, not older than 30 minutes, from a sterile container. If the urine cannot be examined within 30 minutes after sampling, it should be put in the refrigerator for cooling with a minimum exposure to light. Evaluation of samples from the refrigerator can be done for up to 24 hours. It is important to note that the sample needs to be warmed up to room temperature for the analysis.

A morning or fasting sample has higher urine specific gravity and lower pH, whereas a midday sample tends to have a lower specific gravity and higher pH. We should always consider that when reading the results [3, 4].

Physical examination of the urine includes assessment of colour, clarity and specific gravity. The colour of urine is normally colourless or pale yellow. If it is very concentrated, it can be darker yellow. Abnormal colour would be pink-red, red-brown, brown-black, or even yellow-green or yellow-orange. Pink-red or brown-red urine could have possible causes like haematuria, hemoglobinuria or myoglobinuria. Brown-black urine could mean that there is methemoglobin in the urine. Yellow-green urine can contain bilirubin or biliverdin as a possible cause.

The clarity of normal, fresh urine should be clear to clouded. The odour of physiologic urine has an odour of ammonia, but the odour depends on the concentration of the urine and the animal species [4].

The specific gravity is the comparison of the weight of the solution to the weight of the same densitiy of water. For determining the specific gravity, a refractometer is used. There are specific refractometers for dogs and cats that is important to have in mind. The specific gravity has different categories in accordance with their values. Urine-specific gravity is depending on the fluid and electrolyte homeostasis of the animal's body, even medication and serum urea nitrogen and creatinine concentration play an important role. It is called hypersthenuria when the value is ≥1.030 in dogs and ≥1.035 in cats, which means the urine is well concentrated and there is an proper renal response to antidiuretic hormone or it means that more than 1/3 of all nephrons are working. In the range of >1.012 – <1.030 in dogs and >1.012 – <1.035 in cats can be normal in well hydrated animals, but is pathologic in case of dehydration or azotemia. It is called isosthenuria in a range from 1.008 – 1.012 in dogs and cats, which means the urine has not been concentrated or diluted. 1.008 – 1.012 as specific gravity is similar to that of plasma and can be a normal when fluid therapy was applied. It is pathologic in case of dehydration or azotemia. It is called hyposthenuria in a range of <1.008 in dogs and cats. The urine is diluted compared to plasma. It is mostly pathologic in carnivores, especially when there is dehydration or azotemia [3, 4].

The urine dipstick test is also a part of the urinalysis. This test can detect several different variables in the urine. It changes the colour according to the result of the different values. For this test, fresh, well-mixed, and not centrifugated urine should be used. It can detect glucose and ketones, which should be negative in a healthy animal, and bilirubin, which should also be negative (or slightly positive in dogs). The pH of normal urine should be between 5.0 and 7.5, but dipsticks are not very precise with pH. It also tests for protein and blood (or heme) that are physiologically negative as well [4].

Urine sediment examination can also be used to evaluate urine. The urine should be centrifugated, and the sediment should be put on a glass slide. After that, the sample is examined under a microscope at 10x objective for crystals or casts and 40x objective for cells and bacteria. In a healthy urine sample, there are only a few red blood cells, but it depends on how the sample was taken, as with cystocentesis, this can be normal. No cast,

crystals or bacteria should be found in healthy urine. White blood cells are only seen when there is inflammation, infection, trauma or when the sample was taken by voiding [3].

The urine protein-to-creatine ratio (UPC) is a laboratory examination and gold standard in the veterinary field to find out whether a patient has proteinuria or not. For proteinuria, measuring the UPC is the most reliable method. The reason for using the protein-to-creatinine ratio is that creatinine is freely filtered by the glomeruli and not reabsorbed, so creatinine stays more or less constant. The protein is measured in relation to creatinine concentration to consider the degree of concentration in the urine and standardise the protein concentration [6]. The best would be to measure protein, which is excreted from the urine for 24 hours, but this is, in most patients, not realistically doable, so we use the UPC measured from a single spot urine sample [4, 6]. UPC is one of the important factors for proteinuria and is also part of the International Renal Interest Society (IRIS) staging system for chronic kidney disease.

3.2 Proteinuria

3.2.1 Overview

Proteinuria generally means any kind of excessive amount of protein in the urine, for example, albumin, globulins, mucoproteins, or Bence-Jones proteins [7]. A low amount of protein in the urine can be normal in dogs. Clinically, proteinuria has three elements: persistence, localisation, and magnitude. If the proteinuria is detected on three or more occasions, two weeks apart from each other, it is defined as persistent proteinuria and can be categorised as:

- pre-renal
- renal
- post-renal [8].

The definition of localisation is finding the site or mechanism causing the proteinuria. History, physical examination, and urinalysis are important to find the localisation of the proteinuria.

When pre-renal and post-renal proteinuria is ruled out, the magnitude gets important. Magnitude can help to decide if the proteinuria is glomerular or tubulointerstitial. The higher the magnitude, the higher the chance of glomerular disease and mortality [8].

Proteinuria can also be classified as physiological, also called functional, or pathological proteinuria. Physiological proteinuria is typically mild and transient, and no kidney lesions are found. Pathological proteinuria means lesions are found in the kidney and are most likely persistent. Pathological proteinuria can be differentiated between urinary and non-urinary causes. If renal proteinuria is pathological, it can have defects in the glomerular filtration barrier, tubular reabsorption, or interstitial damage. The different categories of persistent proteinuria can have a variety of causes. For example, fever, shock, cardiac diseases, acute pancreatitis, and systemic hypertension can cause pre-renal proteinuria. Pre-renal proteinuria can also be caused by multiple myeloma, drug reactions or hyperadrenocortism. Renal proteinuria can come from acute kidney disease, chronic kidney disease, glomerulopathy, diabetes mellitus, acute pancreatitis, viral diseases, systemic hypertension, endocarditis, drug reaction, hyperadrenocorticism, neoplasia, tick-borne disease, leptospirosis, endocarditis, heartworm disease, exogenous steroid use and any severe inflammatory disease [9, 10]. Post-renal proteinuria can be separated into urinary, such as lower urinary tract diseases or reproductive tract diseases, like vaginitis. Post-renal proteinuria is never persistent; proteinuria should be gone once the underlying illness is treated [9, 11].
Once proteinuria is found, it is recommended to repeat UPC measurement after some weeks to confirm persistent proteinuria. Clinical examination and blood work should also be done, including CBC, chemistry panel, blood pressure measurement, and urine culture. A urine sample should be retaken if the proteinuria is only very mild 2-4 months later. It is always important to look for underlying diseases [11].

Patients with chronic renal disease and proteinuria have a worse prognosis and a shorter expected life span, especially when the UPC is greater than 1.0 [8].

3.2.2 Detection of proteins in the urine

If we suspect the patient has proteinuria, a basic clinical examination should be performed, and clinical signs should be considered [9]. After that, various tests are available to detect proteinuria, such as a

- urine dipstick colourimetric test,
- sulfosalicylic acid test (SSA),
- urine protein-to-creatine ratio (UPC).

