SCREENING FOR EARLY FELINE CHRONIC KIDNEY DISEASE:

Limitations of currently available tests and possible solutions

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LIST OF ABBREVIATIONS

ACVIM	American College of Veterinary Internal Medicine
ALT	Alanine aminotransferase activity
ANOVA	Analysis of variance
AST	Aspartate aminotransferase activity
AUC	Area under the plasma concentration-versus-time curve
BCS	Body condition score
BP	Blood pressure
bpm	beats per minute
CIN	Chronic interstitial nephritis
CKD	Chronic kidney disease
Creat	Creatinine concentration
CysC	Cystatin C
DKD	Diabetic kidney disease
DM	Diabetes mellitus
ECVDI	European College of Veterinary Diagnostic Imaging
ELISA	Enzyme-linked immunosorbent assay
Endo	Endo-iohexol concentration
Exo	Exo-iohexol concentration
FeLV	Feline leukemia virus
FIV	Feline immunodeficiency virus
GFR	Glomerular filtration rate
GGT	γ-glutamyl transpeptidase activity
IRIS	International Renal Interest Society
IV	Intravenous
LPF	Low-power field
LSS	Limited sampling strategy
N	Number
NAG	N-acetyl-β-glucosaminidase activity
NPV	Negative predictive value
PEC-ICT	Plasma exogenous creatinine-iohexol clearance test
PKD	Polycystic kidney disease
PPV	Positive predictive value
RI	Reference interval
RBP	Retinol-binding protein
RCB	Ragdoll Club Benelux
ROC	Receiver-operating-characteristic
SBP	Systolic blood pressure
SCL	Segmental cortical lesion
sCreat	Serum creatinine concentration
sCysC	Serum Cystatin C concentration
SD	Standard deviation
Sens	Sensitivity
Spec	Specificity
SRC	Scandinavian Ragdoll Club
SSA	Sulfosalicylic acid
STT	Schirmer tear test
sUrea	Serum urea concentration
t60	60 minutes after marker injection
t120	120 minutes after marker injection
t180	180 minutes after marker injection
TOD	Target organ damage
TT4	Total thyroxine concentration
UAC	Urinary albumin: creatinine ratio
uCreat	Urinary creatinine concentration
uCysC	Urinary Cystatin C concentration
USG	Urine specific gravity
UPC	Urinary protein: creatinine ratio
UTI	Urinary tract infection
WSAVA	World Small Animal Veterinary Association

CHAPTER 1

GENERAL INTRODUCTION

FELINE CHRONIC KIDNEY DISEASE: DIAGNOSIS, STAGING AND SCREENING

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Paepe D and Daminet S. Feline CKD. Diagnosis, staging and screening – what is recommended? *Journal of Feline Medicine and Surgery* 2013; 15 (Suppl 1): S15 – S27.

1.1 INTRODUCTION

Chronic kidney disease (CKD) is a common disease in cats. In veterinary practices or colleges of the United States, the overall prevalence of feline CKD varies between 1 and 3%. The prevalence increases to approximately 7.5% in cats over 10 years and reaches between 15 and 30% in cats over 15 years of age (Lulich *et al* 1992, Lund *et al* 1999, Polzin 2010, Lefebvre 2011). Hence, feline CKD is frequently encountered by veterinarians, mainly in older cats.

Establishing a correct diagnosis is mandatory for an adequate management of these patients, but diagnosing CKD may be challenging, particularly in early disease stages or in cats with concurrent diseases.

This introduction gives an overview of necessary diagnostic tests, the classification system proposed by the *International Renal Interest Society* (IRIS; IRIS 2009, Polzin 2010) and the importance and possibilities to improve early detection of CKD.

1.2 DIAGNOSIS

Feline CKD is diagnosed based on the presence of combined renal azotemia and poorly concentrated urine (urine specific gravity (USG) ≤ 1.035), with compatible historical or physical examination findings (Grauer 1998, Bartges 2012). If the urinary bladder is palpable, urine can be collected easily by cystocentesis (Fig 1.1).

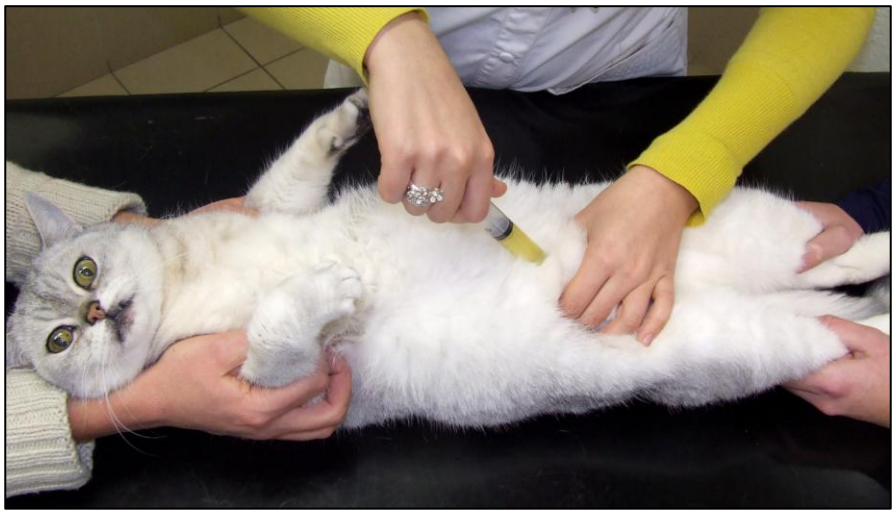

Fig 1.1.
Cystocentesis in an unsedated cat. The cat is restrained in dorsal recumbency and the bladder is palpated and immobilized during aspiration of urine.

1.2.1 Signalment, history and physical examination

Idiopathic CKD may affect cats of all ages and obvious breed and sex predispositions have not been reported. This contrasts with the clear breed predispositions that have been recognized for other renal diseases such as polycystic kidney disease (PKD) and amyloidosis. However, the signalment might be helpful for diagnosing feline idiopathic CKD. Veterinarians should have an increased awareness for CKD in senior and geriatric cats as CKD typically is a disease of older cats. Some breeds have been overrepresented in some studies, such as Siamese, Persian, Abyssinian, Maine Coon, Russian Blue, and Burmese cats (DiBartola *et al* 1987, Lulich *et al* 1992, Elliott and Barber 1998, Boyd *et al* 2008). Websites of European Ragdoll breed clubs warn for an increased susceptibility of Ragdoll cats for renal problems (SRC 2012, RCB 2013). However, scientific literature does not support a predisposition of Ragdoll cats for CKD.

Cats with CKD are presented to veterinarians in various stages of illness. Some are incidentally diagnosed during health screening, others demonstrate mild clinical signs, while others suffer from end-stage CKD with severe signs such as emaciation or dehydration (DiBartola *et al* 1987, Elliott and Barber 1998). The illness duration prior to admission is highly variable. Although feline CKD is typically chronic, an acute history of illness is not uncommon (DiBartola *et al* 1987). The most common clinical signs are nonspecific and include inappetence, polyuria, polydipsia, weight loss, lethargy, halitosis, and vomiting (DiBartola *et al* 1987, Lulich *et al* 1992, Elliott and Barber 1998, King *et al* 2006). Signs associated with nephrotic syndrome or hypertension are uncommon in cats with CKD (DiBartola *et al* 1987, Elliott and Barber 1998, King *et al* 2006).

Physical examination findings depend on the disease stage and consist of thin body condition, dehydration, periodontal disease, unkempt hair coat, abnormal kidney palpation (small, irregular or enlarged) and pale mucous membranes (Fig 1.2). Physical examination can be unremarkable in the early disease stage (DiBartola *et al* 1987, Elliott and Barber 1998).

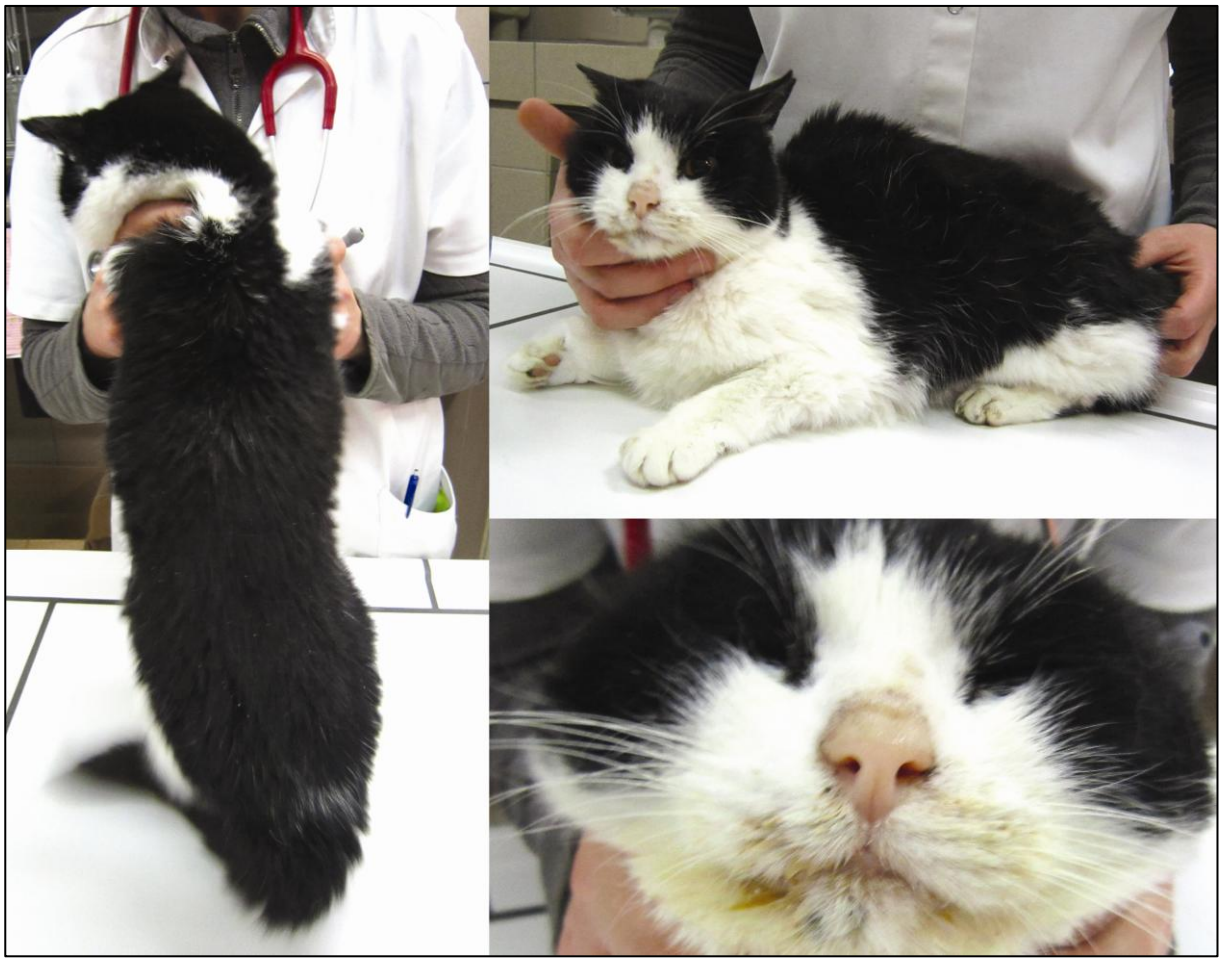

Fig 1.2. A cat presented with severe clinical signs due to acute-on-chronic kidney disease. A thin body condition (body condition score 3/9) and an unkempt haircoat with scaling are visible (left and upper right figure). The cat also had pale mucous membranes and was salivating because of uremic stomatitis and nausea (bottom right figure). After stabilization with infusion, supportive therapy (nasoesophageal tube feeding, antiemetics, antacids, analgesics) and antibiotics, the cat was discharged. Follow-up two weeks later revealed chronic kidney disease IRIS end-stage 3.

1.2.2 Minimum laboratory database

As feline CKD is diagnosed based on the presence of compatible clinical signs and renal azotemia, the minimum laboratory database to confirm CKD consists of measuring serum creatinine and urea concentrations and USG.

Renal azotemia is defined as increased serum creatinine and urea concentrations due to intrinsic renal pathology (Table 1.1) (Stockham and Scott 2008a). Creatinine is more reliable than urea as an indirect marker for glomerular filtration rate (GFR) because creatinine is less influenced by extrarenal factors (e.g. intestinal protein content, liver function) and only undergoes glomerular filtration without tubular reabsorption. The daily production rate of creatinine depends on muscle mass, which may be of clinical importance in geriatric cats with age-related muscle wasting or when muscle mass gradually declines during CKD progression (Lees 2004, Polzin 2010, DiBartola 2010). The (modified) Jaffe and enzymatic assays are currently the only used commercial creatinine assays. Both methods correlate well with a reference method. However, the Jaffe assay may overestimate low concentrations and underestimate high concentrations of feline serum creatinine. The enzymatic assay only slightly overestimates feline creatinine and appears to be the preferred method (Le Garreres *et al* 2007). Although the (modified) Jaffe assay is being replaced gradually by enzymatic assays, many diagnostic laboratories still use the (modified) Jaffe assay to measure serum creatinine concentrations. Reference intervals (RIs) for serum creatinine can widely differ between laboratories which may lead to misclassification of samples as normal or abnormal (Boozer *et al* 2002, Ulleberg *et al* 2011). Because assays and RIs often differ between laboratories, clinicians are encouraged to consistently determine serum creatinine in a laboratory with good quality control (Lees 2004).

Table 1.1. Characteristics of prerenal, renal and postrenal azotemia (Grauer 1998, Stockham and Scott 2008a, DiBartola 2010).

PRERENAL AZOTEMIA	**RENAL AZOTEMIA**	**POSTRENAL AZOTEMIA**
Due to decreased renal blood flow secondary to dehydration, hypovolemia, shock or cardiac failure	Due to intrinsic renal damage/pathology resulting in decreased glomerular filtration rate	Due to urinary obstructive disease or urinary tract rupture leading to accumulation of urine in the body
Signs of dehydration, hypovolemia, shock or cardiac failure	Usually no clinical signs of dehydration or hypovolemia, except if renal azotemia is complicated by prerenal azotemia	History often reveals dysuria, stranguria or oliguria if lower urinary tract obstruction
Usually mild azotemia (serum creatinine < 400 µmol/L)	Mild to severe azotemia	Palpable renal asymmetry if upper urinary tract obstruction
USG > 1.030 – 1.040, unless primary disease interferes with urinary concentrating ability or prerenal azotemia complicates renal azotemia	USG < 1.030 – 1.040, often isosthenuria (1.007 – 1.015)	Distended abdomen and positive undulation if uro-abdomen
Rapid resolution of azotemia with correction of hypovolemia or dehydration	Slower or only partial or no improvement with infusion therapy	Mild to severe azotemia
Can evolve to renal azotemia if no timely management		Variable USG
		Resolution of azotemia with correction of underlying disease
		Can evolve to renal azotemia if no timely management

(USG = Urine specific gravity)

In veterinary medicine, USG is routinely measured by refractometry. Human hand-held optical refractometers can overestimate feline USG (George 2001). However, these errors are not clinically relevant and are unlikely to change clinical decision making (Bennett *et al* 2011). Veterinary refractometers with a separate feline USG scale avoid these errors (George 2001). Alternatively, a conversion calculation is available: *feline USG = (0.846 x SG of human refractometer) + 0.154*, a formula that is dating from 1956 (George 2001, Bennett *et al* 2011). Most CKD cats have isosthenuric urine (USG 1.007 – 1.015) (Fig 1.3) (DiBartola *et al* 1987, Elliott and Barber 1998), but this is less consistent in cats compared with dogs (Finco 1995). Some cats, with spontaneous as well as with experimentally induced CKD, can retain their urine concentrating ability despite being azotemic, particularly in early disease stages (Ross and Finco 1981, DiBartola *et al* 1987, Elliott and Barber 1998). With disease progression, USG usually gradually declines (Elliott and Barber 1998, Elliott *et al* 2003).

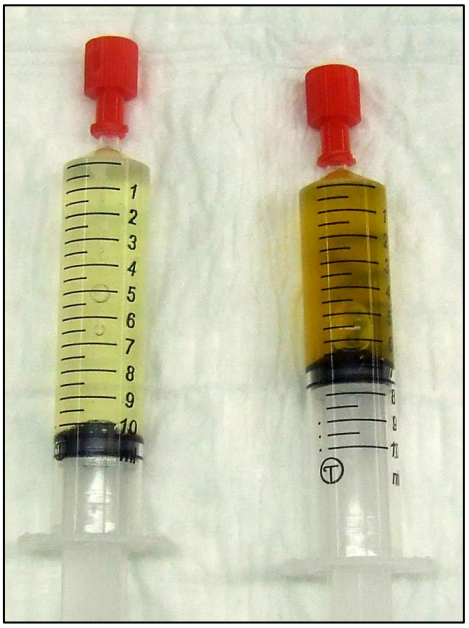

Fig 1.3. Urine samples taken by cystocentesis of a cat with chronic kidney disease IRIS stage 3 (left) and a healthy geriatric cat (right). The CKD cat had poorly concentrated urine with urine specific gravity (USG) 1.014, the healthy cat concentrated urine with USG 1.045. In the absence of bilirubinuria, the difference in urinary concentration is macroscopically visible.

1.2.3 Proteinuria

Although severe proteinuria is uncommon, low-level proteinuria (urinary protein: creatinine ratio (UPC) < 1) commonly affects feline CKD patients and is an important prognostic factor and therapeutic target (King *et al* 2006, Kuwahara *et al* 2006, Syme *et al* 2006, King *et al* 2007). Therefore, quantifying and longitudinal monitoring of proteinuria is very important in all cats with CKD.

Proteinuria may be of prerenal, renal or postrenal origin and persistent renal proteinuria indicates CKD. A step-wise diagnostic approach (Fig 1.4) must be followed to eliminate prerenal (e.g. hemoglobinuria, myoglobinuria, Bence-Jones proteinuria), postrenal urinary (e.g. urolithiasis, cystitis, ureteritis, bladder or urethral neoplasia) and postrenal extraurinary (e.g. genital tract inflammation) proteinuria. The severity of proteinuria may help to localize renal proteinuria: UPC values above 2 are likely glomerular, but lower values might be glomerular, tubular or interstitial in origin (Lees *et al* 2005, Segev 2010).

Several methods exist to evaluate whether CKD cats are proteinuric. However, the UPC, that provides an index of total urinary protein loss and correlates closely with the gold standard of 24-hour urinary protein excretion, is the only reliable method to determine its clinical implications (Adams *et al* 1992, Lees *et al* 2005). Because UPC can vary depending on the methodology used, monitoring of UPC requires the same laboratory assay (Fernandes *et al* 2005). In practice, dipstick tests are often used as a measure of urinary protein. Urine dipstick tests primarily measure albumin; are easy, rapid, in-house tests; and provide semiquantitative assessment of proteinuria. Unfortunately, urine dipsticks only reliably detect severe feline proteinuria. In cats with low-level proteinuria, false positive and false negative results are common (Syme 2009, Lyon *et al* 2010). A positive dipstick test can be confirmed by the more sensitive and specific semiquantitative sulfosalicylic acid turbidity (SSA) test that has a lower detection limit (5 mg/dL) compared with dipstick tests (30 mg/dL). Interpretation of dipstick and SSA results must be done in light of USG, because positive results in concentrated urine reflect less severe protein loss than in diluted urine (Lees 2004, Segev 2010). If a false negative dipstick result is suspected, the SSA test or a species-specific microalbuminuria test (see below) can be employed (Grauer 2007, Segev 2010). Nevertheless, measurement of UPC is recommended in all animals with positive semiquantitative proteinuria tests (Lees 2004, Lees *et al* 2005, Grauer 2007).

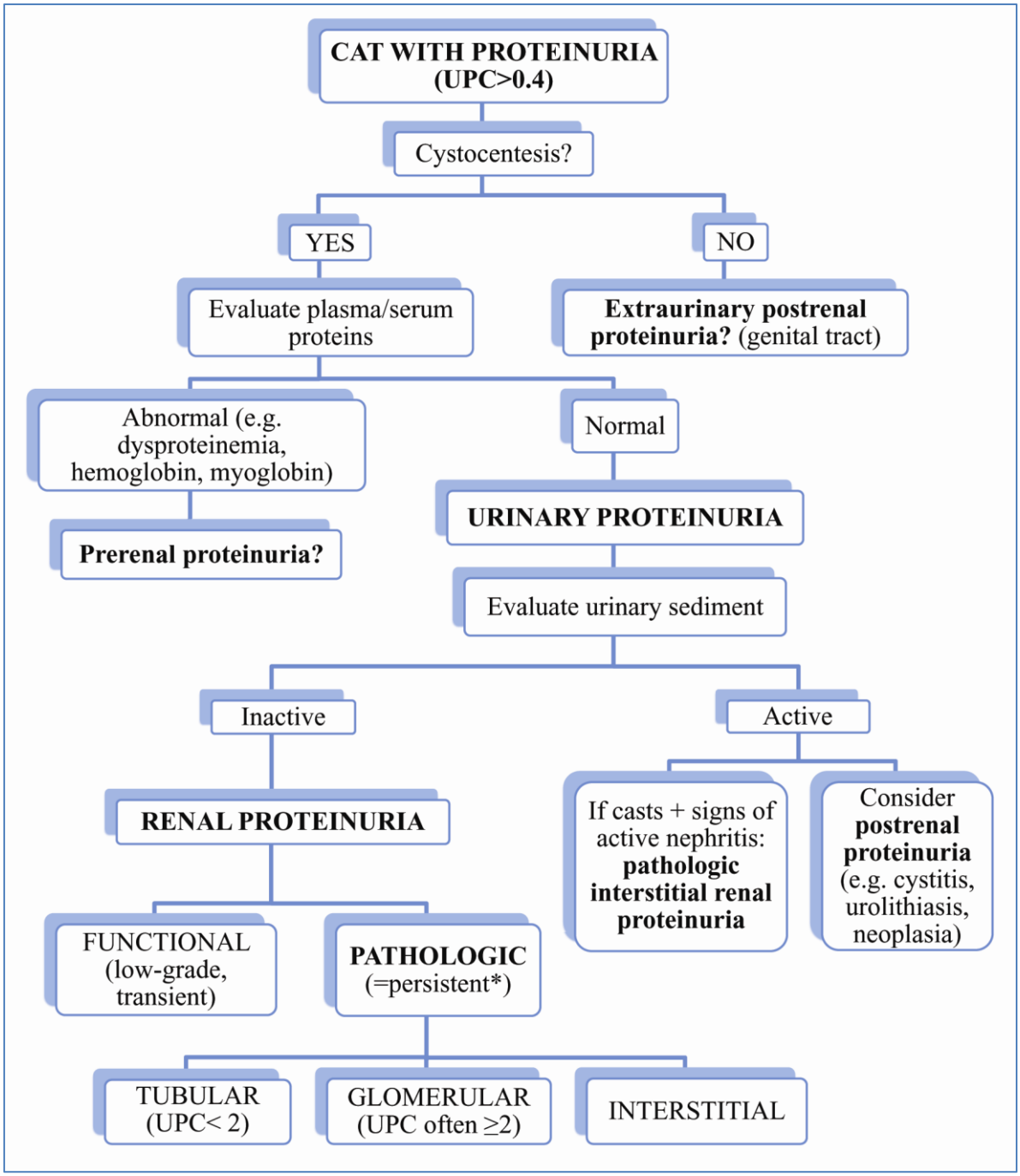

Fig 1.4. Flow-chart to assess the origin of proteinuria in cats, based on Lees *et al* (2005). *To evaluate for persistence of proteinuria: testing on 3 or more occasions, 2 or more weeks apart is recommended.

1.2.4 Additional diagnostic tests

1.2.4.1 Blood and urine examination

Additional blood and urine parameters need to be monitored carefully in CKD cats, mainly to improve early recognition and treatment of complications.

Because 30 – 65% of CKD cats develop anemia during their disease course and chronic anemia implies decreased quality of life, timely diagnosis of *anemia of renal disease* is important (DiBartola *et al* 1987, Lulich *et al* 1992, Elliott and Barber 1998, Boyd *et al* 2008, Chalhoub *et al* 2011). The anemia of CKD, usually non-regenerative, worsens with increasing disease severity (Elliott and Barber 1998, Elliott *et al* 2003, King *et al* 2006). Although not consistently found, anemia at diagnosis might be associated with more rapid disease progression and shorter survival (Kuwahara *et al* 2006, King *et al* 2007, Chakrabarti *et al* 2012).

Renal secondary hyperparathyroidism commonly complicates feline CKD, being more common and more severe with higher degrees of renal dysfunction (Barber and Elliott 1998). Higher phosphate concentration at diagnosis has been associated with shorter survival times and disease progression (King *et al* 2007, Boyd *et al* 2008, Chakrabarti *et al* 2012). Thus, phosphate and – preferably ionized – calcium concentrations should be assessed in CKD cats, though, these are relatively insensitive tests to detect renal secondary hyperparathyroidism. Measuring parathyroid hormone would provide more information regarding parathyroid status, but parathyroid hormone assays are expensive, not widely available and correct sample handling is difficult (Barber and Elliott 1998, Finch *et al* 2012, Geddes *et al* 2013a). Both parathyroid hormone and fibroblast growth factor 23, a phosphaturic hormone secreted in response to hyperphosphatemia, progressively increase with more advanced CKD and may be increased before azotemia develops (Finch *et al* 2012, Finch *et al* 2013a, Geddes *et al* 2013b). Also, fibroblast growth factor 23 was higher in hyperphosphatemic azotemic cats compared with normophosphatemic azotemic cats of the same IRIS stage (Geddes *et al* 2013b). Further studies looking at the potential value of fibroblast growth factor 23 for early diagnosis of renal secondary hyperparathyroidism are warranted (Finch *et al* 2013a). Approximately 15% of cats with mild CKD (IRIS stage 2) and up to 100% of end-stage CKD cats have hyperphosphatemia (DiBartola *et al* 1987, Lulich *et*

al 1992, Barber and Elliott 1998, King *et al* 2006). Total and ionized calcium concentrations in CKD cats vary from increased, normal to decreased (Barber and Elliott 1998, Elliott *et al* 2003, Schenck and Chew 2010). Ionized calcium concentrations tend to decline with increasing severity of CKD (Barber and Elliott 1998, Elliott *et al* 2003). Because total calcium poorly predicts ionized calcium concentration, particularly in CKD cats, measurement of total calcium is of little value and ionized calcium determination is required to assess calcium status in CKD cats (Barber and Elliott 1998, Schenck and Chew 2010).

Hypokalemia affects 15 to 30% of CKD cats, especially IRIS stage 2 and 3 cats. Hypokalemia is less common in cats with more severe CKD and cats with end-stage CKD (IRIS stage 4) may develop hyperkalemia (12 – 22%) due to reduced potassium excretion (DiBartola *et al* 1987, Lulich *et al* 1992, Elliott and Barber 1998, Elliott *et al* 2003, King *et al* 2006). Monitoring potassium concentrations is recommended for CKD cats of all stages.

Metabolic acidosis characterized by decreased pH, low bicarbonate concentration, increased anion gap and/or decreased to normal chloride concentration, occurs frequently in feline CKD patients, especially in cats with advanced CKD (DiBartola *et al* 1987, Lulich *et al* 1992, Elliott *et al* 2003). Cats with mild to moderate CKD usually maintain their acid-base balance (Elliott *et al* 2003). Determining the acid-base status and appropriate treatment of acid-base disturbances is recommended in cats with advanced CKD (IRIS end stage 3 and 4). Blood gas testing in cats with CKD can be performed on venous samples drawn into syringes that are coated with 1:1000 heparin or into specialized blood gas syringes containing pelleted heparin (Kerl 2010). The amount of heparin in the syringe must be minimized by an evacuation technique to avoid preanalytical errors due to dilution of the sample (Hopper *et al* 2005). After sampling, the syringe must be made airtight immediately and sample analysis must be done within 15 minutes on a bench-top analyzer that is developed or validated to perform blood gas analysis in veterinary patients (Kerl 2010). As an alternative to blood gas testing, the bicarbonate concentration may be determined as decreased bicarbonate concentrations are usually associated with metabolic acidosis (Stockham and Scott 2008b).

Routine urine bacterial culture, at diagnosis of CKD and during follow-up, is recommended because *bacterial urinary tract infections* (UTIs), commonly affect CKD cats, independently of the severity of CKD (Mayer-Roenne *et al* 2007, Bailiff *et al* 2008, Martinez-Ruzafa *et al* 2012, White *et al* 2013). During a 3-year follow-up study in which urine cultures were routinely performed in cats with CKD, 30% developed a positive urine culture (White *et*

al 2013). Many CKD cats with UTI have an occult UTI, which means that they do not show lower urinary tract signs (up to 72%) and/or urinary sediment abnormalities (up to 25%). Therefore, UTI may be overlooked based on history, physical examination and routine urinalysis (Mayer-Roenne *et al* 2007, Bailiff *et al* 2008, Martinez-Ruzafa *et al* 2012, White *et al* 2013).

A significant relationship has been shown between *feline immunodeficiency virus* (FIV) and CKD and between FIV and azotemia. Also, proteinuria is common in FIV-infected cats (Thomas *et al* 1993, Avila *et al* 2010, White *et al* 2010, Baxter *et al* 2012). In contrast, a relationship between *feline leukemia virus* (FeLV) and CKD has not yet been shown, but FeLV can cause serious clinical syndromes leading to considerable negative effects on quality and quantity of life (Hartmann 2012). Determination of the FIV- and FeLV-status is therefore recommended in cats with CKD.

1.2.4.2 Blood pressure (BP)

Hypertension frequently complicates CKD (20 – 65%) (Kobayashi *et al* 1990, Stiles *et al* 1994, Syme *et al* 2002) and renal dysfunction is the most common underlying cause of feline hypertension (31.9 – 87%) (Littman 1994, Maggio *et al* 2000, Elliott *et al* 2001, Jepson *et al* 2007). In humans, hypertension is considered to be both cause and consequence of CKD and contributes to progressive CKD (Tedla *et al* 2011). Similarly, regardless of the underlying cause for hypertension, azotemia is observed in many hypertensive cats (Littman 1994, Maggio *et al* 2000, Chetboul *et al* 2003, Jepson *et al* 2007). Idiopathic hypertension is diagnosed in 13 – 20% of hypertensive cats, however, whether these non-azotemic and non-hyperthyroid cats have primary hypertension or hypertension secondary to early, subclinical, non-azotemic CKD is uncertain (Maggio *et al* 2000, Elliott *et al* 2001, Jepson *et al* 2007, Syme 2011). Although an association between hypertension and progressive kidney disease is generally presumed, it remains uncertain whether feline systemic hypertension might cause CKD and which role it plays in CKD progression (Syme 2011). Nevertheless, BP should be measured in all cats with kidney disease and renal function should be assessed in all hypertensive cats (Maggio *et al* 2000, Chetboul *et al* 2003, Stepien 2011).

Techniques for BP measurement are reviewed in detail elsewhere (Brown *et al* 2007, Stepien 2011). Because it is inexpensive, easy and accurate, the Doppler ultrasonic technique

(Fig 1.5) is the most suitable method for indirect systolic blood pressure (SBP) measurement in practice. Oscillometric techniques are less accurate in cats, but may be advantageous in cats that prefer minimal restraint (Stepien 2010, Stepien 2011). Hypertension is considered and further diagnostic tests are advised if SBP, measured with Doppler device, exceeds 160 mmHg on repeated occasions or on a single occasion with clinical manifestations of hypertension (Lin *et al* 2006, Brown *et al* 2007, Stepien 2011). Uncontrolled hypertension leads to end-organ damage at the level of the kidneys, heart, brain and eyes and the majority of hypertensive cats have ocular or cardiac abnormalities on physical examination (Littman 1994, Maggio *et al* 2000, Elliott *et al* 2001, Syme *et al* 2002, Chetboul *et al* 2003, Brown *et al* 2007).

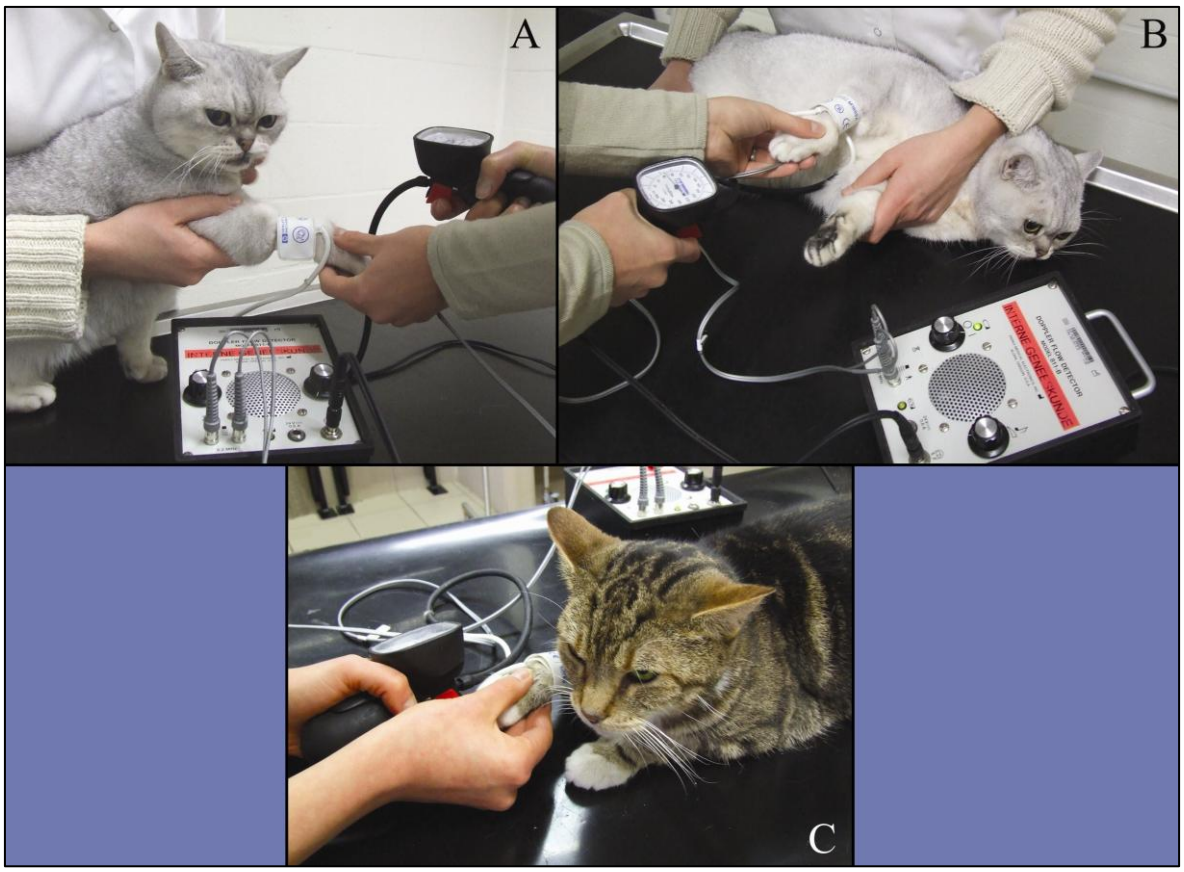

Fig 1.5. Systolic blood pressure measurement in a cat using the Doppler ultrasonic technique, in sitting position (A), in lateral (B) and sternal recumbency (C). Points of attention are to restrain the cat gently in a comfortable position and to held the cuff at the level of the heart base. In cats, we always use headphones to avoid stress hypertension due to the sounds of the Doppler machine and to improve hearing of the Doppler sounds by the clinician.

1.2.4.3 Medical imaging

Once CKD is diagnosed, medical imaging studies may reveal an underlying cause, particularly in cats with unilateral or bilateral renomegaly or obvious asymmetry in kidney size. Causes that may be detected are PKD, nephrocalcinosis, urinary obstructive disease and renal neoplasia (Fig 1.6 – 1.8). Additionally, signs of feline infectious peritonitis or pyelonephritis may be identified (Polzin 2010, Bartges 2012). Abdominal radiography allows assessment of kidney size and presence of radiopaque uroliths (Fig 1.9). Contrast radiography may improve urolith detection and localization. More detailed information regarding internal renal architecture can be obtained with ultrasonography (DiBartola 2010). Typical renal ultrasonographic findings in CKD cats are small (< 3.2 cm) and irregularly outlined kidneys, heterogenous renal parenchyma, focally or diffusely increased cortical and/or medullar echogenicity, loss of corticomedullary demarcation, areas of mineralization and poor visualization of internal architecture (Fig 1.10) (Grooters and Biller 1995, Widmer *et al* 2004, d'Anjou 2008, Debruyn *et al* 2012). However, there is no correlation between ultrasonographic findings and the degree of renal dysfunction (Grooters and Biller 1995). Furthermore, the frequency of renal ultrasonographic abnormalities in healthy cats is unknown.

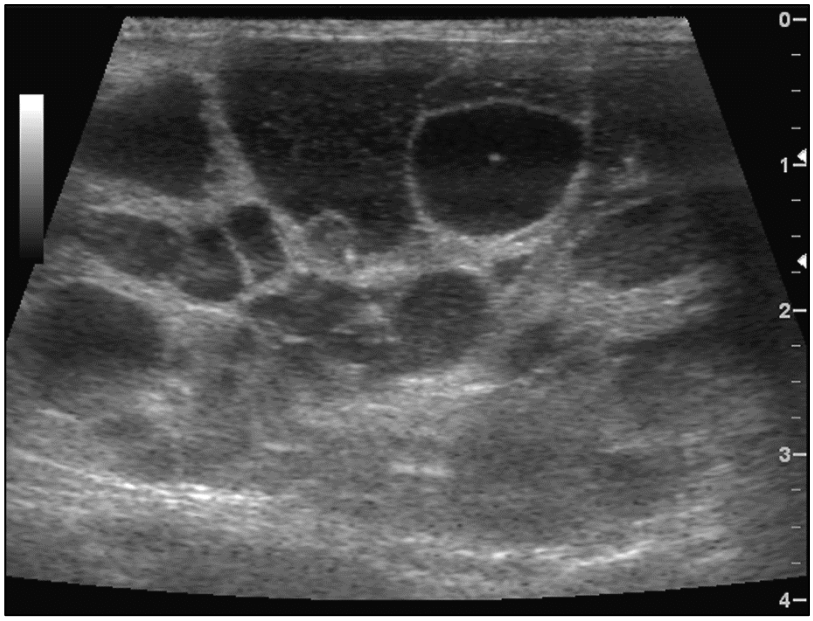

Fig 1.6. Ultrasonographic dorsal plane of the right kidney of a 13-year-old Persian cat with polycystic kidney disease. The kidney is severely enlarged (length > 6 cm) (normal 3.2-4.2 cm; Widmer *et al* 2004) and contains multiple, well-defined cysts of different sizes. The presence of the multiple cysts in the cortex and medulla results in complete distortion of the normal kidney structure.

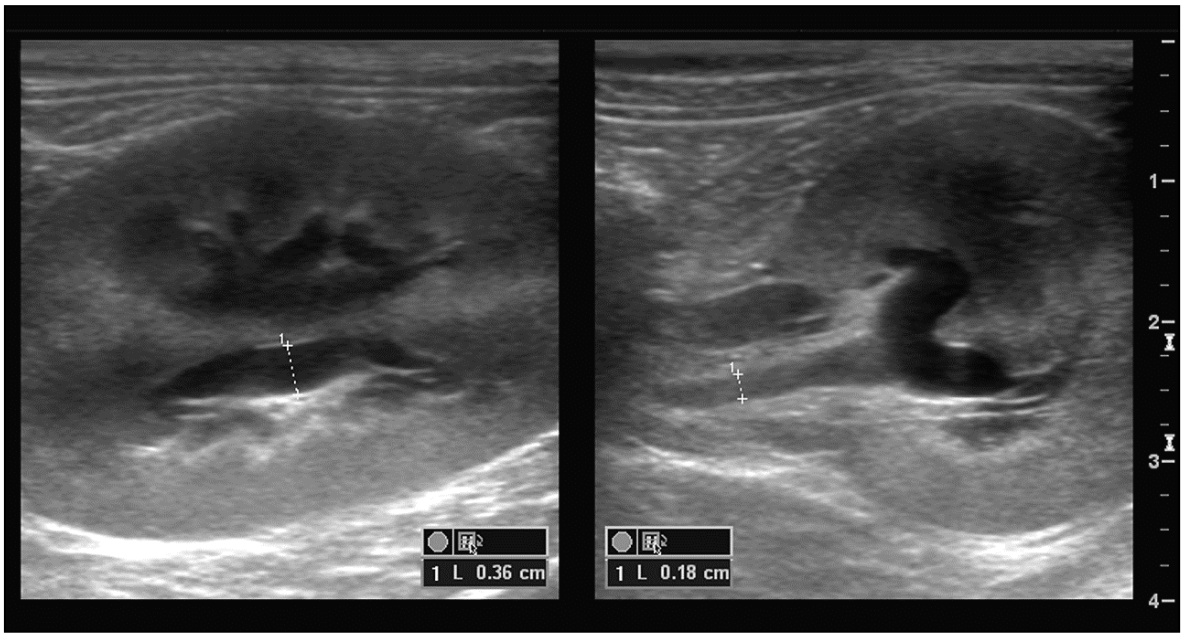

Fig 1.7. Sagittal and transverse ultrasound images of the left kidney of a 1-year-old domestic shorthair cat with ureteral obstruction due to ligation. Note the moderate pyelectasia (3.6 mm) and dilation of the proximal ureter (1.8 mm). The size of the kidney (4.6 cm) was mildly enlarged.

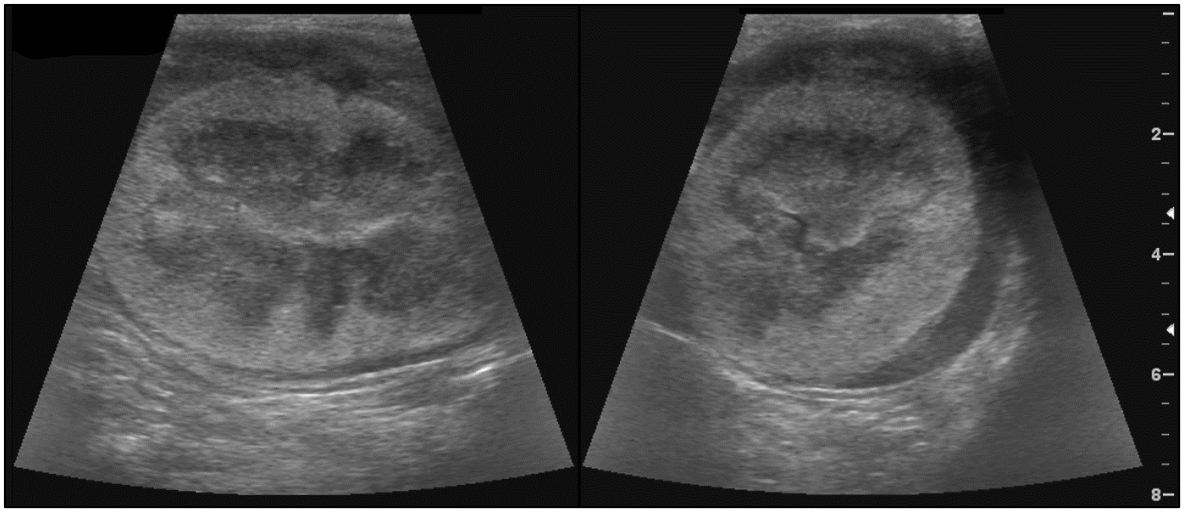

Fig 1.8. Dorsal and transverse ultrasound images of the right kidney of a 9-year-old domestic shorthair cat with renal lymphoma. The hyperechoic cortex is surrounded by a thick hypoechoic subcapsular halo. The kidney was severely enlarged (6.5 cm).

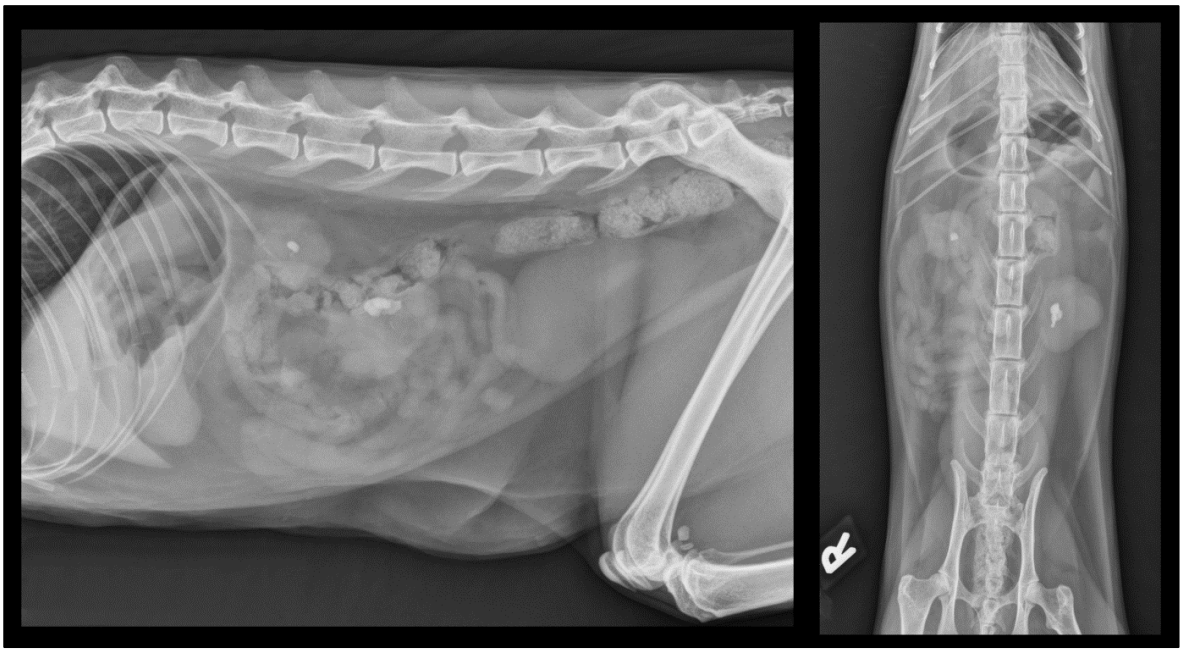

Fig 1.9. Left-right lateral and ventrodorsal projections of the abdomen of a 4-year-old domestic shorthair cat with acute-on-chronic renal failure and nephrolithiasis. Both kidneys are small and markedly irregularly outlined. An asymmetry in kidney size is visible. Both kidneys contain radiopaque, well-defined, mineralized elements (nephroliths) in the pelvic area.

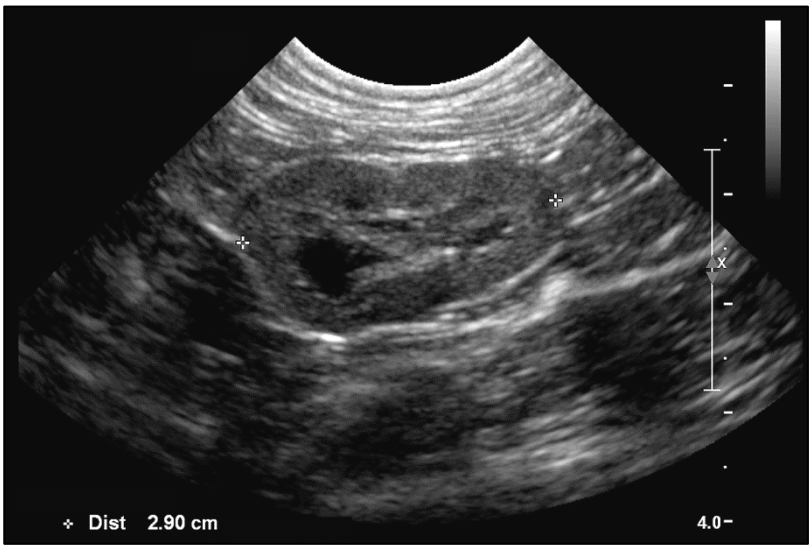

Fig 1.10.
Dorsal ultrasound image of the left kidney of a 7- year-old British shorthair cat with chronic kidney disease (IRIS stage 3). The kidney is decreased in size (2.9 cm), irregularly outlined and has a hyperechoic cortex.

1.2.4.4 (Micro)albuminuria

Microalbuminuria is defined as the presence of a small amount (1 – 30 mg/dL) of albumin in the urine, beneath the limit of detection of urinary dipstick tests (Langston 2004, Grauer 2007). Microalbuminuria may also remain undetected by UPC determination (Lyon *et al* 2010). Higher urinary albumin concentrations (> 30 mg/dL) are termed (overt) albuminuria and are usually detected using urinary dipstick tests or UPC (Langston 2004, Grauer 2007). Persistent renal (micro)albuminuria may be indicative for renal disease (Langston 2004, Lees *et al* 2005), however, (micro)albuminuria has been observed in healthy cats and in cats with a wide variety of non-renal diseases (e.g. infectious, inflammatory, endocrine, neoplastic, urinary tract disease) (Langston 2004, Mardell and Sparkes 2006, Whittemore *et al* 2007, Vaden *et al* 2010, Al-Ghazlat *et al* 2011). It is currently unknown whether microalbuminuria serves as negative prognostic marker in cats as in humans (Langston 2004, Vaden *et al* 2010).

(Micro)albuminuria can be measured with the urinary albumin: creatinine ratio (UAC) (Langston 2004), but an apparent benefit for UAC measurement over UPC has not yet been found (Syme and Elliott 2005a, Syme *et al* 2006, Jepson *et al* 2009). Alternatively, feline microalbuminuria can be detected with a commercial in-house semi-quantitative enzyme-linked immunosorbent assay (ELISA)-based dipstick test (E.R.D-Health Screen, Heska Corporation, Fort Collins, Colorado, United States) (Langston 2004, Syme and Elliott 2005b, Mardell and Sparkes 2006). Although, most cats with negative microalbuminuria dipstick test have UPC < 0.4 (Syme and Elliott 2005b, Mardell and Sparkes 2006, Hanzlicek *et al* 2012), a negative microalbuminuria dipstick does not preclude elevated UPC (Mardell and Sparkes 2006, Al-Ghazlat *et al* 2011). On the other hand, microalbuminuria is unlikely in CKD cats with UPC < 0.2. Nevertheless, quantification of UPC or UAC is recommended in cats with a positive microalbuminuria test (Hanzlicek *et al* 2012).

Routine evaluation for presence of (micro)albuminuria in cats is not warranted because (micro)albuminuria occurs with various diseases, UAC measurement is not widely commercially available, UAC lacks benefit over UPC, semiquantitative test interpretation might be difficult and negative microalbuminuria tests do not rule out proteinuria (Lees 2004, Syme 2009). However, there are some indications to assess for (micro)albuminuria, particularly in cats at risk for renal disease without overt proteinuria (Lees 2004, Grauer 2007). It is important to remember that (micro)albuminuria is not necessarily associated with

CKD and diagnostic tests to define the underlying cause are recommended (Langston 2004, Whittemore *et al* 2007).

1.2.4.5 Glomerular filtration rate

Determination of GFR – i.e. the volume of ultrafiltrate produced per unit of time – is considered to be the gold standard to evaluate kidney function (Braun and Lefebvre 2008). Glomerular filtration rate is mostly determined by plasma clearance of a filtration marker. Appropriate filtration markers are freely filtered through the glomerulus, not protein-bound, not toxic, do not undergo tubular secretion or absorption, and do not alter GFR (Heiene and Moe 1998, DiBartola 2010, Von Hendy-Willson and Pressler 2011, Sandilands *et al* 2013).

For research purposes, plasma clearance of iohexol or creatinine administered by single intravenous injection is frequently used in cats to estimate GFR (Brown *et al* 1996, Miyamoto 1998, Miyamoto 2001a, Miyamoto 2001b, Goy-Thollot *et al* 2006a, Goy-Thollot *et al* 2006b, Le Garreres *et al* 2007, van Hoek *et al* 2007, van Hoek *et al* 2008a, Heiene *et al* 2009, van Hoek *et al* 2009a, van Hoek *et al* 2009b, Miyagawa *et al* 2010a, Miyagawa *et al* 2010b, Finch *et al* 2011, Finch *et al* 2013b). Unfortunately, iohexol assays and injectable creatinine are not commercially available. Also inulin and radioisotopes have been used as clearance markers but inulin assays are technically challenging and not widely available, while radioisotopes require specialized equipment and carry the risk of radiation exposure (DiBartola 2010, Von Hendy-Willson and Pressler 2011). Multi-sample techniques for GFR estimation are labor-intensive, time-consuming and may be stressful or painful for the patient, which limits their practical use in cats (Finch *et al* 2013b). However, GFR determination might be valuable for cats with doubtful renal function (e.g. unexplained weight loss or polyuria/polydipsia; CKD IRIS stage 1 patients; idiopathic hypertension; azotemia, poorly concentrated urine or pathologic renal proteinuria as a single laboratory abnormality). Determination of GFR is also recommended for dosage adjustment in companion animals receiving potentially toxic drugs that primarily undergo renal excretion (Lees 2004, Polzin 2010).

In human medicine, GFR is usually estimated using equations based on serum creatinine concentration and demographic variables. Yet, these equations do not provide an accurate GFR estimate in certain patient groups, namely patients at extremes of ages and body size, patients with unusual diet, patients without CKD and patients with rapidly changing kidney function (de Jong and Gansevoort 2005, Stevens *et al* 2006, Stevens and Levey 2009, Salgado *et al* 2010). Trends in kidney function are often more important than the true GFR value, limiting the impact of these inaccuracies in the clinical setting. Nevertheless, the implications of this imprecision might be important in some clinical situations (e.g. GFR based dose adjustment for nephrotoxic drugs) and, particularly, in research trials (Munar and Singh 2007, Sandilands *et al* 2013). Measuring GFR using exogenous markers is recommended to assess kidney function in these patient groups (Stevens *et al* 2006, Stevens and Levey 2009, Salgado *et al* 2010).

Because GFR determination is cumbersome, time-consuming and consequently expensive and potentially stressful or painful for the patient (de Jong and Gansevoort 2005, Finch *et al* 2013b), efforts have been taken to simplify GFR determination in humans by using the least number of possible blood samples, particularly in children (Schwartz and Work 2009). Limited sampling strategies (LSS) – i.e. clearance techniques based on a reduced number of blood samples – are a suitable compromise between practical convenience and clinical accuracy for GFR determination (Swinkels *et al* 2000). In comparison, several LSS have been described to estimate feline GFR. Unfortunately, in most veterinary studies reported to date, no or only few renal-impaired cats were evaluated and none of these methods is sufficiently validated in CKD cats to be used in practice (Barthez *et al* 2000, Barthez *et al* 2001, Goy-Thollot *et al* 2006b, Vandermeulen *et al* 2008, Heiene *et al* 2009, Miyagawa *et al* 2010a, Vandermeulen *et al* 2010, Finch *et al* 2011, Katayama *et al* 2012, Katayama *et al* 2013). One group recently developed a single sample method for estimating feline GFR in both nonazotemic and azotemic cats (Finch *et al* 2013b), using a modification of the Jacobsson method that was originally developed for human patients (Jacobsson 1983). Yet, it is unknown whether the assumptions that are inherent to this Jacobsson method are applicable to cats as well. The limitations of current feline LSS underline the need for further research on simplified methods to estimate GFR in cats.

1.2.4.6 Renal biopsies

Kidney histology of CKD cats often cannot reveal the underlying cause. However, some primary causes such as renal lymphoma, amyloidosis, and feline infectious peritonitis usually can be identified on renal biopsies, regardless of the disease stage (DiBartola *et al* 1987). Renal biopsies should be considered when knowledge of morphologic alterations in renal structure will substantially influence patient management, for example in cats with renal lymphoma that could not be identified with fine-needle aspiration, amyloidosis or glomerulonephritis. However, this is not true for the majority of CKD cats that suffer from chronic generalized tubulointerstitial nephritis, glomerulosclerosis, tubular necrosis, or PKD nor for cats with significant azotemia or end-stage CKD regardless of the underlying cause (Lulich *et al* 1992, Polzin 2010). Maximal information will be obtained by evaluating kidney biopsies with light, electron and immunofluorescent microscopy, which is particularly recommended in patients with persistent severe proteinuria (UPC $\geq$ 2) without severe azotemia (IRIS stages 1 to early 3). Potential underlying diseases leading to proteinuria should be ruled out before taking kidney biopsies (Segev 2010, Vaden 2010).

1.3 STAGING

Analogous to the situation in human medicine, a classification system to stage dogs and cats with CKD has been developed by the *International Renal Interest Society* and accepted by *American* and *European Societies of Veterinary Nephrology and Urology* (IRIS 2009, Polzin 2010). Goals of staging are to standardize and simplify nomenclature, to facilitate treatment and monitoring by providing therapeutic recommendations adapted for each disease stage, and to facilitate estimating the prognosis of CKD patients (NKF 2002, IRIS 2009, Polzin 2010).

In human medicine, five CKD stages were developed according to the level of GFR of the patient, mostly estimated using creatinine-based equations (NKF 2002). In veterinary medicine, the CKD stage is based on serum creatinine concentration, assessed on at least two occasions, and further substaging is based on proteinuria, assessed by UPC, and SBP (Fig 1.11). Only stable patients can be staged (i.e. well-hydrated, normal eating/drinking behavior), preferably after 12-hour fasting with free access to water. Prerenal or postrenal azotemia needs to be corrected prior to staging to prevent allocating cats incorrectly to a higher disease stage and worsened prognosis (Boyd *et al* 2008, IRIS 2009). For proteinuria substaging, only persistent renal proteinuria is of importance. The SBP substaging system reflects the risk that end-organ injury arises in the eyes, brain, kidneys or heart. Efforts must be taken to minimize white-coat hypertension and SBP must be determined 2 to 3 times over several weeks (IRIS 2009, Brown *et al* 2007, Polzin 2010, Polzin 2011).

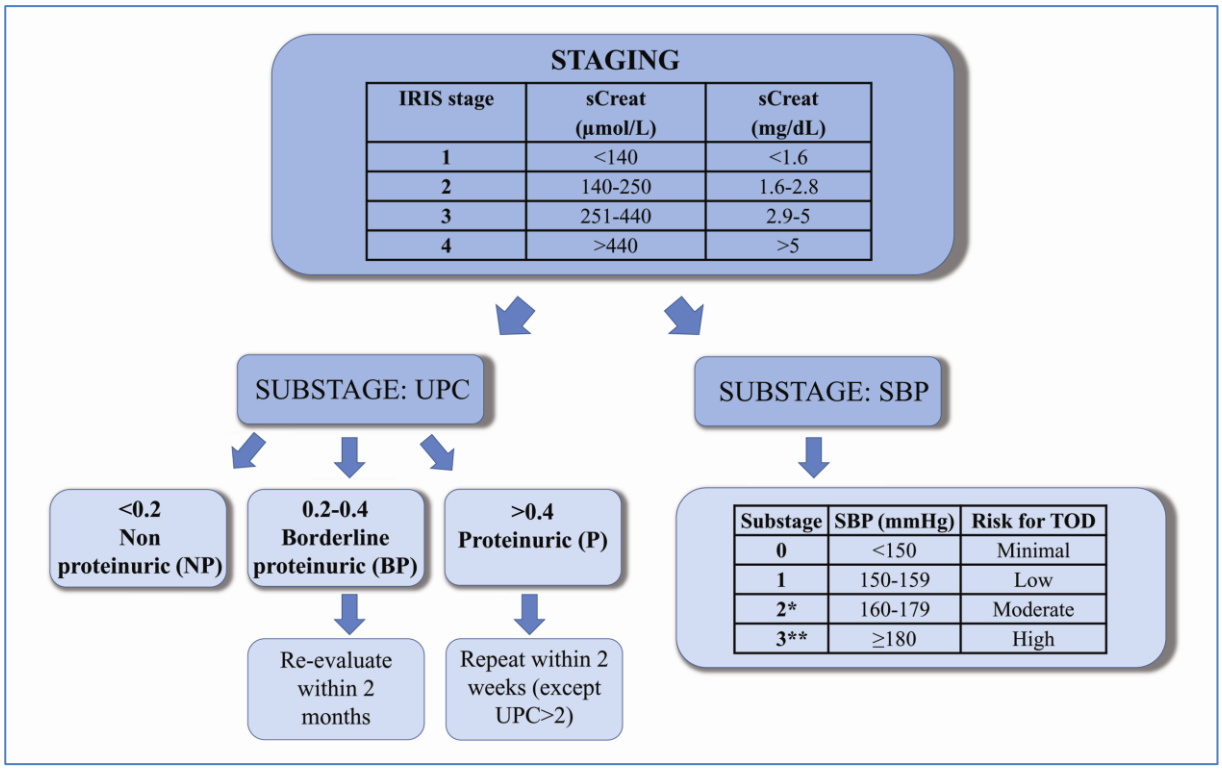

Fig 1.11. Overview of the International Renal Interest Society (IRIS) staging for feline patients with chronic kidney disease (IRIS 2009, Polzin 2010, Polzin 2011). The serum creatinine concentration (sCreat) determines the IRIS stage. Further substaging is based on the degree of proteinuria, assessed by the urinary protein :creatinine ratio (UPC) and the systolic blood pressure (SBP). The SBP substaging system reflects the risk for target organ damage (TOD).

* Persistence of elevation should be judged on multiple blood pressure measurements over a period of 2 months.

** Persistence of elevation should be judged on multiple blood pressure measurements over a period of 1 to 2 weeks.

1.4 SCREENING FOR EARLY CHRONIC KIDNEY DISEASE

1.4.1 Importance of early detection of chronic kidney disease

Survival rates for CKD cats are significantly associated with azotemia and proteinuria and cats diagnosed early in the disease live longer than cats diagnosed with more severe azotemia (Syme *et al* 2006, Boyd *et al* 2008). Consequently, an even better prognosis can be expected for cats diagnosed in the non-azotemic disease stage (IRIS stage 1) because timely therapeutic intervention might prevent or delay disease progression and complications (Fig 1.12) (Lees 2004, Grauer 2005). Similarly, in humans, many adverse events of CKD such as progressive deterioration of kidney function, complications of decreased kidney function, and cardiovascular disease can be prevented or delayed by early detection and treatment (Maschio *et al* 1996, NKF 2002, Remuzzi *et al* 2002, Levey *et al* 2003, Levin and Stevens 2011). Hence, screening is regarded to be an important public health tool for early detection of CKD in people. Screening is strongly recommended in patient groups at-risk for CKD such as patients diagnosed with hypertension or diabetes and close relatives of patients with nephropathy (Li *et al* 2005, Narva 2007).

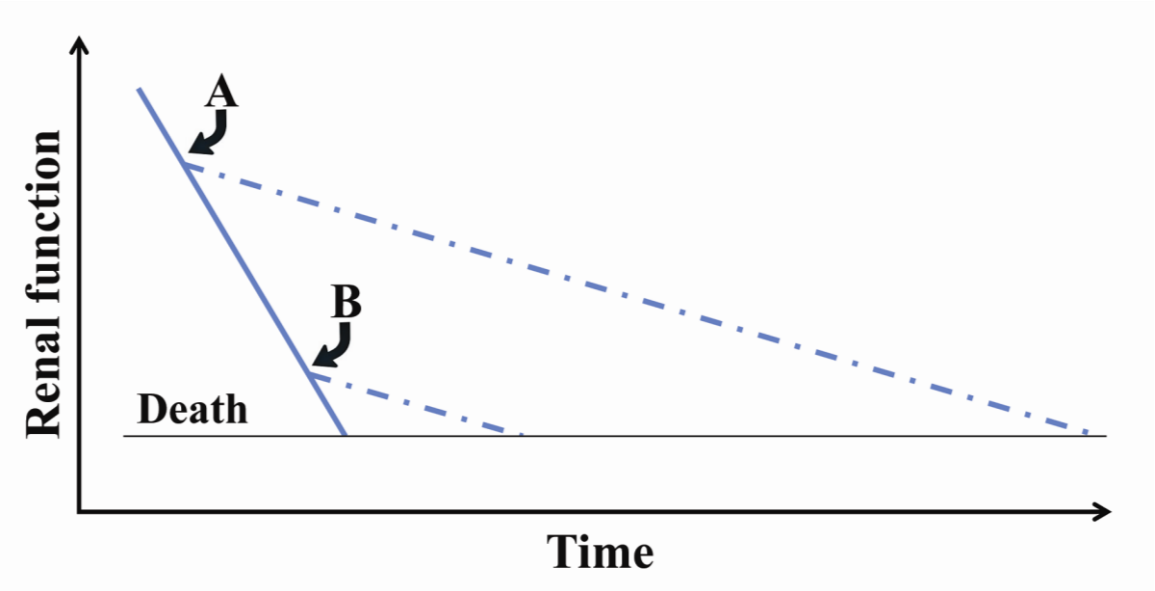

Fig 1.12. Hypothetic effects of altering the rate of disease progression at early (point A) or later (point B) time in the course of renal disease. Equally effective treatments to slow progression of renal dysfunction result in a longer prolongation of survival if early intervention (at point A) compared to later intervention (point B). (Based on Lees 2004)

1.4.2 Screening for chronic kidney disease based on routine diagnostic tests

Unfortunately, diagnosing early feline CKD is challenging. It is assumed that over two-thirds of functional renal mass must be lost before kidneys lose their urinary concentrating ability and over three-quarters must be lost before azotemia develops. Thus, serum creatinine and urea concentrations and USG are often within RIs in cats with early CKD, particularly because some cats may maintain their urinary concentrating ability (Braun and Lefebvre 2008, Stockham and Scott 2008a, DiBartola 2010). Practical, inexpensive and accurate methods to detect early feline CKD are urgently needed. Also, veterinarians should improve owner awareness for early signs of CKD because poor body condition, weight loss and polyuria/polydipsia are not always recognized by cat owners (Hughes *et al* 2002, Pittari *et al* 2009, Bartlett *et al* 2010).

The minimum laboratory database for CKD screening consists of measuring serum creatinine, USG and proteinuria or eventually (micro)albuminuria (FAB 2008, Vogt *et al* 2010). In comparison, a spot urine sample for protein and an estimate of GFR based on serum creatinine concentration are the recommended tests to screen human patients for CKD (NKF 2002). Physical and laboratory parameters should be compared with values of previous health screenings to detect clinically relevant changes. Increasing serum creatinine concentrations, even within RI, may indicate early kidney dysfunction, particularly in cats with weight loss or muscle wasting or USG consistently below 1.035 (Lees 2004, Grauer 2005, Pittari *et al* 2009). However, many factors influence USG and daily USG fluctuations can be seen in healthy animals. Thus, low USG without other indications for CKD does not necessarily suggest kidney dysfunction (Lees 2004, Stockham and Scott 2008a). In healthy non-azotemic geriatric cats, plasma creatinine concentration combined with UPC were predictive of azotemia developing, indicating that high-normal creatinine concentrations and/or UPC values consistent with borderline or overt proteinuria might indicate early kidney dysfunction (Jepson *et al* 2009).

1.4.3 Advanced diagnostic tests for detection of early chronic kidney disease

More advanced tests to evaluate kidney function might be considered in cats with doubtful routine blood and urine tests. As discussed above, GFR estimation would be ideal but has important practical limitations. Some of these limitations might be avoided by LSS, but further research is needed.

Also, the value of serum Cystatin C (sCysC) as indirect GFR marker in cats is under investigation. Cystatin C, a low-molecular-weight protein produced at constant rate by all nucleated cells, meets criteria required for endogenous GFR markers (Dharnidharka *et al* 2002). Serum CysC is superior to serum creatinine to detect renal dysfunction in humans (Dharnidharka *et al* 2002) and also has some advantages over serum creatinine in dogs (Wehner *et al* 2008). Cats with CKD have higher sCysC concentrations compared with healthy cats (Poświatowska-Kaszczyszyn 2012, Ghys *et al* In Press), but evidence showing advantages of sCysC over serum creatinine to detect early feline CKD is currently not available.

Another pathway to identify kidney disease is by urinary biomarkers for tubular or glomerular damage (Price 2002, Cobrin *et al* 2013, De Loor *et al* 2013). Retinol-binding protein (RBP), N-acetyl-β-glucosaminidase activity (NAG), urinary Cystatin C (uCysC), transforming growth factor-β1, interleukin-8 and (micro)albuminuria (see above) are promising candidate urinary biomarkers for cats (Langston 2004, Arata *et al* 2005, Syme and Elliott 2005a, Mardell and Sparkes 2006, van Hoek *et al* 2008b, van Hoek *et al* 2009c, Jepson *et al* 2010, De Loor *et al* 2013, Habenicht *et al* 2013, Ghys *et al* In Press). Low-molecular-weight proteins (NAG, uCysC, RBP) and tubular enzymes (NAG) are not present in the urine of healthy animals. Secondary to tubulointerstitial damage or inflammation, patients with CKD might have detectable urinary concentrations. Also, tubulointerstitial inflammation or fibrosis might result in overexpression and increased urinary concentrations of inflammatory cytokines (transforming growth factor-β1, interleukin-8) (Price 2002, De Loor *et al* 2013). In humans, careful selection of biomarkers allows detection of site specific changes (glomerular versus tubular) (Price 2002). Whether the latter is true in cats and whether these urinary biomarkers have benefit over routine parameters to detect early feline CKD is currently unknown.

1.4.4 Cat populations to consider screening for chronic kidney disease

1.4.4.1 Healthy aged cats

Because old cats are susceptible to many chronic diseases, routine health screening of aged cats is important for early detection of these conditions, such as CKD (FAB 2008, Vogt *et al* 2010). According to the senior and geriatric care guidelines developed by the *American Association of Feline Practitioners, American Animal Hospital Association* and *Feline Advisory Bureau*, 'geriatric health care packages' should consist of a thorough history (preferably by detailed owner questionnaire), physical examination (including oral cavity examination and thyroid palpation), BP measurement, ophthalmic examination and laboratory tests (FAB 2008, Pittari *et al* 2009, Vogt *et al* 2010). Unfortunately, the interpretation of results is difficult because scientific information regarding clinical and laboratory abnormalities in older animals is scarce. One study demonstrated significant but minor age effects on biochemistry values in a purebred cat population with wide age range (0.5 – 15.6 years). These authors found mildly increasing values for urea, creatinine and total protein concentrations with increasing age (Reynolds *et al* 2010). Also, specific RIs to interpret laboratory parameters of senior or geriatric animals may be warranted (Gunn and Alleman 2005). Several physiological changes can be expected with aging, resulting in age-related but clinically insignificant changes (Fig 1.13) (Dowling 2005). As the creatinine concentration depends on muscle mass and as muscle wasting is common in aged cats, this might be particularly true for serum creatinine. Finally, the age at which cats are defined as geriatric varies widely in scientific literature, from 8 years and older (Simpson *et al* 2009), to 14 years (Metzger 2005) or even 15 years and older (FAB 2008, Pittari *et al* 2009, Vogt *et al* 2010). As geriatric medicine is forming an increasing part of the case load of first opinion and referral practices (Caney 2009), improved scientific data on this age group would help veterinarians to manage and treat senior or geriatric cats.

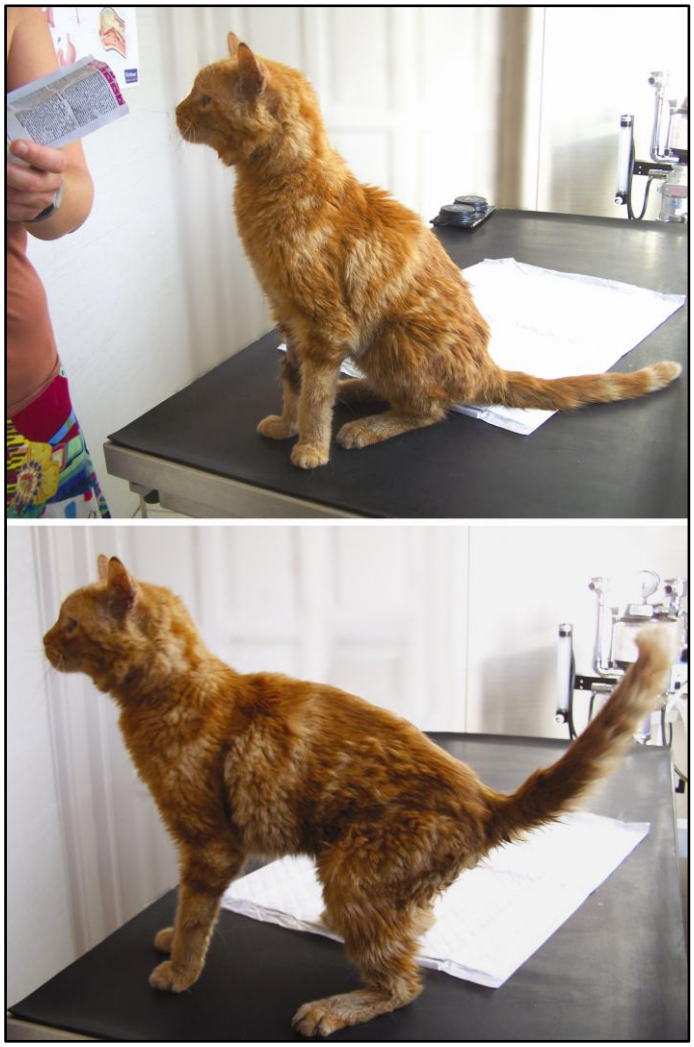

Fig 1.13. A 20-year-old cat that was presented because of constipation. The cat was otherwise healthy and had normal blood (including serum total thyroxine) and urine examinations.
Some age-related changes such as muscle atrophy and unkempt haircoat are visible on the pictures.

1.4.4.2 Ragdoll cats

Several Ragdoll breeder organizations such as the *Scandinavian Ragdoll Club* (SRC) and the *Ragdoll Club Benelux* (RCB) forewarn owners that renal problems may develop due to PKD, chronic interstitial nephritis (CIN), familial renal dysplasia or nephrocalcinosis (SRC 2012, RCB 2013). Based on recommendations of these breed clubs, Ragdoll cats are screened for PKD and CIN prior to breeding in several European countries such as Belgium, the Netherlands, Sweden and Finland (SRC 2012, RCB 2013). Several tests are part of this screening program, including abdominal ultrasonography to identify renal and/or hepatic cysts and evidence of CIN, measurement of serum urea and creatinine concentrations, and genetic testing for the PKD-1 mutation.

Polycystic kidney disease is an inherited condition that results in the formation of fluid-filled renal and, occasionally, hepatic cysts. This condition mainly affects Persian and

Persian-related cats and recent European studies report a prevalence between 31 and 42% for these breeds (Biller and DiBartola 1995, Barthez *et al* 2003, Bonazzi *et al* 2007, Domanjko-Petrič *et al* 2008, Wills *et al* 2009). Because the Ragdoll is one of the breeds that have been outcrossed with Persians, Ragdoll cats could be at risk for PKD (Beck and Lavelle 2001). Affected cats are heterozygous for a stop mutation in the PKD-1 gene that is inherited in an autosomal dominant manner (Biller *et al* 1996, Lyons *et al* 2004, Helps *et al* 2007). In total, scientific literature describes PKD testing of 9 Ragdolls (Beck and Lavelle 2001, Lyons *et al* 2004, Lee *et al* 2010): 4 were only evaluated by ultrasonography and 5 solely by genetic testing. One of the genetically tested Ragdolls had the mutant PKD-1 allele (Lyons *et al* 2004), the others were PKD negative. This indicates that the PKD-1 mutation does occur in Ragdolls, but the PKD prevalence in this breed is unknown.

Chronic interstitial nephritis is a nonspecific inflammatory condition that is classified among the tubulointerstitial renal diseases. It can be primary or secondary to glomerular or systemic diseases, but the underlying cause is often unclear. It results in fibrosis, tubular atrophy and loss of healthy renal tissue. The consequence is progressive renal disease and it is considered to be a common cause of azotemic CKD in cats (DiBartola *et al* 1987, Lulich *et al* 1992, Minkus *et al* 1994, Maxie and Newman 2007). The definitive diagnosis of CIN can only be made on kidney biopsies, but renal histology often does not reveal the underlying cause of the nephritis (DiBartola *et al* 1987, Lulich *et al* 1992, Polzin 2010).

Although screening of Ragdoll cats for PKD and CIN has been performed for many years, there is only minimal scientific evidence about the occurrence of kidney disease within this breed. In addition, the criteria that give rise to a diagnosis of PKD or CIN are not specified on the Ragdoll breeder websites. For these reasons, it is important to elucidate whether the concerns of the breed organizations are justified or not. If Ragdoll cats are indeed at risk for renal disease, adequate and regular monitoring of the renal function may allow early disease detection and intervention.

1.4.4.3 Cats with endocrine disease

Feline endocrine diseases such as hyperthyroidism and diabetes mellitus (DM) might affect kidney function. These endocrine diseases as well as CKD are common in older cats and they may exist concurrently (Mooney and Peterson 2004, Bloom and Rand 2013).

An extensive linkage exists between thyroid and kidney function which becomes mainly important in cats if hyperthyroidism develops. The importance and difficulties to closely evaluate kidney function before, during (e.g. medical treatment) or after treatment (e.g. surgery, radioiodine treatment) have been extensively studied in veterinary medicine and summarized by van Hoek and Daminet (2009) and Daminet *et al* (2014).

In contrast, whether or not feline DM causes renal lesions or dysfunction has not yet been studied in detail.

Diabetic kidney disease (DKD) or diabetic nephropathy is a very common and serious complication in human diabetics, particularly in type 2 DM. Diabetic nephropathy in humans is characterized by glomerular alterations, resulting in altered GFR and micro- or macroalbuminuria, tubular damage and hypertension (Reutens 2013, Ritz 2013, van Buren and Toto 2013).

As feline diabetic patients mostly suffer from type 2 DM, cats might be susceptible to develop DKD (Bloom and Rand 2013, Rand 2013) and regular screening of diabetic cats for kidney disease might be required. However, scientific evidence whether or not feline diabetics are at risk for kidney disease is currently scarce and insufficient to recommend more intensive monitoring of renal function in diabetic cats compared with non-diabetic cats of the same age. Also, CKD and DM may be concurrently present in cats as both CKD and DM are frequent diseases in cats with growing prevalence (Reynolds and Lefebvre 2013, Osto *et al* 2013). Concurrent CKD is reported in 13 to 31% of diabetic cats (Bloom and Rand 2013). For those cats with concurrent DM and CKD, the question whether DM (partially) causes CKD or whether both diseases are unrelated cannot be answered yet.

An additional problem is that the hyperglycemic state of cats with DM may hamper the diagnosis of CKD. Weight loss, polyuria, polydipsia and lethargy – common signs of feline CKD – are also frequent in cats with poorly controlled DM (Feldman and Nelson 2004). Additionally, osmotic diuresis due to hyperglycemia that exceeds the renal threshold and leads to glucosuria might influence the interpretation of routine tests to evaluate kidney

function. The glucosuria itself may falsely elevate USG and an increase of 0.004 – 0.005 for every 1 g/dL of glucose in urine is reported (Stockham and Scott 2008a). On the other hand, osmotic diuresis results in unconcentrated urine (USG < 1.035) and, if severe, may also lead to mild dehydration and mild prerenal azotemia (Feldman and Nelson 2004). So, diagnosing early CKD in a poorly controlled diabetic cat is challenging.

1.4.4.4 Other cat populations to consider screening for chronic kidney disease

Next to the cat groups mentioned above, screening for CKD is also recommended in cats with diseases that might lead to CKD such as infectious diseases (e.g. pyelonephritis, FIV, FeLV), metabolic conditions (e.g. hypercalcemia, hypokalemia), renal neoplasia (e.g. lymphoma, carcinoma), urolithiasis (e.g. ureterolithiasis, nephrolithiasis), conditions that may be associated with renal ischemia (e.g. dehydration, cardiovascular disease) and diseases that may be associated with glomerulopathy (e.g. feline infectious peritonitis, pancreatitis, cholangiohepatitis, neoplasia). Further, screening for CKD must also be considered in cats that need treatment with potentially nephrotoxic agents (e.g. non steroidal anti-inflammatory drugs, doxorubicine). Also, cats that have been treated for acute kidney injury must be monitored carefully for persistent CKD (Polzin 2010, Vaden 2010). Finally, pre-breeding screening is advised for other breeds predisposed for PKD, namely Persian and related breeds, and might be warranted for other familial renal diseases such as amyloidosis in Abyssinian, Siamese and Oriental shorthair cats (Biller and DiBartola 1995).

1.5 CONCLUSION

Feline CKD is typically diagnosed based upon compatible clinical signs, azotemia and poorly concentrated urine (USG ≤ 1.035). The diagnosis of advanced feline CKD and associated complications is usually straightforward based on complete blood and urine examinations. Medical imaging may identify specific underlying causes for CKD.

To facilitate communication, treatment, monitoring and prognosis estimation, all CKD patients must be classified according to the IRIS system, using creatinine for staging and proteinuria and SBP for substaging.

In contrast, early or non-azotemic CKD is more challenging to diagnose. Because early CKD diagnosis might improve the prognosis, screening of at-risk populations is recommended. A scientific basis for geriatric screening of cats in daily practice is required and studies evaluating whether Ragdoll cats and diabetic cats should be routinely screened for kidney disease are needed. Additionally, research regarding feline CKD should focus on development of simple, inexpensive and accurate methods for early disease diagnosis. Possible future techniques are LSS to estimate GFR, methods to identify cats with low GFR, new indirect GFR markers and urinary biomarkers.

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CHAPTER 2

SCIENTIFIC AIMS

Chronic kidney disease (CKD) is a common condition in companion animals, particularly in cats. Advanced feline CKD is usually straightforward to diagnose based on compatible clinical signs and renal azotemia. Since feline CKD is a progressive disease, the main therapeutic goal is slowing down further decline in kidney function and postponing disease complications (Polzin 2010).

As the median survival time shortens with more severe azotemia and proteinuria (Syme *et al* 2006, Boyd *et al* 2008), a better prognosis is expected for cats diagnosed in early disease stages (e.g. non-azotemic or *International Renal Interest Society* (IRIS) stage 1 CKD) (Lees 2004, Grauer 2005). Unfortunately, confirming early CKD is challenging, because azotemia and impaired urine concentrating ability may be absent (Braun and Lefebvre 2008, Stockham and Scott 2008, DiBartola 2010). Therefore, screening of high-risk patients for CKD is highly recommended (FAB 2008, Vogt *et al* 2010, Taylor and Sparkes 2013). Although such screening nowadays is common practice in geriatric cats and – in some countries – in Ragdoll cats, the scientific basis for screening for early CKD is limited.

Therefore, the final goal of this thesis was to evaluate the results and limitations of currently performed screening practices for feline CKD and to develop possible solutions to improve early CKD detection.

Specific objectives of this thesis were:

1. To evaluate results and limitations of routinely used tests for health screening – including screening for early feline CKD – in apparently healthy middle-aged and old cats

2. To evaluate results and limitations of routinely used tests to screen Ragdoll cats for CKD prior to breeding

3. To evaluate if cats with diabetes mellitus are susceptible for diabetic kidney disease and whether routine monitoring of diabetic cats for CKD is required

4. To develop simple and cost-effective methods to estimate glomerular filtration rate (GFR) in cats and to identify cats with low or borderline GFR

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CHAPTER 3

HEALTH SCREENING IN MIDDLE-AGED AND OLD CATS

GENERAL HEALTH SCREENING OF APPARENTLY HEALTHY MIDDLE-AGED TO OLD CATS

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Summary

Veterinary practitioners often perform geriatric health screening in cats. Unfortunately, scientific information regarding clinical and laboratory abnormalities and normal blood pressure (BP) values in elderly cats is scarce.