The urine dipstick colourimetric test has moderate sensitivity but poor specificity. This test is a chemical strip, where a few drops of urine have to be put on it. A colour change occurs according to the protein content of the urine sample. This test has poor specificity, and therefore, false positive results often occur. However, it is easy to use, and we see results fast [12]. It can come to false negative results with urine dipstick colourimetric test if the albumin concentration is very low or, if the patient has Bence-Jones proteinuria, or if the urine is diluted or acidic. This is more common in the urine of cats than dogs [11]. Also, it happens that the test is false positive if the urine is alkalic or urine with haematuria, pyuria, or bacteriuria [10, 12].

The SSA test is semi-quantitative and simple but can be false positive if there are any radiographic contrast agents or antibiotics like penicillin or cephalosporins in the urine. The SSA works by mixing urine supernatant and 5% SSA and we record the turbidity that results from the precipitation of proteins. It is evaluated on a scale from 0 to 4+. SSA test can detect albumin, globulins and Bence-Jones proteins. A false negative does not happen as often as with the dipstick test, as the SSA test is more sensitive [7].

Most laboratories use both tests as they are easy to use, give fast results and are inexpensive. If they are positive, proteinuria should be confirmed by measuring UPC [7].

The UPC test is the gold standard nowadays [11, 12]. According to the IRIS classification of proteinuria with chronic kidney disease, UPC less than 0.2 is non-proteinuric, between 0.2-0.5 in dogs and 0.2-0.4 in cats is borderline proteinuric, and more than 0.5 or 0.4 is proteinuric [8].

All the previously named tests are more sensitive to albumin than other proteins. There are also particular tests for albumin detection, like the Heska ERD semiquantitative test [7], or it can be measured by laboratory methods. Albuminuria is generally expressed by the urinary albumin-to-creatinine ratio (UAC) in the same way as UPC. The UAC is measured from a single urine sample, often taken by cystocentesis. In humans, the UAC ratio is considered accurate for detecting microalbuminuria [13]. If an abnormal elevation in albumin concentration is not detectable by the dipstick method, it is called microalbuminuria or subclinical albuminuria [14]. In people, the albumin concentration > 1.0mg/dL is defined as microalbuminuria; if the albumin concentration is above 30 mg/dL, it is called albuminuria [7].

In the study of Falus et al. 2022, the canine reference interval of UAC was established: 0-19 mg/g (confidence interval 13-28 mg/g). They discovered that the animal RI is very similar to the human RI (0-30 mg/g) but narrower [15].

Proteinuria can be further differentiated with the help of sodium dodecyl sulfate-agarose gel electrophoresis. In this procedure, urine is loaded on the gel, and an electric current separates the urine proteins based on their molecular weight and charge. Glomerular leakage is indicated if there are high molecular weight proteins. If there are lower molecular weight proteins, it speaks for tubular cells which are not working correctly [16].

3.3 Albuminuria

Albuminuria is an abnormal loss of albumin in the urine and can also be caused by renal, pre- or post-renal disorders [12, 15]. Albuminuria is an early marker for renal disease.

Similarly, to proteinuria, various reasons can cause albuminuria. The most important is renal damage, which can have infectious, inflammatory, or even neoplastic reasons. It is important to note that albuminuria is not always an indication of kidney disease. For example, it can be detected in Dirofilaria immitis infection, diabetes mellitus, or osteosarcoma, too. Also, some medications are under suspicion to increase albuminuria, like corticosteroids [12].

There are different indications for testing for microalbuminuria, for example, when a chronically ill animal shows clinical signs of chronic kidney disease or when conventional screening methods like SAA have conflicting results [7]. Albuminuria tests should be done regularly for a patient genetically predisposed to glomerular disease like Wheaten Terriers, as albuminuria can precede proteinuria and thus be the earliest sign of glomerulopathy. Also, animals with hypertension should be checked for albuminuria [14, 15]. The study of Bacic et al. 2010 aimed to determine if there is an elevation in albuminuria in chronic kidney disease patients with hypertension, like in humans. They detected that the UAC ratio was higher in dogs with hypertension than those with normotension [14].

3.4 Glomerular diseases

Kidney failure is one of the most common organ failures in animals. Glomerular disease is a frequently occuring form of chronic kidney disease in dogs, but the true prevalence is unknown. If microalbuminuria is detected, it’s likely an early sign of glomerular disease [8]. Protein-losing nephropathy is more often seen in middle-aged to older dogs than in cats.

The glomerular capillary wall has three layers: the fenestrated endothelium, basement membrane, and podocyte foot processes. The three layers keep big negatively charged proteins like albumin in the circulation; only smaller and positively charged proteins pass through the wall. The filtration depends on the plasma proteins, blood pressure, and integrity of the layers. It filters 20% of the cardiac output and produces litres of ultrafiltrate daily. Proteinuria and albuminuria are indicators of glomerular disease. Primary glomerular diseases include amyloidosis, glomerulonephritis, and hereditary glomerulonephropathies [11]. The most common form of glomerular disease is immune-mediated glomerulonephritis (IMGN). There is also a non-immune mediated form [16, 17]. In the background of immune complex glomerulonephritis often there is a chronic, extrarenal disease, for example, neoplastic processes like leukaemia, lymphosarcoma, and other neoplasms; bacterial infectious processes like borreliosis, bartonellosis, brucellosis, endocarditis, pyometra, or pyoderma; viral infectious processes like canine adenovirus; parasitic infections like dirofilariasis, babesiosis leishmaniosis or non-infectious processes like pancreatitis, inflammatory bowel disease or polyarthritis. In these cases, circulating immune complexes deposit within the glomeruli. In fewer cases, immune-complex formation happens within the glomeruli against the glomerular basement membrane [17]. The non-immune-mediated form of glomerulonephritis is often congenital or is caused by toxins or hypertension. Amyloidosis has an acquired form, which comes from an underlying inflammation or neoplastic disease but is often also idiopathic. Amyloidosis is also a congenital form, often found in Shar-Pei dogs. Some breeds have familial glomerulopathy more often in their family history than others, such as Labrador retrievers and Golden retrievers. Beagles, Collies, and English foxhounds are more predisposed to amyloidosis. These breeds may have a higher incidence of glomerular disease, but if that represents the whole population of these breeds needs further investigation [11].

3.4.1 Clinical symptoms

Clinical signs of patients with glomerular disease can be very different. Especially in the beginning, patients are often asymptomatic. They can also show signs of kidney disease like polyuria and polydipsia, vomiting, or non-specific symptoms like weight loss or lethargy. Often, when they show symptoms, it is already late in the disease. If an animal with suspected glomerular disease comes into the clinic, the physical examination is often unremarkable unless one of the systemic illnesses leads to the glomerular disease. If CKD

stage 4 is already reached, oral ulcerations, pale mucous membranes, or dehydration can be seen. Hypertension, ocular diseases, cardiac murmur, or neurological abnormalities can occasionally be seen, too [17]. It can also come to nephrotic syndrome. The nephrotic syndrome has four criteria: hypoalbuminemia, proteinuria, hyperlipidemia, and extravascular fluid accumulation. If nephrotic syndrome is already present, the glomerular disease is already in a late stage, and the prognosis is poor [16, 18].

3.4.2 Diagnostic workup for glomerulopathies

The diagnosis of a glomerular disease is made based on persistent and severe proteinuria. The proteinuria should be quantified by UPC. Glomerular diseases are often accompanied by hypoalbuminemia and hypercholesterolemia [16]. First, it is important to find out if any underlying diseases cause proteinuria. Pre- and postrenal causes of proteinuria have to be excluded by general bloodwork, urine sediment and culture examination, and diagnostic imaging if needed. If found, the underlying cause should be treated, so generally, the proteinuria will resolve [8]. Diagnostic imaging in glomerular disease is not specific. The kidneys can even look normal on diagnostic imaging. In some cases, kidneys are enlarged, which can be seen with ultrasonography or radiography.

The severity of chronic kidney disease should be categorised in patients with glomerular disease as well. The IRIS staging system of chronic kidney diseases is based on blood creatinine and symmetric dimethylarginine (SDMA) concentrations, which are ideally taken on two occasions in hydrated, stable patients. The staging system has four stages, which show the different severity of chronic kidney disease (see Tables 1 and 2).

Table 1. IRIS staging of chronic kidney disease in dogs

Stage	Blood creatinine (mg/dl; µmol/l)	SDMA (µg/dl)
1	<1.4 mg/dl; <125 µmol/l	<18
2	1.4-2.8 mg/dl; 125-250 µmol/l	18-35
3	2.9-5.0 mg/dl; 251-440 µmol/l	36-54
4	>5.0 mg/dl; >440 µmol/l	>54

[19].

Table 2. IRIS staging of chronic kidney disease in cats

Stage	Blood creatinine (mg/dl; µmol/l)	SDMA (µg/dl)
1	< 1.6 mg/dl; <140 µmol/l	<18
2	1.6-2.8 mg/dl;140-250 µmol/l	18-25
3	2.9-5.0 mg/dl; 251-440 µmol/l	26-38
4	>5.0 mg/dl >440 µmol/l	>38

[19].

CKD patients are subcategorised based on the severity of the proteinuria and blood pressure.

Blood pressure should always be measured in dogs with acute or chronic glomerular diseases as it is highly likely that patients with glomerular disease also have high blood pressure [20]. Normotensive blood pressure is <140 mmHg, prehypertensive is 140-159 mmHg, hypertensive is 160-179 mmHg and severely hypertensive is over 180 mmHg [19]. Systolic blood pressure of >180 mmHg has a high risk of target organ damage, whereas systolic blood pressure of 150 has already low risk of target organ damage. The target organs of hypertension are the kidneys, the eyes, the heart and the brain. Target organ damage is seen in an increase of frequency uremic crisis or proteinuria or even higher mortality [20].

A renal biopsy is often suggested to diagnose the exact glomerular disease. Only a biopsy can show the precise severity of the lesions. Still, a renal biopsy is contraindicated in patients with hypertension, CKD with serum creatinine greater than 5 mg/dL (440 µmol/l), anaemia, pyelonephritis, renal cystic disease, or coagulopathy. Renal biopsy can be done ultrasound-guided, by laparoscopy, or surgically. The purpose of renal biopsy is to find out if active immunopathogenesis is ongoing in the glomeruli or not. Samples are histopathologically evaluated traditionally by light microscopy. The World Small Animal Veterinary Association recommends the use of transmission electron microscopy and immunostaining to complete the evaluation, although it is only available in a limited number of laboratories. The study by Cianciolo et al. 2016 aimed to determine the most detailed and accurate diagnostic tool to detect immunocomplexes in animals. In this study, they used dogs with proteinuria indicative of the presence of glomerular disease. They did a renal biopsy and

used either light microscopy, transmission electron microscopy and immunofluorescence staining or only light microscopy. They found that misdiagnosis happened with the light microscope in 22 out of 89 cases, so it is also advised to use more advanced diagnostic tools [13].

3.4.3 Treatment options

The treatment of glomerular disease depends on the origin of the proteinuria. The standard therapy is based on a renal diet with omega-3 fatty acid supplementation, inhibition of the renin-angiotensin-aldosterone system (RAAS), antithrombotic therapy and treatment of hypertension. Proteinuria treatment aims to get the proteinuria as low as possible [20]. A protein-restricted diet plays a huge role, and as it helps to reduce the protein amount in the circulation, there is less risk of an overload of proteins within the glomeruli. It is always important to look for body weight changes in the patient, as a low protein diet can cause weight loss as a side effect. A diet alone is, in most cases, not enough, drug therapy is indicated [16].

Often, patients with glomerular disease also have hypertension; in this case, it is important to give medication to control hypertension. Drugs that block the RAAS are the best way to reduce proteinuria while they also reduce blood pressure. For example, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), and aldosterone receptor antagonist drugs. ACEIs are widely used to decrease proteinuria. Studies have shown ACE inhibitors like benazepril to increase the life span and decrease the progression of azotemia in dogs [11, 17]. ACEIs and ARBs are contraindicated in animals with hypovolemia and dehydration. Angiotensin receptor blockers like losartan or telmisartan have shown great results in humans with proteinuria [17]. Recently, IRIS changed its recommendations, and telmisartan has become the first choice in the treatment of proteinuria for dogs, while ARBs and ACEIs stayed at the same level for cats [21]. Aldosterone antagonists like spironolactone are effective in humans, but there is not much data about the same effect in dogs, in dogs where the serum aldosterone concentration is increased and persistent proteinuria is present, it could be worth a try [11, 20]. Still, it could also be considered if the ACEIs and/or ARBs are not working. Although, it should not be used in animals with hyperkalemia or hypotension [11].

Hyperkalemia is often a side effect of drugs acting on the RAA system; if hyperkalemia is suspected, we have to rule out pseudohyperkalemia by checking the potassium concentration in lithium heparin plasma. Serum potassium levels over 6mmol/L should be monitored [20]. In most cases, hyperkalemia resolves by reducing the drug dosage or feeding a low-potassium diet.

Amlodipine, a calcium channel blocker, is the most potent antihypertensive drug for dogs and cats. ACEIs and ARBs are also antihypertensive agents but weak ones. In a proteinuric and hypertensive patient, often a combination of these medications is needed.

Fluid therapy should always be administrated with caution, as animals with nephrotic syndrome are likely to have a fluid overload. Fluid therapy should only be administered in animals with dehydration or poor tissue perfusion [20].

When the animal is diagnosed with nephrotic syndrome, it should be considered to give diuretic therapy like furosemide [20].

Thrombophilia is part of the pathogenesis of glomerulonephritis. Thromboembolism has been reported to be present in 25% of dogs with glomerulopathies. It is up to this point not fully understood, but in humans, it is often a venous thromboembolism rather than an arterial thromboembolism, and some clinical observations show the same result for dogs. Low-dose aspirin or clopidogrel can be used to prevent thromboembolism as they both inhibit platelet aggregation. Vitamin K antagonists could help as well, but unfortunately, there are not enough studies which show the most effective antithrombotic therapy for dogs [20].