This prospective study evaluated routine health screening tests in apparently healthy middle-aged and old cats. One hundred cats of 6 years and older underwent BP measurement; physical, blood and urine examinations; indirect fundoscopy; and bilateral Schirmer tear tests (STT).

The mean systolic blood pressure (SBP) was 133.6 ± 21.5 mmHg. Increased SBP (> 160 mmHg) was observed in eight, submandibular lymphadenopathy in 32, gingivitis in 72, heart murmur in 11, thyroid goiter in 20, increased creatinine in 29, hyperglycemia in 25, increased total thyroxine in three, feline immunodeficiency virus (FIV) seropositivity in 14, crystalluria in 41, borderline proteinuria in 25 and overt proteinuria in two cats. The mean tear production was very similar for both eyes and none of the cats had ocular lesions secondary to hypertension.

Old cats (> 10 years) had a significantly higher SBP, heart rate, murmur frequency, thrombocyte count, urinary protein: creatinine ratio (UPC) and serum urea and bilirubin concentrations in addition with significantly lower body condition score (BCS), hematocrit and albumin and total calcium concentrations than middle-age cats (6 – 10 years).

In conclusion, physical examination as well as laboratory abnormalities are common in apparently healthy old cats underlining the need for regular health checks and development of age dependent laboratory reference intervals (RIs).

Introduction

In the last few decades, the expected lifespan of pet cats in Europe and the United States has increased and the population of senior and geriatric cats has grown concurrently (Gunn-Moore 2006). Old cats are susceptible to many chronic diseases and senior care guidelines have been developed to improve early disease detection and promote longevity and quality of life. These "geriatric health care packages" should consist of a thorough history – preferably by detailed owner questionnaire –, physical examination – including oral cavity examination and thyroid palpation –, BP measurement, ophthalmic examination and laboratory tests (Epstein *et al* 2005, FAB 2008, Pittari *et al* 2009). Unfortunately, the interpretation of these results is difficult because scientific information regarding clinical and laboratory abnormalities in older animals is scarce. With aging, several physiologic changes can be expected, resulting in age-related but clinically insignificant changes (Dowling 2005). Therefore, specific RIs for senior or geriatric animals may be warranted (Gunn and Alleman 2005). Although routine BP screening in middle-aged to old cats is advised (Stepien 2011), normal BP values in elderly cats have not been reported. Several studies evaluated indirect BP measurements in healthy adult cats (Bodey and Sansom 1998, Sparkes *et al* 1999, Sansom *et al* 2004, Lin *et al* 2006). However, only small numbers of old cats were included and these studies yielded conflicting results regarding the association between age and SBP. The STT to evaluate tear production can also be part of the minimum database for senior pets (Epstein *et al* 2005). STT-results are poorly documented in older cats. Several reports described STT-results in healthy cats, but mostly included young or middle-aged cats (Margadant *et al* 2003, Cullen *et al* 2005, Ghaffari *et al* 2010). In normal dogs tear production decreases with age (Hartley *et al* 2006), but a similar age effect has not been examined in cats.

Also, the age at which cats are defined as geriatric varies widely, from 8 years and older (Simpson *et al* 2009), to 14 years (Metzger 2005) or even 15 years (FAB 2008, Pittari *et al* 2009, Vogt *et al* 2010) and older. In general, it is accepted that a geriatric animal is one that has reached 75% of its expected life span, which obviously depends on species and breed (Carpenter 2005).

All these questions make interpretation of clinical and laboratory parameters of healthy and diseased old cats complex. Because geriatric medicine is forming an increasing

part of the case load of first opinion and referral practices (Caney 2009), improved scientific data on this age group would help veterinarians to manage and treat senior or geriatric cats.

Therefore, this study aimed to evaluate the presence of abnormal findings on SBP measurement, physical examination, ophthalmic examination and routine blood and urine examinations prospectively in middle-aged and old cats that were apparently healthy according to their owners.

Materials and methods

Study population

One hundred healthy cats of 6 years and older were included. To evaluate an age effect, the cats were allocated to two groups, namely group 1 (middle-aged cats) between 6 and 10 years and group 2 (old cats) older than 10 years. We aimed to include an equal number of cats in both groups. All cats were fasted for 12 hours, water was offered ad libitum.

Health was defined as 'being healthy for the owner', namely no changes in general behavior or habits, normal eating and drinking behavior, stable body weight and absence of clinical signs. Cats needed to be free of medication (except preventive medication) for at least two months before inclusion. Preventive medication was allowed except during the week before inclusion. To check the health status, owners completed a questionnaire with questions related to their cat's health, living environment, daily activity, feeding practices, vaccination status, parasite control and disease history.

The study was completed at the Department of Medicine and Clinical Biology of Small Animals, Ghent University between May and October 2010. All cats were privately owned, the owners signed an informed consent and the study was approved by the local and national ethical committees (EC2010/27).

Procedures

All procedures were performed in the same order by the same author (GV) without using sedation or anesthesia.

First, SBP was measured using the Doppler ultrasonic technique and standardized procedure following the consensus statement of the *American College of Veterinary Internal Medicine* (ACVIM) (Brown *et al* 2007). Hypertension was defined as SBP > 160 mmHg and hypotension as SBP < 80 mmHg (Brown *et al* 2007, Stepien 2010, Waddell 2010).

A standard physical examination was performed to evaluate the general demeanor, BCS on a 9-point scale (Laflamme 1997), rectal temperature, color and capillary refill time of the mucous membranes, visual oral inspection, peripheral lymph node palpation, respiratory

rate, heart rate, cardiac and pulmonary auscultation (Fig 3.1), abdominal palpation and thyroid gland palpation. The thyroid glands were palpated as previously described (Fig 3.2; Paepe *et al* 2008, Boretti *et al* 2009), using the classic technique (Feldman and Nelson 2004) and semi-quantitative scoring system initially proposed by Norsworthy *et al* (2002).

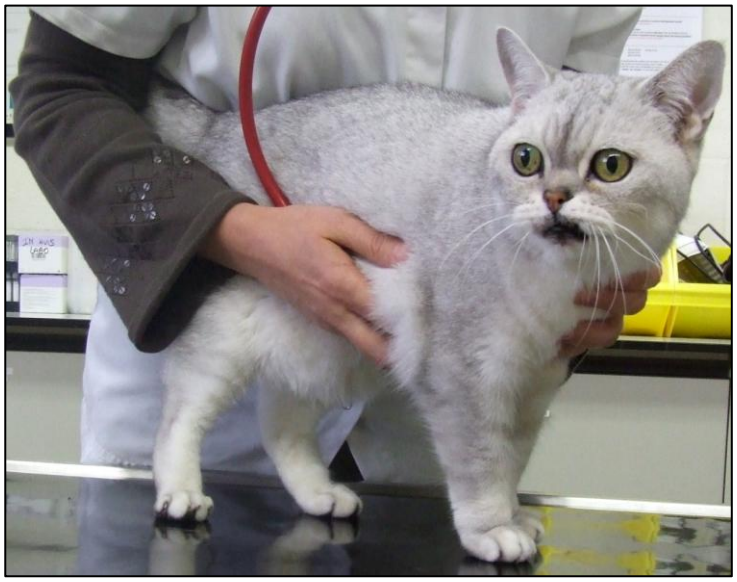

Fig 3.1. Auscultation of the right ventral thorax in a cat. Standing behind the cat to restrain the cat gently in a comfortable position allows auscultation in almost all cats.

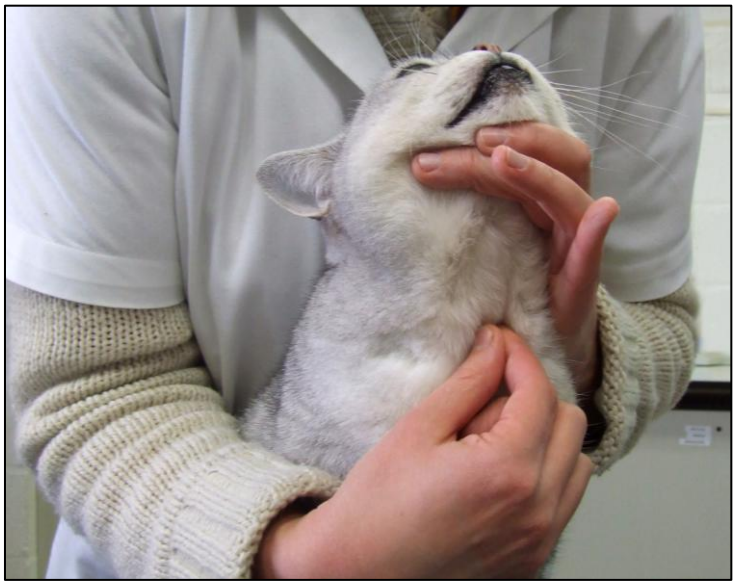

Fig 3.2. Thyroid gland palpation in a cat using the classic thyroid palpation technique. The cat is restrained in sitting position. The neck of the cat is extended and the clinicians' thumb and forefinger are placed on each side of the trachea and swept downwards from the larynx to the sternal manubrium.

All cats underwent an ophthalmic examination. The tear production was evaluated by placing a STT test-strip in the ventral conjunctival sac for 60 seconds and was recorded in mm/minute for both eyes (Fig 3.3). Afterwards, indirect fundoscopy was performed, paying special attention for signs secondary to systemic hypertension, such as retinal edema, vascular tortuosity, hemorrhage or detachment and papilledema.

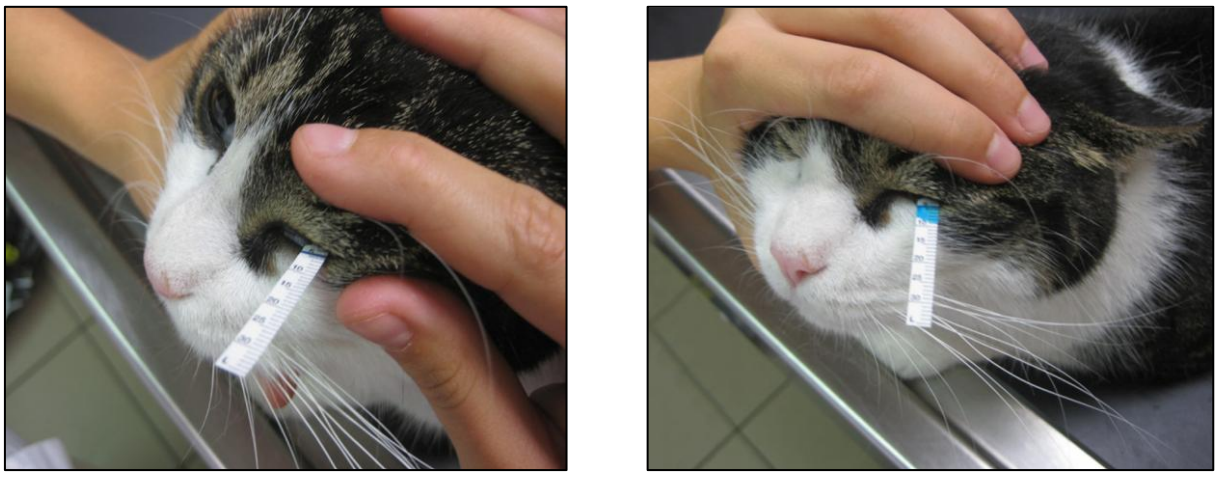

Fig 3.3. One of the study cats just after the placement of the Schirmer tear test strip in ventral conjunctival sac of the left eye (a) and 60 seconds after placement of the Schirmer tear test strip (b). The tear production for the left eye is 9 mm/minute.

Five mL of blood was taken from the jugular vein (Fig 3.4) and 10 mL of urine was collected by cystocentesis. To obtain serum, the coagulated tubes were centrifuged within 30 minutes at 2431 x *g*. All samples were preserved at 4°C and analyzed on the day of collection[a].

A complete blood count[b] and serum biochemistry profile[c] were performed and the total thyroxine (TT4) concentration was measured using a previously validated immunoassay[d] (Singh *et al* 1997). All cats were screened for infection with FIV and feline leukemia virus (FeLV) using an in-house enzyme-linked immunosorbent assay (ELISA) test[e].

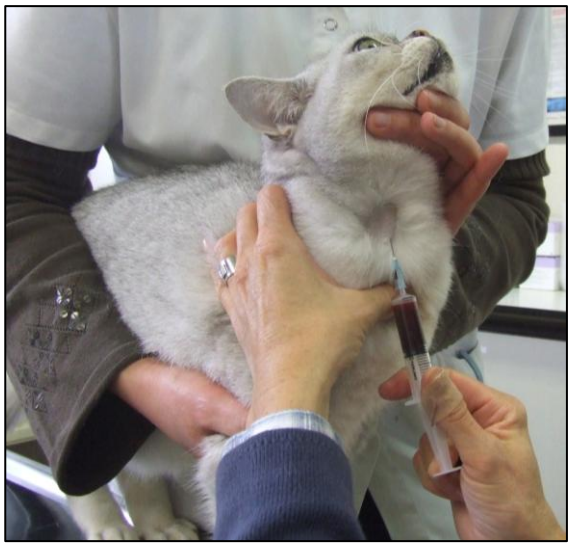

Fig 3.4. Blood sampling from the jugular vein of a cat. By keeping the neck and front legs extended, the jugular vein becomes visible or easily palpable in most cats.

Urinalysis consisted of a urinary dipstick test; measurement of urine specific gravity (USG) with a manual refractometer, urinary pH and UPC[f]; sediment examination and urine bacterial culture[g]. The sediment was prepared by centrifugation of 5 mL of urine in a conical-tipped tube for 3 minutes at 447 x *g* and decanting the supernatant to leave an equal amount of sediment and urine. This sediment was resuspended by flicking the tube several times and one unstained drop was placed on a clean glass slide and covered with a coverslip (Meyer 2001). The sediment (cells, casts, crystals) was evaluated under the microscope within 30 minutes of collection. Crystalluria was evaluated semi-quantitatively and expressed per low-power field (LPF, 10x objective) as mild (< 1/LPF), moderate (1 – 3/LPF), or severe (> 3/LPF).

Statistical methods

All statistical analyses were performed with the statistical software package SAS[h]. Different response variables were compared between two groups (FIV+ versus FIV-, middle-aged versus old cats, normal versus abnormal UPC). The response variables that were normally distributed were analyzed by the *t*-test. For binary response variables, such as UPC (normal = UPC < 0.2 and abnormal = UPC ≥ 0.2), Fisher's exact test was used. For discrete response variables with more than two outcomes, such as BCS ((underweight (BCS < 5), ideal (BCS = 5) and overweight (BCS > 5)), the Wilcoxon rank sum test was used. The Pearson's correlation coefficient was used to evaluate the correlation between continuous variables. The significance level was set at 0.05.

Results

Study population

One hundred cats, 56 middle-aged and 44 old cats, were included. The population consisted of 44 males (five intact, 39 neutered) and 56 females (seven intact, 49 neutered). Most (n = 93) were domestic short- or long-haired cats, seven were pure breed cats (two British shorthairs, two Ragdolls, one Norwegian Forest cat, one Maine coon, one Persian cat). The majority of cats had both in- and outdoor access (n = 61), some were strictly indoor (n = 19) or strictly outdoor (n = 20) cats. The age and body weight of the complete population and age groups are presented in Table 3.1.

Blood pressure measurement, physical and ophthalmic examination

The descriptive statistics for the SBP measurements and physical examination findings for the total population and age groups are summarized in Table 3.1.

Eight cats had a mean SBP exceeding 160 mmHg. One of these cats had tachypnea, which was thought to be stress-related, one had mild leukocytosis, but none of these cats had hyperglycemia or glucosuria. No significant correlation between SBP and parameters that could be influenced by stress, namely respiratory rate (r = -0.144), heart rate (r = -0.118), serum glucose concentration (r = -0.127) and total leukocyte count (r = -0.033) was found. SBP ranged from 160 to 170 mmHg in four cats (two middle-aged, two old cats) and one of these was borderline proteinuric and had a serum creatinine concentration at the upper limit of the RI but with hypersthenuric urine. SBP exceeded 180 mmHg (with a maximum of 237 mmHg) in the other four cats (one middle- aged, three old cats). The middle-aged cat had a grade 3/6 systolic heart murmur and the three old cats were borderline proteinuric (one with USG 1.051 and normal serum creatinine; one with USG 1.020 and normal serum creatinine; one with USG 1.027 and mildly increased serum creatinine). In the other hypertensive cats target organ abnormalities or an underlying cause for the hypertension were not found based on the diagnostic tests carried out in this study.

Table 3.1. Descriptive statistics for all included cats and for the two age groups. All parameters are expressed as mean ± standard deviation.

	Global population (n = 100)	**Group 1 (n = 56)**	**Group 2 (n = 44)**	**Unit**
Age	9.9 ± 2.7	7.9 ± 1.4	12.5 ± 1.5	years
Body weight	4.6 ± 1.2	4.7 ± 1.2	4.4 ± 1.2	kg
SBP	133.6 ± 21.5	128.3 ± 16.7	140.4 ± 25.0	mmHg
Heart rate	191.4 ± 17.4	187.6 ± 18.4	196.2 ± 14.8	bpm
Respiratory rate	49.6 ± 16.2	48.0 ± 15.9	51.6 ± 16.6	/min
Body temperature	38.2 ± 0.5	38.3 ± 0.5	38.1 ± 0.5	°C
STT left eye	13.4 ± 5.5	12.8 ± 5.1	14.2 ± 6.0	mm/min
STT right eye	14.0 ± 5.7	14.1 ± 5.7	14.0 ± 5.9	mm/min
Hematocrit	36.8 ± 4.6	37.9 ± 4.7	35.5 ± 4.2	%
Leukocytes	9,828 ± 4,575	9,162 ± 4,048	10,677 ± 5,091	/µL
Thrombocytes	293,140 ± 144,685	266,179 ± 120,856	327,455 ± 165,416	/µL
Sodium	152.9 ± 1.9	153.0 ± 1.8	152.8 ± 2.0	mmol/L
Potassium	4.4 ± 0.5	4.4 ± 0.4	4.5 ± 0.5	mmol/L
Total calcium	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	mmol/L
Phosphate	1.4 ± 0.3	1.4 ± 0.2	1.5 ± 0.3	mmol/L
Urea	9.2 ± 2.4	9.1 ± 1.7	10.0 ± 2.9	mmol/L
Creatinine	122.7 ± 30.8	126.0 ± 26.0	118.5 ± 35.9	µmol/L
Total protein	73.7 ± 6.1	73.2 ± 5.8	74.4 ± 6.4	g/L
Albumin	37.5 ± 3.5	38.4 ± 3.3	36.3 ± 3.4	g/L
Glucose	5.4 ± 2.1	5.4 ± 2.1	5.4 ± 2.2	mmol/L
ALT	40.2 ± 33.9	36.0 ± 14.6	45.5 ± 48.2	U/L
AST	19.7 ± 10.5	18.3 ± 8.3	21.4 ± 12.7	U/L
Total thyroxine	29.1 ± 18.4	28.0 ± 8.0	30.6 ± 27.3	nmol/L
Urinary pH	6.7 ± 0.6	6.8 ± 0.6	6.6 ± 0.4	
USG	1.046 ± 0.010	1.047 ± 0.010	1.044 ± 0.012	
UPC	0.18 ± 0.11	0.13 ± 0.05	0.21 ± 0.15	

(Group 1 = Middle-aged cats (6 to 10 years); Group 2 = Old cats (older than 10 years); n = Number of cats; SBP = Systolic blood pressure; bpm = Beats per minute; STT = Schirmer tear test; ALT = Alanine aminotransferase activity; AST = Aspartate aminotransferase activity; USG = Urine specific gravity; UPC = Urinary protein: creatinine ratio)

Forty-nine cats had an ideal BCS, 11 cats (two middle-aged, nine old cats) were too thin (BCS < 5) and 40 cats (24 middle-aged, 16 old cats) too heavy (BCS > 5). Most cats (n = 96) had a body temperature between 37.5 and 39.3°C. Four old cats had a body temperature below 37.5°C (minimum 37°C). The mucous membranes were moist and pink with normal capillary refill time in all cats. Mild local lymphadenopathy was detected in 34 cats: 32/34 had submandibular and 2/34 popliteal lymphadenopathy. Thirty cats with submandibular lymphadenopathy had gingivitis. In total, 72 out of 100 cats had gingivitis. Pathological tachycardia (heart rate > 240 beats per minute (bpm)) or bradycardia (heart rate < 140 bpm) was not observed, but 11 cats had stress-related tachypnea. One cat even exhibited open-mouth breathing initially, but calmed down after a prolonged acclimatization period, allowing further examinations. Auscultation revealed a cardiac murmur in 11 cats (two middle-aged, nine old cats), all systolic and with following murmur intensity: grade 1/6 (n = 3), 2/6 (n = 4), 3/6 (n = 2), 4/6 (n = 2). These eleven cats were normotensive, normothermic and had a normal hematocrit, but one was hyperthyroid. Cardiac arrhythmias or gallop sounds were not heard. Lung auscultation was normal in 98 cats; two cats had a diffuse mild increase in lung sounds. Abdominal palpation did not reveal significant abnormalities in any of the cats. The majority (n = 92) had a soft abdomen, the others had a tensed but not painful abdomen. A thyroid goiter (score > 0) was palpated in 20 cats with a maximum score of 3: score 1 in 13/20, score 2 in 4/20, score 3 in 3/20.

The mean tear production was 14.0 ± 5.7 mm/min for the right eye and 13.5 ± 5.7 mm/min for the left eye. A STT result < 5 mm/minute was found in both eyes of one cat and in the left eye of another cat. One cat showed mild corneal edema and one had papillary membrane remnants in both eyes. Fundoscopic abnormalities secondary to systemic hypertension were not found in any of the cats.

Laboratory parameters

The descriptive statistics for the laboratory parameters are presented in Table 3.1 and the number of animals having laboratory parameters within, below or above the RI in Table 3.2. The distribution of the serum phosphorus concentration of the cats across different categories based on the '2006 Phosphate Roundtable Guidelines' (Elliott 2007) is shown in Table 3.3.

Table 3.2. Distribution of the 100 included cats below, within and above the reference interval for certain laboratory parameters.

Parameter	Reference interval	Below RI		Within RI	Above RI	
		n	Min	n	n	Max
Hematocrit (%)	24 – 45	1	20.5	95	4	51.8
Leukocytes (/µL)	5,000 – 15,000	5	3,950	82	13	26,990
Platelets (/µL)	175,000 – 500,000	26	22,000	67	8	909,000
Urea (mmol/L)	6.2 – 11.0	1	6.0	84	15	25.6
Creatinine (µmol/L)	44.2 – 132.6	0	/	71	29	280.2
Total protein (g/L)	53 – 76	1	51	69	30	94
Albumin (g/L)	25 – 4	0	/	98	2	45.8
Glucose (mmol/L)	3.1 – 5.6	0	/	75	25	18.6
Sodium (mmol/L)	146 – 154	0	/	80	20	159
Potassium (mmol/L)	3.6 – 5.3	1	3.3	96	3	6.3
Total calcium (mmol/L)	2.2 – 2.7	2	2.1	97	1	2.7
Phosphorus (mmol/L)	1.4 – 3.0	40	0.87	60	0	/
TT4 (nmol/L)	14.2 – 45.2	4	6.5	93	3	> 193.5
ALT (U/L)	< 70	0	/	94	6	333
AST (U/L)	< 42	0	/	98	2	88
GGT (U/L)	< 4	0	/	100	0	/
Bilirubin (µmol/L)	< 6.8	0	/	99	1	14.1

(RI = Reference interval; n = Number of cats; Min = Minimum value observed for this laboratory parameter; Max = Maximum value observed for this laboratory parameter; TT4 = Total thyroxine concentration; ALT = Alanine aminotransferase activity; AST = Aspartate aminotransferase activity; GGT = γ-glutamyl transpeptidase activity)

Table 3.3. Distribution of the complete population and age groups for the serum phosphorus concentration across four different categories. These categories were defined based on the 2006 Phosphate Roundtable Guidelines for dogs and cats with chronic kidney disease (Elliott 2007).

	Global population (n = 100)	**Group 1 (n = 56)**	**Group 2 (n = 44)**
Phosphorus 0.81 – 1.45 mmol/L	56	33 (58.9%)	23 (52.3%)
Phosphorus 1.45 – 1.61 mmol/L	24	14 (25%)	10 (22.7%)
Phosphorus 1.61 – 1.94 mmol/L	18	9 (16.1%)	9 (20.5%)
Phosphorus > 1.94 mmol/L	2	0 (0%)	2 (4.5%)

(Group 1 = Middle-aged cats (6 to 10 years); Group 2 = Old cats (older than 10 years); n = Number of cats)

The differential leukocyte count of all five leukopenic cats revealed a decrease in mature neutrophils only. Of the 13 cats with a leukocytosis, none had a left shift or a total leukocyte count exceeding the expected value for cats with steroid leukograms (< 30,000/µl) (Stockham and Scott 2008). The majority of cats with thrombocytopenia (18/26) showed platelet aggregates on their blood smear, suggesting pseudothrombocytopenia. Of the 29 cats with serum creatinine concentration above the RI, two had isosthenuric urine and six a USG between 1.015 – 1.035, six were borderline proteinuric (UPC 0.2 – 0.4), two were hypertensive, seven had an increased serum urea concentration and one a decreased TT4 concentration. The others had a USG above 1.035, were nonproteinuric (UPC < 0.2) and normotensive and had serum urea and TT4 concentrations within RI. Both cats with isosthenuric urine were old cats (9 and 13 years) and nonproteinuric. They were diagnosed with chronic kidney disease (CKD) IRIS stage 2 and stage 3, respectively. Serum glucose exceeded 10 mmol/L in 3/25 cats with hyperglycemia. Two of these and one other cat (glucose 9.6 mmol/L) also had glucosuria. The increase in alanine aminotransferase activity (ALT) was mild (< 1.5 times the upper limit of the RI) in 5/6 cats, but serious in one cat (almost five times the upper limit of the RI). This cat (13 years old) was hyperthyroid and also the only cat with a significant elevation of aspartate aminotransferase activity (AST; twice the upper limit of the RI). The bilirubin concentration was increased in only one cat (11 years). This cat also had a very mildly increased ALT without increase of other liver enzymes and a decreased hematocrit. Of the three cats with an increased TT4 concentration, the TT4 was seriously elevated in one old and just above the upper limit of the RI in two middle-aged cats

(6 and 9 years). The hyperthyroid cat and one out of 2 cats with a mildly increased TT4 had a palpable thyroid gland (score 1).

Fourteen cats were FIV seropositive (eight middle-aged, six old cats), no cats tested positive for FeLV antigen. Three FIV infected cats were hypertensive and, additionally, all FIV seropositive cats had a significantly higher SBP (mean 145.9 ± 29.8 mmHg) than FIV seronegative cats (mean 131.6 ± 19.4 mmHg; $P = 0.02$). Three of five cats with leukopenia were FIV seropositive and FIV seropositive cats had significantly lower total leukocyte counts (mean 6,899 ± 2,068 cells/µL) than FIV seronegative cats (mean 10,305 ± 4,699 cells/µL; $P = 0.009$).

Urinalysis was performed in all cats except one, which had an empty bladder. Fifteen cats had a USG below 1.035, with isosthenuria detected in three of them (one middle-aged, two old cats). More detailed information regarding USG is given in Table 3.4. Urinary pH ranged from 5.1 to 7.5 in 91/99 cats. The remaining eight had a pH > 7.5 (maximum 9) with a positive urine culture in one of these. The distribution of cats in proteinuria categories according to the ACVIM consensus statement (Lees *et al* 2005) is shown in Table 3.5. Of the 27 cats with an abnormal UPC (UPC > 0.2) none had overt renal failure, isosthenuria or macroscopic hematuria; four were hypertensive; five had USG below 1.035; six an increased serum creatinine concentration; and the amount of urinary crystals did not differ significantly from cats with UPC < 0.2. Of the 25 cats with borderline proteinuria, three had microscopic hematuria and one a positive urine culture. Both cats with UPC > 0.4 had normal SBP and normal serum urea and creatinine concentrations. One had microscopic hematuria and the other was hyperthyroid. Casts were not detected on urinary sediment analysis. Crystalluria was present in 41/99 cats and was mild in 28/41, moderate in 8/41 and severe in 5/41 cats. Amorphous crystals were mostly (33/41) detected, struvite crystals in 5/41 and calcium oxalate crystals in 3/41 cats. For cats with crystalluria, 17/41 cats mainly received dry food, 1/41 mainly canned food, 15/41 a combination of dry and canned food and for 8/41 the owners did not specify the diet type. For cats without crystalluria, 20/58 mainly received dry food, 2/58 mainly canned food, 20/58 a combination of dry and canned food, 2/58 a combination of dry and table food and for 12/58 the owner did not specify the diet type. The distribution of the diet type did not significantly differ between cats with and without crystalluria and between cats with different types of urinary crystals. On urinary dipstick

examination a trace of ketonuria, glucose positivity and a trace of urobilinogen was each present in 3/99 cats, 28/99 cats were hemoglobin positive, 98/99 cats were leukocyte-esterase positive, and no cats were bilirubin or nitrites positive. Only one cat had a positive urine bacterial culture with an *Enterococcus* species. This was a 9-year-old, intact female, mixed-breed cat that had serum glucose, TT4, urea and creatinine concentrations within the RIs. It had alkaline urine, mild pyuria, and moderately concentrated urine (USG 1.020).

Table 3.4. Distribution of the complete population and age groups for the urine specific gravity (USG) across three categories: USG below 1.035, USG 1.035 – 1.040 and USG above 1.040.

	Global population (n = 99)	**Group 1 (n = 56)**	**Group 2 (n = 43)**
USG < 1.035	15	6 (10.7%)	9 (20.9%)
USG 1.035 – 1.040	8	7 (12.5%)	1 (2.3%)
USG > 1.040	76	43 (76.8%)	33 (76.7%)

(Group 1 = Middle-aged cats (6 to 10 years); Group 2 = Old cats (older than 10 years); n = Number of cats; USG = Urine specific gravity)

Table 3.5. Distribution of the complete population and age groups across the three proteinuria categories according to the ACVIM consensus statement (Lees *et al* 2005).

	Global population (n = 99)	**Group 1 (n = 56)**	**Group 2 (n = 43)**
UPC < 0.2	72	50 (89.3%)	22 (51.2%)
UPC 0.2 – 0.4	25	6 (10.7%)	19 (44.2%)
UPC > 0.4	2	0 (0%)	2 (4.7%)

(Group 1 = Middle-aged cats (6 to 10 years); Group 2 = Old cats (older than 10 years); n = Number of cats; UPC = Urine protein: creatinine ratio; UPC < 0.2 = No proteinuria; UPC 0.2 – 0.4 = Borderline proteinuria; UPC > 0.4 = Proteinuria)

Age group comparison

Old cats had a significantly higher SBP ($P = 0.0049$), heart rate ($P = 0.014$), murmur frequency ($P = 0.026$), platelet count ($P = 0.035$), urea concentration ($P = 0.042$), bilirubin concentration ($P = 0.025$) and UPC ($P < 0.001$) and a significantly lower BCS ($P = 0.031$), hematocrit ($P = 0.009$), albumin concentration ($P = 0.002$) and calcium concentration ($P = 0.049$) than middle-aged cats. The other parameters did not differ significantly between the two age groups.

Underweight cats

Of the 11 underweight cats, one was diagnosed with CKD IRIS stage 3, one with hyperthyroidism, and one was FIV seropositive. The serum creatinine, urea, TT4 concentrations and UPC did not significantly differ between the three BCS categories (underweight, ideal and overweight). However, underweight cats had significantly lower USG (mean 1.038 ± 0.016) compared to cats with an ideal BCS (mean 1.045 ± 0.011; $P = 0.037$) and to overweight cats (mean 1.048 ± 0.008; $P = 0.007$).

Discussion

This study is the first to describe health screening results of an apparently healthy cat population. The study population was balanced for sex and age and the breed distribution reflected the general cat population in Belgium. Domestic short- or longhair cats were the predominant breed also in other clinical studies performed in Belgium (Defauw *et al* 2011).

The mean SBP of our population was 133.6 ± 21.5 mmHg which is very similar to two recent reports (mean SBP 133.6 ± 16 mmHg and 131.1 ± 17.8 mmHg). These studies also evaluated healthy conscious client-owned cats with the indirect Doppler technique, but contained cats with a wider age distribution (Lin *et al* 2006, Paige *et al* 2009). Eight cats had a mean SBP that exceeded the cut-off value above which further diagnostics are advised (160 mmHg) (Lin *et al* 2006, Stepien 2010, Stepien 2011). We tried to limit the white-coat effect by measuring the SBP in presence of the owner after acclimatization in a quiet room and before performing the physical examination (Belew *et al* 1999). However, white-coat hypertension cannot be excluded in our cats because the SBP was only measured on a single occasion (Brown *et al* 2007). Values of SBP above 180 mmHg, as recorded in four of our cats, are less likely to reflect white-coat hypertension (Belew *et al* 1999, Stepien 2011). Also, the SBP did not correlate with other physical and laboratory parameters that can be influenced by stress. An obvious underlying cause for the hypertension in our cats was not found. Further work-up was advised but declined by the owners. Several cats were borderline proteinuric or had moderately concentrated urine which can be consequences of the hypertension or indicative of early renal insufficiency (Brown *et al* 2007, Jepson 2011). Assessment of the glomerular filtration rate (GFR) could be helpful in such cats to diagnose non-azotemic kidney disease (Jepson 2011), but this was outside the scope of the present study. Several of our hypertensive cats were FIV seropositive and these cats had a significantly higher SBP than FIV negative cats. In human medicine, seropositivity for human immunodeficiency virus is a known risk factor for hypertension, cardiovascular disease and nephropathy (Weiner *et al* 2003, Jung *et al* 2004, Bloomfield *et al* 2011). To the best of our knowledge, an association between hypertension and FIV infection is not reported and further studies are needed to elucidate if this is an incidental finding or if FIV seropositive cats are at risk for hypertension.

According to the BCS, which is useful for assessing the body fat percentage of pet cats (Bjornvad *et al* 2011), less than half of this apparently healthy cat population had an ideal body condition. This indicates that cat owners do not appreciate under- or overweight as a problem (Courcier *et al* 2010). Improved owner awareness of normal feline body condition and regular nutritional assessments by veterinarians is important to increase the proportion of cats with an optimal body condition (Freeman *et al* 2011). Forty percent of our cats were too heavy, which is comparable to a recent UK study (39%) (Courcier *et al* 2010) and somewhat higher than in a recent French study (27%) (Colliard *et al* 2009). However, all three studies confirm that overweight and obesity is common in pet cats. As in another study (Courcier *et al* 2010), underweight cats were significantly older than cats with an ideal or overweight body condition. This may be explained by reduced fat and protein digestion with age in cats (Laflamme 2005). Only a few of our underweight cats were diagnosed with a condition that could explain weight loss. However, we must also consider that our underweight cats could have had occult systemic disease. The significantly lower USG might indicate decreased renal function at least in some underweight cats. Assessment of the GFR in underweight cats with poorly concentrated urine would have helped identifying cats with non-azotemic kidney disease.

The most common abnormalities on physical examination were gingivitis, submandibular lymphadenopathy and a cardiac murmur. The majority of our cats (72%) showed gingivitis which is very similar to the 73.2% of gingivitis found by Verhaert and Van Wetter (2004) in a large cat population. Both studies took place in the same geographic area (Flanders, Belgium) and in both studies most owners (in the present study none) did not brush their cats' teeth. Because we only performed visual oral inspection on conscious cats, we cannot comment on the number of cats with periodontitis, stomatitis or odontoclastic resorptive lesions. The presence of gingivitis was associated with mild submandibular lymphadenopathy in many cats.

A heart murmur was auscultated in 11% of our cats which is lower than the 21% murmur prevalence in a young to middle-aged healthy domestic cat population (Côté *et al* 2004). In both studies, all murmurs were systolic with intensity between 1/6 and 4/6. Differences in murmur prevalence between studies may be the result of differences in study populations (e.g. age), interobserver variation or geographic differences (Côté *et al* 2004).

One of our cats with a murmur was hyperthyroid, but in 10 cats there was no evidence of a systemic condition that could explain the murmur. In these cats the murmur could be caused by subclinical structural heart disease, which was a common finding in other studies that evaluated apparently healthy cats with a murmur (Côté *et al* 2004, Dirven *et al* 2010, Nakamura *et al* 2011). Although echocardiography is strongly recommended in healthy cats with a murmur (Côté *et al* 2004, Dirven *et al* 2010, Nakamura *et al* 2011), auscultation of a heart murmur had only poor sensitivity but moderate specificity to detect cardiomyopathy (Paige *et al* 2009).

Several euthyroid cats had a palpable goiter. The maximum score of 3 is in line with other recent studies in which euthyroid cats mostly had small thyroid gland nodules (Norsworthy *et al* 2002, Paepe *et al* 2008, Boretti *et al* 2009). The mean STT results of the present study are very comparable to the STT results reported for young adult and middle-aged normal cats (Margadant *et al* 2003, Cullen *et al* 2005, Ghaffari *et al* 2010). In contrast to dogs (Hartley *et al* 2006), we did not detect decreased tear production with increasing age. Only two cats had a STT < 5 mm/min in one or both eyes, which could be consistent with keratoconjunctivitis sicca (Moore 2000).

Major findings on blood examination were leukocytosis, thrombocytopenia, increased serum urea concentration, increased serum creatinine concentration, hyperproteinemia, hyperglycemia, hypernatremia and hypophosphatemia. The leukocytosis and hyperglycemia were probably stress-related and the thrombocytopenia was probably pseudothrombocytopenia in the majority of cases. The 25% of cats with hyperglycemia is lower than recently published percentages for ill cats at admission to an emergency service (40%) (Chan *et al* 2002) or during hospitalization (64%) (Ray *et al* 2009). For the other laboratory parameters where many cats were outside the RI (Table 3.2), some of the abnormal values could truly be the result of occult disease, but we should also bear in mind that the RI may not be appropriate.

One of the most striking findings was the increased serum creatinine concentration in almost one third of cats. Although some cats could have had early CKD, the increase was probably not clinically relevant in the majority of cases because most cats only had a mildly increased serum creatinine and hypersthenuric urine. The most likely explanation for the high proportion of cats with an increased serum creatinine concentration is the laboratory RI. For

serum creatinine, RIs can vary markedly between laboratories influencing the classification of samples as normal or abnormal (Boozer *et al* 2002, Ulleberg *et al* 2011). Finally, some cats could have had an increased creatinine production rate, resulting in mild azotemia despite normal renal function (Le Garreres *et al* 2007).

Also many cats had mild hyperproteinemia, hypophosphatemia or hypernatremia which questions the appropriateness of these RIs. To avoid misinterpretation of clinical data, RIs need to reflect the population for which it is used (Friedrichs 2010). The laboratory that analyzed our samples used healthy young cats to calculate RIs which is not representative for our study population. An obvious example of an inappropriate RI in our study is the serum phosphorus concentration. In veterinary literature, typically the RI for serum phosphorus concentration in cats is between 0.81 and 1.94 mmol/L (Bates 2008, Kidder and Chew 2009). In contrast, the lower limit of our RI was 1.35 mmol/L, which resulted in a high proportion (40%) of cats with hypophosphatemia. The fact that young cats (6 months – 1 year) were used to determine the RI might explain this inappropriately high low reference limit. Indeed, enhanced renal tubular phosphate reabsorption results in increased serum phosphorus concentrations in growing animals (Corvilain and Abramow 1964). All but two of our cats had serum phosphorus within the RI if the published RI is used (Table 3.3). In addition, the majority of our cats had a serum phosphorus concentration in the lower half of the published RI and many of these cats were incorrectly classified as hypophosphatemic. This indicates the need to calculate age-dependent RIs to improve the interpretation of laboratory parameters in all age categories.

We found a 14% FIV seroprevalence. Recent FIV seroprevalences vary greatly (2.5 – 33.9%), depending on area and the cat population studied (Levy *et al* 2006, Norris *et al* 2007, Gleich *et al* 2009, Little *et al* 2009, Duarte *et al* 2010, Nakamura *et al* 2010, Al-Kappany *et al* 2011). Our cats were all client-owned cats living in and around Ghent. Urban stray cats of the same area had a seroprevalence of 11.2% (Dorny *et al* 2002). This means that the FIV seroprevalence has not decreased despite stray cat programs have been running for years in this area. In contrast, FeLV antigen was detected in 3.8% of stray cats (Dorny *et al* 2002), but not in our cats. Possible explanations are different study populations (stray versus client-owned cats) and different ages as FeLV mainly affects young cats (Gleich *et al* 2009).

On urinalysis, borderline proteinuria and crystalluria were common. The majority of cats with borderline proteinuria (except one with a positive culture) had an inactive sediment, and the amount of crystals was similar in cats with a normal (< 0.2) versus abnormal (≥ 0.2) UPC. Therefore and because the urine was taken by cystocentesis, this borderline proteinuria was probably of renal origin (Lees *et al* 2005). Although none of these cats showed overt renal failure, early CKD cannot be ruled out. Secondly, borderline proteinuria could be associated with occult or subclinical systemic disease because further diagnostics were not performed in any of the cats (Mardell and Sparkes 2006, Whittemore *et al* 2007). Finally, because the UPC was measured only once, we cannot determine if these cats were transiently or persistently borderline proteinuric (Lees *et al* 2005). As in Whittemore *et al* (2007), an age effect on the degree of urinary protein excretion was found with a significantly higher UPC in old versus middle-aged cats. Although older cats may be more prone for early CKD or occult systemic disease, the 0.2 cut-off value may also not be appropriate for UPC evaluation in old cats. Further research to explain borderline proteinuria in old cats is definitively warranted.

Crystalluria was detected in almost half of our cats and was mostly mild and caused by amorphous crystals. All types of crystals that we detected (amorphous, struvite, calcium oxalate) can occur in normal urine samples. Although it is generally accepted that crystals are commonly present in feline urine (DiBartola 2010), we are not aware of scientific studies assessing how often crystalluria affects client-owned healthy cats. Although we cannot rule out urolithiasis, the crystalluria was probably non-pathogenic in the majority because none of our cats had lower urinary tract signs. Veterinarians should be aware that crystalluria is common in healthy cats and not sufficient to start feeding a calculolytic diet. In a previous study in specific pathogen free (SPF) cats, struvite crystals were found more commonly in cats fed a mixed diet compared to cats fed solely canned food (Sturgess *et al* 2001). This contrasts with the lack of association between diet type and presence or type of urinary crystals in our study. However, the absence of an association must be interpreted cautiously because diet information was not available in several of our cats and because we only found low number of cats with anything other than amorphous crystals.

Another remarkable finding on urinalysis was the positive leukocyte-esterase dipstick test in all but one cats. This confirms that this test is nonspecific and cannot replace microscopic urine sediment examination (Holan *et al* 1997).

One cat was diagnosed with an occult bacterial urinary tract infection (UTI). In cats, spontaneous bacterial UTIs occur most frequently in older cats (Lekcharoensuk *et al* 2001, Mayer-Roenne *et al* 2007, Bailiff *et al* 2008), mainly because common metabolic diseases such as CKD, diabetes mellitus, and hyperthyroidism predispose to UTIs (Mayer-Roenne *et al* 2007, Bailiff *et al* 2008). However, a recent study found that cats of all ages were equally affected by UTIs (Martinez-Ruzafa *et al* 2012). Cats with UTIs often do not show lower urinary tract signs (= occult bacterial UTI) (Bailiff *et al* 2006, Mayer-Roenne *et al* 2007, Bailiff *et al* 2008, Martinez-Ruzafa *et al* 2012). A recent study revealed that occult bacterial UTIs particularly affect older female cats, mostly have *Enterococcus faecalis* isolated and can occur in cats without a history or clinical signs of a predisposing disease, as was the case in our cat (Litster *et al* 2009). Decreased USG, which was present in our cat, was recently found to be associated with UTI in cats (Martinez-Ruzafa *et al* 2012).

Comparison of age groups resulted in significant differences for several parameters. A larger spread of UPC values in old versus middle-aged cats was obvious. For the other parameters, there was moderate to severe overlap between both groups which limits the clinical relevance for these differences. The significantly higher SBP, heart rate and murmur frequency in the old cats may be consequences of cardiovascular changes that occur with aging (Carpenter *et al* 2005).

Conclusion

Based on the screening we performed in 100 cats, we diagnosed FIV infection in 14 cats, CKD in two cats, hyperthyroidism in one cat and a UTI in one cat. In addition, several cats had a suboptimal SBP or BCS, gingivitis, heart murmur or laboratory abnormalities (e.g. increased serum creatinine, increased bilirubin, increased ALT, increased TT4, borderline proteinuria, glucosuria) for which further diagnostic investigation, treatment and/or follow-up were indicated. A minor limitation was that our cats were only evaluated at a single time point. Also further diagnostic tests to explain abnormal SBP, abnormal physical examination and laboratory findings were not always performed, although advised to the owners. However, this study clearly indicates the need and value of regular health checks of apparently healthy cats to improve early disease detection and allow early therapeutic intervention. This health screening should contain comprehensive history and thorough physical examination, including BCS assessment and oral inspection. Because most relevant abnormal laboratory findings were observed in group 2 cats – except FIV seropositivity –, we advise to perform FIV/FeLV testing in middle-aged cats with outdoor access and complete blood and urine examinations in old cats (above 10 years). Our findings support the widely accepted advice to measure the BP of cats that are ≥ 10 years of age (Brown *et al* 2007). To improve the interpretation of geriatric screening, small animal laboratories should make efforts to develop age depended RIs for certain parameters and further research is warranted to reveal the clinical importance of proteinuria in the borderline range.

In conclusion, physical examination and laboratory abnormalities are common in apparently healthy older cats which emphasizes the need for regular health checks and age dependent laboratory RIs.

End notes

[a]MEDVET Algemeen Medisch Laboratorium Diergeneeskunde, Antwerp, Belgium

[b]Advia 2120, Siemens, Brussels, Belgium

[c]Architect C16000, Abbott, Wiesbaden, Germany

[d]Immulite 2000 systems, Siemens, Brussels, Belgium

[e]SNAP[*]Combo Plus, IDEXX Europe BV, Hoofddorp, The Netherlands

[f]Iricell IQ, Instrumentation Laboratory, Zaventem, Belgium

[g]BioMerieux Media Square, Brussels, Belgium

[h]SAS version 9.2, SAS Institute Inc, North Carolina, USA

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CHAPTER 4

SCREENING OF RAGDOLL CATS FOR CHRONIC KIDNEY DISEASE

Introduction

In some countries, Ragdoll cats are routinely screened for kidney disease prior to breeding, based on recommendations of Ragdoll breed clubs. As explained in the general introduction of this thesis (Chapter 1), there is a need to gain scientific information on the occurrence of kidney disease within this breed and to evaluate the results of this screening.

In Belgium and the Netherlands, many Ragdoll breeders perform pre-breeding screening on a voluntary basis. Although this screening is not obligatory to obtain a pedigree, it is highly recommended by breed clubs and there exists a strong social pressure between Ragdoll breeders to perform this screening.

In our institution, this screening starts with controlling the cats' identity by scanning the microchip and comparing this scanned microchip number with the original pedigree. A photocopy of the original pedigree is kept in the patient file. History and physical examination are performed to assess general health of the cats. Depending on the wishes of the breeder, pre-breeding screening consists of various combinations of echocardiography, assessment of feline immunodeficiency virus and feline leukemia virus status, measurement of serum urea and creatinine concentrations, blood typing, genetic testing for hypertrophic cardiomyopathy and/or polycystic kidney disease (PKD) and performing ultrasonography of the liver and kidneys. Most breeders perform all these tests in all their breeding cats. In cats that undergo screening, the results for hypertrophic cardiomyopathy and PKD are printed on the pedigree. Results of abdominal ultrasonography to evaluate for chronic kidney disease or chronic interstitial nephritis are not mentioned on the pedigree.

At first (**Section §4.1**), we performed a retrospective study in which we evaluated the data of Ragdolls that were presented at our institution for screening between September 2001 and November 2009. Because of limitations that were inherent to the retrospective nature of this first study, we also performed a prospective study (**Section §4.2**) in which the results of screening tests of Ragdoll cats were compared with those of age-matched non-Ragdoll cats.

SECTION §4.1

SCREENING OF RAGDOLL CATS FOR CHRONIC KIDNEY DISEASE:

A RETROSPECTIVE EVALUATION

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Summary

This study aimed to assess the prevalence of renal abnormalities in Ragdoll cats. Ragdoll breeders often warn clients to watch for future renal problems, mainly due to chronic interstitial nephritis (CIN) and polycystic kidney disease (PKD). Therefore, Ragdoll screening by abdominal ultrasonography, measurement of serum creatinine and urea concentrations and genetic testing is often performed without documented scientific evidence of increased risk of renal disease.

Data of Ragdoll screening for renal disease at one institution over an 8 year period were retrospectively evaluated.

Renal ultrasonography was performed in 244 healthy Ragdoll cats. Seven cats were positive for PKD, 21 were suspected to have chronic kidney disease (CKD), 8 had abnormalities of unknown significance, and 2 cats had only one visible kidney. Cats suspected to have CKD were significantly older and had significantly higher serum urea and creatinine concentrations than cats with normal renal ultrasonography. All 125 genetically tested cats were negative for PKD. However, only one of the seven ultrasonographically positive cats underwent genetic testing for PKD.

In conclusion, ultrasonographic findings compatible with CKD were observed in almost 10% of cats, and PKD occurred at a low prevalence (< 3%) in this Ragdoll population. Further studies are required to elucidate if Ragdoll cats are predisposed to CKD.

Introduction

According to the *Cat Fanciers' Association*, the Ragdoll is one of the most popular cat breeds worldwide (CFA 2010). Several Ragdoll breeder organizations such as the *Scandinavian Ragdoll Club* (SRC) and the *Ragdoll Club Benelux* (RCB) forewarn owners that renal problems may develop due to PKD, CIN, familial renal dysplasia or nephrocalcinosis (RCB 2003, SRC 2004). Based on recommendations of these breed clubs, Ragdoll cats are screened for PKD and CIN prior to breeding in several European countries such as Belgium, the Netherlands, Sweden and Finland (RCB 2003, SRC 2004). Several tests are part of this screening program, including abdominal ultrasonography to identify renal and/or hepatic cysts and evidence of CIN, measurement of serum urea and creatinine concentrations, and genetic testing for the PKD-1 mutation. The results of these screening tests can be found on the *Ragdoll Health Database* on the internet (RHD 2011).

Although these screening tests have been performed for many years, there is only minimal scientific evidence of a specific increased risk for kidney disease within this breed. In Ragdoll cats, PKD and the PKD-1 nonsense mutation have been identified (Lyons *et al* 2004), but information about the prevalence of PKD in this breed is lacking. Furthermore, there is no evidence that Ragdoll cats are predisposed to CIN, CKD, or renal dysplasia. For that reason, it is important to elucidate whether the concerns of the breed organizations are justified or not. If Ragdoll cats are indeed at risk for renal disease, adequate and regular monitoring of renal function may allow early disease detection and intervention. In addition, screening of breeding cats may then be warranted.

Scientific evidence on the occurrence of renal disease in Ragdoll cats would appear beneficial to provide breeders with sound recommendations. Therefore, this study was undertaken to retrospectively evaluate the results of the screening tests performed on Ragdoll cats.

Materials and Methods

The medical records of Ragdoll cats that were presented to the companion animal clinic at the Faculty of Veterinary Medicine of Ghent University (Belgium) between September 2001 and November 2009 were retrospectively evaluated. Healthy Ragdoll cats presented for CIN or PKD screening and evaluated by renal ultrasonography were included. Health was defined as clinically healthy for their owner and without significant abnormalities on physical examination. In addition to ultrasound, some Ragdoll owners also requested measurement of serum urea and creatinine concentrations or a genetic PKD test to complete their cat's data in the 'Ragdoll Health Database'. These blood results were also evaluated retrospectively. Cats that were only evaluated by renal ultrasonography, without a concurrent genetic PKD test, needed to be at least 10 months old to be included as PKD negative to avoid false negative results.

Abdominal ultrasonography was performed by a Diplomate of the *European College of Veterinary Diagnostic Imaging* (ECVDI) or supervised ECVDI Resident. The cats were manually restrained in dorsal recumbency, and the hair was not or only minimally clipped. In the region of the kidneys and the liver, the hair was parted to expose the skin. To improve skin contact and conduction of ultrasound waves, the hair was soaked with alcohol and water before ultrasound coupling gel was applied. The kidneys and liver were evaluated with a 7.5 MHz convex or sectorial transducer (Logic 7 GD Medical Systems, USA) using a ventrolateral and ventral approach.

Serum creatinine and urea concentrations were measured with a colorimetric assay (Spotchem®, Menarini, Belgium). The reference intervals (RIs) (80 – 164 µmol/L for creatinine; 4.9 – 11.9 mmol/L for urea) were determined by the machine manufacturer by evaluating over 100 healthy cats of different ages and weights. At our institution, the PKD genetic test was completed as described by identifying the C > A transversion in exon 29 of the PKD-1 gene (Helps *et al* 2007). Owners of cats not genetically tested at our institution, were asked for the result of genetic PKD testing at other laboratories.

A cat was defined as PKD positive if one or more clearly defined spherical anechoic cavities were detected in at least one kidney or the liver (Beck and Lavelle 2001, Cannon *et al* 2001) or if the cat was heterozygous for the PKD-1 nonsense mutation. A cat was suspected

of being affected by CKD if the kidneys showed ultrasonographic changes compatible with CKD, such as small kidneys (< 3.2 cm), irregular or undulating kidney shape or surface, reduced corticomedullary distinction, heterogeneous renal parenchyma, focal or diffuse cortical hyperechogenicity, focal or diffuse medullar hyperechogenicity, medullary rim sign, renal infarct and/or renal parenchymal mineralization. The presence of a single infarct, medullary rim sign, hyperechoic cortex or medulla, renal parenchymal mineralization or heterogeneity, as the only abnormality, was not sufficient to classify a cat as suspected of having CKD (Grooters and Biller 1995, Widmer *et al* 2004, d'Anjou 2008). These cats were classified as having ultrasonographic abnormalities of unknown significance. The serum urea and creatinine concentrations were not used to classify cats as having CKD or not because of the lack of urinalysis.

Owners were contacted by telephone to follow up on cats suspected of having CKD. Owners were asked to complete a questionnaire evaluating the cat's health, eating and drinking behavior, body weight and the cause of death when relevant. They were also asked if serum creatinine and urea concentrations and/or a urinalysis had been repeated since the time of inclusion in the study.

All statistical analyses were performed with statistical software (PASW® Statistics 18, USA). Cats without ultrasonographic abnormalities were compared to cats suspected of having CKD for age (nonparametric Mann-Whitney *U* rank sum test) and for serum urea and creatinine concentrations (independent two-samples *t*-test). All statistical tests were performed at the 0.05 significance level.

Results

The study population consisted of 244 Ragdoll cats: 172 females (5 neutered) and 72 males (4 neutered). The population characteristics are presented in Table 4.1. Only 4 cats were younger than 10 months, and these cats were all PKD positive based on the presence of renal cysts on ultrasonography. Renal ultrasonography was performed in all cats, serum urea and serum creatinine were measured in 141 cats, and the in-house genetic PKD-test was performed in 21 cats. Fifty-five cats were not genetically PKD tested, 104 cats were tested in various laboratories and for 64 cats there was no information if they underwent genetic PKD testing. The descriptive statistics for serum urea and serum creatinine concentrations are presented in Table 4.1. Four cats had an increase of both serum urea and creatinine concentrations, 11 only had a serum creatinine and two only a serum urea concentration exceeding the RI. All genetically tested cats were homozygous for the wild-type PKD-1 allele, which is consistent with a PKD-negative status.

Table 4.1. Descriptive statistics for the age, body weight, serum urea concentration and serum creatinine concentration of the included Ragdoll cats.

	N	Mean ± SD	Median	Range	RI
Age (years)	244	2.2 ± 1.4	1.8	0.3 – 8.7	/
Body weight (kg)	45	4.2 ± 1.2	4.2	1.8 – 7.9	/
Urea (mmol/L)	141	8.5 ± 2.0	8.2	4.9 – 18.9	4.9 – 11.9
Creatinine (µmol/L)	141	127.3 ± 30.8	124.0	70.0 – 222.0	80 – 164

(N = Number of cats for which the parameter was found in the medical record; SD = Standard deviation; RI = Reference interval)

Renal ultrasonography showed abnormalities in 38 cats (Table 4.2). Seven cats were diagnosed with PKD based on the presence of renal cysts on ultrasound. Hepatic cysts were not detected in any of the cats. One of the cats with renal cysts was genetically PKD negative. On repeat examination of this cat 5 years after inclusion, cysts were not observed on ultrasound, and blood and urine examinations were normal. In two cats, the right kidney could not be visualized, suggesting unilateral renal agenesis or aplasia. In one of these cats, the right kidney was also not visible on a ventrodorsal abdominal radiograph and on recheck ultrasound (4.5 years after inclusion). At this recheck, blood and urine examinations did not

reveal renal azotemia. Ultrasonographic abnormalities that could be compatible with CKD were observed in 21 cats and eight cats showed other renal ultrasonographic abnormalities. The significance of these other lesions is unknown. The CKD suspected cats were significantly older (median 2.7 (range 1 – 8.7) versus 1.7 (0.9 – 7.7) years; $P = 0.002$) and had significantly higher serum urea (mean ± SD 11.1 ± 3.6 versus 8.2 ± 1.3 mmol/L; $P = 0.006$) and creatinine concentrations (mean ± SD 155.9 ± 30.9 versus 124.7 ± 29.2 μmol/L; $P < 0.001$) compared to cats without ultrasonographic abnormalities. In the CKD suspected cats, both serum urea and creatinine concentrations were normal in eight cats, increased in three cats and not measured in five cats. Four cats only had a serum creatinine and one cat only a serum urea concentration that exceeded the RI.

Table 4.2. Overview of the observed ultrasonographic abnormalities and the frequency of their observation in Ragdoll cats with ultrasonographic abnormalities.

The Ragdoll cats with ultrasonographic abnormalities (n = 38) are subdivided into cats that showed abnormalities suggestive of chronic kidney disease (CKD), cats that were diagnosed with polycystic kidney disease (PKD), cats with unilateral renal agenesis or aplasia and cats with ultrasonographic abnormalities for which the significance was uncertain. The majority (18/21) of CKD suspected cats had more than one ultrasonographic abnormality, which explains why the total number of ultrasonographic abnormalities exceeds the number of cats suspected of CKD (n = 21).

Ultrasonographic conclusion	**N**	**Age (years)**	**Ultrasonographic abnormalities and frequency of occurrence**	
Suspected of CKD	21	Median 2.7 (range 1.1 – 8.7)	Small kidney(s)	10/21
			Reduced corticomedullary distinction	7/21
			Undulating or bumpy cortical surface	6/21
			Renal mineralizations	5/21
			Hyperechoic renal cortex	4/21
			Renal infarct(s)	4/21
			Heterogeneous cortex	4/21
			Other findings	7/21
PKD	7	Median 0.4 (range 0.3 – 2.9)	Renal cysts	7/7
Unilateral renal agenesis/aplasia	2	1.4 and 1.9	Absent right kidney	2/2
Unknown significance	8	Median 2.5 (range 0.9 – 7.2)	Hyperechoic renal cortex	3/8
			Corticomedullary rim sign	2/8
			Cortical hyperechoic triangle/spots	2/8
			Slight undulation of cortical surface	1/8

(CKD = Chronic kidney disease; N = Number of cats; PKD = Polycystic kidney disease)

Follow-up was only available for 14 of 21 CKD suspected Ragdoll cats. Three developed clinical signs and laboratory abnormalities consistent with chronic renal failure. Two are still alive and were in IRIS stage 3 CKD at the last follow-up (14 months and 5 years after inclusion). The third cat was euthanized at 12 years of age (4 years after inclusion) after being treated for several years with a renal diet and benazepril. According to the owners, ten CKD suspected cats were still healthy at the time of writing (mean follow-up 3.4 ± 1.1 years), with normal eating and drinking behavior and stable body weight. Repeated measurements of serum urea and creatinine were only performed in one of these 10 cats (1 year after inclusion), and the results were within the RI. Two months after inclusion, a wedge-biopsy of the left kidney of one non-azotemic cat was taken during ovariohysterectomy. This cat had a small left kidney with an undulating cortical surface and an abnormal rounded shape. Routine histology indicated only very mild degeneration in the distal renal tubules, without glomerular or interstitial changes. The owner of one cat noted polydipsia. A recheck evaluation (6 years after inclusion) revealed mildly increased serum creatinine, hypersthenuric urine and microscopic renal hematuria. On ultrasonography, a small (3 cm) and irregularly shaped right kidney, infarcts in the left kidney and left ureteral dilation were detected.

Discussion

In the popular European literature, there is a strong belief that Ragdoll cats are predisposed to renal disease, mainly due to CIN and PKD (RCB 2003, SRC 2004).

CIN is a nonspecific inflammatory condition that is classified among the tubulointerstitial renal diseases. CIN can be primary or secondary to glomerular or systemic diseases, but the underlying cause is often unclear. It results in fibrosis, tubular atrophy and loss of healthy renal tissue. The consequence is progressive renal disease and it is considered to be a common cause of azotemic CKD in cats (DiBartola *et al* 1987, Lulich *et al* 1992, Minkus *et al* 1994, Maxie and Newman 2007). The definitive diagnosis of CIN is not possible without taking kidney biopsies and renal histology often does not reveal the underlying cause of the nephritis (DiBartola *et al* 1987, Lulich *et al* 1992, Polzin 2010). Ragdoll breed organizations recommend measuring serum urea and creatinine and performing renal ultrasonography to screen for the presence of CIN. The usefulness of ultrasonography in the diagnosis of CIN is limited, because CIN is a histological diagnosis and there are no pathognomonic ultrasonographic features for feline CIN (Grooters and Biller 1995, d'Anjou 2008, DiBartola 2010). Therefore, in this study, cats were only evaluated for ultrasonographic features of CKD and not of CIN. Another limitation is that kidneys of cats with CIN occasionally have a normal ultrasonographic appearance (Grooters and Biller 1995, d'Anjou 2008, DiBartola 2010). Finally, ultrasonography only provides information on organ structure, without evaluating organ function (Grooters and Biller 1995). An important limitation of measuring serum urea and creatinine to evaluate renal function is that these parameters are difficult to interpret without concurrent urinalysis. In addition, they only provide a rough estimate of kidney function and do not allow detection of early kidney dysfunction (DiBartola 2010).

In this study, 8.6% of the screened Ragdoll cats showed ultrasonographic abnormalities that could be compatible with CKD. These CKD suspected cats were significantly older and had significantly higher urea and creatinine concentrations compared to cats without ultrasonographic abnormalities. It is important to recognize that the ultrasonographic findings do not imply that these cats were affected by CIN because several other diseases, such as glomerulonephritis, glomerulosclerosis, amyloidosis and nephrocalcinosis, can result in similar ultrasonographic abnormalities (Widmer *et al* 2004).

Although 8.6% appears to be a fairly high percentage of structural renal abnormalities for this population of young healthy cats, it is difficult to draw strong conclusions because the prevalence of renal ultrasonographic abnormalities in healthy cats of other breeds is currently unknown. The significantly higher age of the CKD suspected cats could be expected because CKD is a slowly progressive disease. However, the age difference between both groups was rather small, and the ages overlapped significantly. The significantly higher serum urea and creatinine concentrations of CKD suspected cats may indicate decreased renal function. However, because urinalysis was not performed in our cats, we cannot determine how many cats actually suffered from renal azotemia. Also, it is widely accepted, despite a lack of evidence, that ultrasonography is not a useful tool to predict which cats will develop azotemic kidney disease (Grooters and Biller 1995). Further studies will need to determine if the observed ultrasonographic abnormalities are clinically relevant. Urinalysis and renal function tests will help to identify cats with decreased renal function (DiBartola 2010). Renal biopsies may reveal the underlying cause of the ultrasonographic abnormalities. It is important to consider that kidney biopsies are not recommended in most cats with advanced or end-stage CKD. However, at an early CKD stage, renal biopsies can help to identify the underlying cause of CKD, mainly to evaluate if specific therapy directed at a primary cause makes sense (Polzin 2010). In this study, only one non-azotemic CKD suspected cat underwent a kidney biopsy which makes valid conclusions difficult.

A small number of the cats had ultrasonographic abnormalities for which the clinical significance was unclear. Most had diffuse or focal hyperechoic renal cortices as a single abnormality. It has been shown that the cortical echogenicity of normal cat kidneys increases with the amount of fat present in the proximal tubular epithelial cells, which can result in cortical hyperechogenicity, especially in intact male cats (Yeager and Anderson 1989). On the other hand, hyperechogenicity of the renal cortex is the most common ultrasonographic finding in diffuse parenchymal renal diseases (Grooters and Biller 1995). A corticomedullary rim sign was present in two cats. Previous studies indicated that rim signs can occur in healthy and renal-diseased cats and dogs. Currently, it is still unknown if cortical hyperechogenicity or medullary rim signs can be early indicators of renal disease (Yeager and Anderson 1989, Biller *et al* 1992, Widmer *et al* 2004, d'Anjou 2008).

PKD is an inherited condition that results in the formation of fluid-filled renal and, occasionally, hepatic cysts. This condition mainly affects Persian and Persian-related cats (Biller and DiBartola 1995). Because the Ragdoll is one of the breeds that have been

outcrossed with Persians, Ragdoll cats could be at risk for PKD (Beck and Lavelle 2001). Affected cats are heterozygous for a stop mutation in the PKD-1 gene that is inherited in an autosomal dominant manner (Biller *et al* 1996, Lyons *et al* 2004, Helps *et al* 2007). This mutation has been identified in a limited number of Ragdoll cats (Lyons *et al* 2004). The present study is the first to evaluate PKD prevalence in a large number of Ragdoll cats. A prevalence of less than 3% was found, which is considerably lower than the 31% to 42% prevalence of PKD described in recent European studies in Persian and related cats (Barthez *et al* 2003, Bonazzi *et al* 2007, Domanjko-Petrič *et al* 2008, Wills *et al* 2009). Five of the seven PKD-positive cats were presented in 2001 or 2002, which means that only one PKD-positive and one doubtful PKD-positive (cysts on ultrasonography, negative genetic test) Ragdoll cat were observed at this institution during the last 7 years of the study. This may indicate that PKD screening prior to breeding is effective at eradicating PKD in this breed. However, the PKD prevalence found in this study is only an estimate of the true prevalence. A selection bias can have resulted in an underestimation of the actual prevalence because most Ragdoll breeders already screened their cats for PKD over several generations.

All genetically tested cats tested negative for PKD, however, one was considered PKD positive on renal ultrasonography. In a previous study, several cats (5.7%) that showed renal cysts on ultrasonography were homozygous for the wild-type PKD-1 alleles (Bonazzi *et al* 2009). There are several possible explanations for this discrepancy. The small cysts that were visualized by ultrasound could have been acquired instead of inherited. The cat could have also been affected by inherited PKD caused by another mutation, other than the one that is evaluated by the PKD test, or a technical error resulting in a false negative PKD test or a false positive ultrasonography could have occurred (Helps *et al* 2007, d'Anjou 2008, Bonazzi *et al* 2009). As no cysts were observed five years later, a false positive ultrasonography is the most likely explanation in this case.

In two cats, the ultrasonographer could not identify the right kidney. This can indicate unilateral renal agenesis or severe renal hypoplasia or dysplasia, resulting in a kidney-like remnant that is too small to be detected by routine medical imaging modalities (Toolan 1993, Greco 2001, Chang *et al* 2008). Further diagnostic tests to differentiate between these conditions were not performed. Both feline and canine unilateral renal agenesis are often associated with other developmental urogenital tract abnormalities or compensatory hypertrophy of the contralateral kidney (Greco 2001, Agut *et al* 2002, Taney *et al* 2003, Morita *et al* 2005, Chang *et al* 2008). The ultrasound reports of both cats, and abdominal

radiographs and recheck ultrasonography of one cat did not reveal other abnormalities besides the absent right kidney.

Almost 11% of our Ragdoll cats had serum creatinine concentrations exceeding the RI. Because urinalysis or clearance tests to determine the glomerular filtration rate were not performed, it is not possible to determine how many cats actually had decreased renal function. At least three cats developed overt renal failure, but follow-up was not available for many cats or was limited to phone contact with the owner. Next to kidney dysfunction, other reasons may explain the increased serum creatinine concentration. One is misclassification due to an inappropriate RI. The issue of inappropriate RIs for small animal laboratory parameters has recently been emphasized in veterinary literature (Archer, 2010, Friedrichs 2010). The fact that we used the RI of the machine manufacturer, instead of developing a laboratory-specific RI, was not ideal (Friedrichs 2010). Both in dogs and in cats, RIs are often not appropriate to assess serum or plasma creatinine concentrations, which can influence the classification of samples as normal or abnormal (Boozer *et al* 2002, Ulleberg *et al* 2011). The increased creatinine concentrations can be a breed-specific feature, as was reported for Birmans (Gunn-Moore *et al* 2002, Reynolds *et al* 2010). The Ragdoll was founded by cross-bred cats, but has been outcrossed with Persians, Birmans, Balinese and maybe other breeds (Beck and Lavelle 2001, RFCI 2006). Although Gunn-Moore *et al* (2002) reported hypercreatinemia in one third of adult Birmans, Reynolds *et al* (2010) only described a small number of Birmans with a creatinine concentration that exceeded the RI. Further research is required to determine if a breed-specific RI is warranted for assessment of serum creatinine in Ragdoll cats.

This study had several limitations, mainly due to its retrospective nature. First, several cats only underwent renal ultrasonography without a genetic PKD test or measurement of serum urea and creatinine concentrations. Therefore, the PKD-positive status of all but one cat that showed renal cysts at ultrasonography was not confirmed genetically. Second, renal ultrasonography was performed by different ultrasonographers with or without minimal clipping of the cats' hair. Therefore, it is possible that mild ultrasonographic abnormalities were missed or not noted in the patient records or that the renal echogenicity was underestimated (Walter *et al* 1987). However, as in previous studies, adequate images could be obtained in most cases by preparing the skin and hair coat well before scanning (Cannon *et al* 2001, Wills *et al* 2009). Third, interpretation of the serum urea and creatinine concentrations was limited by the lack of urinalyses. Finally, disease prevalence is affected by

the specific characteristics of the studied population (Hahn and Overley 2010), such as geography. All Ragdoll cats included in this study resided in Belgium or the Netherlands and the majority was born in the same area. Only a minority of cats were imported from other European countries, the United States or Australia.

Despite these limitations, this study has clear value because kidney disease screening results were evaluated for the first time in a large population of Ragdoll cats. It can be concluded that ultrasonographic findings compatible with CKD occurred in almost 10% of this healthy Ragdoll population. Further research is needed to elucidate if this is clinically relevant and whether these cats were affected by CIN. In addition, PKD occurs at a low prevalence in Ragdoll cats residing in Belgium and the Netherlands.

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SECTION §4.2

SCREENING OF RAGDOLL CATS FOR CHRONIC KIDNEY DISEASE:

A PROSPECTIVE EVALUATION

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Summary

Ragdoll breeder organizations often forewarn Ragdoll cat owners that renal problems may develop due to polycystic kidney disease (PKD), chronic interstitial nephritis (CIN), familial renal dysplasia or nephrocalcinosis.

Healthy Ragdoll and non-Ragdoll cats were prospectively evaluated by measuring serum creatinine and urea concentrations, routine urinalysis and abdominal ultrasonography. All Ragdoll cats also underwent genetic PKD testing.

One hundred and thirty-three Ragdoll and 62 control cats were included. Ragdoll cats had significantly lower serum urea concentrations and higher urine specific gravity (USG). However, median creatinine concentration, median urinary protein: creatinine ratio (UPC) and the proportion of cats with serum creatinine or urea concentration exceeding the reference interval (RI) did not differ. One or more renal ultrasonographic changes were detected in 66/133 (49.6%) Ragdoll and in 25/62 (40%) control cats. Ragdoll cats showed significantly more frequent segmental cortical lesions (SCLs; 7.5% versus 0%), abnormal renal capsule (19.5% versus 8%) and echogenic urine (51.9% versus 25.8%). Chronic kidney disease (CKD) was ultrasonographically suspected in 7/133 (5.3%) Ragdoll and in none of the control cats, which approached significance. Laboratory parameters confirmed kidney dysfunction only in 1/7 of these Ragdoll cats. All Ragdoll cats were PKD negative.

In conclusion, breed-specific serum creatinine RIs are not likely required for Ragdoll cats. Secondly, renal ultrasonographic abnormalities are common, both in Ragdoll and non-Ragdoll cats. Thirdly, healthy young Ragdoll cats are uncommonly affected by PKD and CKD, but an increased susceptibility of Ragdoll cats to develop CKD cannot be excluded. Finally, Ragdoll cats are predisposed for SCLs, which may indicate renal infarction or cortical scarring.

Introduction

According to the *Cat Fanciers' Association*, the Ragdoll is one of the most popular cat breeds worldwide (CFA 2012). Several Ragdoll breeder organizations such as the *Ragdoll Club Benelux* and the *Scandinavian Ragdoll Club* forewarn owners that renal problems may develop due to PKD, CIN, familial renal dysplasia or nephrocalcinosis (RCB 2003, SRC 2004). Based on recommendations of these breed clubs, Ragdoll cats are screened for PKD and CIN prior to breeding in several European countries such as Belgium, the Netherlands, Sweden and Finland (RCB 2003, SRC 2004). Several tests are part of this screening program, including abdominal ultrasonography to identify renal and/or hepatic cysts and evidence of CIN, measurement of serum urea and creatinine concentrations, and genetic testing for the PKD-1 mutation. The results of these screening tests can be found on the *Ragdoll Health Database* on the internet (RHD 2012).

Recently our group retrospectively evaluated the results of these screening tests performed on Ragdoll cats at our institution. Ultrasonographic findings compatible with CKD were observed in 8.6% and PKD in 2.9% of included healthy Ragdoll cats. However, this study was limited by the lack of urinalysis, incomplete screening tests in many Ragdoll cats and the lack of information about the prevalence of renal ultrasonographic abnormalities in healthy non-Ragdoll cats (Paepe *et al* 2012).

To further elucidate if the concerns of the Ragdoll breed organizations are justified or not, we performed a prospective study to compare serum creatinine and urea concentrations, routine urinalysis and renal ultrasonographic findings between Ragdoll cats and an age-matched control group.

Materials and methods

Study population

Ragdoll cats that were presented by their owner for CIN or PKD screening were considered for inclusion. Age matched non-Ragdoll cats were actively recruited as control cats by contacting colleagues, friends and veterinary students. Both pure- and mixed-breed cats were considered for inclusion as control cats, but maximum five cats of each pure cat breed were allowed. Both Ragdoll and control cats needed to be 10 months or older to be included. To avoid bias towards kidney disease in the Ragdoll population, Ragdoll cats that were presented by their owner with an already diagnosed CKD were excluded from this study. Because cats presented for screening are usually healthy, only healthy Ragdoll and control cats were included. Health was defined as clinically healthy for their owner and without significant abnormalities on physical examination, complete blood count[a,b] and serum biochemistry profile[a,c], except for serum creatinine and urea concentrations. All cats were fasted for 12 hours, water was offered ad libitum.

The study was completed at the Department of Medicine and Clinical Biology of Small Animals, Faculty of Veterinary Medicine, Ghent University between October 2010 and March 2012. All cats were privately owned, the owners were thoroughly informed about the study aims and protocol, and the study was approved by local and national ethical committees (EC2010/104).

Procedures

The cats underwent measurement of serum creatinine[d] and urea[d] concentrations, complete urinalysis and abdominal ultrasonography. Only the Ragdoll cats underwent a genetic PKD test[e] that was completed as described by identifying the C > A transversion in exon 29 of the PKD-1 gene (Helps *et al* 2007).

Urinalysis consisted of a urinary dipstick test; measurement of USG with a manual refractometer, urinary pH and UPC[a,f]; and sediment examination. The sediment was prepared as previously described (Meyer 2001, Paepe *et al* 2013) and evaluated under the microscope within 30 minutes of collection. Crystalluria was evaluated semi-quantitatively and expressed

per low-power field (LPF, 10x objective) as mild (< 1/LPF), moderate (1 – 3/LPF), or severe (> 3/LPF). Bacterial culture[a,g] of the urine was only performed if considered necessary based on routine urinalysis or on the presence of sediment in the urinary bladder during ultrasonography.

Abdominal ultrasonography was performed by a Diplomate of the *European College of Veterinary Diagnostic Imaging* (ECVDI) or supervised ECVDI Resident, using a multifrequency (6 – 10 MHz) microconvex or multifrequency (7.5 – 12 MHz) linear transducer[h]. The cats were manually restrained in dorsal recumbency. The hair was not or only minimally clipped and parted to expose the skin. To improve skin contact and conduction of ultrasound waves, the hair was soaked with alcohol and water before ultrasound coupling gel was applied. The kidneys, urinary bladder and liver were evaluated according to a standard protocol in longitudinal and transverse scanning planes (see Addendum). A ventrolateral and ventral approach was used for the kidneys and liver, a ventral approach for the urinary bladder. Parameters assessed at the level of the kidneys were the renal capsule (normal or abnormal), renal shape (regular, irregular or other), kidney length in a sagittal plane, cortical echogenicity (diffuse hypo-, iso- or hyperechogenic compared to the spleen or liver or focal abnormalities), medullar echogenicity (diffuse hypo-, iso- or hyperechogenic compared to the renal cortex or focal abnormalities), corticomedullary demarcation (well-delineated or reduced), renal pelvis (normal, enlarged, or presence of uroliths), proximal ureter (normal, enlarged, or presence of uroliths) and the presence or absence of medullary rim sign, dystrophic mineralization, cavitary lesion, solid mass, nodule, segmental cortical lesion and retroperitoneal fluid. For the urinary bladder, bladder filling, echogenicity of the urine and presence or absence of uroliths was noted. The liver was carefully evaluated for the presence or absence of cysts or other parenchymal changes. Additional changes in the liver, kidneys, urinary bladder and the remainder of the abdomen were also noted. Because CIN is not an ultrasound diagnosis, the radiologist was asked to judge if the observed abnormalities could be indicative of CKD possibly caused by CIN. In addition, the radiologist was asked if the cat could be affected by renal agenesis, hypoplasia or dysplasia.

Statistical methods

All statistical tests were performed with statistical software[i] and at the 0.05 significance level. To compare the Ragdoll and the control group, student's *t*-test was used for continuous variables that approached normal distribution, Mann-Whitney *U*-test for non-normally distributed continuous variables and the Fisher's exact test or the Pearson Chi-Square test for discrete variables.

Results

Study population

In total, 133 Ragdoll and 62 control cats were included. Seven Ragdoll cats with known CKD and one Ragdoll cat for which it was already known that it only had one kidney were excluded. Two other Ragdoll cats were excluded, one because of dyspnea and muffled lung sounds due to pleural effusion and one because of ascites. Further diagnostic tests revealed feline infectious peritonitis in both cats. One control cat was excluded because of ulcerative and scaly swellings of the footpads consistent with plasma cell pododermatitis.

Age and body weight did not differ between groups (Table 4.3). The gender distribution differed significantly between groups ($P < 0.001$; Ragdoll: 80 intact female, 5 neutered female, 40 intact male, 8 neutered males; control: 16 intact females, 20 neutered females, 0 intact males, 26 neutered males).

Ragdoll pedigrees indicated that 43 Ragdoll cats originated from Belgium, 37 from the Netherlands, 15 from other European countries, 28 from the United States of America, seven from Australia or New-Zealand, two from Canada and one from Israel.

Laboratory parameters

The results for serum creatinine and urea concentrations, USG and UPC for both groups are summarized in Table 4.3. Urine was not available in four Ragdoll cats because of empty bladder or pregnancy. Additionally, in nine Ragdoll and three control cats the amount of urine was insufficient to allow microscopic sediment examination.

The median creatinine concentration and proportion of cats with serum creatinine (three Ragdoll, one control cat) or serum urea (two Ragdoll, one control cat) concentration exceeding the RI did not significantly differ between groups. However, control cats had significantly higher serum urea concentrations compared to Ragdoll cats ($P = 0.010$).

Median USG was significantly lower in control cats ($P < 0.001$) and the number of cats that had USG below 1.035 was significantly higher in control (8/62) compared to Ragdoll (4/129) cats ($P = 0.013$). Median UPC and the proportion of cats with borderline proteinuria

(UPC = 0.2 – 0.4 (Lees *et al* 2005); 36/129 Ragdoll, 14/62 control cats) or overt proteinuria (UPC > 0.4 (Lees *et al* 2005); 14/129 Ragdoll, 4/62 control cats) did not significantly differ between groups. The urinary dipstick was positive for hemoglobin in 37/129 Ragdoll and 17/62 control cats, for acetone in 9/129 Ragdoll and 1/62 control cats, for protein in 128/129 Ragdoll and 56/62 control cats, for urobilinogen in 36/129 Ragdoll and 19/62 control cats and for glucose in 0/129 Ragdoll and 5/62 control cats. Bilirubin or nitrites positivity was not observed in any cat. Protein positivity was observed significantly more often in Ragdoll cats (P = 0.005) and glucose positivity more often in control cats (P = 0.003). Urinary crystals were observed in 61/121 Ragdoll (40 mild, 17 moderate, 4 severe crystalluria; 46 amorphous, 14 struvite, 1 calcium oxalate crystalluria) and 29/58 control (17 mild, 7 moderate, 5 severe crystalluria; 19 amorphous, 10 struvite crystalluria) cats. Urinary casts were seen in 3/121 Ragdoll and 2/58 control cats. All had rare (< 1 per high-power field) granular casts. A urine culture was performed in 70 Ragdoll and 45 control cats. *Escherichia coli* cystitis was diagnosed in one Ragdoll and one control cat. The amount and type of urinary crystals and the proportion of cats with casts or positive urine culture did not differ between groups.

Based on the laboratory parameters, one Ragdoll cat was diagnosed with IRIS stage 2 CKD. All Ragdoll cats were homozygous for the wild-type PKD-1 allele, which is consistent with a PKD-negative status.

Ultrasonography

A summary of the renal ultrasonographic findings is presented in Table 4.4. Descriptive statistics for the left and right kidney size and the absolute size difference between both kidneys is shown in Table 4.3. Only renal capsular abnormalities (P = 0.029), presence of SCL(s) (P = 0.019) and abnormal ultrasonographic appearance of the urine (P < 0.001) were significantly different between groups and observed more frequently in Ragdoll cats. For the complete cat population, a significant influence of gender was seen on the presence of echogenic urine. Intact male cats were predisposed to have echogenic urine compared to the other genders (P < 0.001). Five of 10 Ragdoll cats with SCL only had one lesion (three right kidney, two left kidney), the other five had two lesions (1/5 had two in right kidney, 1/5 had two in left kidney, 3/5 had one in both kidneys). All 15 SCLs were positioned in the cortex, were triangular to wedge-shaped with peripheral broad base and hyperechoic compared to the surrounding cortex. These lesions were observed in the cranial (5/15) or

caudal pole (7/15) or in the middle (3/15) of the kidney. The presence of the lesion resulted in an irregular kidney shape and indentation of the renal capsule in all but one lesion. One of the Ragdoll cats with SCL(s) was the cat with IRIS stage 2 CKD. The other cats all had serum creatinine and urea concentrations within the RIs and USG above 1.035. Three of the cats with SCL(s) had borderline proteinuria and one overt proteinuria. Urinary sediment examination revealed hematuria in two, mild hematuria and rare granular casts in one, severe struvite crystalluria in one, mild amorphous crystalluria in two and no abnormalities in four cats. Urine culture was negative in all six cats in which it was assessed. Brief pedigree analysis of the Ragdoll cats with SCL(s) showed that one sire and his daughter and one dam and her son all had SCL(s). In addition, this dam and another cat with SCL(s) had two common grandparents. Furthermore, two other cats with SCL(s) had two common grandparents.