In humans, immunosuppressive drugs are widely used and seem to work very well in glomerular diseases, but no study showed the same effect in animals. Immunosuppressive agents like mycophenolate should be considered only in confirmed cases of immune-mediated glomerulonephritis by renal biopsy. Immunosuppressant drugs should be used with caution [11]. To prevent amyloid formation, it is suggested to give colchicine. The already present amyloid depositions cannot be resolved, but colchicine can prevent further depositions. There are no veterinary studies that support this presumption of function from colchicine [16].

The prognosis of glomerular diseases can be very variable and depends on many different factors and how early the disease is diagnosed. Some studies show that dogs with glomerular disease and azotemia survive less than three months, and the median survival time of dogs

that already show signs of nephrotic syndrome is only 12.5 days. In contrast to this, dogs with glomerulopathy but without azotemia or nephrotic signs can live up to many years (median 605 days). More studies with higher case numbers are needed to confirm the exact survival times [22].

As some protein-losing nephropathies (PLNs) have genetic backgrounds, the question arises if some diseases could be prevented with special genetic DNA markers. Some DNA tests are already available. Another prevention measurement could be more regular check-ups, with serum albumin and urine microalbumin tests [16].

3.5 Examination of the effect of different sampling methods on the UPC

In the last few years, a few studies have dealt with different urine sample methods in relation to different urine values.

For example, the goal of the study from Beatrice et al., 2010 was to find out whether taking urine samples via cystocentesis or by free catch makes any difference concerning the UPC. Until then, UPC ratio values were only considered reliable when collected via cystocentesis. In the study, they evaluated the UPC values from 230 urine samples of 115 dogs taken via the two sampling methods. Then, they categorised them after the IRIS staging system. The study showed no significant difference between the UPC values taken by cystocentesis or free catch. 92.6% of the dogs were categorised in the same IRIS substage with both methods. They stated that the urine sediment must be inactive when using the free catch method [1, 16].

The study from Mortier et al., 2023 is about comparing UPC and urine specific gravity in two different sampling methods: cystocentesis versus home catch urine samples in cats. The study found that the UPC was slightly higher in the cystocentesis group, but the urine specific gravity was slightly lower than in the voiding samples. The conclusion is that sampling at home done by the owner is a good alternative to cystocentesis, although always using the same method for monitoring is advised [2].

4 Materials and methods

4.1 Animals

The study was performed at the Small Animal Clinic of the University of Veterinary Medicine in Budapest, Hungary. We started to look for healthy, suitable dogs for the study at the beginning of 2022. The first collection date was in February 2022, and the last collection of urine samples was in the summer of 2022.

To find participants for the study, I asked many different people. Mainly, international students from the University of Veterinary Medicine, Budapest, with dogs were asked to participate. Students with dogs walking on campus were asked to participate, and posts on different social media platforms were made to find suitable dogs with owners for the study. Many of them said no because they did not think their animals could be handled for cystocentesis. A few persons did not want to try to collect the urine and come to the clinic with their dog.

The dogs were all privately owned, and no urinary tract infections were known at that point. There was always a conversation about the whole procedure to explain how the cystocentesis would be done and the aim of the study. Also, it was important to explain how to collect the free catch urine sample. After explaining the whole procedure of cystocentesis, we asked if they thought the animal was suitable and calm enough for this. If the owners understood and agreed, they got a container for the free catch sample and a date for the urine sampling day.

A written owner consent form, approved by the National Scientific Ethical Committee on Animal Experimentation, was signed by each owner.

4.2 Urine sample collection technique and urinalysis

Dog owners were asked on a specific date to collect urine from their dogs in the morning via free catch. All participants received a suitable sterile container for the free catch, and they were asked to come right after collecting the sample to the clinic to perform an ultrasound-guided cystocentesis. We asked for a minimum of 5 ml urine in the container, which is necessary to be able to perform the different urinalyses.

Picture 1: Free catch and cystocentesis samples from our first sample day

At the clinic, before the cystocentesis, we took the urine containers and put them in the fridge. Before the cystocentesis, a physical exam was done, and a few questions about the health status of the animal were asked to make sure no health issues were known. After that, we put the dog on the table on their back and started with the ultrasound examination. With the ultrasound, we checked the urinary bladder for any abnormalities. After that, the cystocentesis was performed using a 22G or 23G needle with a 5-10 ml syringe. On most collection dates, we sampled 5-6 dogs after each other. All cystocentesis samples were put into a refrigerator after collection, and immediately after all the samples were taken, we sent them out to an outside laboratory for urine examination.

A complete urinalysis was carried out on all samples by the Praxislab Ltd. laboratory. It included specific gravity measurement with a refractometer, dipstick analysis, urine sediment examination and the measurement of UPC and UAC. Urinary albumin was measured by an immunoturbidimetric method. It uses a chemistry autoanalyser and a dedicated reagent kit (OSR6167). In this reagent kit, monoclonal antibodies generate an agglutination against human albumin. It can detect urinary albumin in the range of 0.5 – 30 mg/dL. Pooled canine sera was used for the calibration, as Gentilini et al. described it previously.

An enzymatic assay (Diagnosticum Kreatinin) was used for the measurement of urinary creatinine on the same chemistry analyser.

Urinary albumin to creatinine ratio was calculated using the following formula:

$$UAC\ (without\ unit)\ = \frac{urinary\ albumin\ mg/L/10}{urinary\ creatinine\ \mu mol/L\ *0.0113} = \frac{urinary\ albumin\ mg/dL}{urinary\ creatinine\ mg/dL}$$

$$UAC\ \left(\frac{mg}{g}\right) = 1000 * UAC\ (without\ unit)$$

4.3 Statistical analysis

The UAC, UPC and urine specific gravity (USG) results of the two sampling methods (cystocentesis and free catch) were compared. Due to the small sample size, we used a non-parametric test, the paired sign test. For the descriptive statistics we used Microsoft Excel and for the paired signed test we used an online calculator [23]. At the paired sign test the p-value was defined as $p < 0.05$, and a two-tailed hypothesis was used for all variables.

Picture 2: Free catch and cystocentesis samples shortly before sending them to the laboratory

5 Results

Twenty-seven dog owners agreed to participate in the study. From 81% of the dogs, we managed to get a free catch sample and to take urine via cystocentesis; the other 19% we needed to exclude for different reasons. Two dogs did not tolerate the cystocentesis, as we could not even put the dog calm on his back, so it was not possible to perform cystocentesis at all, but they managed to catch the urine at home. The dogs who did not tolerate the cystocentesis were stressed, so one even bit one of the assistants. These owners obviously could not evaluate their animals well, as we asked them before the cystocentesis if they thought their animals would tolerate the procedure. One owner could not catch urine in the morning, as the dog would not urinate, and if she tried to put the container under the animal, she would immediately stop and run. Another owner managed to catch urine, but when they arrived for the ultrasound, we could see that the bladder was empty, as it was an uncastrated male dog, and he marked with his urine on the way to the clinic. Unfortunately, they had no time to wait for the dog to produce more urine. So, this dog was excluded as well.

In the end, we managed to take urine from 22 dogs successfully. Of the 22 dogs who participated in the study, ten were male, and twelve were female. All male dogs except two were castrated, and only one female dog was not castrated. Their body weight ranged from 6.8 to 27.0 kg (average 16.9 ± 7.0), and their age ranged from 7 months to 14.2 years (average 5.6 ± 3.6). Various breeds and mixed-breed dogs were represented (see Table 3).

Table 3. Data and results of the examined dogs. F=female, FC=female castrated, M=male, MC= male castrated.

Name of the animal	Breed	Gender	Age (years)	Bodyweight (kg)	UPC Cystocentesis (g/g)	UPC free catch (g/g)	UAC cystocentesis (mg/g)	UAC free catch (mg/g)
Bolle	Puggle	M	12.8	12.0	0.17	0.19	0	1
Pille	Beagle	FC	8.9	10.4	0.12	0.09	0	0
Cosmo	Australian Shephard	MC	2.10	25.0	0.08	0.07	0	0
Eddy	Mix breed	MC	4.3	10.5	0.06	0.08	0	0
Molly	Mix breed	FC	8.1	12.0	0.10	0.09	0	0
Ludwig	Beagle	MC	6.2	14.4	0.07	0.08	1	0
Balu	Wolf spitz	MC	7.7	19.5	0.00	0.06	0	0
Gigi	Mix breed	FC	2.7	27.0	0.08	0.08	0	0
Daffy	German shorthair pointer	FC	6.8	25.0	0.08	0.07	0	0
Ida	Australian Shephard	F	0.7	13.0	0.14	0.1	0	0
Bogi	Mix breed	FC	8.3	11.0	0.11	0.09	1	0
Pepe	Dalmatian	M	1	25.0	0.36	0.12	11	0
Bodza	Mix breed	FC	4.4	8.0	0.11	0.11	1	1
Tobi	Mix breed	MC	8.1	18.0	0.12	0.06	0	0
Elsa	Mix breed	FC	14.1	24.0	0.10	0.08	1	1
Darwin	Beagle	MC	5.4	9.8	0.35	27	11	9
Marley	Mix breed	FC	2.5	25.0	0.08	0.08	0	0
Abby	Mix breed	FC	2.8	10.0	0.08	0.07	0	0

Name of the animal	Breed	Gender	Age (years)	Bodyweight (kg)	UPC Cystocentesis (g/g)	UPC free catch (g/g)	UAC cystocentesis (mg/g)	UAC free catch (mg/g)
Cinkei Dio	Magyar Vizsla	M	5.2	22.0	0.19	0.13	0	0
Sunny	Labrador Mix	MC	9.2	27.0	0.44	0.07	2	0
Remus	Mix breed	FC	1.3	17.0	0.09	0.07	0	0
Jodie	Mix breed	FC	2.8	6.8	0.08	0.08	0	0

USG was significantly higher in the free catch group than in the cystocentesis group (p=0.008; median [range]: 1.048 [1.018-1.050] vs. 1.040 [1.005-1.050]) (see Table 4). The UPC was significantly higher in the cystocentesis group than in the free catch group (p=0.02; median [range]: 0.1 [0-0.44] vs 0.08 [0.06-0.27]) (see Table 4). No dogs had overt proteinuria (UPC >0.5). Three dogs had borderline proteinuria (UPC 0.2-0.5). One dog had borderline proteinuria with both sampling methods, while two dogs only had borderline proteinuria in the cystocentesis sample but not in the free catch. In Table 4, you can see that the result shows that there was no difference in the UAC between the two groups (p=0.1 median [range]: 0 [0-9] for free catch vs. 0 [0-11] for cystocentesis). None of the dogs had albuminuria (UAC >19 mg/g).

Table 4. Statistical analysis results about SG, UPC and UAC from cystocentesis and free catch samples. Free=free catch, Cysto=cystocentesis

	USG		UPC (g/g)		UAC (mg/g)	
	Cysto	Free	Cysto	Free	Cysto	Free
Mean	1035.41	1043.68	0.14	0.10	1.27	0.55
SD	15.05	8.67	0.11	0.05	3.19	1.92
Min	1005	1018	0	0.06	0	0
Max	1050	1050	0.44	0.27	11	9
Median	1040	1048	0.1	0.08	0	0
1st quartile	1022.75	1038	0.08	0.07	0	0
3rd quartile	1050	1050	0.15	0.10	1	0

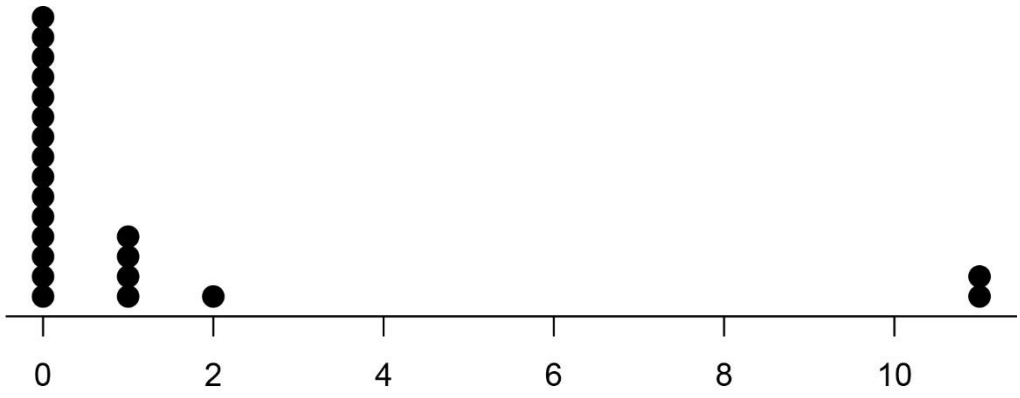

Figure 1: UAC results from the cystocentesis samples (each dot meaning the result of one (dog)

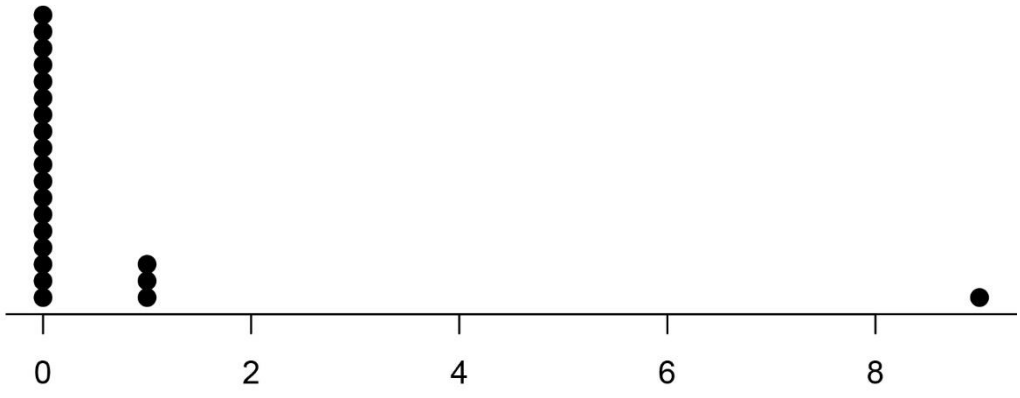

Figure 2: UAC results from the free catch samples (each dot meaning the result of one dog)