The radiologist concluded that CKD was likely present in seven Ragdoll cats. The radiologist diagnosed PKD in one and suspected renal dysplasia in another control cat. One of these seven cats was the Ragdoll cat with IRIS stage 2 CKD. The other six cats that were suspected of CKD had hypersthenuric urine (USG > 1.035), three had borderline proteinuria and one had a serum urea concentration exceeding the RI. None of the control cats showed ultrasonographic abnormalities that were indicative for CKD. Although not significant, a trend towards a significantly different proportion of CKD suspected cats between groups was observed ($P = 0.065$). In two Ragdoll cats, PKD could not be ruled out based on the presence of a single anechoic cyst or a single cyst with echogenic debris. The control cat suspected of renal dysplasia was a one-year old cat with normally sized kidneys that showed a diffusely hypoechoic cortex with multiple small to large cortical hypoechoic areas that completely deformed the renal shape and contours. Fine-needle aspiration of the kidneys did not reveal significant abnormalities, so lymphoma was ruled out as a differential for this cat.

The number of renal ultrasonographic abnormalities in Ragdoll and control cats is presented in Table 4.5 and does not differ between groups. Of the seven Ragdoll cats suspected of CKD all had five or more renal ultrasonographic abnormalities. The only Ragdoll with five renal ultrasonographic abnormalities not suspected of CKD was a Ragdoll with an abnormally shaped right kidney that was smaller than the left kidney due to the presence of two segmental cortical lesions. Both kidneys showed mildly increased cortical echogenicity and a medullary rim sign. The only control cat with five renal ultrasonographic abnormalities was suspected of renal dysplasia.

Table 4.3. Descriptive statistics for continuous variables age, body weight, serum creatinine concentration, serum urea concentration, urine specific gravity (USG), urinary protein: creatinine ratio, size of the left kidney, size of the right kidney, difference in size between left and right kidney (in absolute values) for Ragdoll cats (group 1) and control cats (group 2).

Parameter	Group	N	Mean ± SD	Median	Range
Age (years)	1	133	2.7 ± 1.8	2.1	0.9 – 8.8
	2	62	2.7 ± 1.6	2.4	0.8 – 8.2
Body weight (kg)	1	133	4.2 ± 0.9	4.1	2.8 – 7.5
	2	62	4.1 ± 0.8	4.2	2.5 – 6.2
Creatinine (µmol/L)	1	133	145.5 ± 29.1	141	80 – 266
	2	62	139.6 ± 33.1	137.5	80 – 227
Urea (mmol/L)*	1	133	7.6 ± 1.5	7.4	4.8 – 15.8
	2	62	8.0 ± 1.3	7.9	5.0 – 13.3
USG*	1	129	1.056 ± 0.009	1.055	1.019 – 1.080
	2	61	1.049 ± 0.013	1.052	1.012 – 1.075
UPC	1	129	0.21 ± 0.1	0.15	0.04 – 0.68
	2	62	0.18 ± 0.1	0.17	0.05 – 0.52
Size left kidney (cm)	1	133	3.8 ± 0.5	3.7	2.8 – 5.7
	2	62	3.7 ± 0.3	3.7	3.0 – 4.5
Size right kidney (cm)	1	133	3.9 ± 0.5	3.9	2.7 – 5.0
	2	62	3.8 ± 0.4	3.8	3.0 – 4.5
Size difference (cm)	1	133	0.21 ± 0.22	0.15	0 – 1.28
	2	62	0.22 ± 0.18	0.17	0 – 0.76

(N = Number of cats in each group for which the parameter was available; SD = Standard deviation; *Significant difference between group 1 and group 2 ($P < 0.05$))
Reference interval for serum creatinine concentration: 71 – 212 µmol/L.
Reference interval for serum urea concentration: 5.7 – 12.9 mmol/L.

Table 4.4. Abnormalities observed during ultrasonographic evaluation of the kidneys, liver and urinary bladder in 133 Ragdoll and 62 control cats. For each evaluated ultrasonographic parameter, the proportion of cats that showed abnormalities (N) and a brief description and localization of the abnormalities are presented in the table.

	RAGDOLL		CONTROL	
	N	**Abnormalities**	**N**	**Abnormalities**
Renal capsule*	26/133 (19.5%)	Undulating or irregular (15 bilateral, 9 only right, 2 only left)	5/62 (8%)	All bilateral undulating or irregular
Renal shape	18/133 (13.5%)	Irregular or bumpy shape (8 bilateral, 6 only right, 4 only left)	6/62 (9.7%)	5 bilateral irregular or bumpy shape, 1 more rounded but regular left kidney
Cortical echogenicity	20/133 (15.0%)	12 diffuse hyperechoic (1/12 hyperechoic striation radiating to the medulla), 7 focal hyperechoic (5/7 focal triangular area due to segmental cortical lesion, 1/7 hyperechoic spots, 1/7 marbled appearance right kidney), 1 diffuse hypoechoic	8/62 (12.9%)	5 diffuse hyperechoic, 2 focal hyperechoic speckles, 1 diffuse hypoechoic with focal round hypoechoic areas
Medullar echogenicity	7/133 (5.3%)	6 diffuse hyperechoic, 1 medulla in two parts (echogenicity outer border between cortex and inner medulla)	2/62 (3.2%)	2 diffuse hyperechoic
Corticomedullary demarcation	10/133 (7.5%)	8 bilateral reduced (1/8 also irregular), 1 only right reduced, 1 only left reduced	5/62 (8.1%)	4 bilateral reduced, 1 bilateral increased
Medullary rim sign	27/133 (20.3%)	21 bilateral present (1/21 very thick and ill-defined), 2 only right present, 4 only left	11/62 (17.7%)	7 bilateral present (1/7 thick and very echoic), 4 only left present
Dystrophic mineralization	4/133 (3.0%)	3 focal in both kidneys (1/3 in both pelvices and left diverticuli, 1/3 cortex, 1/3 both pelvices), 1 focal only in right (corticomedullary junction)	0/62 (0.0%)	/
Cavitary lesion	2/133 (1.5%)	1 with one (2.9 mm) cyst lesion with small echogenic structure in the cyst, 1 with one (4.5 mm) anechoic cyst cranial in left kidney	1/62 (1.6%)	Several cysts in cortex of both kidneys (largest cyst right kidney: 16 mm; largest cyst left kidney: 6.9 mm)

*Significant difference between group 1 and group 2 ($P < 0.05$)

Table 4.4 Continued.

	RAGDOLL		CONTROL	
	N	**Abnormalities**	**N**	**Abnormalities**
Solid mass/nodule	1/133 (0.8%)	1 cat with 1 small nodular lesion in left and 2 in right kidney	1/62 (1.6%)	1 cat with 5 ill-defined round to oval hypoechoic areas in the cortex of both kidneys, deforming the renal contours
Segmental cortical lesion*	10/133 (7.5%)	3 bilateral, 4 only right, 3 only left	0/62 (0.0%)	/
Renal pelvis / proximal ureter	3/133 (2.3%)	1 with 1.8 mm of right pelvis and hyperechoic spots in right proximal ureter, 2 focal mineralizations in both pelvices	0/62 (0.0%)	/
Small kidney (< 3.2 cm)	11/133 (8.3%)	2 bilateral, 2 only right, 7 only left kidney	6/62 (9.7%)	1 bilateral, 2 only right, 3 only left kidney
Large kidney size difference (> 0.7 cm)	5/133 (3.8%)	2 right, 3 left kidney larger	1/62 (1.6%)	1 right kidney larger
Urine*	69/133 (51.9%)	49 sediment, 17 speckles, 3 other	16/62 (25.8%)	3 sediment, 13 speckles
Bladder uroliths	3/133 (2.3%)	1 sediment with acoustic shadow, 1 with one bladder urolith, 1 with three bladder uroliths	1/62 (1.6%)	1 with one bladder urolith
Retroperitoneal space	0/133 (0.0%)	/	0/62 (0.0%)	/
Liver: cysts	0/133 (0.0%)	/	0/62 (0.0%)	/
Liver: parenchymal changes	0/133 (0.0%)	/	0/62 (0.0%)	/
Other findings	5/133 (3.8%)	3 pregnant, 1 small liver, 1 enlarged uterus and possible metritis	5/62 (8%)	3 pregnant, 1 pancreas cyst surrounded by hyperechoic parenchyma, 1 splenomegaly

*Significant difference between group 1 and group 2 ($P < 0.05$)

Table 4.5. The sum of abnormalities observed on ultrasonography of the kidneys in 133 Ragdoll and 62 control cats. The parameters that were assessed to calculate this sum were abnormalities in renal capsule, renal shape, cortical echogenicity, medullar echogenicity, corticomedullary demarcation and renal pelvis; presence of small left kidney (< 3.2 cm), small right kidney (< 3.2 cm), large size difference between left and right kidney (absolute difference > 0.7 cm), medullary rim sign(s), dystrophic mineralization(s), cavitary lesion(s), solid mass(es)/nodule(s) and segmental cortical lesion(s). Multiple abnormalities of the same category (e.g. multiple segmental cortical lesions, multiple cavitary lesions) in the same cat were counted only once.

N	Ragdoll cats	Control cats
0	67	37
1	35	13
2	11	5
3	8	5
4	4	1
5	3	1
6	3	0
7	0	0
8	1	0
9	1	0

(N = number of abnormalities)

Discussion

The main finding of this prospective study, in which serum creatinine and urea concentrations, urinalysis and renal ultrasonography were compared between healthy Ragdoll and age-matched non-Ragdoll control cats, was that SCLs occur more commonly in Ragdoll cats. Also, 5.3% of Ragdoll cats had ultrasonographic abnormalities suggestive of CKD and none of the Ragdoll cats was affected by PKD. Furthermore, the serum creatinine concentration and UPC did not differ between Ragdoll and control cats. In contrast, Ragdoll cats had lower serum urea concentrations and higher USG than control cats, but the clinical relevance of these findings is unknown.

An important finding of this study is that serum creatinine concentrations did not differ between Ragdoll and control cats. In a retrospective study performed by our group, almost 11% of Ragdoll cats had serum creatinine concentrations exceeding the RI (Paepe *et al* 2012). This raised the question if a breed-specific RI for serum creatinine should be developed for Ragdoll cats, as was reported for the Birman breed (Reynolds *et al* 2010). The low number of Ragdoll cats with serum creatinine concentration exceeding the RI in addition to similar serum creatinine concentrations in both cat populations in this study, suggest that this is not necessary for the Ragdoll breed.

Crystalluria was observed in half of our Ragdoll and control cats, which is comparable to the 41% apparently healthy middle-aged and aged cats with crystalluria in a recent study (Paepe *et al* 2013). The low number of cats with urolithiasis in the present study confirms that crystalluria occurs commonly in healthy cats and that crystalluria per se is not a sufficient reason to start a calculolytic diet.

The statistical differences in laboratory parameters between Ragdoll and control cats are probably not clinically relevant. The control cats had significantly higher serum urea concentrations, however all but one control cats had serum urea within RI. There were more control cats with poorly concentrated urine (USG < 1.035), resulting in significantly lower USG for control cats. However, none of the control cats with poorly concentrated urine had azotemia. Both in dogs and in cats, the USG can fluctuate over the day, many factors influence USG and a low USG without other indications for kidney disease does not

necessarily suggest decreased renal function (van Vonderen *et al* 1997, Stockham and Scott 2008). It must be mentioned that USG in this study was measured with a traditional optical refractometer without separate scale for feline USG. Although it had been reported that these refractometers can overestimate the actual USG in feline urine (George 2001), a recent report has shown that this is not clinically relevant (Bennett *et al* 2011). Control cats were more likely to have glucosuria. However, none of the control cats with glucosuria showed or developed clinical signs of diabetes mellitus or showed other evidence (casts, mild proteinuria) for tubular dysfunction. Higher frequency of protein positivity on urinary dipstick in Ragdoll cats is probably not relevant because significant differences in UPC and in the proportion of cats with borderline and overt proteinuria were not observed between both populations. In addition, urinary dipstick tests are not very reliable to identify non-severe proteinuria, especially in cats with concentrated urine (Syme 2009).

Segmental cortical lesions were seen more commonly in Ragdoll versus control cats. The ultrasonographic aspect of these lesions was in line with the ultrasonographic description of kidney infarcts in veterinary literature. Renal infarcts are described as linear or wedge-shaped, well defined lesions in the renal cortex that are located perpendicular to the capsule, and may cause a dimple in the adjacent serosal surface. Initially, kidney infarcts are hypoechoic, but may become hyperechoic in the chronic state (Grooters and Biller 1995, Widmer *et al* 2004, d'Anjou 2008).

Other pathogenic explanations besides renal infarction must be considered to explain the SCLs. Hyperechoic triangular to wedge-shaped cortical lesions, irregular kidney shape and cortical outline, as were seen in our cats with SCLs, also are ultrasonographic features of renal scarring in humans (Barry *et al* 1998). Segmental cortical scarring causing depression of the renal cortical surface has been described in humans and dogs with reflux nephropathy (Cargollo and Diamond 2007, Kolbjørnsen *et al* 2008). In humans, these renal scars mostly develop due to chronic nonobstructive pyelonephritis secondary to primary vesico-ureteral reflux (Cargollo and Diamond 2007). None of our cats with SCLs had a positive urine culture or a history of urinary tract infection. Whether sterile reflux results in renal damage remains controversial in humans (Cargollo and Diamond 2007). Renal scarring in humans occurs mostly in the polar segments of the kidney (Cargollo and Diamond 2007). In our study, cats were affected by single or double SCLs and the location varied from unilateral to bilateral and

from cranial to the caudal part of the kidney and most SCLs were observed in one of the kidney poles.

Because SCLs were only observed in the Ragdoll and not in the control cat population, Ragdoll cats may have a breed-dependent increased susceptibility for SCL. Interestingly, brief pedigree analysis of the Ragdoll cats with SCLs showed two parent-offspring combinations and several other cats that were related to each other. This may indicate a hereditary explanation for the SCLs.

Major complications of renal scarring due to vesico-ureteral reflux and renal infarction in humans are hypertension and renal failure (Racusin and Pollack 2005, Cargollo and Diamond 2007, Tsai *et al* 2007). In addition, microscopic hematuria is commonly present in humans with acute renal infarction (Racusin and Pollack 2005, Tsai *et al* 2007). Indeed, some of our cats with SCLs showed mild to moderate urinary sediment abnormalities, such as microscopic hematuria. However, azotemic kidney disease and overt proteinuria was uncommon. Blood pressure was not evaluated in our cats, so hypertension cannot be excluded as underlying cause or consequence for SCLs in Ragdoll cats. Further studies will need to reveal the clinical significance and underlying cause for the SCLs observed in the Ragdoll cats.

None of the control cats but 5.3% of the Ragdoll cats in this study showed ultrasonographic abnormalities suggestive of CKD. This percentage is mildly lower than the 8.6% prevalence of CKD in Ragdoll cats in a recent retrospective study (Paepe *et al* 2012). Although we only found a trend towards significance ($0.5 < P < 1$), we cannot exclude that Ragdoll cats are predisposed to show ultrasonographic abnormalities compatible with CKD. However, it is important to remember that ultrasonography does not correlate with renal function and is not a useful tool to predict which cats will develop azotemic disease (Grooters and Biller 1995). In addition, the ultrasonographic findings do not imply that these cats were affected by CIN as several other diseases, such as glomerulonephritis, glomerulosclerosis, amyloidosis and nephrocalcinosis can result in similar ultrasonographic abnormalities (Widmer *et al* 2004). Follow-up of Ragdoll cats with ultrasonographic abnormalities suggestive of CKD is warranted to determine the clinical relevance of these findings.

In this study, PKD was only diagnosed in one Persian cat of the control group and in none of the included Ragdoll cats. In our retrospective study, PKD prevalence in Ragdoll cats of 2.9% was found (Paepe *et al* 2012). Although Ragdoll cats have been outcrossed with Persian cats in the past and could be at risk for PKD (Beck and Lavelle 2001), both studies indicate that PKD is uncommon in this population of Ragdoll cats.

Ragdoll cats more frequently showed an undulating or irregular renal capsule and echogenic urine. The renal capsular abnormalities may in part be explained by the predisposition of Ragdoll cats for segmental cortical lesions or the presence of CKD in some cats. The predisposition for echogenic urine in Ragdoll cats may be explained by the different gender distribution of both populations. Intact male cats were predisposed for echogenic urine in this study, possibly because the presence of sperm, mucus or fat droplets resulted in turbid or cloudy urine (Stockham and Scott 2008).

A remarkable finding of this study is that ultrasonographic abnormalities at the level of the kidney are very common in healthy cats, especially if the young age of our cat population is taken into account. To the authors' knowledge, this is the first study that evaluated kidney ultrasonography in a large healthy cat population. Also, several commonly cited studies reporting ultrasonographic features of feline kidneys date from the eighties or early nineties (Walter *et al* 1987a, Walter *et al* 1987b, Walter *et al* 1988, Yeager and Anderson 1989, Biller *et al* 1992). Since then, the ultrasound devices and expertise have improved which may explain why minor changes nowadays are commonly detected. In half of the Ragdoll and 40% of the control cats at least one ultrasonographic abnormality was observed. Abnormalities that were seen in more than 10% of cats of both groups were the presence of a medullary rim sign and changes in cortical echogenicity, especially hyperechoic renal cortices. Although the significance of these abnormalities is still unknown (Yeager and Anderson 1989, Biller *et al* 1992, Widmer *et al* 2004, d'Anjou 2008), it is important that clinicians are aware that renal ultrasonographic abnormalities often occur in healthy cats. Further studies to evaluate which renal ultrasonographic abnormalities are clinically relevant are needed.

It is important to realize that disease prevalences are affected by the specific characteristics of the studied population (Hahn and Overley 2010), such as geography. All Ragdoll cats included in this study resided in Belgium or the Netherlands and the majority was born in the same area. However, 40% of Ragdoll cats were imported from different areas such as the United States, other European countries, Australia or New Zealand, Canada, or Israel. This means that the findings of this study are not just applicable for Ragdoll cats in the Benelux, but may be of interest for Ragdoll breeders all over the world. Secondly, a selection bias may have resulted in an underestimation of actual disease prevalence because most Ragdoll breeders that participated to this study had screened their cats over several generations.

Although precise breeding recommendations cannot be made based on this study, it seems reasonable to discourage intensive breeding with Ragdoll cats with SCL(s) and obvious renal ultrasonographic changes. However, if Ragdoll breeders want to screen their Ragdoll cats for the presence of kidney disease, ultrasonography and measuring serum creatinine and urea concentrations must be combined with routine urinalysis. Concurrent urinalysis will facilitate the interpretation of serum urea and creatinine concentrations and is needed to detect kidney dysfunction (DiBartola 2010).

Conclusion

Based on this population, breed-specific serum creatinine RIs are not required for Ragdoll cats. Furthermore, renal ultrasonographic abnormalities are common in young healthy cats, both in Ragdoll and non-Ragdoll cats. Ragdoll cats are predisposed to segmental triangular to wedge-shaped cortical changes. Further studies are required to elucidate whether these lesions may represent renal infarction or cortical scarring and to determine the clinical implications of these SCLs. None of the Ragdoll cats was diagnosed with PKD, but 5.3% Ragdoll cats had clinically significant renal lesions based on ultrasonographic findings. Further studies are needed to identify if these cats are affected by CIN and whether they will develop azotemic kidney disease or not.

End notes

[a]MEDVET Algemeen Medisch Laboratorium Diergeneeskunde, Antwerp, Belgium

[b]Advia 2120, Siemens, Brussels, Belgium

[c]Architect C16000, Abbott, Wiesbaden, Germany

[d]IDEXX Catalyst Dx Analyzer, IDEXX Europe BV, Hoofddorp, the Netherlands

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[f]Iricell IQ, Instrumentation Laboratory, Zaventem, Belgium

[g]BioMerieux Media Square, Brussels, Belgium

[h]Logic 7 GE Medical Systems, Milwaukee, Wisconsin, USA

[i]SPSS® Statistics version 20, SPSS Inc., Chicago, Ilinois, USA

Addendum: Ultrasound protocol to evaluate the urinary system and liver

Ultrasonographer:

US parameters/lesions	**Patient number: Name:**
Renal capsule	Smooth / irregular / undulating / thickened
Renal shape	Left kidney: regular / irregular Other: Right kidney: regular / irregular Other:
Renal size (length in cm)	Left kidney: Right kidney:
Renal echogenicity	Cortex: *Diffuse: hypoechoic / normal / hyperechoic *Focal abnormalities: Medulla: *Diffuse: hypoechoic / normal / hyperechoic *Focal abnormalities:
Corticomedullary distinction	Good / reduced
Medullary rim sign	Absent / present
Dystrophic mineralization	Absent / present *linear / patchy / focal *location:
Cavitary lesions	Absent / cysts / abscess / hematoma *number: *size: *location:
Solid mass / nodule	Absent / present *number and size: *location: *description:
Renal infarct	Absent / present *number: *location: *description:
Renal pelvis and proximal ureter	Normal / enlarged *diameter: *remarks:

Bladder	Filling: empty / moderately / severely distended Urine: anechoic / sediment / blood clot / other: Uroliths: absent / present *number: *size: *location: renal pelvis / ureter / bladder / urethra
Retroperitoneal space	Fluid: absent / present *anechoic / echoic Other:
Liver	Cysts: absent / present *number *size *location Parenchymal changes: absent / present *description:
Additional findings not in the list	*urinary tract: *other:

After performing the US, the ultrasonographer must make conclusions.

1) No abnormalities / abnormalities
2) If abnormalities:
   - Are abnormalities clinically relevant? Yes / no / uncertain
   - Are abnormalities indicative of chronic kidney disease/chronic interstitial nephritis? Yes / no / uncertain
   - Is the cat affected by PKD? Yes / no
   - Comment why this is concluded:

Renal agenesis or hypoplasia	No / yes *left / right kidney *compensatory enlargement of other kidney? Yes / no
Renal dysplasia	No / yes

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CHAPTER 5

EVALUATION OF CATS WITH DIABETES MELLITUS FOR DIABETIC KIDNEY DISEASE

ROUTINE KIDNEY VARIABLES, GLOMERULAR FILTRATION RATE AND URINARY CYSTATIN C IN CATS WITH DIABETES MELLITUS, CATS WITH CHRONIC KIDNEY DISEASE AND HEALTHY CATS

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Paepe D, Ghys LFE, Smets P, Lefebvre HP, Croubels S, Delanghe J, Meyer E and Daminet S. Routine kidney variables, glomerular filtration rate and urinary Cystatin C in cats with diabetes mellitus, cats with chronic kidney disease and healthy cats. *In preparation for submission.*

Summary

Diabetic kidney disease (DKD) is a frequent and serious complication in human diabetic patients, but data are limited in cats.

Kidney function was compared between cats with diabetes mellitus (DM), cats with chronic kidney disease (CKD) and age-matched healthy cats and between recently (< 1 month) and not-recently diagnosed diabetic cats by measuring routine kidney variables (serum creatinine, serum urea, urine specific gravity (USG), urinary protein: creatinine ratio (UPC)), urinary Cystatin C: creatinine ratio (uCysC/uCreat) and glomerular filtration rate (GFR).

Thirty-six diabetic cats (15 recently, 21 not-recently diagnosed), 10 cats with CKD and 10 healthy cats were prospectively recruited. Glomerular filtration rate was evaluated by exo-iohexol clearance in 17 diabetic cats, all cats with CKD and all healthy cats. In all cats but two diabetic cats, uCysC was measured with a human particle-enhanced nephelometric immunoassay, validated to measure feline Cystatin C (CysC).

Diabetic cats had significantly lower serum creatinine (mean ± SD 123 ± 38 versus 243 ± 80 µmol/L), serum urea (11 ± 3 versus 18 ± 7 mmol/L) and uCysC/uCreat (6 ± 31 versus 173 ± 242 mg/mol) and significantly higher USG (1.033 ± 0.012 versus 1.018 ± 0.006) and GFR (2.0 ± 0.7 versus 0.8 ± 0.3 mL/min/kg) compared with cats with CKD. Compared with healthy cats, diabetic cats only had significantly lower USG (1.033 ± 0.012 versus 1.046 ± 0.008). None of these parameters significantly differed between recently and not-recently diagnosed diabetic cats.

Based on evaluation of routine kidney variables, GFR and uCysC as a tubular marker at a single time point, a major impact of feline DM on kidney function could not be demonstrated.

Introduction

Both humans and cats are frequently affected by DM and the prevalence is rapidly increasing (Osto *et al* 2013, Reutens 2013). Diabetic kidney disease or diabetic nephropathy is a common and serious complication in human diabetics, particularly in type 2 DM. Diabetic nephropathy is characterized by glomerular alterations, resulting in altered GFR and micro- or macroalbuminuria, tubular damage and hypertension. In the prediabetic or early diabetic phase, GFR is often increased (glomerular hyperfiltration), whereas decreased GFR is a typical finding for patients with more prolonged diabetes (Reutens 2013, Ritz 2013, van Buren and Toto 2013). Although the detection of persistent renal (micro)albuminuria is often used as early marker for DKD, recent findings have revealed that renal impairment without albuminuria has become an increasingly common presentation of DKD in type 2 diabetic patients. Also, many patients with microalbuminuria never progress to renal dysfunction. Hence, more sensitive and specific markers for early detection of DKD are needed (Matheson *et al* 2010, Reutens 2013, Ritz 2013, Dwyer and Lewis 2013, Moresco *et al* 2013, Tramonti and Kanwar 2013). Many human patients with DKD have increased concentrations of urinary biomarkers indicating tubular damage, such as retinol-binding-protein (RBP), N-acetyl-β-D-glucosaminidase, *β*-2 microglobulin, neutrophil-gelatinase associated lipocalin, kidney injury molecule 1 and liver-fatty acid-binding protein (Hong and Chia 1998, Matheson *et al* 2010, Moresco *et al* 2013, Tramonti and Kanwar 2013). Recent data show that uCysC, another marker of tubular dysfunction (Kim *et al* 2012, Togashi and Miyamoto 2013), might be a promising biomarker for early detection of DKD and for prediction of progression of renal impairment in type 2 diabetic patients (Kim *et al* 2011, Jeon *et al* 2011, Kim *et al* 2013, Togashi and Miyamoto 2013).

As feline diabetic patients mostly suffer from type 2 DM, cats might be susceptible to develop DKD (Rand 2013, Bloom and Rand 2013). However, evidence whether or not feline diabetics are at risk for kidney disease is scarce. Nevertheless, a high prevalence of microalbuminuria and proteinuria has been described in diabetic cats (Al-Ghazlat *et al* 2011). On the other hand, hypertension is considered to be uncommon in diabetic cats (Norris *et al* 1999, Sennello *et al* 2003, Al-Ghazlat *et al* 2011), in contrast to human diabetic patients. Additionally, histological lesions of the kidney were not more frequent in diabetic cats

compared with cats that died from other diseases (Zini *et al* 2012). To the authors' knowledge, data on GFR and urinary biomarkers in cats with DM are currently lacking.

Therefore, this study mainly aimed to evaluate GFR and uCysC in cats with DM. These parameters and routine kidney variables, i.e. serum creatinine and urea concentrations, USG and UPC, were prospectively compared between cats with DM, cats with CKD and healthy cats. The second objective was to evaluate the influence of duration of DM on kidney function, by comparing the same parameters between recently and not-recently diagnosed diabetic cats.

Materials and Methods

Animals

Cats with DM, cats with CKD and healthy cats were prospectively included. Diabetes mellitus was diagnosed based on compatible clinical signs, persistent hyperglycemia and glucosuria. Recently diagnosed DM cats were defined as cats that had received insulin therapy for less than 1 month. Not-recently diagnosed DM cats had received insulin for more than 1 month. Cats with intermittent episodes of diabetic remission (i.e. no insulin required to maintain normoglycemia for at least 4 weeks) and relapse of overt DM were included in the not-recently diagnosed DM group, also if the current insulin treatment duration was less than 1 month. Glycemic control was evaluated based on the combination of history, bodyweight, blood glucose level, serum fructosamine concentration, and, if available, blood glucose curve in the hospital or at home. Glycemic control was considered good if all these parameters indicated good metabolic control (e.g. absence of polyphagia, polydipsia, polyuria; stable bodyweight; blood glucose level of 10 – 15 mmol/L with serum fructosamine < 470 µmol/L). Glycemic control was considered poor if all of these parameters indicated poor glycemic control (e.g. presence of polyphagia, polydipsia, polyuria, weight loss; blood glucose > 15 mmol/L; serum fructosamine > 600 µmol/L). The diabetes was considered moderately controlled in cats with several (at least 2) parameters indicating poor glycemic control and the other parameters indicating good glycemic control. The diagnosis of CKD was based on compatible clinical signs and renal azotemia. Healthy was defined as absence of clinical signs and significant abnormalities on physical examination and routine laboratory analysis (see below). Efforts were taken to age-match the healthy cats to the diabetic cats.

Routine physical examination (including thyroid gland palpation), complete blood count, serum biochemistry profile (including total thyroxin concentration in cats older than 6 years), and urinalysis (including UPC and bacterial culture) were performed to assess the general health status of all included cats. Exclusion criteria for all groups were presence of hyperthyroidism or concurrent significant systemic disease and treatment with angiotensin-converting enzyme inhibitors and antihypertensive drugs at the time of inclusion. The presence of azotemia was not an exclusion criterion for the DM group. Diabetic cats that were in diabetic remission and did not receive insulin therapy were not included. In the CKD

group, only cats with *International Renal Interest Society* (IRIS) stages 2 – 3 were included (IRIS 2009).

The study was completed at the Department of Small Animal Medicine and Clinical Biology, Faculty of Veterinary Medicine, Ghent University. All cats were privately owned, the owners were thoroughly informed about the study aims and protocol, and the study was approved by local and national ethical committees (EC2010_029).

Procedures

Routine kidney variables were measured in all cats, namely serum creatinine (modified Jaffe assay[a]; reference interval (RI) 44 – 133 µmol/L), serum urea (enzymatic assay[a]; RI 6.2 – 11 mmol/L), USG (traditional hand-held optical refractometer) and UPC[b]. To evaluate if proteinuria was of renal origin, urine bacterial culture[c] was performed and the urinary sediment was evaluated as previously described (Paepe *et al* 2013a, Paepe *et al* 2013b). The tubular marker uCysC was determined in all cats. Urine was frozen at -80°C until batched analysis. The uCysC concentration was measured with a human particle-enhanced nephelometric immunoassay[d], previously validated to measure feline CysC (Ghys *et al* In press), and expressed as uCysC: urinary creatinine ratio (uCysC/uCreat). Serum fructosamine concentration (colorimetric assay[a]; RI < 290 µmol/L) was measured only in diabetic cats.

A combined plasma exogenous creatinine-iohexol clearance test (PEC-ICT) was performed in cats of ≥ 3 kg bodyweight with easy, unstressed and nonaggressive behavior and for which the owner gave permission. Cats with CKD IRIS stage 4 were not eligible for GFR determination. The PEC-ICT was performed as previously reported (van Hoek *et al* 2007, van Hoek *et al* 2008a). Briefly, all cats received 40 mg/kg creatinine and 64.7 mg/kg iohexol intravenously. Blood samples were taken in tubes with ethylenediaminetetraacetic acid as anticoagulant before and 5, 15, 30, 60, 120, 180, 360, 480 and 600 minutes after injection. Plasma was frozen at -80°C until analyzed. For this study, the data of the exo-iohexol clearance test was used to compare GFR between the 3 groups of cats. Exo- and endo-iohexol concentrations were determined by a validated high-performance liquid chromatography method with ultraviolet detection (van Hoek *et al* 2007, De Baere *et al* 2012). Pharmacokinetic analyses were performed using WinNonlin[e]. The plasma data were subjected to noncompartmental analysis with a statistical moment approach. The area under the plasma

concentration-versus-time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity, as described by Watson *et al* (2002). Plasma clearance of exo-iohexol was determined by dividing exo-iohexol dose administered by AUC and indexed to bodyweight (mL/min/kg).

The study design included systolic blood pressure (SBP) measurement in all cats that underwent a clearance test for GFR determination. Whether or not SBP was measured in the other cats depended on the responsible clinicians' decision. To measure SBP, the Doppler ultrasonic technique and a standardized procedure following the consensus statement of the *American College of Veterinary Internal Medicine* (ACVIM) (Brown *et al* 2007) were used. Hypertension was defined as SBP > 160 mmHg (Brown *et al* 2007, Stepien 2010).

Statistical analysis

All statistical tests were performed with statistical software[f] and at the 0.05 significance level.

The effect of the disease (DM, CKD, healthy) on age, bodyweight, SBP, serum creatinine, serum urea, USG, UPC, uCysC/uCreat and GFR was tested by ANOVA. Within the DM group, ANOVA was also used to evaluate the effect of history (recently versus not-recently diagnosed DM) on the same parameters and on serum fructosamine concentration. In case of a global significant difference, post-hoc pairwise comparisons were performed using the Tukey test. For cats with uCysC concentrations below the limit of quantification, the uCysC concentration was considered to be "zero" for the statistical analysis.

Results

In total, 56 cats were included, namely 36 diabetic cats (15 recently diagnosed, 21 not recently diagnosed), 10 cats with CKD and 10 healthy cats. Routine kidney variables (serum creatinine, serum urea, USG and UPC) and uCysC/uCreat were measured in all cats, except for serum urea in one diabetic cat and uCysC/uCreat in two diabetic cats because of insufficient sample volume. The exo-iohexol GFR was determined in 17 cats with DM (8 recently diagnosed, 9 not recently diagnosed), all cats with CKD and all healthy cats. Because of technical problems SBP could not be measured in 3 of the diabetic and 1 healthy cat(s) that underwent a clearance test. In total, SBP was measured in 20 diabetic cats, all CKD cats and 9 healthy cats. Serum fructosamine concentration was unavailable in 3 cats with DM. Eight of the 15 recently diagnosed diabetic cats were not yet treated with insulin at time of inclusion, the other 7 received insulin for a period between 2 and 4 weeks. The mean ± SD insulin treatment duration for the not recently diagnosed diabetic cats was 12.9 ± 15.3 months. Six cats were treated longer than 1.5 years, the others were treated between 1 month and 1 year. Four cats intermittently received insulin, but all of the not recently diagnosed diabetic cats were receiving insulin treatment at the time of inclusion. Of the 21 not recently diagnosed diabetic cats, glycemic control was good in 4, moderate in 6 and poor in 11 patients.

Breed distribution consisted of 29 domestic short- or longhaired cats and 7 purebred cats (2 Burmese, 1 Siamese, 1 Russian Blue, 1 Oriental, 1 British shorthair, 1 Norwegian forest cat) in the diabetic group; 7 domestic short- or longhaired cats and 3 purebred cats (1 British shorthair, 1 Persian, 1 Siamese cat) in the group of CKD cats and 10 domestic short- or longhaired cats in the group of healthy cats. The diabetic group involved 27 male (25 neutered, 2 intact) and 9 female (7 neutered, 2 intact) cats and the CKD and healthy cat groups involved 3 male (all neutered) and 7 female (all neutered) cats.

Because the available RI is inappropriate for interpretation of certain laboratory parameters in aged cats (Paepe *et al* 2013a), the mean concentration + 2 SD of the healthy cats was used as upper reference limit. Four diabetic cats had serum creatinine concentration > 160 µmol/L. Similarly, 5 diabetic cats had serum urea concentration > 14 mmol/L. Four diabetic, 8 CKD but no healthy cats had USG between 1.007 – 1.020; 17 diabetic, 2 CKD and 1 healthy cat had USG between 1.021 – 1.035 and 15 diabetic, 0 CKD and 9 healthy cats had USG between 1.036 – 1.060. Glucosuria was detected in 29 diabetic cats but all other cats

were negative on urine dipstick for glucose. Ketonuria was not detected in any of the cats. According to the ACVIM Consensus Statement on proteinuria (Lees *et al* 2004), 23 cats did not have proteinuria (UPC < 0.2; 14 DM, 3 CKD, 6 healthy cats), 16 had borderline proteinuria (UPC 0.2 – 0.4; 8 DM, 4 CKD, 4 healthy cats) and 17 had overt proteinuria (UPC > 0.4; 14 DM, 3 CKD, 0 healthy cats). The urine bacterial culture was unavailable in one diabetic cat but was negative in all other cats. Urinary sediment analysis revealed microscopic hematuria in 2 cats (1 DM, 1 CKD) and a moderate amount of struvite crystals (6 per low power field) in 1 healthy cat. The urinary sediment of the other cats did not show significant abnormalities.

Hypertension was present in 2 diabetic cats (SBP 165 and 220 mmHg), none of the cats with CKD and in 2 healthy cats (SBP 180 and 190 mmHg). All other cats were normotensive. The diabetic cat with SBP 165 mmHg also had mild proteinuria (UPC 0.5) but otherwise normal kidney parameters, including GFR. Although recommended, repeated SBP measurement was not performed. This cat was euthanized approximately 1 year after inclusion because of relapse of overt DM after a period of diabetic remission. Clinical signs of CKD were never noticed, but laboratory tests were not repeated after inclusion. The other hypertensive diabetic cat (SBP 220 mmHg) had mild azotemia with USG 1.020 and proteinuria (UPC 1.1), GFR was not measured in this cat. Repeated SBP measurements confirmed hypertension and further work-up revealed iatrogenic hypothyroidism. Both healthy cats with hypertension were very anxious during the examination which makes white-coat hypertension most likely.

The descriptive statistics for the parameters age, bodyweight, SBP, serum creatinine, serum urea, USG, UPC, uCysC/uCreat and GFR of the 3 groups are presented in Table 5.1. The box-plots for the GFR values of the 3 groups is shown in Fig 5.1. Between the 3 groups (DM, CKD, healthy) significant differences were not detected for the parameters age, bodyweight, SBP, and UPC. Cats with DM had significantly lower serum creatinine and serum urea ($P < 0.001$), higher USG ($P = 0.001$), lower uCysC/uCreat ($P < 0.001$) and higher GFR ($P < 0.001$) than cats with CKD. The same parameters also significantly differed between healthy cats and cats with CKD, which was expected based on our inclusion criteria. Diabetic cats only had significantly lower USG compared to healthy cats ($P = 0.002$).

Table 5.1. Mean ± SD [median (range)] age, bodyweight, systolic blood pressure (SBP), serum creatinine concentration (sCreat), serum urea concentration (sUrea), urine specific gravity (USG), urinary protein: creatinine ratio (UPC), urinary Cystatin C: creatinine ratio (uCysC/uCreat) and exo-iohexol glomerular filtration rate (GFR) for 36 cats with diabetes mellitus (DM), 10 cats with chronic kidney disease (CKD) and 10 healthy cats. Age, bodyweight, sCreat, USG and UPC were available in all cats, sUrea in all cats except for 1 DM cat, uCysC/uCreat in all cats except for 2 DM cats, GFR in 17 diabetic cats and in all CKD and healthy cats and SBP in 20 diabetic cats, all CKD and 9 healthy cats.

	DM	**CKD**	**Healthy**
Age (years)	10.7 ± 3.0 [10.9 (5-17.4)]	11.0 ± 6.0 [9.9 (2.3-20)]	10.5 ± 3.8 [11.0 (3-14.6)]
Bodyweight (kg)	5.1 ± 1.4 [4.9 (2.4-8.5)]	4.4 ± 1.3 [4.1 (3.0-7.5)]	4.5 ± 1.1 [4.5 (3-6.6)]
SBP (mmHg)	133 ± 28 [130 (91-220)]	133 ± 14 [136 (106-150)]	143 ± 28 [132 (103-190)]
sCreat (µmol/L)[*$]	123 ± 38 [114 (68-229)]	243 ± 80 [197 (158-383)]	108 ± 26 [98 (80-162)]
sUrea (mmol/L)[*$]	10.9 ± 3.3 [10.3 (5.3-20.2)]	18.3 ± 6.8 [16.2 (8.2-28.3)]	9.4 ± 2.3 [9.0 (6.7-14.5)]
USG[*§$]	1.033 ± 0.012 [1.030 (1.010-1.060)]	1.018 ± 0.006 [1.017 (1.010-1.030)]	1.046 ± 0.008 [1.047 (1.030-1.060)]
UPC	0.39 ± 0.28 [0.33 (0.09-1.12)]	0.41 ± 0.39 [0.23 (0.07-1.21)]	0.19 ± 0.06 [0.19 (0.06-0.29)]
uCysC/uCreat (mg/mol)[*$]	6.2 ± 30.6 [< LOQ (< LOQ-177.7)]	172.7 ± 241.7 [3.4 (< LOQ-585.6)]	< LOQ
GFR (mL/min/kg)[*$]	2.0 ± 0.7 [1.9 (1.0-3.8)]	0.8 ± 0.3 [0.7 (0.5-1.4)]	2.1 ± 0.4 [2.2 (1.4-2.6)]

(< LOQ = uCysC below limit of quantification (0.049 mg/L) (Ghys *et al* In Press); [*]Significant difference between cats with DM and CKD; [§]Significant difference between cats with DM and healthy cats; [$]Significant difference between cats with CKD and healthy cats)

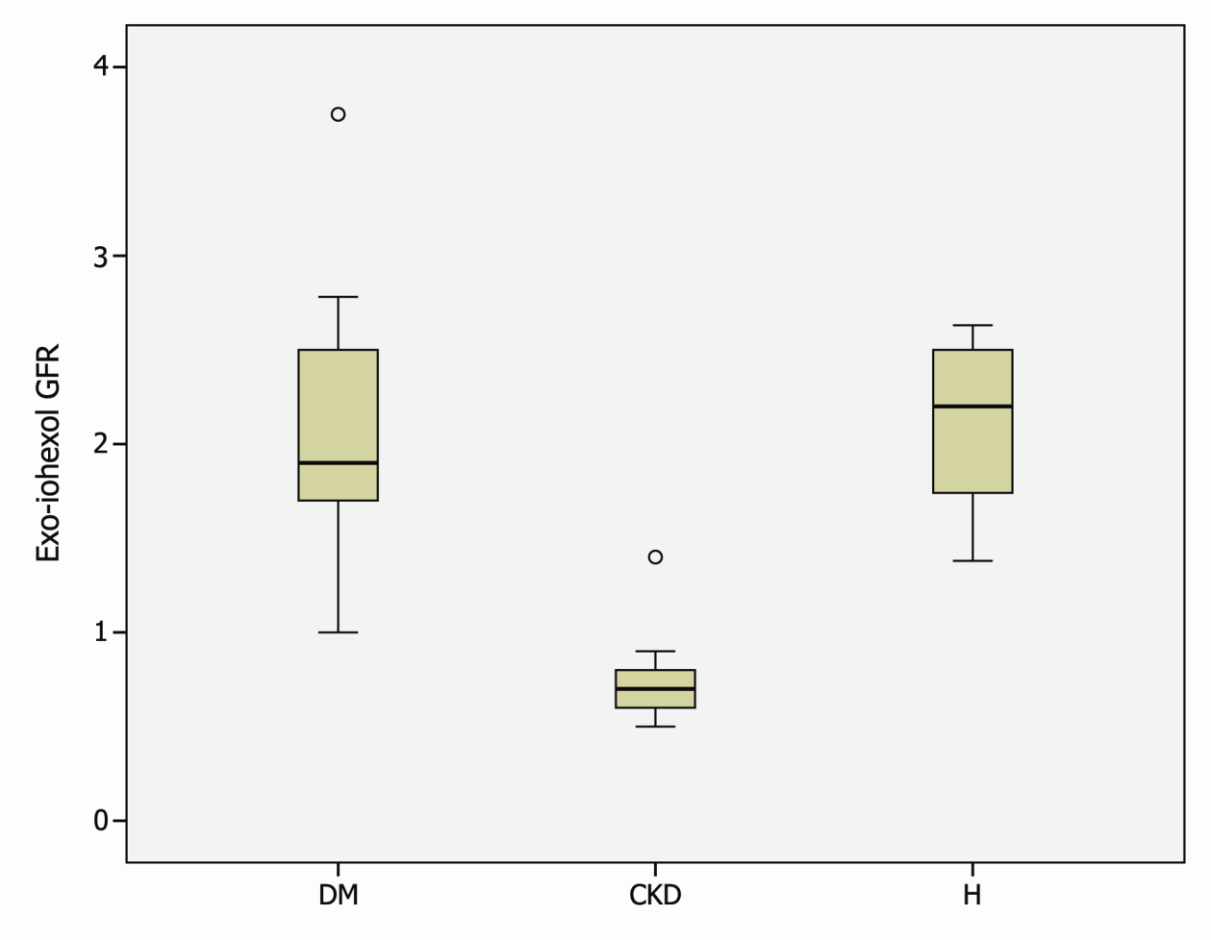

Fig 5.1. Box-plots of exo-iohexol glomerular filtration rate (GFR; in mL/min/kg) for cats with diabetes mellitus (DM; n = 17), cats with chronic kidney disease (CKD; n = 10) and healthy cats (H; n = 10).

The descriptive statistics for the parameters age; bodyweight; SBP; serum creatinine; serum urea; serum fructosamine concentration; USG; UPC; uCysC/uCreat and GFR for recently and not recently diagnosed diabetic cats are presented in Table 5.2. Mean age was significantly lower in recently diagnosed than in not recently diagnosed diabetic cats (P = 0.026). The other evaluated parameters did not significantly differ between both groups.

Table 5.2. Mean ± SD [median (range)] age, bodyweight, systolic blood pressure (SBP), serum creatinine concentration (sCreat), serum urea concentration (sUrea), serum fructosamine concentration, urine specific gravity (USG), urinary protein: creatinine ratio (UPC), urinary Cystatin C: creatinine ratio (uCysC/uCreat) and exo-iohexol glomerular filtration rate (GFR) for 15 cats with recently diagnosed diabetes mellitus (DM) and 21 cats with not-recently diagnosed DM. Age, bodyweight, sCreat, USG and UPC were available in all cats. SBP was measured in 9 recently and 11 not-recently and GFR in 9 recently and 8 not-recently diagnosed DM cats. sUrea was unavailable in 1 not-recently diagnosed DM cat, serum fructosamine in 1 recently and 2 not-recently diagnosed DM cats, uCysC/uCreat in 1 recently and 1 not-recently diagnosed DM cat.

	Recently diagnosed DM	**Not-recently diagnosed DM**
Age (years)[*]	9.4 ± 3.6 [9.0 (5.0-17.4)]	11.6 ± 2.2 [12.0 (6.0-14.3)]
Bodyweight (kg)	5.0 ± 1.5 [4.9 (2.4-7.8)]	5.2 ± 1.3 [4.9 (3.1-8.5)]
SBP (mmHg)	128 ± 20 [130 (100-165)]	136 ± 33 [130 (91-220)]
sCreat (µmol/L)	111 ± 34 [95 (72-197)]	131 ± 40 [124 (68-229)]
sUrea (mmol/L)	10.0 ± 3.1 [10.3 (5.7-17.7)]	11.5 ± 3.4 [10.5 (5.3-20.2)]
Fructosamine (µmol/L)	555 ± 130 [544 (364-891)]	487 ± 177 [445 (262-914)]
USG	1.032 ± 0.012 [1.035 (1.010-1.050)]	1.033 ± 0.013 [1.030 (1.010-1.060)]
UPC	0.37 ± 0.18 [0.40 (0.14-0.80)]	0.40 ± 0.33 [0.26 (0.09-1.12)]
uCysC/uCreat (mg/mol)	13.3 ± 47.4 [< LOQ (< LOQ-177.7)]	1.1 ± 5.1 [< LOQ (< LOQ-22.9)]
GFR (mL/min/kg)	2.2 ± 0.7 [1.9 (1.7-3.8)]	1.9 ± 0.6 [1.8 (1-2.8)]

(< LOQ = uCysC below limit of quantification (0.049 mg/L) (Ghys *et al* In Press);
[*]Significant difference between recently and not-recently diagnosed DM cats)

Discussion

This study evaluated if DKD complicates feline DM as it does in human medicine. Therefore routine kidney variables, a tubular urinary marker and GFR of diabetic cats were compared with those of healthy cats and cats with CKD at a single time point. A major impact of feline DM on kidney function could not be demonstrated.

Most parameters significantly differed between cats with DM and CKD, which indicates that our diabetic cats did not have obvious renal dysfunction. Compared to healthy cats, our diabetic cats only had significantly lower USG. As most of our diabetic cats had glucosuria, the lower USG is probably related to osmotic diuresis. This hypothesis is strengthened by the fact that most of our diabetic cats had USG > 1.020 which reflects renal concentrating ability in polyuric glucosuric animals. On the other hand, it should be kept in mind that marked glucosuria may falsely increase USG (Stockham and Scott 2008).

In our diabetic group, an increased serum creatinine concentration and serum urea concentration was detected in 11.1% and in 14.3% of the cats, respectively. Our findings are somewhat lower compared to a recent study, where 17% of newly diagnosed cats with DM or diabetic ketoacidosis had an increased serum creatinine concentration (Callegari *et al* 2013). In the latter study, having a higher serum creatinine concentration at diagnosis appeared to be associated with decreased survival time, but whether the azotemia of these diabetic cats was pre-renal or renal in origin was not reported.

Although the mean UPC was higher in diabetic cats than in healthy cats and approximately 40% of diabetic cats had proteinuria compared to none of the healthy cats, this did not result in a statistically significant different UPC between both groups. The prevalence of proteinuria in diabetic cats has been evaluated in 2 studies. Sennello *et al* (2003) did not detect proteinuria in 12 diabetic cats, but a cut-off for proteinuria of 1 was used, which is nowadays considered high based on the ACVIM Consensus on proteinuria (Lees *et al* 2005). In contrast, in a more recent study in 66 cats, 75% of feline diabetics had UPC > 0.4 and the UPC of diabetic cats was significantly higher compared to UPC of healthy and sick control cats (Al-Ghazlat *et al* 2011). Differences in proteinuria prevalence between the latter and our study might be explained either by technical or methodological differences in UPC measurement (Rossi *et al* 2012) or by differences in study population such as duration of DM

and degree of glycemic control. The conflicting results of studies evaluating proteinuria in diabetic cats might argue for further investigation, for instance by measuring urinary albumin: creatinine ratio (UAC). In human studies, proteinuria is more commonly quantified by measuring UAC than by UPC, both in diabetic as in non-diabetic patients. Although microalbuminuria is useful as early marker for DKD, there is no obvious evidence that UAC is superior to UPC once overt albuminuria is present (Reutens 2013, Fisher *et al* 2013). The effect of DM on UAC is poorly studied in veterinary medicine and data are currently unavailable in cats. In one study in diabetic dogs, an increase in UPC and UAC was commonly present and some diabetic dogs only had an increased UAC with normal UPC value. Thus UAC might have additional value to UPC for the detection of early renal damage in diabetic dogs (Mazzi *et al* 2008). An important remaining question is whether this low-level proteinuria (UPC 0.4 – 1), that commonly affects feline diabetic patients, is an early marker for more severe renal dysfunction and whether these cats will develop DKD with more prolonged DM? Indeed, in cats with CKD, it is accepted that low-level proteinuria is a negative prognostic factor (Syme *et al* 2006, Kuwahara *et al* 2006, King *et al* 2007). On the other hand, the prognostic significance of low-level proteinuria in nonazotemic cats is less studied. However, preliminary data indicate that it might be associated with reduced survival times (Walker *et al* 2004). Borderline proteinuria was detected in 22% of diabetic cats and 40% of healthy cats. A high prevalence of borderline proteinuria in apparently healthy cats has recently been reported by our group, but the clinical significance of this finding remains unknown (Paepe *et al* 2013a, Paepe *et al* 2013b).

Altered GFR, initial glomerular hyperfiltration and in a later stage glomerular hypofiltration, is typical for DKD in humans (Mogensen *et al* 1983, Reutens 2013). In contrast, our diabetic cats did not show significant changes in GFR compared to healthy cats. Only one of the diabetic cats, diagnosed with DM the day before entering the study, was suspected of glomerular hyperfiltration (Fig 5.1). The GFR of this cat exceeded the GFR values for healthy cats of the current study and a previous study from our group (Paepe *et al* Submitted; see Chapter 6) and also exceeded the mean GFR value for hyperthyroid cats (3.3 mL/min/kg; van Hoek *et al* 2009a). Only one diabetic cat had GFR below the low GFR cut-off (1.2 mL/min/kg) for exo-iohexol clearance of the same previous study (Paepe *et al* Submitted; see Chapter 6). This indicates that almost all our diabetic cats had normal GFR.

In contrast to human diabetic patients that frequently suffer from hypertension, particularly patients with type 2 DM (Van Buren and Toto 2013), only 10% of our diabetic

cats had hypertension. This is comparable to 15% previously found in cats (Al-Ghazlat *et al* 2011). In two other reports, hypertension was not detected in any of the diabetic cats, but a cut-off of 180 mmHg was used to define hypertension (Norris *et al* 1999, Sennello *et al* 2003). Although the latter two studies are limited by this inappropriately high cut-off to define hypertension (Brown *et al* 2007), the current veterinary literature suggests that hypertension is uncommon in feline diabetic patients.

In our study, we could not detect significant differences in uCysC levels between diabetic and healthy cats. However, this parameter needs to be interpreted cautiously, because most diabetic cats (n = 31) had undetectable uCysC concentrations. Somewhat unexpected, uCysC also could not be found in several cats with CKD (n = 5). In contrast, in a previous study – using the same human nephelometric assay – all cats with CKD, but none of the healthy cats, had measurable uCysC concentrations (Ghys *et al* In Press). The urine samples of our study were stored up to 3 years at -80°C before CysC analysis, compared to maximum 1.4 years in the study of Ghys *et al*. Cystatin C is considered to be a stable protein in human medicine and freezing or freeze/thaw cycles do not affect its concentrations (Herget-Rosenthal *et al* 2004, Séronie-Vivien *et al* 2008, Gislefoss *et al* 2009). Therefore, stability of uCysC was assumed, but the stability of feline CysC in serum and urine is still under investigation. Although uCysC is a good marker for early detection of diabetic nephropathy in human medicine, it is possible that uCysC is a less ideal tubular marker in cats. Another tubular marker that has been studied in cats is retinol-binding protein (RBP) (van Hoek *et al* 2008b, van Hoek *et al* 2009b). In an in-house pilot study, significant differences in exo-iohexol GFR and urinary RBP: uCreat ratio were not detected between 7 diabetic and 5 age-matched healthy cats (Paepe *et al* 2007). The combined results of both urinary markers might indicate that cats with DM are less sensitive to tubular damage compared to human diabetics.

Humans with DKD may have variable structural changes of the kidneys, even before renal dysfunction occurs. The most typical structural renal changes of DKD are thickening of the glomerular and/or tubular basement membrane and mesangial expansion (Dalla Vestra and Fioretto 2003, Fioretto and Mauer 2007). Although a small study of cats with persistent hyperglycemia detected similar changes in some cats (Nakayama *et al* 1990), a recent study did not detect more frequent histological glomerular, tubulointerstitial and vascular lesions in the kidney of diabetic cats compared with cats that died from other diseases (Zini *et al* 2012). The latter study is in line with our findings suggesting that DKD does not seem to be of major importance in feline diabetic patients. Unfortunately, both veterinary studies only evaluated

the kidneys by light microscopy (Nakayama *et al* 1990, Zini *et al* 2012). Maximum information can only be obtained by evaluating kidney biopsies with a combination of light-, electron- and immunofluorescent microscopy (Segev 2010, Vaden 2010) as is routinely performed in human diabetic patients (Suzuki *et al* 2001, Penescu and Mandache 2010, Zhuo *et al* 2013).

Our study was limited by not measuring GFR and SBP in all diabetic cats. Secondly, the patients were evaluated only at a single time point without performing follow-up. We were also not able to evaluate the influence of glycemic control on kidney function because only a small number of cats had good glycemic control at the time of inclusion. Finally, the different gender and breed distribution between groups may be potential confounding factors.

Conclusion

By evaluating routine kidney variables, GFR and uCysC as a tubular marker at a single time point, we could not demonstrate a major impact of feline DM on kidney function. In cats with concurrent DM and CKD, the question whether DM (partially) causes CKD or whether both diseases are unrelated cannot be fully answered. Still, current veterinary literature does not support a strong relationship between both diseases. However, follow-up studies, mainly to reveal the clinical significance of low-level proteinuria, are required in diabetic cats.

End notes

[a]Architect C16000, Abbott, Wiesbaden, Germany

[b]Iricell IQ, Instrumentation Laboratory, Zaventem, Belgium

[c]BioMerieux Media Square, Brussels, Belgium

[d]Behring Nephelometer (BN) ProSpec, Siemens Healthcare Diagnostics, Marburg, Germany

[e]WinNonlin Version 4.0.1, Scientific Consulting Inc Apex, NC, USA

[f]Systat 12, Systat Software Inc, San Jose, CA, USA

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CHAPTER 6

SIMPLIFIED METHODS TO ESTIMATE GLOMERULAR FILTRATION RATE AND TO IDENTIFY CATS WITH DECREASED GLOMERULAR FILTRATION RATE

SIMPLIFIED METHODS FOR ESTIMATING GLOMERULAR FILTRATION RATE IN CATS AND FOR DETECTION OF CATS WITH LOW OR BORDERLINE GLOMERULAR FILTRATION RATE

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Summary

Simplified methods to detect patients with decreased glomerular filtration rate (GFR) are needed.

Data of a 9-sample combined plasma exogenous creatinine-iohexol clearance test (PEC-ICT) of 73 cats were used. Limited sampling strategies (LSS) were developed by comparing all sampling time combinations to the entire 9 sampling times set and selecting the best sampling time combinations based on maximum relative error. By regression analysis, the ability of routine blood and urine parameters to predict GFR or identify cats with low or borderline GFR was examined. Cut-off clearance marker concentrations to predict low or borderline GFR were determined at three time points after marker injection. All procedures were analyzed for three clearance markers (exo-iohexol, creatinine, endo-iohexol).

For reliable GFR estimation, at least 3 blood samples for clinical purpose and 5 blood samples for research purpose are required. Regression formulae based on routine variables did not reliably predict GFR, but accurately identified cats with low or borderline GFR. Clearance marker concentrations exceeding given marker cut-off concentrations also identified cats with low or borderline GFR with high sensitivities and specificities.

These simplified methods will facilitate detection of early kidney dysfunction in cats. The methodology used might also be valuable for human medicine.

Introduction

Chronic kidney disease (CKD) is a serious health problem in humans and early identification of kidney dysfunction is important to delay progression to end-stage renal disease (Stevens *et al* 2006, Salgado *et al* 2012). Glomerular filtration rate is considered the best overall measure of kidney function. However, GFR determination is cumbersome, time-consuming and expensive and therefore difficult to do in routine clinical practice (de Jong and Gansevoort 2005, Stevens *et al* 2006). Equations based on serum creatinine concentration and demographic variables are routinely used to estimate GFR (de Jong and Gansevoort, Stevens *et al* 2006, Salgado *et al* 2010). Unfortunately, these equations provide a less accurate GFR estimate in certain patient groups, including patients at extremes of ages and body size, severe under- or overweight patients, patients without CKD and patients with rapidly changing kidney function. Measuring GFR using exogenous markers is recommended to assess kidney function in these patient groups (Stevens *et al* 2006, Stevens and Levey 2009, Salgado *et al* 2010).

Similarly, routine blood and urine variables namely serum creatinine concentration, serum urea concentration, urine specific gravity (USG) and urinary protein: creatinine ratio (UPC), do not allow detection of early kidney dysfunction in small animals. It is generally accepted that more than two-thirds of functional renal mass must be lost before kidneys lose their ability to concentrate urine, and more than three-fourths must be lost before an animal becomes azotemic (Braun and Lefebvre 2008, Stockham and Scott 2008). Plasma clearance of an intravenously administered marker is commonly used in cats to estimate GFR (DiBartola 2010, Von Hendy-Willson and Pressler 2011). However, multi-sampling techniques to determine GFR are labor-intensive, time-consuming, expensive and may be stressful or even painful which limits their practical use, particularly in cats (Paepe and Daminet 2013). Several LSS have been described to estimate feline GFR (Barthez *et al* 2000, Barthez *et al* 2001, Goy-Thollot *et al* 2006, Vandermeulen *et al* 2008, Heiene *et al* 2009, Miyagawa *et al* 2010, Vandermeulen *et al* 2010, Finch *et al* 2011, Katayama *et al* 2012, Finch *et al* 2013, Katayama *et al* 2013), but none of these methods is sufficiently validated in cats with CKD to be used in practice.

Thus, reliable simplified methods to identify humans or cats with early kidney dysfunction are needed (Salgado *et al* 2010, Paepe and Daminet 2013). For research purpose,

estimating the true GFR value with an acceptable margin of error is important. Conversely, knowledge of the actual GFR value is often not needed in daily practice. More important, clinicians need to be able to predict which patients have a decreased GFR based on routine blood and urine variables or based on other methods, requiring only a minimal number of blood samples.

In this study, a population of cats with wide GFR range was evaluated. At first, we aimed to develop LSS, both for daily practice as for research purposes for creatinine, exo-iohexol, and endo-iohexol clearances. Secondly, we aimed to evaluate if routine variables (serum urea, serum creatinine, USG, UPC, systolic blood pressure (SBP)) can predict the actual GFR value of a cat or can identify cats with low or borderline GFR. Finally, we aimed to develop cut-off concentrations for creatinine, exo-iohexol, and endo-iohexol at three time points after IV bolus of creatinine and iohexol to identify cats with low or borderline GFR.

Materials and Methods

Data of cats that underwent a combined PEC-ICT at the Department of Small Animal Medicine and Clinical Biology, Ghent University, Belgium were used. All animal work was conducted according to guidelines for animal care, with consent of the Ethical Committee of the Faculty of Veterinary Medicine from Ghent University, Belgium and with informed owner consent. Data of several cats were previously published (van Hoek *et al* 2007, van Hoek *et al* 2008, van Hoek *et al* 2009). If cats underwent several clearance tests, only one clearance test was used for the present study. The PEC-ICT had been performed as previously reported (van Hoek *et al* 2007, van Hoek *et al* 2008). Briefly, all cats received 40 mg/kg creatinine and 64.7 mg/kg iohexol intravenously. Blood samples were taken in tubes with ethylenediaminetetraacetic acid as anticoagulant before and 5, 15, 30, 60, 120, 180, 360, 480 and 600 minutes after injection. Plasma creatinine was assayed by an in-house validated enzymatic method[a] and exo-iohexol and endo-iohexol concentrations were determined by a validated high-performance liquid chromatography method with ultraviolet detection (van Hoek *et al* 2007, De Baere *et al* 2012). Pharmacokinetic analyses were performed using WinNonlin[b]. The plasma data were subjected to noncompartmental analysis with a statistical moment approach. The area under the plasma concentration-versus-time curve (AUC) was calculated by the trapezoidal rule with extrapolation to infinity, as described by Watson *et al* (2002). Plasma clearance of creatinine, exo- and endo-iohexol was determined by dividing dose administered by AUC and indexed to bodyweight (mL/min/kg).

Following information was retrieved from the medical records of these cats: health status, signalment, serum creatinine, serum urea, total thyroxine concentration (TT4), USG, UPC, and SBP. Healthy was defined as absence of clinical signs and significant abnormalities on physical examination, complete blood count, serum biochemistry profile (including TT4 in cats older than 6 years) and routine urinalysis (including UPC and bacterial culture). CKD was diagnosed based on compatible clinical signs and renal azotemia. Cats that had both CKD and diabetes mellitus (DM), both CKD and hyperthyroidism or cats that developed CKD after treatment for hyperthyroidism were included in the CKD group and excluded from the hyperthyroid or DM group. Hyperthyroidism was diagnosed based on compatible clinical signs and increased TT4. The diagnosis of DM was made based on compatible clinical signs, hyperglycemia, glucosuria and increased serum fructosamine concentrations. Cats without

DM or hyperthyroidism that were suspected of renal disease but with doubtful routine blood (serum urea, serum creatinine) and urine (USG, UPC) parameters were allocated to a separate group of cats with doubtful renal status.

Statistical analysis

All statistical tests were performed with statistical software[c,d] and at the 0.05 significance level.

Simplified methods for estimating GFR

To develop LSS, a total of 510 sampling time combinations (i.e. all possible combinations) were compared to the entire 9 blood sampling times set for each clearance marker (creatinine, exo-, and endo-iohexol) by calculation of AUC values by the trapezoidal rule. The maximum relative error was calculated and used to select the best sampling time combination for each number of samples.

Secondly, a general linear model was used to evaluate if routine variables (SBP, serum urea, serum creatinine, USG, UPC) could predict feline GFR values.

Simplified methods to identify cats with borderline or low GFR

Method 1: Logistic regression analysis was used to evaluate if routine parameters (SBP, serum urea, serum creatinine, USG, UPC) are able to differentiate cats with GFR below a certain threshold from cats with GFR equal to or above this threshold. By looking at the GFR data and health status of the cats, the range in which GFR results of cats with CKD overlapped with GFR results of other groups was identified for each clearance marker (creatinine, exo-, and endo-iohexol). Within these ranges of GFR values, several GFR cut-off values were evaluated by binary logit analysis. Clinically useful GFR cut-off values to discriminate between cats with GFR below and above these cut-off GFR values were determined based on sensitivity, specificity and receiver-operating-characteristic (ROC) curve analysis.

Method 2: To develop cut-off concentrations for creatinine, exo-iohexol and endo-iohexol after marker injection to identify cats with decreased GFR, borderline and low GFR cut-off values were selected for each clearance marker. The *borderline GFR cut-off values* were arbitrarily selected by looking at the serum creatinine-GFR relationship curves and selecting a cut-off value in the area where the curve started to bend (i.e. where decreasing GFR resulted in increasing serum creatinine concentrations) and where GFR values of CKD cats and cats with doubtful renal function overlapped with the other groups. We assured that GFR values of all CKD cats were below this cut-off (except the outlier for endo-iohexol clearance) (Fig 6.1). Borderline GFR cut-off values were defined as 1.7 mL/min/kg for exo-iohexol and 1.9 mL/min/kg for creatinine and endo-iohexol clearances. The *lower GFR cut-off value* was selected based on GFR results of CKD and healthy cats in a previous study using the same PEC-ICT (van Hoek *et al* 2009). A GFR cut-off value between GFR results of CKD and healthy cats was selected, resulting in low GFR cut-off values of 1.2 mL/min/kg for exo-iohexol and 1.4 mL/min/kg for creatinine and endo-iohexol clearances. Using these selected borderline and lower GFR cut-off values, sensitivities, specificities, positive (PPV) and negative (NPV) predictive values were calculated for various creatinine, exo-iohexol, and endo-iohexol concentrations 60 (t60), 120 (t120) and 180 (t180) minutes after marker injection. The sensitivities and specificities were used to draw ROC curves for each clearance marker at these three time points for both the borderline and low GFR cut-off value. For PPV and NPV calculation (Bourdaud'hui 2012), the pre-test probability that the animal is diseased was set between 40 and 60% because a veterinarian will only evaluate kidney function more thoroughly in cats for which routine blood and urine parameters give doubtful results. Clinically useful cut-off creatinine, exo-, and endo-iohexol concentrations at t60, t120 and t180 were identified based on their sensitivities, specificities, PPV and NPV.

Results

Study population

Seventy-three cats were included, namely 16 healthy, 20 CKD (13 only CKD, 6 CKD after treatment for hyperthyroidism, 1 CKD and DM), 19 DM, 16 untreated hyperthyroid cats and 2 cats with doubtful renal status. None of the cats had combined DM and hyperthyroidism. According to the *International Renal Interest Society* (IRIS) staging system (IRIS 2009), 10 CKD cats were in IRIS stage 2 and 10 in IRIS stage 3. The study population involved 63 domestic short- or long-haired and 9 purebred (3 Siamese, 3 British shorthairs, 1 Burmese, 1 Persian, 1 Oriental) cats and 34 female (1 intact, 33 neutered) and 38 male (3 intact, 35 neutered) cats. For one cat, breed, gender and age were not recorded. The mean ± SD age was 10.4 ± 4.6 (range 1-20) years and mean ± SD bodyweight 4.6 ± 1.2 (range 2.3-7.8) kg. The mean ± SD SBP was 137 ± 24 (range 91-210; n = 59) mmHg, mean ± SD serum urea 11.0 ± 4.7 (range 5.3-28.3; n = 68) mmol/L, mean ± SD serum creatinine 135.1 ± 70.9 (range 40.7-382.8; n = 68) µmol/L, mean ± SD TT4 51.7 ± 60.6 (range < 6.5-200; n = 58) nmol/L, mean ± SD USG 1.034 ± 0.015 (range 1.008-1.060; n = 68) and mean ± SD UPC 0.34 ± 0.30 (range 0.06-1.29; n = 69). For the 5 cats for which serum creatinine (modified Jaffe assay) was not recorded, the baseline creatinine concentration of the PEC-ICT was used to assess renal status. These cats were excluded from the general linear model to predict GFR and from the logistic regression analysis to identify cats with decreased GFR.

Clearance

The GFR values for the complete population, healthy cats, hyperthyroid cats, CKD cats, DM cats and cats with doubtful renal status are presented in Table 6.1. The GFR-versus-serum creatinine relationship for the three clearance markers are shown in Fig 6.1. The mean ± SD (range) extrapolated area of the AUC was 5.7 ± 5.5% (0.2-20.7%) for exo-iohexol, 6.3 ± 4.9% (0.6-19.0%) for endo-iohexol and 14.9 ± 14.9% (0.2-61.4%) for creatinine clearance. This extrapolated area exceeded 20% for exo-iohexol clearance in 2 CKD cats and for creatinine clearance in 16 CKD cats, two diabetic cats, one healthy old cat and one of the cats with unknown renal status. The relationships between GFR versus serum urea, USG, UPC, SBP for exo-iohexol clearance are presented in Fig 6.2. For creatinine and endo-iohexol clearances, comparable relationships were found (data not shown).

Table 6.1. Overview of glomerular filtration rates for the complete study population and the subgroups.

Glomerular filtration rates (in mL/min/kg) for creatinine, exo-iohexol and endo-iohexol clearance for the complete population (n = 73) and subgroups of healthy cats (n = 16), untreated hyperthyroid cats (n = 16), cats with chronic kidney disease (CKD; n = 20), cats with diabetes mellitus (DM; n = 19), cats with doubtful renal status based on routine blood and urine parameters (n = 2). Results are presented as mean ± standard deviation (range), except for the cats with doubtful renal status where the actual GFR values are shown.

Group	Creatinine clearance	Exo-iohexol clearance	Endo-iohexol clearance
Complete	2.33 ± 1.29 (0.4-6)	1.99 ± 1.16 (0.5-5.64)	2.55 ± 1.36 (0.52-6.2)
Healthy	2.44 ± 0.78 (1.3-4.3)	1.82 ± 0.37 (1-2.5)	3.05 ± 0.76 (1.1-4.1)
Hyperthyroid	3.95 ± 1.26 (2.1-6)	3.59 ± 1.12 (2-5.64)	3.69 ± 1.21 (1.94-6.2)
CKD	1.02 ± 0.37 (0.4-1.6)	0.85 ± 0.22 (0.5-1.4)	1.02 ± 0.41 (0.52-2.4)
DM	2.31 ± 0.61 (1.5-3.8)	2.05 ± 0.61 (1.2-3.6)	2.86 ± 1.16 (1-5)
Doubtful	1.6 and 1.8	1.1 and 1.6	1.8 and 1.9

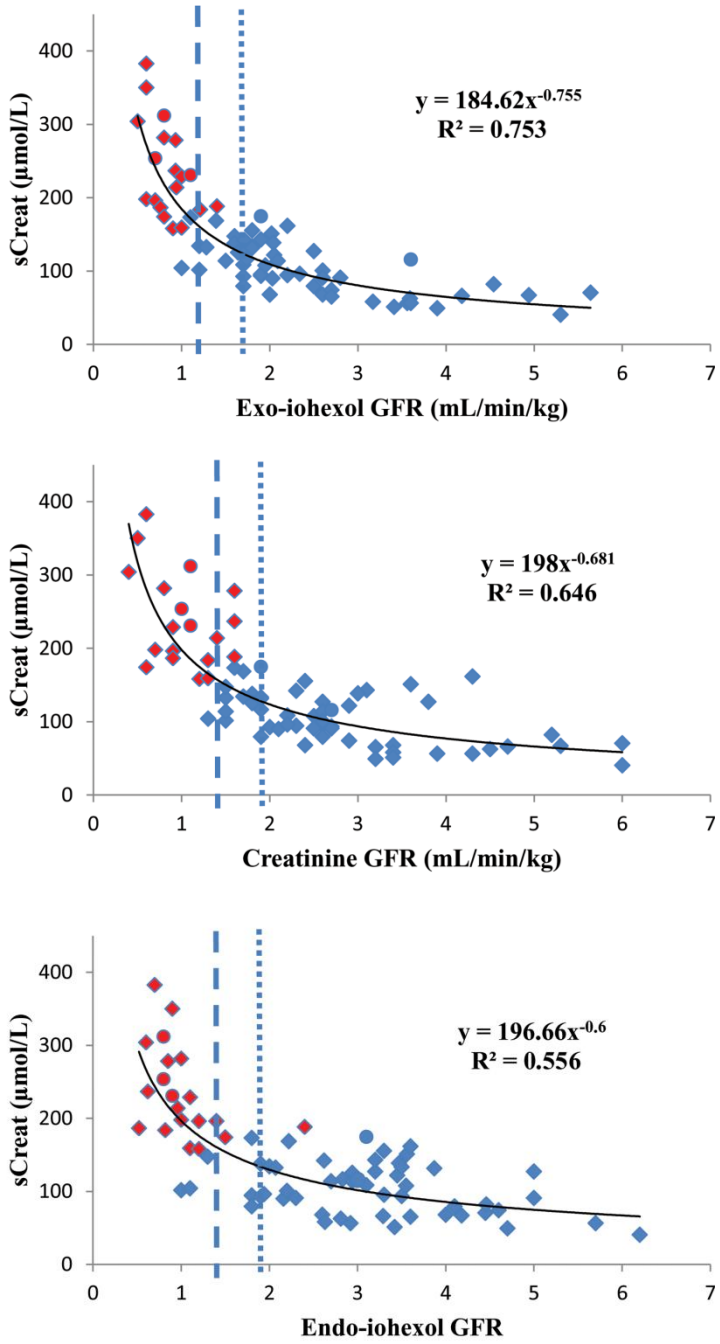


Fig 6.1. Relationship between glomerular filtration rate and serum creatinine concentration.

The relationship between glomerular filtration rate (GFR) and serum creatinine concentration (sCreat) measured by a modified Jaffe assay for exo-iohexol, creatinine and endo-iohexol clearances for the complete study population (n = 73 cats). Each cat represents a dot on the figure. The cats with CKD are presented with a red dot, the cats of the other groups with a blue dot. The argyle-shaped dots represent cats for which sCreat was measured with a modified Jaffe assay (n = 68). For cats for which sCreat measured with this modified Jaffe assay was not recorded (n = 5), the baseline creatinine concentration of the clearance test, measured with an enzymatic assay, was used. These cats are presented as circles on the figure. The GFR cut-off values that were selected based on these GFR-versus-sCreat curves (borderline GFR cut-off; dotted line) and based on the literature (low GFR cut-off; striped line) are also shown.

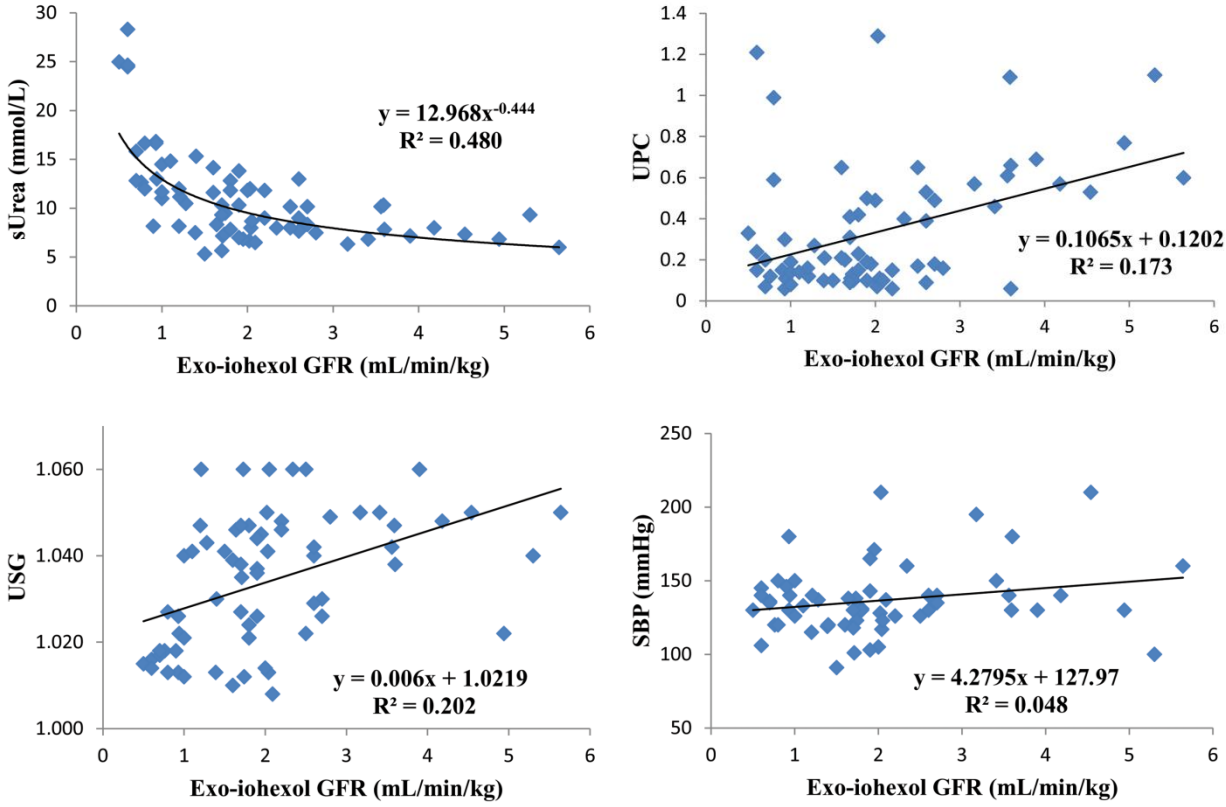


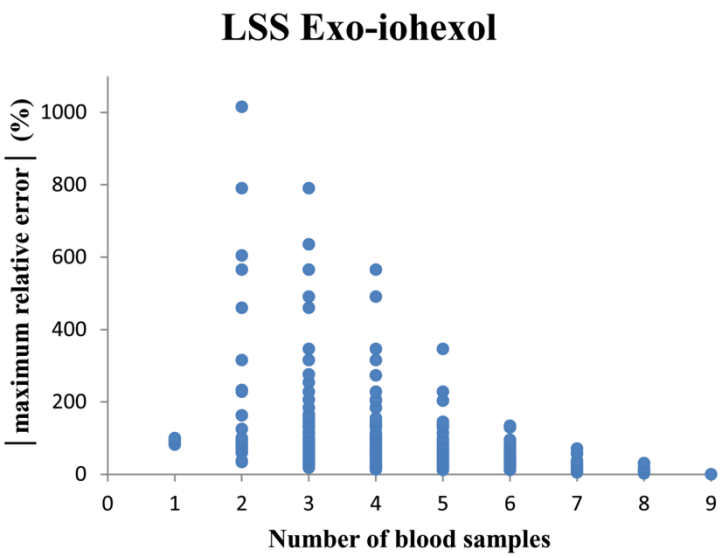
Fig 6.2. Relationship between glomerular filtration rate and other routine parameters.

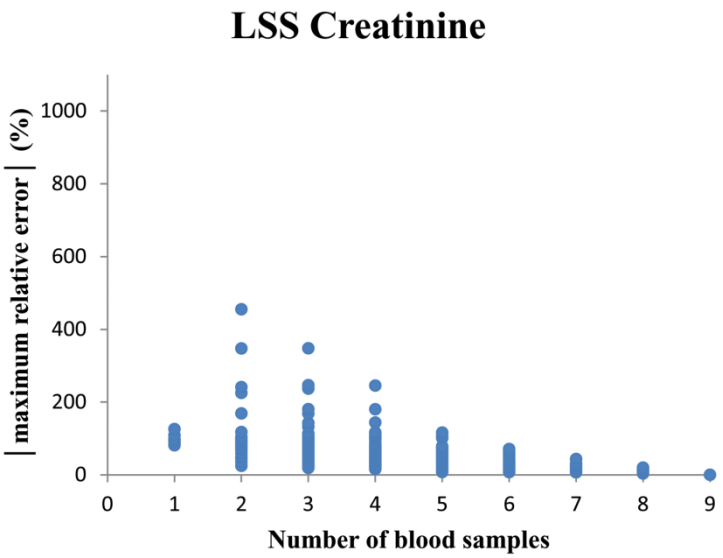
The relationship between glomerular filtration rate (GFR) and serum urea concentration (sUrea), urine specific gravity (USG), urinary protein: creatinine ratio (UPC) and systolic blood pressure (SBP) for exo-iohexol clearance for the complete study population (n = 73 cats). Each cat represents a blue argyle-shaped dot on the graphs. Cats for which sUrea (n = 5), USG (n = 5), UPC (n = 4) or SBP (n = 14) was not available, are not shown on these graphs.

Simplified methods for estimating GFR

The absolute value of maximum relative errors on the GFR calculation for all limited sampling time combinations compared with the GFR based on the complete 9 sample data set is shown in Fig 6.3. The best sampling time combinations and maximum relative errors for LSS with 1 – 8 blood samples for all three clearance markers are presented in Table 6.2.

The best regression formulae to predict GFR values based on routine parameters (SBP, serum urea, serum creatinine, USG, UPC) and associated R^2 values are shown in Table 6.3.





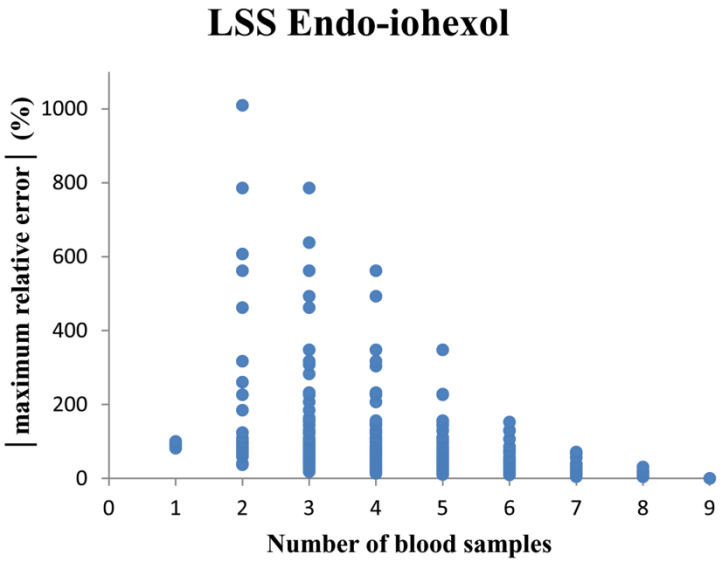


Fig 6.3. Maximum errors for limited sampling strategies.