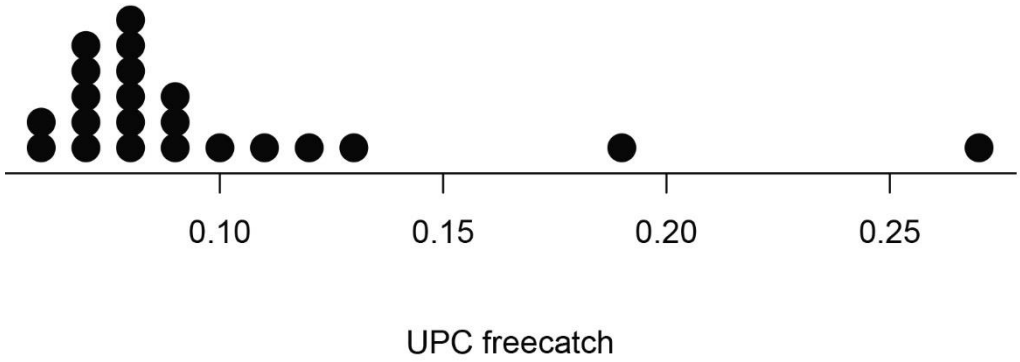

Figure 3: UPC results from the free catch samples (each dot meaning the result of one dog)

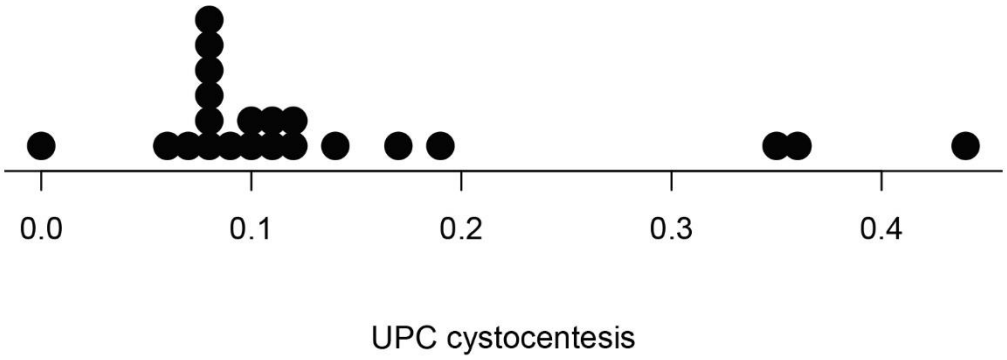

Figure 4: UPC result from the cystocentesis samples (each dot meaning the result of one dog)

6 Discussion

UPC is one of the most important variables when it comes to diagnosing proteinuria. UAC can be the earliest sign of protein-losing nephropathy. The earlier the increasing values of UPC and UAC are noticed, the better, as in the early stages of CKD, we can manage the disease with diet and medications. The sooner proteinuria and CKD are diagnosed, the better we can manage it and give the animal a better quality of life and, most likely, also a longer survival time [9].

The important question is now if the only way to get a correct UPC and/or UAC result is cystocentesis or if it is also possible with free catch sampling. Having the possibility of different methods of sampling could increase the number of urinalyses, as the most suitable method could be individually adapted to the animal's and owner's wishes. Free catch at home is often less stressful for the animal, as the dog does not need to come to the clinic for the sampling and is also cheaper than cystocentesis. Suppose the free catch is also a good alternative for UPC and/or UAC values. In that case, more owners will let it be tested more regularly with their pets, which could increase the quality of life for patients suffering from renal diseases.

Our study is the first to examine UAC values in urine samples taken by different methods. The UAC values were not significantly different in the free catch and cystocentesis samples. All of the UAC results were within the normal range (0-19 mg/g) with both sampling methods. This means it makes no difference for the UAC value if we take the sample via free catch or cystocentesis, the method can be chosen up to the preference of the dog owner or the veterinarian.

In contrast to UAC, the UPC was significantly higher in the cystocentesis group than in the free catch group. Beatrice et al. found no significant difference between the UPC values collected by cystocentesis and free catch in dogs. A similar study was also made on cats, where they found a difference between the two groups. A different IRIS substage based on proteinuria was diagnosed in 28% of the cats when cystocentesis and free catch samples were compared.

Although the difference between the two sampling methods was significant in our study, these differences (median [range]: 0.1 [0-0.44] vs.0.08 [0.06-0.27]) are clinically unimportant. 20/22 dogs were classified into the same IRIS substages based on proteinuria.

Only 2/22 dogs were classified into different IRIS substages. In both cases, cystocentesis resulted in ‘borderline proteinuria ‘(0.36 and 0.44), while free catch resulted in ‘no proteinuria’ (0.27 and 0.07). In these cases, it is possible that the mild proteinuria was induced by stress. Duffy et al. found in their study that UPC was higher in free catch urine samples collected at the hospital than at home. An increase in epinephrine or cortisol levels or blood pressure caused by stress could temporarily affect glomerular filtration [24]. Stress at the clinic could also affect our cystocentesis results. Preanalytical factors can also affect UPC levels, such as leaving the sample at room temperature. Rossi et al. determined that UPC values increase after 4 hours at room temperature. It is unlikely that this affected our results as free catch urine samples were refrigerated within 1-2 hours, and the cystocentesis samples immediately [25].

The USG value showed a significant difference between the free catch and cystocentesis samples, the specific gravity was higher in the free catch group. 7/22 dogs were classified into different specific gravity categories. On 5/22, the dog's USG of cystocentesis samples were in the minimally concentrated range (1012-1030), while the free catch sample was in the hyperstenuric range (>1030). There were 2/22 dogs where the free catch sample showed hyperstenuria, but the cystocentesis sample showed isostenuria (1008-1012) and hypostenuria (<1008). At one of these dogs, the owner admitted that he gave the dog lots of water before the appointment, as he was afraid that the bladder would be too small when arriving at the clinic. Another reason for the more concentrated urine could be that free catch samples were collected in the morning, while cystocentesis samples were collected some hours later. It is known that the first-morning urine has the highest specific gravity, usually, as dogs eat and drink in the morning, the urine gets more diluted [26].

The changes in USG should not influence the UAC and UPC results because albumin and protein are measured in relation to creatinine concentration. Creatinine is freely filtered through the glomeruli, and its concentration in the urine is dependent on the specific gravity of the urine [6].