Absolute value of maximum errors (%) on the calculation of plasma exo-iohexol, creatinine, and endo-iohexol clearances with a limited sampling strategy (LSS) compared with the clearances calculated based on the complete set of 9 blood samples. Each blue circle on the plots represents the maximum relative error for a given number of blood samples.

Table 6.2. The best sampling time combinations for the limited sampling strategies.

The best sampling time combinations and maximum relative errors for limited sampling strategies (LSS) with 1-8 blood samples for three clearance markers (creatinine, exo-iohexol, endo-iohexol), compared with the entire 9 blood sampling times set of the plasma exogenous creatinine-iohexol clearance test of 73 cats. A negative maximum error indicates that the LSS underestimates the glomerular filtration rate (GFR), a positive maximum error indicates that the LSS overestimates the GFR compared with the entire 9 blood sampling times set.

	Exo-iohexol clearance		**Creatinine clearance**		**Endo-iohexol clearance**	
N	**T (min)**	**ME (%)**	**T (min)**	**ME (%)**	**T (min)**	**ME (%)**
1	60	-82.0%	180	-81.1%	60	-81.6%
2	60, 480	33.8%	60, 480	-24.6%	60, 360	-36.9%
3	30, 120, 480	-18.2%	60, 480, 600	-18.9%	30, 120, 480	-18.2%
4	15, 60, 180, 480	-13.3%	30, 120, 480, 600	-15.7%	15, 60, 180, 480	-13.3%
5	15, 30, 120, 360, 600	11.7%	15, 120, 180, 360, 600	7.5%	15, 60, 120, 360, 600	-10.5%
6	15, 30, 120, 360, 480, 600	11.7%	15, 30, 180, 360, 480, 600	7.2%	15, 30, 120, 360, 480, 600	-9.6%
7	5, 30, 60, 120, 180, 360, 600	5.0%	5, 30, 60, 120, 180, 360, 600	6.6%	5, 30, 60, 120, 180, 360, 600	4.6%
8	5, 15, 30, 60, 120, 180, 360, 600	2.5%	5, 15, 30, 60, 120, 180, 360, 600	3.5%	5, 30, 60, 120, 180, 360, 480, 600	4.3%

(N = Number of samples; T = Optimal timing of the blood samples for this LSS; min = Minutes; ME = Maximum relative error)

Table 6.3. Regression formulae to predict glomerular filtration rate.

The best regression models to predict the glomerular filtration rate (GFR) based on the routine parameters systolic blood pressure, serum creatinine concentration (sCreat), serum urea concentration (sUrea), urine specific gravity and urinary protein: creatinine ratio (UPC). The associated R-square values to judge the goodness of fit of the models are also shown.

Clearance	**Regression model**	**R^2**
Exo-iohexol GFR	= 3.141 – (0.011 * sCreat) + (0.961 * UPC)	0.582
Creatinine GFR	= 3.817 – (0.071 * sUrea) – (0.007 * sCreat) + (1.085* UPC)	0.513
Endo-iohexol GFR	= 4.338 – (0.013 * sCreat)	0.447

Simplified methods to identify cats with borderline or low GFR

For prediction of borderline or low GFR based on routine parameters (serum urea, serum creatinine, USG, UPC), the regression formulae, the GFR range for which binary logit regression analysis was evaluated, the most appropriate cut-off values and associated sensitivities, specificities and area under ROC curve are shown in Table 6.4. Adding SBP to these routine parameters gave very similar results (data not shown).

The sensitivities, specificities, PPV and NPV for clinically useful cut-off creatinine, exo-, and endo-iohexol concentrations at t60, t120 and t180 after marker injection to predict borderline or low GFR are presented in Table 6.5. The ROC curves to identify cats with borderline or low GFR for the evaluated exo-iohexol, creatinine, and endo-iohexol concentrations at t60, t120, and t180 are shown in Fig 6.4. The creatinine concentration for one cat at t60 and exo- and endo-iohexol concentrations for two cats at t180 were not available because of insufficient sample. Thus, the calculations of cut-off concentrations were based on the data set of 73 cats, except for t60 for creatinine clearance (n = 72) and for t180 for exo- and endo-iohexol clearances (n = 71).

Table 6.4. Regression formulae to predict borderline or low glomerular filtration rate.

The regression formulae for prediction of low glomerular filtration rate (GFR; mL/min/kg) for three clearance markers (creatinine, exo-iohexol, endo-iohexol) based on routine blood (serum urea concentration in mmol/L, sUrea; serum creatinine concentration in µmol/L, sCreat) and urine (urine specific gravity, USG; urinary protein: creatinine ratio, UPC) parameters in a population of cats (n = 67) for which all these parameters were available. The GFR range for which the logistic regression was performed and two clinically useful GFR cut-off values (*low* and *borderline GFR cut-off*) are presented. If $e^u/1+e^u < 0.5$, the cat has a high probability to have a GFR below the given cut-off. The associated sensitivities (Sens; %), specificities (Spec; %) and area under receiver-operating-characteristic (ROC) curves (%) are also shown.

Marker	GFR range	GFR Cut-off	Sens	Spec	Area under ROC curve	Regression formula
Exo-iohexol	0.9-1.6	1.1	98.0	91.3	97.2	*u*=-77.799 – 0.265 * sUrea – 0.040 * sCreat + 86 * USG – 0.162 * UPC
		1.5	91.1	81.8	96.5	*u*=6.770 – 0.201 * sUrea – 0.065 * sCreat + 5 * USG – 0.928 * UPC
Creatinine	1.0-2.0	1.2	96.5	60.0	97.4	*u*=-84.997 – 0.016 * sUrea – 0.033 * sCreat + 91 * USG – 3.241 * UPC
		1.7	93.3	81.8	95.8	*u*=22.479 – 0.363 * sUrea – 0.05 * sCreat – 11 * USG + 1.009 * UPC
Endo-iohexol	1.0-1.6	1.1	98.2	90.0	98.8	*u*=102.494 – 0.288 * sUrea – 0.073 * sCreat – 81 * USG – 0.251 * UPC
		1.5	96.0	76.5	97.2	*u*=-21.674 – 0.396 * sUrea – 0.037 * sCreat + 31 * USG + 4.406 * UPC

Table 6.5. Cut-off marker concentrations to predict borderline or low glomerular filtration rate.

Sensitivities , specificities, PPV and NPV to identify cats with GFR above a certain threshold if marker concentrations are similar to or exceed the presented cut-off creatinine, exo-, and endo-iohexol concentrations 60, 120, and 180 minutes after marker injection. For the predictive values, the pre-test probability was set at 40-60% and the mean PPV and NPV for this range of pre-test probabilities is shown. In Table 6.5a *low GFR cut-off value*s are used as GFR threshold and set at 1.2 mL/min/kg for exo-iohexol and at 1.4 mL/min/kg for creatinine and endo-iohexol clearances. In Table 6.5b *borderline GFR cut –off values* are used as GFR threshold and set at 1.7 mL/min/kg for exo-iohexol and at 1.9 mL/min/kg for creatinine and endo-iohexol clearances.

Table 6.5a.

	Exo-iohexol clearance					**Creatinine clearance**					**Endo-iohexol clearance**				
Time	**Exo**	**Sens**	**Spec**	**PPV**	**NPV**	**Creat**	**Sens**	**Spec**	**PPV**	**NPV**	**Endo**	**Sens**	**Spec**	**PPV**	**NPV**
t60	**160**	95.0	86.8	87.8	94.6	**505**	94.1	65.5	73.2	91.8	**24**	95.0	75.5	79.5	93.8
	180	95.0	92.5	92.6	94.9	**555**	94.1	85.5	86.6	93.6	**27**	90.0	81.1	82.7	89.0
	205	50.0	98.1	96.4	66.2	**655**	70.6	96.4	95.1	76.6	**32**	80.0	98.1	97.7	83.1
t120	**100**	100.0	88.7	89.8	100	**340**	94.1	76.8	80.2	92.9	**15**	100.0	84.9	86.9	100
	120	95.0	96.2	96.2	95.1	**400**	88.2	92.9	92.5	88.8	**19**	95.0	96.2	96.2	95.1
	135	80.0	98.1	97.7	83.1	**460**	70.6	94.6	93.0	76.3	**22**	80.0	98.1	97.7	83.1
t180	**80**	100.0	96.1	96.2	100	**270**	94.1	78.6	81.5	93.0	**10**	100.0	90.2	91.1	100
	85	95.0	98.0	98.0	95.2	**315**	94.1	89.3	89.8	93.8	**14**	90.0	98.0	97.9	90.7
	90	75.0	100.0	100	80.0	**400**	64.7	96.4	94.8	73.2	**18**	70.0	100.0	100	76.9

(t60 = Blood sample 60 minutes after clearance marker injection; t120 = Blood sample 120 minutes after clearance marker injection; t180 = Blood sample 180 minutes after clearance marker injection; Creat = Creatinine concentration (µmol/L); Exo = Exo-iohexol concentration (µg/mL); Endo = Endo-iohexol concentration (µg/mL); Sens = Sensitivity (%); Spec = Specificity (%); PPV = Positive predictive value (%); NPV = Negative predictive value (%))

Table 6.5b.

	Exo-iohexol clearance					Creatinine clearance					Endo-iohexol clearance				
Time	**Exo**	**Sens**	**Spec**	**PPV**	**NPV**	**Creat**	**Sens**	**Spec**	**PPV**	**NPV**	**Endo**	**Sens**	**Spec**	**PPV**	**NPV**
t60	**130**	100.0	65.1	74.1	100	**465**	96.7	71.4	77.2	95.5	**23**	96.0	77.1	80.7	95.1
	143	93.3	83.7	85.2	92.6	**505**	90.0	81.0	82.5	89.0	**27**	88.0	87.5	87.6	87.9
	160	83.3	97.7	97.3	85.4	**560**	70.0	95.2	93.6	76.1	**30**	76.0	93.8	92.4	79.6
t120	**80**	96.7	86.1	87.4	96.3	**290**	100.0	67.4	75.4	100	**10**	100.0	75.0	80.0	100
	84	96.7	93.0	93.3	96.5	**320**	93.3	83.7	85.2	92.6	**15**	96.0	91.7	92.0	95.8
	90	86.7	97.7	97.4	88.0	**350**	80.0	93.0	92.0	82.3	**19**	80.0	97.9	97.5	83.0
t180	**45**	100.0	90.2	91.1	100	**210**	100.0	67.4	75.4	100	**7**	100	87.0	88.5	100
	50	96.7	95.1	95.2	96.6	**250**	96.7	90.7	91.2	96.5	**10**	92.0	95.7	95.5	92.3
	55	90.0	100.0	100	90.9	**290**	83.3	97.7	97.3	85.4	**13**	76.0	100.0	100	80.6

(t60 = Blood sample 60 minutes after clearance marker injection; t120 = Blood sample 120 minutes after clearance marker injection; t180 = Blood sample 180 minutes after clearance marker injection; Creat = Creatinine concentration (µmol/L); Exo = Exo-iohexol concentration (µg/mL); Endo = Endo-iohexol concentration (µg/mL); Sens = Sensitivity (%); Spec = Specificity (%); PPV = Positive predictive value (%); NPV = Negative predictive value (%))

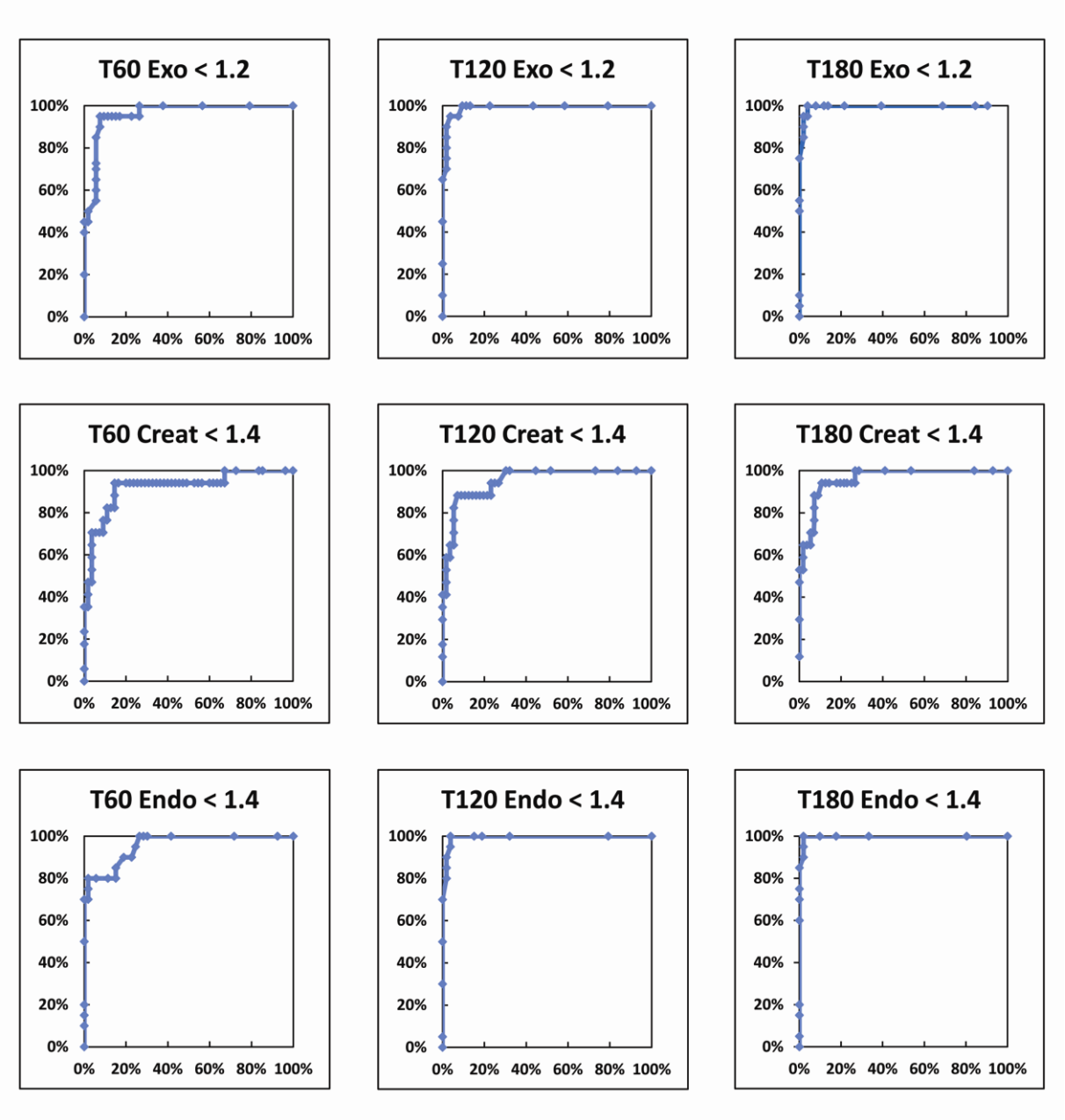


Fig 6.4a. Cut-off marker concentrations to predict low glomerular filtration rate.

The receiver-operating-characteristic (ROC) curves to identify cats with GFR below *low GFR cut-off value*s for the evaluated concentrations of exo-iohexol, creatinine and endo-iohexol at 60, 120, and 180 minutes after creatinine and iohexol injection. The GFR threshold is set at 1.2 mL/min/kg for exo-iohexol and at 1.4 mL/min/kg for creatinine and endo-iohexol clearances. For all graphs, '1 – specificity' (%) is shown at the X-axis and 'sensitivity' (%) at the Y-axis.

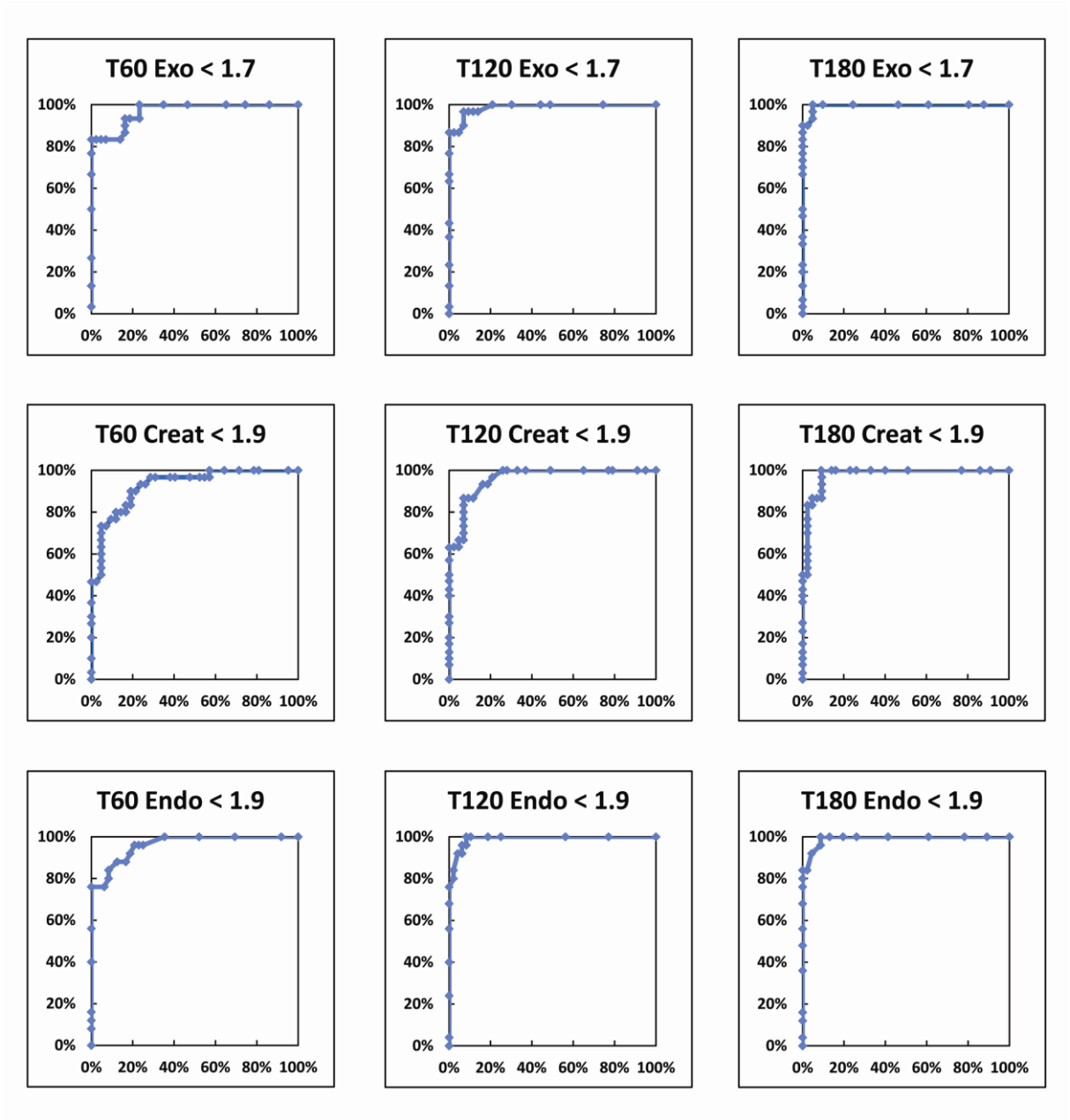


Fig 6.4b. Cut-off marker concentrations to predict borderline glomerular filtration rate.

The receiver-operating-characteristic (ROC) curves to identify cats with GFR below *borderline GFR cut-off value*s for the evaluated concentrations of exo-iohexol, creatinine and endo-iohexol at 60, 120, and 180 minutes after creatinine and iohexol injection. The GFR threshold is set at 1.7 mL/min/kg for exo-iohexol and at 1.9 mL/min/kg for creatinine and endo-iohexol clearances. For all graphs, '1 – specificity' (%) is shown at the X-axis and 'sensitivity' (%) at the Y-axis.

Discussion

One of the major goals of this study was to develop LSS to estimate GFR with an acceptable margin of error. The present study has important advantages compared with other studies that have described feline LSS. Firstly, cats over a wide GFR range were involved, namely cats with glomerular hyperfiltration (hyperthyroid cats), normal GFR (healthy cats, most diabetic cats) and glomerular hypofiltration (CKD cats). Therefore, our study population is representative for the GFR range that can be encountered in practice. In other reports concerning simplified methods for GFR estimation, no or only few renal impaired or hyperfiltrating cats had been included (Barthez *et al* 2000, Barthez *et al* 2001, Goy-Thollot *et al* 2006, Vandermeulen *et al* 2008, Heiene *et al* 2009, Vandermeulen *et al* 2010, Finch *et al* 2011, Katayama *et al* 2012, Katayama *et al* 2013). Secondly, all possible time combinations were evaluated. This contrasts with other studies evaluating LSS with blood sampling on arbitrarily selected time points, often without judging if the selected time points were the most appropriate ones for the GFR method used (Heiene *et al* 2009, Miyagawa *et al* 2010, Katayama *et al* 2012, Katayama *et al* 2013). However, our data clearly showed that, for a fixed number of samples, the maximum relative error can seriously differ based on the timing of blood sampling (Fig 6.3). Thirdly, LSS were compared with a GFR estimate that was calculated using a noncompartmental approach and based on multiple blood samples over a 10-hour period, both in the distribution and elimination phases of the clearance marker. In several previous studies evaluating single or two-sample approaches, the reference GFR estimate was based on maximum five blood samples over a relatively short time period (4 – 5 hours) and/or calculated using a one- or two-compartmental model (Heiene *et al* 2009, Miyagawa *et al* 2010, Vandermeulen *et al* 2010, Katayama *et al* 2012, Katayama *et al* 2013). Noncompartmental analysis is assumption free, unlike compartmental analysis. Both pharmacokinetic approaches are scarcely compared in cats, but it is known that a one-compartmental model overestimates true feline GFR (Finch *et al* 2011). This means that in some of these studies, single- or two-sample approaches to estimate GFR have been compared with another GFR estimate that was prone to errors. To correct for these errors, correction formulae have been used (Heiene *et al* 2009, Miyagawa *et al* 2010, Katayama *et al* 2012). However, these formulae were based on human medicine or on dogs and their use in cats is not properly evaluated. Recently, a feline correction formula was reported to accurately predict multisample clearance in cats and with smaller errors than human- or dog-based

formulae. Unfortunately, only healthy cats were enrolled in that study (Finch *et al* 2011). Finally, LSS were developed for several clearance markers, which was previously reported only in one study and only in healthy cats (Heiene *et al* 2009).

Our findings indicate that, based on a noncompartmental approach, GFR cannot be reliably estimated based on a single sample for all three markers. At least 3 or 4 blood samples after injection of the clearance marker are needed to estimate GFR with an acceptable margin of error (errors below 20%). For research purposes smaller margins of errors are required, preferably 10% or less, and 5 or more samples after marker injection are needed to maintain the error in the GFR estimate ≤ 10% in most cats. Our results also indicate that the optimal timing for blood sampling for LSS depends on the marker that is used for the clearance test. Also, a blood sample 10 hours after marker injection is almost always part of the optimal sampling time combination. This implies that, by performing LSS, the number of blood samples can be reduced but not the time needed to perform the clearance test. The same has been found in dogs (Watson *et al* 2002) and this may be explained because the timing of the last sample determines the percentage of AUC extrapolated. Also, the limit of quantification of the analytical method may influence the percentage of AUC extrapolated (Beal 2001, Fang *et al* 2011). The larger this proportion, the more inaccurate the GFR estimate and the extrapolated area should never exceed 20% of the total area (Watson *et al* 2002). In our study, this criterion was met for almost all cats for exo- and endo-iohexol clearances, but not for creatinine clearance, particularly not in CKD cats. Prolonged sampling, between 10 and 24 hours after creatinine injection, may be required in cats with renal dysfunction for more accurate GFR estimation based on creatinine clearance, as has been reported for dogs with surgically induced renal impairment (Watson *et al* 2002). Similarly, late sampling is required for accurate GFR determination in humans with advanced renal failure or premature infants (Brion *et al* 1986, Bröchner-Mortensen and Freund 1981, Stake *et al* 1991, Schwartz *et al* 2010).

Besides LSS, we evaluated the ability of routine blood and urine variables SBP, serum creatinine, serum urea, USG and UPC to predict GFR value. However, logistic regression analysis indicated that these parameters did not predict GFR values accurately. A possible explanation is the lack of strong relationships between GFR and these routine parameters (Fig 6.1 and Fig 6.2). We found an inverse curvilinear relationship between GFR and serum creatinine and serum urea for each clearance marker with a stronger relationship for serum creatinine compared with serum urea. This is in agreement with the literature in dogs and cats

(Finco 1995, Miyamoto 2001). The GFR-versus-serum creatinine curves for our population indicated that up to a serum creatinine concentration of 200 µmol/L, minimal change in serum creatinine concentration can be associated with serious change in GFR. Above serum creatinine of 200 µmol/L, further decline of GFR results in concurrent increase of serum creatinine concentration. In this study, mildly positive correlations between GFR and USG were found. However, several cats with normal to high GFR values had poorly concentrated urine (USG < 1.035), which confirms that cats with normal renal function can have wide variations in USG (Finco 1995). Also in previous studies, several healthy cats had USG below 1.035 (Paepe *et al* 2013a, Paepe *et al* 2013b). Conversely, some cats with decreased GFR had concentrated urine (USG ≥ 1.035). This is in line with the finding that some cats with severe experimental loss of renal functional mass retained their concentrating ability (Ross and Finco 1981). Our data also indicated a mildly positive relationship between UPC and GFR. This was unexpected because we suspected more severe proteinuria with decreasing kidney function, as reported in humans (Regeniter *et al* 2009; Wu *et al* 2012) and in dogs (Wehner *et al* 2008). On the other hand, increased UPC could also result from increased filtration pressure associated with higher GFR values. Finally, no relationship between SBP and GFR was found. Also a previous study did not find an association between the severity of azotemia and the presence of hypertension (Syme *et al* 2002).

Because, in daily practice, it is more important to detect cats with early renal dysfunction than knowing the exact GFR value, we also proposed two new methods to identify cats with low or borderline GFR. At first, with the regression formulae that we developed for each clearance marker (Method 1), veterinarians can determine if a cat has a high likelihood to have a GFR value below the proposed cut-off values based on routine parameters (serum urea, serum creatinine, USG, UPC). These regression formulae could predict, with very good sensitivity and moderate to good specificity, if a cat had a GFR value below the proposed GFR cut-offs. Adding SBP to these parameters did not have a major impact on these sensitivities and specificities, probably because there was no clear relationship between SBP and GFR. Also, because of more missing data for SBP than for the other parameters, adding SBP to these parameters reduced the number of cats available for the logistic regression analysis.

The second simplified method (Method 2) to identify cats with borderline or low GFR requires clearance marker injection, one blood sample 60, 120 or 180 minutes after marker injection and determination of the clearance marker concentration in that blood sample.

Important advantages are that multiple sample analysis and mathematics for GFR calculation are not needed, which makes this method both simple and cost-effective. Based on the ROC curves, time points t120 and t180 minutes seem to be more appropriate than t60 for all three clearance markers. Depending on the clearance marker concentration, sensitivities, specificities, PPV and NPV vary. For each time point and for each clearance marker, three cut-off marker concentrations are given, which allows the veterinarian to choose a cut-off marker concentration depending if (s)he wants to predict borderline or low GFR with high sensitivity, high specificity or both. The higher the cut-off marker concentration at a certain time point, the more likely a cat with a value above this threshold has low to borderline GFR (higher specificity and PPV) because higher marker concentrations are associated with slower clearance rates. Important to remember is that predictive values change with disease prevalence or pre-test probability. For example, for disease prevalence lower than 40%, PPV will decrease and NPV will improve compared to the reported values in this study (Erb 2011).

For both new simplified methods, we presented both *low* and *borderline GFR cut-off values*, so that veterinarians can choose which cut-off GFR value they will use, depending on the case. The *low GFR cut-offs* (Method 1: 1.2 mL/min/kg for creatinine clearance, 1.1 mL/min/kg for exo- and endo-iohexol clearance; Method 2: 1.2 mL/min/kg for exo-iohexol clearance, 1.4 mL/min/kg for creatinine and endo-iohexol clearances) indicate renal dysfunction. Initiating treatment for renal disease may be indicated in these cats. The *borderline GFR cut-offs* (Method 1: 1.7 mL/min/kg for creatinine clearance, 1.5 mL/min/kg for exo- and endo-iohexol clearance; Method 2: 1.7 mL/min/kg for exo-iohexol clearance, 1.9 mL/min/kg for creatinine and endo-iohexol clearances) indicate reduced to low-normal renal function. Several of these cats will have routine blood and urine parameters within reference intervals. Thus, both new methods may be additional tests to improve detection of cats with early kidney dysfunction. If these simplified methods suggest that a cat has a high likelihood to have GFR below this borderline cut-off value, more close monitoring of routine blood and urine parameters or additional tests to estimate GFR are warranted. The reason that the cut-off GFR concentrations differ for both methods to identify cats with borderline or low GFR is that these cut-offs were defined prior to statistical analysis only for Method 2, but not for Method 1.

Possible strategies to identify renal dysfunction in cats with doubtful routine blood and urine variables are shown in Fig 6.5. A cost-effective approach is to combine the second simplified method to identify borderline or low GFR with GFR estimation based on LSS. The

veterinarian can perform a 3- or 4-sample clearance test on the time points presented in Table 6.2 and initially analyze creatinine, exo- or endo-iohexol concentration only in one sample (t120 for 3-sample exo- and endo-iohexol clearance and for 4-sample creatinine clearance; t180 for 4-sample exo- and endo-iohexol clearance). If the marker concentration suggests that this cat likely has GFR below the cut-off value, clearance marker concentrations should be determined in the other 2 or 3 samples and GFR calculated. Currently, routine use of these methods is hampered because iohexol assays are expensive and not widely available and because injectable creatinine is not available for practitioners. As creatinine assays are inexpensive and easily accessible, a medical-grade formulation of creatinine should be commercialized for use in clearance tests.

A study limitation is that the cut-offs were developed and evaluated in the same cat population which may result in overestimation of the ability to correctly predict new observations (Kutner *et al* 2005). An important further step is to evaluate the accuracy of these cut-offs in a different cat population.

Conclusion

In this cat population that is representative for the GFR range that can be encountered in practice, we developed simplified methods to estimate GFR or to identify cats with decreased GFR. These simplified methods will facilitate detection of cats with early kidney dysfunction allowing timely treatment and improved prognosis of CKD cats. The simplified methods to identify low or borderline GFR are a new and practical approach to identify kidney dysfunction. The methodology used might be valuable to detect early CKD in humans, particularly in patients in which equations to estimate GFR are less reliable and determination of GFR has practical limitations (e.g. pediatric patients).

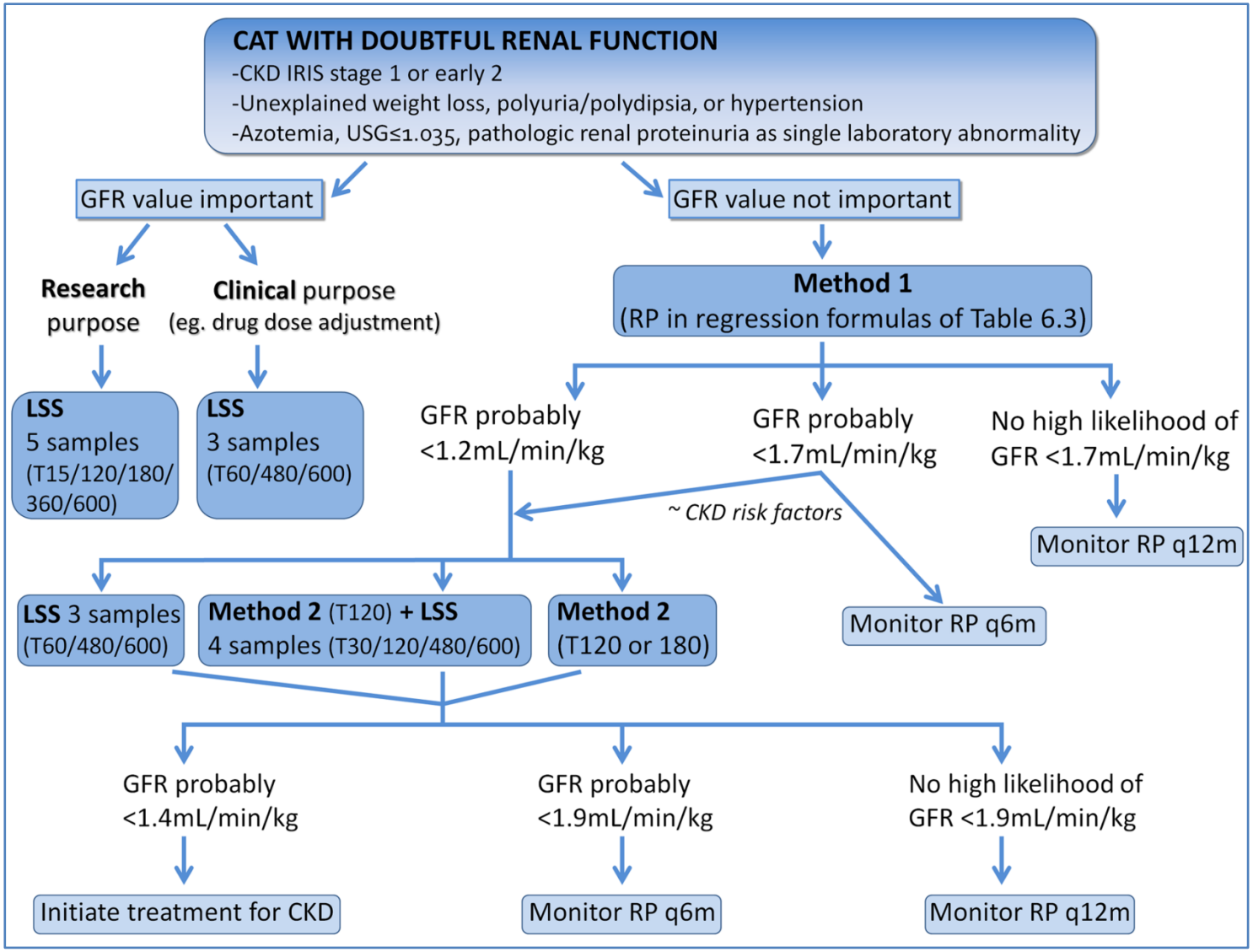


Fig 6.5. Tentative diagnosis algorithm to detect early kidney dysfunction

Tentative diagnosis algorithm to screen for renal dysfunction in cats that need further assessment of kidney function. This scheme is designed for creatinine clearance. For exo- and endo-iohexol clearances, the cut-offs for the glomerular filtration rate and time points for sampling for limited sampling strategies need to be adjusted.

(GFR = Glomerular filtration rate; CKD = Chronic kidney disease; IRIS = International Renal Interest Society; USG = Urine specific gravity; RP = Routine parameters, namely serum creatinine concentration, serum urea concentration, urine specific gravity and urine protein: creatinine ratio; LSS = Limited sampling strategy; T = Time point of blood sampling after clearance marker injection, in minutes)

End notes

[a]Vettest analyzer, Idexx Laboratories Europe BV, Amsterdam, the Netherlands

[b]WinNonlin Version 4.0.1, Scientific Consulting Inc Apex, NC, USA

[c]Systat 12, Systat Software Inc, San Jose, CA, USA

[d]Excel® 2007, Microsoft® Office, Washington, USA

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CHAPTER 7

GENERAL DISCUSSION

7.1 INTRODUCTION

Feline CKD is typically diagnosed based on the presence of combined renal azotemia and poorly concentrated urine (urine specific gravity (USG) ≤ 1.035), with compatible historical and/or physical examination findings (Grauer 1998, Bartges 2012). Unfortunately, diagnosing early feline CKD based on these criteria is challenging (Braun and Lefebvre 2008, Stockham and Scott 2008).

Usually cats do not suffer from clinical signs of CKD until they have reached advanced disease stages. The most common clinical symptoms, namely polyuria, polydipsia, weight loss, partial anorexia and lethargy usually develop in cats with *International Renal Interest Society* (IRIS) stages 2 and 3. Further, polyuria/polydipsia – one of the earliest signs of CKD – is often not recognized by cat owners (Bartlett *et al* 2010, Polzin 2010). More severe and more frequent clinical signs, including vomiting and weakness, are seen in cats with IRIS stage 4 (i.e. end-stage) CKD (Elliott and Barber 1998, King *et al* 2007, Polzin 2010).

It is generally assumed that over two-thirds of functional renal mass must be lost before kidneys lose their urine concentrating ability and over three-quarters must be lost before azotemia develops. Thus, serum creatinine, urea concentrations and USG are often within reference intervals (RIs) in cats with early CKD (IRIS stages 1 and early 2) (Braun and Lefebvre 2008, Stockham and Scott 2008, DiBartola 2010). The limitations of these routine diagnostic tests can be overcome by measuring glomerular filtration rate (GFR), usually by performing a multi-sample clearance test (Braun and Lefebvre 2008). However, multi-sample clearance tests are labor-intensive, time-consuming and may be stressful or painful for the patient (Finch *et al* 2013). Although some simplified techniques to estimate GFR had been described at the start of this thesis (Barthez *et al* 2000, Barthez *et al* 2001, Goy-Thollot *et al* 2006, Vandermeulen *et al* 2008, Heiene *et al* 2009), none of these simplified techniques had been sufficiently validated in cats with CKD to be routinely used in practice.

Because clinical signs often develop late in the disease course and early clinical signs are often not recognized by cat owners, screening of at-risk populations for CKD is highly recommended in companion animal medicine (FAB 2008, Vogt *et al* 2010, Taylor and Sparkes 2013). However, prior to this thesis, scientific data to interpret this screening were

limited. Additionally there was a need for convenient diagnostic methods to detect mild feline kidney dysfunction. These may facilitate the diagnosis of early CKD, allowing timely therapeutic intervention and possibly an improved prognosis (Lees 2004, Grauer 2005).

A more thorough summary of the diagnostic tests for cats with CKD and screening tests applicable to cats with increased risk for CKD can be found in **Chapter 1** of this thesis.

The final goal of this thesis (**Chapter 2**) was to evaluate limitations of currently performed screening practices for feline CKD and to develop possible solutions to improve detection of early CKD.

Therefore, the actually used screening procedures were evaluated in two cat populations, namely middle-aged to old cats and Ragdoll cats. Also, we investigated if there is a need to routinely screen cats with diabetes mellitus (DM) for CKD. Finally, simple and cost-effective methods were developed to improve detection of early feline CKD.

7.2 SCREENING FOR CHRONIC KIDNEY DISEASE IN AGED CATS

In **Chapter 3**, we assessed the results of health screening, as it is currently performed, in a population of aging cats. The rationale to perform 'geriatric health screening' is the increased sensitivity of old cats to many chronic diseases and the assumption that early diagnosis and timely treatment might improve the prognosis (FAB 2008, Vogt *et al* 2010). Chronic kidney disease is a major point of attention for feline geriatric health screening as the prevalence of CKD is increasing with age and reaches up to 30% in cats over 15 years of age (Lulich *et al* 1992). Guidelines established by panels of experts in the veterinary field recommend that geriatric health screening should consist of a combination of clinical and laboratory variables (FAB 2008, Pittari *et al* 2009, Vogt *et al* 2010). However, studies evaluating the results of these screening procedures were not available before this thesis. Thus, it was unknown if age-specific RIs for clinical (e.g. systolic blood pressure (SBP), Schirmer tear test) or laboratory variables were required for aging cats.

Therefore, general health screening was executed in 100 apparently healthy middle-aged and old cats by fulfilling a complete history, SBP measurement, physical examination, ophthalmic examination, complete blood count, serum biochemistry profile and urinalysis. Physical and laboratory abnormalities were frequent and several physical clinical and laboratory variables significantly differed between middle-aged (6 – 10 years) and old cats (> 10 years).

Focusing on variables that are important for screening for CKD, we identified several potential problems. Mainly the findings on blood pressure, body condition and laboratory abnormalities warrant further discussion.

Before the start of this study, the question remained whether normal blood pressure values in cats changed with aging because available reports yielded conflicting results (Bodey and Sansom 1998, Sparkes *et al* 1999, Sansom *et al* 2004, Lin *et al* 2006). The mean SBP of our population was 133.6 ± 21.5 mmHg, SBP exceeded 160 mmHg in 8% of our cats and old cats (> 10 years) had significantly higher SBP than middle-aged cats (6 – 10 years). No ocular lesions secondary to hypertension were detected in any of the cats. The mean SBP of our study corresponded well with two other reports that also evaluated healthy conscious client-owned cats using the indirect Doppler technique (Lin *et al* 2006, Paige *et al* 2009). In the

latter two studies, the age-distribution of the included healthy cats was much wider (less than 1 year to over 16 years) compared to our study. Sansom *et al* (2004) found that SBP increased with age if young (< 5 years), middle-aged (≥ 5 – < 10 years) and old (≥ 10 years) cats were compared, in contradiction to 2 other studies in which age did not correlate with SBP (Sparkes *et al* 1999, Lin *et al* 2006). Similar to Sansom *et al* (2004), we detected an age-effect on SBP as SBP was significantly higher in old cats (> 10 years) when compared to middle-aged (6 – 10 years) cats. This age effect of SBP likely is a consequence of cardiovascular changes that occur with aging (Carpenter *et al* 2005), but an increased occurrence of undiagnosed conditions leading to hypertension in the older cat groups cannot be ruled out. Particularly subclinical CKD may be difficult to diagnose without measuring the GFR because increased blood pressure may induce polyuria and associated low USG (< 1.030) due to pressure diuresis (Brown *et al* 2007). Despite the observed age-effect on SBP, the vast majority of old cats of our study and the study of Sansom *et al* (2004) had SBP below 160 mmHg. This means that only few healthy geriatric cats have SBP above the cut-off to pursue further diagnostic testing (Lin *et al* 2006, Stepien 2010, Stepien 2011).