Most of the samples had inactive urine sediment in both samples, but some of the dogs had active urine sediment. In our study, we did not exclude dogs with haematuria or crystalluria, but we examined their result and decided about them individually, searching for signs of urinary tract infections that would have been a reason for exclusion. 5/22 dogs had mild haematuria in the cystocentesis samples because of the puncture itself. We did not exclude

these animals based on the clinical experience that mild haematuria caused by the puncture does not affect UPC, UAC and USG levels. We also did a statistical evaluation without these dogs, which showed that excluding them would not have altered our results.

In the free catch sample, we found high amounts of calcium oxalate crystals and mild haematuria in one dog. The female dog also had some blood and white blood cells in the urine in the cystocentesis sample, which can be normal, as we needed to puncture many times, and the dog did not tolerate the cystocentesis well. There were no calcium oxalate crystals found in the cystocentesis sample. The first possibility is that this dog had true crystalluria, and the haematuria present in the free catch sample was caused by the crystals. In this case, the crystals were not visible on the cystocentesis sample because they were covered by red blood cells. The other option is that the crystals were only formed during storage as the free catch sample was stored some hours longer. What is interesting to see here is that there is no difference in the UAC (0 and 0 mg/g) and UPC (0.14 and 0.1) values in cystocentesis or free catch samples.

In the sample of the Dalmatian, we found ammonium-urate crystals in both samples, which is not a surprise as the Dalmatians are predisposed to forming crystals and stones if they have a specific genetic mutation. These dogs have a defect in the urate transport into the hepatocytes; thus, they cannot break down the urate. Uric acid will be excreted in their urine, causing this problem [27].

In some free catch samples, we could see bacterial contamination, which is due to the fact of how the owners sampled the urine; some of them needed to open the container more often, maybe even left it open, and so the contamination happened. The contamination can also happen because of the urinary tract or genital tract. As we did not store the samples long, bacterial overgrowth could not alter the UPC or UAC results.

There were some limitations of our study. We recruited only healthy dogs, and none of them had overt proteinuria or albuminuria. Only three dogs had borderline proteinuria. Thus, we could only examine the differences between cystocentesis and free catch UAC and UPC results in the normal and borderline range. Further studies are needed to examine the UAC differences between the two sampling methods in dogs with overt albuminuria. Also, recruiting more dogs would make the statistical significance stronger.

In conclusion, our study revealed that UAC ratios determined in urine samples collected by the free catch method did not differ from the values in cystocentesis samples. Therefore, the free catch collection method can be safely used in clinical practice when determining UAC.

7 Summary

Urinalysis is an important tool to find different diseases. There are various methods to sample urine in dogs; this thesis focuses on the free catch and cystocentesis sampling methods. The aim of our study was to determine if the urinary albumin-to-creatinine (UAC) values in the urine differ between the two methods or if the sampling method is not important. The question is if other factors like feasibility of the sampling, stress caused for the animal or financial issues could influence the sampling method of choice or if the sampling method is the most important factor when it comes to UAC and urinary protein-to-creatinine (UPC) values.

For this study, we recruited healthy, suitable dogs in different age and body weight groups. In the morning, the dog owners caught urine via free catch with a sterile container, and a few hours later, we took urine via ultrasound guided cystocentesis at the clinic. Both samples were sent to a laboratory for the urinalysis. We looked at UAC and UPC values in particular.

The UPC values showed a significant difference between the two sampling methods; the value was higher in the cystocentesis sample group (median [range]: 0.1 [0-0.44] for cystocentesis vs. 0.08 [0.06-0.27] for free catch), but in the end, 20 out of 22 dogs were still classified in the same IRIS substage of proteinuria.

The UAC values showed no significant difference between the two different sampling methods (median [range]: 0 [0-9] for free catch vs. 0 [0-11] for cystocentesis). Therefore, it makes no difference, and the sampling method can be decided by the dog owner or the veterinarian based on what is best for the animal and needed for the diagnosis.

In conclusion, the UAC values can be measured safely from urine samples taken by cystocentesis or free catch sampling method.

8 Összefoglaló

A vizeletvizsgálatnak fontos szerepe van különféle betegség felismerésében. A vizelet gyűjtésére, kutyák esetében, többféle módszer is rendelkezésünkre áll. Jelen kutatásunk ezek közül a spontán ürített és a cisztocentézissel vett vizeletgyűjtésre összpontosít.

Kutatásunk célja annak megállapítása volt, hogy a vizeletbeli albumin/kreatinin arány (UAC) értéke eltér-e a két módszer között. Kérdés, hogy más tényezők, mint például a mintavétel kivitelezhetősége, az állatnak okozott stressz, vagy anyagi okok befolyásolhatják-e a mintavételi módszer kiválasztását, vagy a mintavételi módszer fontos tényező az UAC és az UPC (vizelet fehérje/kreatinin arány) meghatározásakor.

Vizsgálatunkhoz változatos életkorú és testsúlyú, egészséges, a mintavételeket toleráló kutyákat toboroztunk. A mintavétel reggelén a kutyatulajdonosok egy steril tégelybe gyűjtötték kutyáik spontán ürített vizeletét, majd néhány órával később a klinikán ultrahang-vezérelt cisztocentézissel vettünk vizeletmintát. Mindkét mintát laboratóriumba küldtük vizeletvizsgálatra, különös tekintettel az UAC és az UPC értékekre.

Az UPC értékek szignifikáns különbséget mutattak a két mintavételi módszer között; az értékek magasabbak voltak a cisztocentézis csoportban (medián [tartomány]: 0,1 [0-0,44] cisztocentézis, vs. 0,08 [0,06-0,27] spontán ürített). Ugyanakkor a 22 kutyából 20-at a két módszer ugyanabba az IRIS alkategóriába sorolt proteinuria szempontjából.

Az UAC-értékek nem mutattak szignifikáns különbséget a kétféle mintavételi módszer között (medián [tartomány]: 0 [0-9] spontán ürített és 0 [0-11] cisztocentézis). Vagyis, a mintavétel módját a kutya tulajdonosa vagy állatorvos választhatja meg, az alapján, amit az állat számára legjobb módszernek talál.

Összefoglalva, az UAC értékek biztonságosan meghatározhatók mind spontán ürített mind pedig cisztocentézissel vett vizeletmintából.

9 References

1. Beatrice L, Nizi F, Callegari D, Paltrinieri S, Zini E, D'Ippolito P, Zatelli A (2010) Comparison of urine protein-to-creatinine ratio in urine samples collected by cystocentesis versus free catch in dogs. J Am Vet Med Assoc 236:1221–1224. https://doi.org/10.2460/javma.236.11.1221

2. Mortier F, Daminet S, Duchateau L, Marynissen SJJ, Paepe D (2023) Comparison of cystocentesis versus home sampling to determine urinary protein: Creatinine ratio and urine specific gravity in cats. J Vet Intern Med 37:1401–1408. https://doi.org/10.1111/jvim.16800

3. Callens AJ, Bartges JW (2015) Urinalysis. Vet Clin North Am Small Anim Pract 45:621–637. https://doi.org/10.1016/j.cvsm.2015.02.001

4. Grauer GF, Pohlman LM Urinalysis Interpretation in Dogs & Cats | Clinician's Brief. https://www.cliniciansbrief.com/article/urinalysis-interpretation. Accessed 8 Sep 2023

5. Sample collection. In: eClinpath. https://eclinpath.com/urinalysis/sample-collection/. Accessed 16 Aug 2023

6. Chemical constituents. In: eClinpath. https://eclinpath.com/urinalysis/chemical-constituents/. Accessed 12 Jul 2023

7. Grauer GF (2011) Proteinuria: Measurement and Interpretation. Top Companion Anim Med 26:121–127. https://doi.org/10.1053/j.tcam.2011.04.002

8. Vaden SL, Elliott J (2016) Management of Proteinuria in Dogs and Cats with Chronic Kidney Disease. Vet Clin North Am Small Anim Pract 46:1115–1130. https://doi.org/10.1016/j.cvsm.2016.06.009

9. Lees GE, Brown SA, Elliott J, Grauer GF, Vaden SL (2005) Assessment and Management of Proteinuria in Dogs and Cats: 2004 ACVIM Forum Consensus Statement (Small Animal). J Vet Intern Med 19:377–385. https://doi.org/10.1111/j.1939-1676.2005.tb02713.x

10. (2014) The mini vet guide to companion animal medicine. Gerardo Poli, Mt Gravatt East, Qld.

11. Harley L, Langston C (2012) Proteinuria in dogs and cats. Can Vet J Rev Veterinaire Can 53:631–638

12. Grauer GF (2007) Measurement, Interpretation, and Implications of Proteinuria and Albuminuria. Vet Clin North Am Small Anim Pract 37:283–295. https://doi.org/10.1016/j.cvsm.2006.11.003

13. Whittemore JC, Gill VL, Jensen WA, Radecki SV, Lappin MR (2006) Evaluation of the association between microalbuminuria and the urine albumin-creatinine ratio and systemic disease in dogs. J Am Vet Med Assoc 229:958–963. https://doi.org/10.2460/javma.229.6.958

14. Bacic A, Kogika MM, Barbaro KC, Iuamoto CS, Simões DMN, Santoro ML (2010) Evaluation of albuminuria and its relationship with blood pressure in dogs with chronic kidney disease: Albuminuria in dogs with kidney disease. Vet Clin Pathol 39:203–209. https://doi.org/10.1111/j.1939-165X.2009.00207.x

15. Falus FA, Vizi Z, Szabó KÉ, Müller L, Reiczigel J, Balogh N, Manczur F (2022) Establishment of a reference interval for urinary albumin-to-creatinine ratio in dogs. Vet Clin Pathol 51:585–590. https://doi.org/10.1111/vcp.13149

16. Littman MP (2011) Protein-losing Nephropathy in Small Animals. Vet Clin North Am Small Anim Pract 41:31–62. https://doi.org/10.1016/j.cvsm.2010.09.006

17. Vaden SL (2011) Glomerular Disease. Top Companion Anim Med 26:128–134. https://doi.org/10.1053/j.tcam.2011.04.003

18. Klosterman ES, Pressler BM (2011) Nephrotic Syndrome in Dogs: Clinical Features and Evidence-Based Treatment Considerations. Top Companion Anim Med 26:135–142. https://doi.org/10.1053/j.tcam.2011.04.004

19. IRIS Kidney - Guidelines - IRIS Staging of CKD. http://www.iris-kidney.com/guidelines/staging.html. Accessed 21 Apr 2023

20. IRIS Canine GN Study Group Standard Therapy Subgroup, Brown S, Elliott J, Francey T, Polzin D, Vaden S (2013) Consensus Recommendations for Standard Therapy of Glomerular Disease in Dogs. J Vet Intern Med 27:S27–S43. https://doi.org/10.1111/jvim.12230

21. IRIS Kidney - Guidelines - IRIS Treatment Recommendations. http://www.iris-kidney.com/guidelines/recommendations.html. Accessed 21 Apr 2023

22. Klosterman ES, Moore GE, De Brito Galvao JF, DiBartola SP, Groman RP, Whittemore JC, Vaden SL, Harris TL, Byron JK, Dowling SR, Grant DC, Grauer GF, Pressler BM (2011) Comparison of Signalment, Clinicopathologic Findings, Histologic Diagnosis, and Prognosis in Dogs with Glomerular Disease with or without Nephrotic Syndrome: Nephrotic Syndrome in Dogs. J Vet Intern Med 25:206–214. https://doi.org/10.1111/j.1939-1676.2010.0669.x

23. Sign Test Calculator. In: Soc. Sci. Stat. https://www.socscistatistics.com/tests/signtest/default.aspx. Accessed 30 Oct 2023

24. Duffy ME, Specht A, Hill RC (2015) Comparison between Urine Protein: Creatinine Ratios of Samples Obtained from Dogs in Home and Hospital Settings. J Vet Intern Med 29:1029–1035. https://doi.org/10.1111/jvim.12836

25. Rossi G, Giori L, Campagnola S, Zatelli A, Zini E, Paltrinieri S (2012) Evaluation of factors that affect analytic variability of urine protein-to-creatinine ratio determination in dogs. Am J Vet Res 73:779–788. https://doi.org/10.2460/ajvr.73.6.779

26. Vonderen IK, Kooistra HS, Rijnberk A (1997) Intra- and Interindividual Variation in Urine Osmolality and Urine Specific Gravity in Healthy Pet Dogs of Various Ages. J Vet Intern Med 11:30–35. https://doi.org/10.1111/j.1939-1676.1997.tb00070.x

27. Syme HM (2012) Stones in cats and dogs: What can be learnt from them? Arab J Urol 10:230–239. https://doi.org/10.1016/j.aju.2012.06.006

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I hereby confirm that I am familiar with the content of the thesis entitled

Comparison of urinary albumin-to-creatinine ratio in urine samples taken by cystocentesis or free catch method

..... written by Alisha Schnitzer

(student name) which I deem suitable for submission and defence.

Date: Budapest, ...02...day...11...month ...2023...year

DR. FRUZSINA FALUS

Falus Fruzsina

..... Supervisor name and signature

Department and Clinic of Internal Medicine

..... Department

Thesis progress report for veterinary students

Name of student: Alisha Schnitzer.....

Neptun code of the student: IOHI9I.....

Name and title of the supervisor: Dr. Fruzsina Falus.....

Department: Department and Clinic of Internal Medicine.....

Thesis title: Comparison of urinary albumin-to-creatinine ratio in urine samples taken by cystocentesis or free catch method.

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	02	23	Taking urine samples	
2.	2022	04	04	Taking urine samples	
3.	2022	04	25	Taking urine samples	
4.	2022	06	27	Taking urine samples	

Grade achieved at the end of the first semester: .....5.....

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	02	20	Discussion about the literature review	
2.	2023	04	12	Discussion about the results	
3.	2023	05	23	Discussion about the conclusions of the thesis	

Grade achieved at the end of the second semester: .....5.....

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: .....

Signature of the secretary of the department: .....

Date of handing the thesis in..... .....