An important finding was that cat owners did not recognize some physical examination abnormalities, demonstrated by the high number of cats with non-ideal body condition (51%) or with gingivitis (72%). Particularly in aging pets, owners may appreciate clinical problems as a normal aging phenomenon (FAB 2008). The fact that cat owners do not appreciate under- or overweight as a problem had been reported previously (Courcier *et al* 2010). Aging might affect the body condition because fat and protein digestion reduces with increasing age, potentially leading to age-related muscle wasting and decreased body weight (Laflamme 2005). Thus, it was unexpected to find such high proportion of overweight or obese cats (40%), similar or even worse to previous studies of healthy pet cats of various ages (Colliard *et al* 2009, Courcier *et al* 2010, Courcier *et al* 2012). However, our findings confirm that obesity is an important disease with growing incidence in pet cats, at it is in humans and pet dogs (Seidell 1999, Seidell 2000, WHO 2000, Corbee 2013). Overweight in dogs is related to overweight of their owners, but this is not true for cats (Nijland *et al* 2009). Recently, the common occurrence of overweight and obesity in purebred show cats was related to the standards of several cat breeds (Corbée 2014). Another potential reason why cat owners judge overweight as normal might be that cats are depicted as lazy fatty characters in popular cartoons or comics such as *Garfield* or *Dikkie Dik*. In one study, the probability of being obese peaked in the mature age group (7 – 10 years) (Courcier *et al* 2012). Also in our

study, middle-aged cats had significantly higher body condition score (BCS) compared to old cats.

On the other hand, and more important in regard to screening for CKD, 11% of our study cats were underweight. Most underweight cats (n = 9/11) were over 10 years of age, which is in accordance with recent findings that the risk of being underweight increases with each life stage and that cats over 15 years of age have the highest risk (Courcier *et al* 2012). Weight loss and poor body condition are early clinical signs in companion animals with CKD and our findings indicate that these might be overlooked by pet owners (Hughes *et al* 2002, Pittari *et al* 2009, Bartlett *et al* 2010, Polzin 2010). One of our underweight cats had confirmed CKD, but the lower USG in underweight cats compared to cats with normal or overweight body condition might indicate that more underweight cats had early renal dysfunction. Studies that investigate kidney function in detail – preferably by assessing GFR – are needed in non-azotemic underweight cats with poorly concentrated urine. Our study described in Chapter 3 is limited by not assessing the muscle condition score, as recommended by the *World Small Animal Veterinary Association* (WSAVA) Nutritional Assessment Guidelines (Freeman *et al* 2011). However, the muscle condition scoring system proposed by WSAVA was not available at the time of our study. It is important to realize that muscle wasting might occur in cats of any body condition, thus also in overweight or obese cats. As the daily production rate of creatinine depends on the muscle mass, muscle wasting due to aging or concurrent conditions might affect serum creatinine concentrations (Lees 2004, DiBartola 2010, Polzin 2010). Nevertheless, improved owner awareness of normal feline body condition, regular nutritional assessments (diet history, body weight, body and muscle condition score) by veterinarians and, if needed, nutritional support is mandatory to increase the proportion of aging cats with an optimal body condition (Hughes *et al* 2002, Freeman *et al* 2011, Freeman 2012).

As mentioned before, another clinical sign that feline owners do not always recognize as being abnormal is polyuria and polydipsia (Bartlett *et al* 2010). Inherent to the study design, none of our owners reported polyuria or polydipsia as a problem in their cat. However, 15% of the cats had poorly concentrated urine (USG < 1.035) and some of these might have had polyuria and/or polydipsia. Similarly, 13% of control cats in the study of Chapter 4 – Section §4.2 had poorly concentrated urine. Particularly in cats with outdoor access or in multi-cat households, the drinking and urination behavior might be difficult to judge for cat owners. As polyuria/polydipsia is one of the earliest clinical manifestations of

CKD and as patients with CKD show impaired urine concentrating ability prior to the inability to excrete metabolic waste, poorly concentrated urine might indicate early CKD (Lees 2004, Grauer 2005, Polzin 2010). However, it is important to keep in mind that many factors influence USG and daily USG fluctuations can be seen in healthy animals. So, low USG without other indications for CKD does not necessarily suggest kidney dysfunction (Lees 2004, Stockham and Scott 2008). However, USG consistently below 1.035 or USG below 1.035 found together with either azotemia or a physiologic state for which concentrated urine is expected (e.g. dehydration) may indicate impaired urine-concentrating ability and warrants further consideration (Lulich *et al* 1992, Lees 2004, Grauer 2005).

The fact that owners might overlook clinical signs of CKD is confirmed by the two cases with confirmed CKD in this study and the single case with CKD in the prospective study of Ragdoll cats (Chapter 4, Section §4.2). One of these cats already had more advanced CKD (IRIS stage 3). Despite being a chronic disease, cats with CKD are often presented with an acute history of illness or in advanced diseases stages (DiBartola *et al* 1987, Elliott and Barber 1998, Kuwahara *et al* 2006, Boyd *et al* 2008). This also indicates that their owners probably missed the more subtle clinical signs of early CKD. Veterinarians should educate their cat owners properly in order to recognize early and subtle signs of CKD. An improved owner awareness might lead to earlier diagnosis and intervention and potentially better long-term outcome (Hughes *et al* 2002, Bartlett *et al* 2010).

For several laboratory variables, a high proportion of our apparently healthy middle-aged and old cats had values outside the RI (e.g. 40% for serum phosphorus, 30% for total protein, 29% for serum creatinine). It is unlikely that all cats with one or more laboratory variables outside the RI had an occult pathologic condition explaining these abnormalities. Particularly the high number of cats with serum creatinine exceeding the upper reference limit and serum phosphorus below the lower reference limit are important to consider if cats are screened for CKD. For example, most of the cats with serum creatinine outside the RI only had a mild increase in combination with hypersthenuric urine, making early CKD in these cats less likely. Next to the high proportion of cats with laboratory variables outside the RI, we also found significant differences between middle-aged (6 – 10 years) and old (> 10 years) cats for several laboratory variables (serum urea, UPC, serum albumin, serum calcium, serum bilirubin, platelet count, hematocrit). This might be due to a higher prevalence of occult diseases in the older cat group, but an age-effect for several laboratory variables seems to be more plausible. If screening of cats for CKD is undertaken, it is important to keep in mind

that serum urea concentration and UPC are significantly higher in old cats (> 10 years) compared to middle-aged cats (6 – 10 years).

The high proportion of cats with laboratory variables outside the RI and the potential age-effect for certain laboratory variables, confirm the need for age-specific RIs to improve the interpretation of blood and urine examinations of aging cats (Gunn and Alleman 2005). In general, the reference population to establish the RI should reflect the animal population for which the RI is used (Archer 2010, Friedrichs 2010, Friedrichs *et al* 2012). This was not true for the RIs that were used in our study as they were determined in young (6 months – 1 year) healthy cats. Growth hormone increases renal tubular phosphate reabsorption in dogs, explaining increased serum phosphorus concentrations in young, growing animals (Corvilain and Abramow 1964). For the other variables, it is more difficult to clarify the need for age-specific laboratory RIs based on physiologic conditions. An important consequence of using inappropriate RIs is the misclassification of samples as normal or abnormal (Friedrichs 2010, Friedrichs *et al* 2012). A wide variation of laboratory RIs for serum creatinine leading to misclassification of samples had previously been reported, both in dogs and cats (Boozer *et al* 2002, Ulleberg *et al* 2011). Adjacent to the variation in RIs, the variation in assay methodology must be taken into consideration as well (Friedrichs *et al* 2012). Both (modified) Jaffe and enzymatic assays for serum creatinine are still frequently used in commercial laboratories, as discussed in Chapter 1, and one should employ RIs established based on the same assay (Le Garreres *et al* 2007). Misclassification of samples may lead to misdiagnosis and/or improper treatment (Friedrichs *et al* 2012). For example, hyperphosphatemia might be overlooked if the phosphate RI is based on healthy young cats. This is a serious flaw as hyperphosphatemia commonly complicates feline CKD and is considered to be an important therapeutic target and prognostic factor (King *et al* 2007, Boyd *et al* 2008, Chakrabarti *et al* 2012, Geddes *et al* 2013). Finally, technical or methodological factors must also be taken into consideration for comparing laboratory variables to generally accepted decision thresholds (Friedrichs *et al* 2012), as is done for UPC. Values of UPC can vary depending on the assay that is used (Fernandes *et al* 2005). In addition, other factors, such as storage conditions and sample dilution might lead to misclassification of UPC in dogs, mainly for UPC ratios close to the threshold limits 0.2 or 0.5 (Rossi *et al* 2012). These issues might be one reason for the high number of cats with borderline proteinuria in the studies of Chapter 3, Chapter 4 and Chapter 5, as discussed below (Section 7.4).

In conclusion, we encourage clinicians to consistently determine laboratory variables in a laboratory with good quality control and perform patient follow-up in a single laboratory (Lees 2004, Geffré *et al* 2009, Friedrichs *et al* 2012). Published guidelines to determine RIs for veterinary species should be followed (Geffré *et al* 2009, Friedrichs *et al* 2012) and veterinary laboratories should establish age-dependent RIs for certain laboratory variables. Partitioning the RIs for age will improve health screening of aged cats. However, a major challenge in the development of age-dependent RI is the selection of an appropriate reference population because older cats might suffer from subclinical diseases. To overcome the limitations of population-based RIs, subject-based RIs may be considered. These are referred to as reference change values or critical differences (i.e. a meaningful change between 2 successive measurements) and are mainly useful for serial laboratory measurements during patient follow-up (Walton 2012, Baral *et al* 2014). For many biochemical analytes, subject-based reference values are more sensitive than population-based reference values for detecting pathologic changes in an individual, both in humans and in dogs (Fraser 2004, Ruaux *et al* 2012). Whether or not subject-based RIs are more suitable to assess biochemical analytes is determined by the analyte's index of individuality which is based on biological (intra- and inter-individual) and analytical variation (Walton 2012, Baral *et al* 2014). Recent findings indicated that most feline plasma biochemistry variables have intermediate or high individuality (Baral *et al* 2014). For 5 variables with high individuality, amongst others plasma creatinine measured using an enzymatic assay, the use of subject-based reference change value or critical difference would be more appropriate than population based RI (Baral *et al* 2014). Calculation of reference change values is simple since laboratories should know the analytical variation and biological variation can be assessed based on a low number (e.g. as few as 8) of healthy reference individuals over a period of days to weeks (Fraser 2012, Walton 2012). To overcome calibration bias and measurement imprecision between and within laboratories, guidelines for standardization of creatinine measurements have been described in human medicine (Myers *et al* 2006). Similarly, standardization for measurement of urinary albumin in humans is under development (Miller *et al* 2009, Lieske *et al* 2013). Using subject-based reference values and standardization of laboratory tests in veterinary medicine will be important future steps to ameliorate the accuracy of laboratory measurements.

An important benefit of health screening is that it supports identifying conditions that remain unrecognized or that are regarded as normal aging consequences by cat owners (FAB 2008, Pittari *et al* 2009). In our study, health screening allowed us to diagnose feline immunodeficiency virus (FIV) infection in 14 cats, CKD in two cats and hyperthyroidism and urinary tract infection in one cat. In addition, health screening may serve as baseline for future physical and laboratory examinations (FAB 2008, Pittari *et al* 2009). For example, increasing serum creatinine concentrations, even within RI, may indicate early kidney dysfunction, particularly in cats with weight loss or muscle wasting or USG consistently below 1.035 (Lees 2004, Grauer 2005, Pittari *et al* 2009). By knowledge of reference change values, clinically meaningful changes will be more readily detected on regular laboratory assessments of mature and elderly patients (Ruaux et al 2012). The reference change value or critical difference for plasma creatinine measured with an enzymatic assay in healthy cats was determined to be 17.4%. In serial measurements in a feline patient, increases or decreases in plasma creatinine larger than 17.4% are clinically relevant, even if plasma creatinine remains within the RI (Baral *et al* 2014). Our study was not primarily designed to determine which tests should be part of health screening and at what age it should be initiated. However, based on our findings and current literature (FAB 2008, Vogt *et al* 2010), health screening of middle-aged cats should consist of a comprehensive history and thorough physical examination – including thyroid gland palpation, nutritional assessment and oral inspection – and finally FIV and feline leukemia virus (FeLV) testing for cats with outdoor access. For old cats (> 10 years), these tests should be added with blood pressure measurement and complete blood and urine examinations. Health screening is advised yearly in healthy senior cats (> 10 years) and twice yearly in healthy geriatric cats (> 15 years) and more frequently in cats with signs of illness (FAB 2008, Vogt *et al* 2010). Also, a more frequent screening of cats infected with feline immunodeficiency virus (FIV) for CKD seems to be warranted because of significant relationships between FIV and azotemia or CKD and because proteinuria is common in FIV-infected cats (Thomas *et al* 1993, Avila *et al* 2010, White *et al* 2010, Baxter *et al* 2012). As we detected significantly higher SBP in FIV-infected cats, the association between hypertension and FIV should be investigated further.

7.3 SCREENING FOR CHRONIC KIDNEY DISEASE IN RAGDOLL CATS

In **Chapter 4** we evaluated the screening of Ragdoll cats for polycystic kidney disease (PKD) and chronic interstitial nephritis (CIN). For many years, this screening was performed at our institution based on recommendations of Ragdoll breeder organizations. According to these organizations, screening should include measuring serum creatinine and urea concentrations, ultrasonography of liver and kidneys and genetic testing for PKD (SRC 2012, RCB 2013). As mentioned in the general introduction of this thesis (Chapter 1), we already identified a problem prior to the start of our studies. The definitive diagnosis of CIN can only be made by kidney histology because pathognomonic ultrasonographic features for CIN do not exist (Grooters and Biller 1995, d'Anjou 2008, DiBartola 2010). Thus, using the screening tests that are recommended by Ragdoll breeder organizations, we could only evaluate Ragdoll cats for CKD and not for CIN.

At first, we performed a retrospective evaluation of Ragdoll cats presented at our institution over an 8 year period (Section §4.1). Renal ultrasonography of 244 healthy Ragdoll cats revealed a PKD prevalence of 2.9%, a suspicion for CKD in 8.6%, unilateral renal agenesis or severe hypoplasia in 2 cats (0.1%) and abnormalities with unclear significance in 8 cats (3.3%). All genetically tested cats (n = 125) were homozygous for the wild-type PKD-1 alleles indicating a PKD negative state. Almost 11% of the Ragdoll cats for which serum creatinine was measured (n = 141) had serum creatinine concentration exceeding the RI, which raised the question whether a breed-specific creatinine RI for Ragdoll cats would be appropriate.

Because our retrospective study was limited by the lack of urinalysis, incomplete screening tests in many Ragdoll cats and lack of information on the prevalence of renal ultrasonographic abnormalities in healthy non-Ragdoll cats, a prospective study was the next step. A prospective evaluation (Section §4.2) of serum creatinine and urea concentrations, routine urinalysis and abdominal ultrasonography in healthy Ragdoll and age-matched non-Ragdoll cats was subsequently performed. Renal ultrasonographic abnormalities were frequent, in Ragdoll cats (49.6%) as well as in control cats (40%). Approaching significance, CKD was ultrasonographically suspected in 5.3% of Ragdoll cats but in none of the control cats. Ragdoll cats showed significantly more frequent segmental cortical lesions, abnormal renal capsule and echogenic urine and significantly lower serum urea concentration and

higher USG compared to the control cats. The other ultrasonographic and laboratory parameters did not significantly differ between both groups. Blood and urine examinations confirmed CKD in only 1 of the Ragdoll cats and all Ragdoll cats were PKD negative based on genetic testing.

Both studies indicate that PKD is uncommon in the breeding population of healthy juvenile Ragdoll cats and that CKD is ultrasonographically suspected in 5 to almost 10% of the same population. However, these disease prevalences are only estimates of the true prevalence as they are affected by the characteristics of the studied population (Hahn and Overley 2010). Nevertheless, the majority (n = 5) of the PKD-positive cats were presented in 2001 – 2002 and almost no (n = 2) PKD positive cats were seen at our faculty since then. This may indicate that PKD screening prior to breeding is effective at eradicating PKD in this breed. In both studies, predominantly young Ragdoll cats were included, indicated by the mean age of 2.2 years in the retrospective study and 2.7 years in the prospective study. Taking this into account, in addition to the fact that none of the control cats was ultrasonographically suspected of having CKD, the 5 – 9% Ragdoll cats with ultrasonographic suspicion of CKD is a rather large proportion. It is important to keep in mind that it is unknown whether the cats that were suspected of having CKD on ultrasonography will progress to azotemic CKD because ultrasonography does not correlate with renal function (Grooters and Biller 1995). The prevalence of ultrasonographic abnormalities might be underestimated in our retrospective study because no standardized protocol was used for kidney ultrasonography and many ultrasound reports were incomplete. On the other hand, the ultrasound report containing the most complete information regarding the kidneys was retained for inclusion if a cat underwent multiple ultrasonographic examinations. This might have created a bias towards an increased prevalence of abnormal ultrasonography because – if no standardized protocol is used – it is more likely that the radiologist gave detailed information if abnormalities are noticed. In comparison, a recent retrospective study described that ultrasonographic findings compatible with CKD are not uncommon in juvenile Maine Coons, with a prevalence of 5.3%. None of these CKD suspected cats underwent blood or urine testing. It was unclear if these authors used a standard ultrasound protocol and how they defined CKD. The most common findings in cats with ultrasonographic findings compatible with CKD were reduced corticomedullary distinction (60%), irregular kidney contours in addition to other changes compatible with CKD (60%) and mineral sand or reflectors in the renal pelvis (50%) (Gendron *et al* 2013). In our prospective study, all but one Ragdoll cats

suspected of CKD had 6 renal ultrasonographic abnormalities, indicating that our ultrasonographers were stringent in their diagnosis of CKD suspicion. The presence of single renal abnormalities (e.g. reduced corticomedullary demarcation, small kidney size, dystrophic mineralization) was not sufficient for a suspicion of CKD. Although the prospective study design did overcome many limitations of the retrospective study by including urinalysis and using a standard ultrasound protocol, the true prevalence of CKD in Ragdoll cats still remains unknown. Most Ragdoll cats of the prospective study belonged to breeders that had screened their cats over several generations, possibly underestimating the true disease prevalence. A similar influence on disease prevalence might be caused by exclusion of cats with an already diagnosed CKD because we did not only include Ragdoll cats that underwent screening for the first time in their lifetime. Both in Belgium and the Netherlands, many Ragdoll breeders screen their cats yearly prior to breeding and usually only cats without a suspicion of CKD on previous screenings are retained for future screenings. However, the exclusion of cats with an already diagnosed CKD was necessary to avoid bias towards CKD.

A large difference in proportion of Ragdoll cats with serum creatinine concentration exceeding the upper limit of the RI was noticed for both studies, namely almost 11% in the retrospective study versus 1% in the prospective study. The question whether the increased creatinine concentration could be a breed-specific feature for Ragdoll cats, as has been reported for Birmans (Gunn-Moore *et al* 2002, Reynolds *et al* 2010, Paltrineiri *et al* 2014), was rejected by the prospective study. Indeed, in the latter study, the serum creatinine concentration did not differ between Ragdoll and control cats, suggesting that a breed-specific serum creatinine RI is not required for Ragdoll cats. Possible reasons for the large discrepancy between both studies in the proportion of Ragdoll cats with increased serum creatinine concentration might be the assay used, variable RI, or more cats with kidney dysfunction in the retrospective study. Serum creatinine concentrations were measured with a modified Jaffe assay in the retrospective study and with an enzymatic assay in the prospective study. Both methods correlate well with a reference method, but the Jaffe assay may overestimate feline serum creatinine at low concentrations by also measuring noncreatinine chromogens (Le Garreres *et al* 2007, DiBartola 2010). However, this probably does not explain the high proportion of Ragdoll cats with serum creatinine exceeding the RI because the same error will occur when measuring creatinine in the reference population. How many cats of both studies had kidney dysfunction is unknown because serum urea and creatinine concentrations do not allow detection of early kidney dysfunction and GFR was not measured (DiBartola 2010).

The retrospective study was additionally limited by the lack of urinalysis, making interpretation of serum creatinine concentration difficult (DiBartola 2010). However, as most cats of the retrospective study with increased serum creatinine only had a mildly increased concentration and normal serum urea concentration, it is unlikely that many of these Ragdoll cats had kidney dysfunction. Thus, the remaining and most feasible explanation is an inappropriate RI resulting in misclassification of samples as abnormal in the retrospective study. The importance of appropriate RIs to prevent incorrect sample classification as normal or abnormal (Boozer *et al* 2002, Archer 2010, Friedrichs 2010, Ulleberg *et al* 2011, Friedrichs *et al* 2012) has been discussed earlier in this chapter (Section 7.2).

A notable finding of the prospective study was the high number of renal ultrasonographic abnormalities in young healthy cats, both in Ragdoll as in non-Ragdoll cats. In the retrospective study substantially fewer ultrasonographic abnormalities were observed, most likely due to incomplete recording. In that study, abnormalities were probably only noted if the radiologist presumed they could be clinical relevant. Although abdominal ultrasonography is performed daily in companion animal practice, scientific information on feline kidney ultrasonography was scarce prior to our studies. Knowledge on ultrasonographic appearance of kidneys of healthy cats was largely based on studies of the late eighties (Walter *et al* 1987a, Walter *et al* 1987b, Yeager and Anderson 1989). The substantial progress of ultrasound devices and expertise since then, emphasizes the need for updated scientific information. Although our prospective study was not primarily intended to serve this goal, we were able to describe the frequency and type of ultrasonographic renal findings in juvenile healthy cats. In one study based on a very limited number of cats (n = 10), the renal length of healthy cats has been reported to be 3.8 to 4.4 cm (Walter *et al* 1987a). However, other authors prefer normal values of 3.2 to 4.2 cm (Widmer *et al* 2004) or between 3.0 and 4.3 cm (d'Anjou 2008). To our knowledge, our study was the first to report renal length in a large group of healthy cats. The size of the left and right kidney of the control cats was comparable, with mean values of 3.7 ± 0.3 cm and 3.8 ± 0.4 cm respectively. Only three (5%) of our control cats had a kidney smaller than 3.2 cm with a minimum size of 3 cm. One or both kidneys were larger than 4.3 cm in more than 15% of our control cats, but only five (8%) of our control cats had one or both kidneys larger than 4.4 cm with a maximum size of 4.5 cm. Thus, cut-offs of 3.2 cm and 4.4 cm seem to be appropriate to evaluate renal length in healthy cats. Recently, in a study from our institution, no significant differences were found for renal length, cortical thickness, medullary thickness and corticomedullary ratio of healthy cats of

three cat breeds (Debruyn *et al* 2013a). Within the Ragdoll breed, renal length showed a positive correlation with age, bodyweight and male gender, indicating that it might be useful to keep age, bodyweight and gender in mind when evaluating renal length on ultrasonography (Debruyn *et al* 2013b). Renal ultrasonographic abnormalities that were frequently seen in both groups of cats were the presence of a medullary rim sign and changes in cortical echogenicity, particularly hyperechoic renal cortices. Medullary rim signs have been described in healthy and renal-diseased cats, but the question remained whether it could be an early indicator of renal disease (Yeager and Anderson 1998, Biller *et al* 1992, Widmer *et al* 2004, d'Anjou 2008). In our study, a rim sign was identified in approximately one fifth of healthy young non-azotemic Ragdoll and control cats. Because it is unlikely that all these cats will develop CKD in the near future, our study suggests that medullary rim signs as sole abnormality are unlikely to be relevant, as was reported for dogs (Mantis and Lamb 2000). The cortical hyperechogenicity, noticed in approximately 10% of our healthy Ragdoll and control cats, is probably explicable by presence of fat in the proximal tubular epithelial cells (Yeager and Anderson 1989). For other single abnormalities (such as changes in renal capsule, renal shape, unilateral small kidney), the clinical relevance remains uncertain. In the study of Gendron *et al* (2013) four out of nine Maine Coons with irregular renal form without changes in renal echogenicity and available follow-up had died or been euthanized because of CKD at less than six years of age. Thus, longitudinal studies in order to monitor kidney function and renal ultrasonography in cats with ultrasonographic changes of unknown significance are needed to reveal the significance of these changes. Our finding that renal ultrasonographic abnormalities often occur in healthy cats will ameliorate the awareness of clinicians that ultrasonography is not sufficient to diagnose CKD. Consequently, current data do not support feeding a renal diet solely based on ultrasonographic findings. However, the ultrasonographic appearance of kidneys in healthy and diseased cats warrants further studies. Contrast-enhanced ultrasonography might be a valuable additional test for the evaluation of renal diseases in cats, as it is in humans. The pattern of contrast enhancement in normal feline kidneys has been described, but the diagnostic value of contrast-enhanced ultrasonography in ill cats still needs to be studied (Kinns *et al* 2010).

The most important renal ultrasonographic parameter that significantly differed between Ragdoll and age-matched non-Ragdoll cats in the prospective study was the presence of segmental cortical lesion(s) (SCLs). Further studies will need to reveal whether these represent renal infarction or renal scarring due to vesico-ureteral reflux and what is the

underlying cause (Domanovits *et al* 1999, Cargollo and Diamond 2007, Kolbjørnsen *et al* 2008). In humans, the most common causes or risk factors for kidney infarcts are embolization, hypertension, cardiovascular disease, renal trauma, neoplasia, renal artery dissection, renal vein thrombosis, hypercoagulable states, hyperviscosity syndromes, vasculitis or DM (Suzer *et al* 2002, Tsai *et al* 2007, Javaid *et al* 2009). Idiopathic renal infarcts have also been described (Racusin and Pollack 2005). There was no history of trauma or illness in any of the Ragdoll cats with SCLs. The young age, the healthy clinical condition and normal routine blood and urine examinations in our Ragdoll cats excluded DM and made neoplasia unlikely. Cardiac disease was unlikely because echocardiography for pre-breeding screening for hypertrophic cardiomyopathy did not show significant abnormalities in any of these cats. The blood pressure and coagulation profile warrants further investigation in cats with SCLs. Reflux nephropathy in humans is mostly associated with primary vesico-ureteral reflux (Cargollo and Diamond 2007) and it is unknown whether this occurs in Ragdoll cats with SCLs.

In the retrospective study, only 1.6% of Ragdoll cats had SCLs versus 7.5% in the prospective study. As discussed earlier, it is possible that not all SCLs were recorded in the retrospective study, but a growing prevalence must also be considered. In the recent report on renal ultrasonography of healthy juvenile Maine Coons, no infarct-like lesions were described, also not in cats with an irregular shape of the kidney (Gendron *et al* 2013). This might be a further indication that SCLs typically occur in Ragdoll cats, but further studies are required to confirm this.

The suspicion of CKD on ultrasonography in 5 – 9% of Ragdoll cats versus none of the control cats and the predisposition of Ragdoll cats for SCLs indicates that a predisposition of Ragdoll cats for CKD cannot be excluded. Therefore, there seems to be a rationale to continue pre-breeding screening of Ragdoll cats for CKD. However, further research is needed to define what is the optimal screening protocol (i.e. which screening tests and at what age), to elucidate the genetic basis of SCLs or suspicion of CKD on ultrasonography and to determine the clinical relevance of SCLs and whether these represent renal infarction or cortical scarring. Until more data are available, urinalysis should be added to the currently applied screening protocol and a standard protocol (see Addendum of Chapter 4, Section §4.2) should be followed for the abdominal ultrasonography. Based on our studies, it seems wise to avoid breeding with Ragdoll cats with SCLs and with an ultrasonographic suspicion of CKD.

Because PKD is rare in Ragdoll cats and most Ragdoll breeders already screen their cats for PKD over several generations, routine screening for PKD does not appear to be mandatory. Screening of Ragdoll breeding cats for PKD is advised if there is no or dubious information regarding the PKD status of the parents or if cysts are detected on ultrasonography. Since it is known that a small number of ultrasonographically PKD positive cats are homozygous for the wild-type PKD-1 allele, PKD testing should be established on a combination of ultrasonography and genetic testing (Bonazzi *et al* 2009). To avoid false negative results, cats should be at least 10 months to undergo the ultrasonography (Barrs *et al* 2001, Cannon *et al* 2001).

7.4 EVALUATION OF DIABETIC CATS FOR 'DIABETIC KIDNEY DISEASE'

In **Chapter 5** we assessed whether cats with DM show evidence for diabetic kidney disease (DKD) or diabetic nephropathy (DN) which is defined as structural and/or functional abnormalities of the kidney as complication of DM (Bloom and Rand 2013). Diabetic kidney disease is a common complication in human diabetic patients and is characterized both by glomerular changes leading to altered GFR and proteinuria and by tubular damage (Reutens 2013, Ritz 2013). Prior to this study, data on influence of feline DM on kidney function were scarce, especially data on GFR and urinary biomarkers were lacking.

Therefore, a prospective study was performed to evaluate routine kidney variables (serum urea, serum creatinine, USG, UPC), SBP, GFR and urinary Cystatin C (uCysC) as tubular marker in cats with DM, cats with CKD and healthy cats that were age-matched to the diabetic cats. The same variables were compared between recently diagnosed diabetic cats (i.e. diagnosed less than 1 month ago) and cats that were not-recently diagnosed with DM (i.e. 1 month or longer). Cats with DM only had significantly lower USG compared to healthy cats. In contrast, most variables significantly differed between cats with DM and cats with CKD: diabetic cats had significantly lower serum urea concentration, serum creatinine concentration and urinary Cystatin C: creatinine ratio (uCysC/uCreat) and significantly higher USG and GFR. In the diabetic group, 38% had proteinuria (UPC > 0.4) versus 30% in cats with CKD and none of the healthy cats. Despite this, UPC did not significantly differ between these groups. Also SBP did not differ between the 3 groups of cats. None of the variables evaluating kidney function or kidney damage differed significantly between recently and not-recently diagnosed diabetic cats.

Thus, based on a single evaluation of routine kidney variables, GFR and uCysC, a major impact of feline DM on kidney function could not be demonstrated. Nevertheless, the high prevalence of proteinuria in the diabetic group might be important. An increased prevalence of proteinuria in diabetic cats had already been described by Al-Ghazlat *et al* (2011). Low-level proteinuria (UPC 0.4 – 1) is a negative prognostic factor for azotemic cats (Kuwahara *et al* 2006, Syme *et al* 2006, King *et al* 2007) and for hypertensive cats (Jepson *et al* 2007). In addition, proteinuria is a marker for progression of feline CKD (Chakrabarti *et al* 2012). However, the significance in nonazotemic normotensive cats is not yet known. In hyperthyroid cats, proteinuria is not a mediator of progression of CKD, but correlates with all-

cause mortality (Williams *et al* 2010) and the same may be true for diabetic cats. Follow-up studies of diabetic cats with and without proteinuria will need to reveal if it is a negative prognostic factor and whether proteinuric diabetic cats will progress to more severe renal dysfunction.

Several reasons might explain why cats are not as sensitive as humans to develop DKD. The shorter duration of DM in cats might be a major issue. In human patients with type 2 DM, progression to overt nephropathy usually takes over 10 years and progression to end-stage renal disease over 30 years (Ayodele *et al* 2004). In our study population only 6 of 36 diabetic cats were treated for longer than 1.5 years with a maximum of over 5 years. Genetic predisposition, which is an initiator of DKD in humans (MacIsaac and Watts 2005), might be less important in cats, but studies are currently lacking. Several promoters of human DKD, such as hypertension and dyslipidemia, are less common in cats and others, such as smoking, are not relevant for cats (MacIsaac and Watts 2005). Although hypertension is a frequent complication in human diabetic patients (Van Buren and Toto 2013), this does not appear to be true in cats. In our study only 10% of diabetic cats had hypertension, which is comparable to 15% found by Al-Ghazlat *et al* (2011). Also, primary hypertension is a major problem in human medicine but not in feline medicine (Reusch *et al* 2010). Around 18 – 20% of hypertensive cats are reported to have primary hypertension (Maggio *et al* 2000, Elliott *et al* 2001), but subclinical CKD and primary hyperaldosteronism may have been overlooked as underlying causes for hypertension in these studies (Reusch *et al* 2010). Dyslipidemia in humans is associated with atherosclerosis and microvascular dysfunction leading to renal and cardiovascular disease (MacIsaac and Watts 2005, Falk *et al* 2013, Moody *et al* 2013). Hypercholesterolemia has been described in diabetic cats (Crenshaw and Peterson 1996, Nelson *et al* 1999) and obese cats have dyslipidemia, characterized by abnormalities in lipoprotein particle number and size (Jordan *et al* 2008). In addition, atherosclerosis can be induced in cats by feeding a high-fat, cholesterol enriched diet (Ginzinger *et al* 1997). However, spontaneous cases of cats with atherosclerosis have not been described and atherosclerosis is not a feature of feline obesity or diabetes, which contrasts markedly with the situation in humans. The lack of an apparent systemic inflammatory reaction in response to increased fat mass in cats might explain why atherosclerosis and cardiovascular problems do not occur in obese cats (Hoenig *et al* 2013).

Based on our findings and data in literature, there is currently no rationale for more intense screening of cats with DM for kidney damage or dysfunction in comparison to non-diabetic cats of similar age. For diabetic cats of 6 years or older, guidelines of middle-aged or old non-diabetic cats can be followed as mentioned in Chapter 3 and Section 7.2. For younger diabetic cats, it is advised to act upon guidelines as proposed by the *Feline Advisory Bureau* (FAB 2008) or the *American Association of Feline Practitioners* and *American Animal Hospital Association* (Vogt *et al* 2010). It might be prudent to assess UPC in all diabetic cats and monitor UPC in diabetic cats with proteinuria. This will not cause specific harm to diabetic cats as regular urine bacterial culture is advised in cats with DM, because of their predisposition for urinary tract infection (Bailiff *et al* 2006, Mayer-Roenne *et al* 2007).

7.5 JOINT FINDINGS IN DIFFERENT POPULATIONS OF HEALTHY CATS

A remarkable finding in the studies described in **Chapter 3**, **Chapter 4** and **Chapter 5** is the high prevalence of borderline proteinuria in healthy cats. A UPC between 0.2 and 0.4 was detected in 25% of middle-aged and old cats (Chapter 3), in 27.9% of Ragdolls and in 22.6% of control cats of Chapter 4 and in 40% of the healthy cats of Chapter 5. To evaluate if proteinuria is of renal origin, urine should be taken by cystocentesis, urine bacterial culture should be performed, urinary sediment should be analyzed and prerenal proteinuria must be ruled out by evaluating serum/plasma protein concentration (Lees *et al* 2005). This was achieved in all three studies. Urine bacterial culture was positive only in a minority of our cats (approximately 1%). Urinary sediment analysis revealed crystalluria in many cats, but was mostly mild, and evidence of inflammation or microscopic hematuria was uncommonly detected. Also, routine blood examination did not show abnormalities in the serum proteins. This indicates that borderline proteinuria was of renal origin in most cats. In case of proteinuria of renal origin it is important to evaluate persistence, preferably with 3 samples over a period of at least 2 weeks. If persistent borderline proteinuria is noted, re-evaluation and re-classification after 2 months is recommended (Lees *et al* 2005, IRIS 2009). These recommendations were not accomplished in our studies as our cats were only evaluated at a single time point, therefore, the number of cats with persistent borderline proteinuria is unknown. Because of the high number of cats with borderline proteinuria, re-evaluation would have been very impractical and expensive, which raises the question if these recommendations are practically feasible.

A more important question is whether borderline proteinuria in healthy cats is clinically relevant. In azotemic cats, it is accepted that low-level proteinuria is a negative prognostic factor (Kuwahara *et al* 2006, Syme *et al* 2006, King *et al* 2007), but data in non-azotemic cats are scarce. In one study, that was only published in the format of an abstract, the median UPC for apparently healthy cats that died due to any cause was 0.3, whereas median UPC in cats that were still alive at the end of the study was 0.11, indicating that borderline or mild proteinuria could be of prognostic significance (Walker *et al* 2004). As borderline proteinuria might be a sensitive indicator of renal or extrarenal diseases (Segev 2010), the significantly higher UPC in old versus middle-aged cats (Chapter 3), might be explained by the fact that older cats are more prone to early CKD or occult systemic disease.

However, it is unlikely that early CKD was the cause of borderline proteinuria in approximately ¼ of juvenile healthy cats (Chapter 4). As mentioned above (Section 7.2), it is possible that at least some of our cats actually did not have borderline proteinuria, but were incorrectly classified as such. Misclassification might be a consequence of the assay used (Fernandes *et al* 2005) or due to inappropriate storage or dilution of urine samples (Rossi *et al* 2012). Assessing borderline proteinuric cats for microalbuminuria could have been meaningful to decide whether renal damage was indeed present (Grauer 2007), but this was not done in our studies. Further research is needed to reveal the clinical relevance of borderline proteinuria in healthy cats of different ages. Hopefully, these future studies will result in precise and practical recommendations for the assessment and management of borderline proteinuria in non-azotemic cats.

Another mutual finding in the healthy cats investigated in **Chapter 3** and **Chapter 4** was the frequent crystalluria, namely in 41% of the middle-aged to old cats (Chapter 3) and in 50% of both cat groups of Chapter 4. Because we performed the urinary sediment analysis within 30 minutes of urine collection, in vitro crystal formation due to storage is unlikely (Albasan *et al* 2003). Although it is generally accepted that crystals are commonly present in feline urine (DiBartola 2010), we are not aware of scientific studies on the frequency of crystalluria in client-owned healthy cats prior to this thesis. Most of our cats had small amounts of amorphous crystals. Uroliths could not be ruled out in Chapter 3 because medical imaging studies were not performed in these cats, but were detected with ultrasonography only in approximately 2% of Ragdolls and control cats of Chapter 4. Almost all our cats with crystalluria had inactive urinary sediment and none had a history of lower urinary tract signs, indicating that these crystals usually do not cause discomfort to these cats. This indicates that amorphous crystals in small quantities do not necessarily explain clinical signs in cats that are presented with lower urinary tract signs. Also, the finding of crystalluria is not sufficient to recommend a calculolytic diet.

7.6 SIMPLIFIED METHODS TO DIAGNOSE EARLY KIDNEY DYSFUNCTION

Finally, in **Chapter 6** we aimed to develop simple methods to identify cats with early kidney dysfunction, because routine blood and urine variables can be within RIs in cats with early CKD (Braun and Lefebvre 2008, Stockham and Scott 2008, DiBartola 2010). These methods might be valuable mainly to screen feline populations at risk for CKD. In certain situations, such as for dosage adjustment of nephrotoxic drugs or in research situations, knowledge of the exact GFR value of the cat might be important (Lees 2004, Polzin 2010). Because multi-sample techniques for GFR estimation are too labor-intensive, time-consuming and stressful for use in practice (Finch *et al* 2013), there was a need for reliable, simple and cost-effective methods to estimate feline GFR. Conversely, identifying cats with decreased GFR is more important than knowledge of the exact GFR value in daily practice. Hence we also aimed to develop simple methods to identify cats with GFR below certain thresholds.

Data of a 9-sample combined plasma exogenous creatinine-iohexol clearance test of 73 cats were used. Regression formulae based on routine variables (serum urea and creatinine concentrations, USG, UPC) could not reliably estimate the actual GFR value, but were trustworthy to identify cats with GFR below low or borderline thresholds. For reliable GFR estimation, at least 3 blood samples for clinical purpose and 5 blood samples for research purpose are required and optimal timing for these samples was determined. Finally, cut-off marker concentrations at three time points after marker injection were defined and these were able to identify cats with GFR below low and borderline thresholds with high sensitivities, specificities, positive and negative predictive values.

Their relationship with GFR confirmed that routine variables might be within RIs in cats with low GFR (Fig 6.1 and 6.2). Several cats with low GFR had normal serum creatinine or urea concentration and USG $\geq$ 1.035. The fact that some cats with CKD retained their urine concentrating ability had already been described in cats with experimental loss of renal functional mass (Ross and Finco 1981). On the other hand, poorly concentrated urine or serum creatinine or urea concentrations exceeding the RI were seen in some cats with normal GFR. This is in line with the general opinion that USG below 1.035 – without other indications for CKD – does not necessarily suggest kidney dysfunction (Lees 2004, Stockham and Scott 2008). As discussed above (Section 7.2), poorly concentrated urine was found in 15% of cats of Chapter 3 and in 3% of Ragdolls and 13% of control cats of Chapter 4. It is

unlikely that all these cats had early CKD. The same is true for cats of Chapter 3 and Chapter 4 with increased serum creatinine and urea concentrations.

Prior to this thesis, reported limited sampling strategies (LSS) in cats were insufficiently validated to be used in practice (Barthez *et al* 2000, Barthez *et al* 2001, Goy-Thollot *et al* 2006, Vandermeulen *et al* 2008, Heiene *et al* 2009). Since the study of Chapter 6 was finalized, other reports describing LSS in cats were published (Miyagawa *et al* 2010, Vandermeulen *et al* 2010, Finch *et al* 2011, Katayama *et al* 2012, Finch *et al* 2013, Katayama *et al* 2013). As mentioned in Chapter 6, many of these studies are predominantly limited by including no or only few renal-impaired cats (Barthez *et al* 2000, Barthez *et al* 2001, Goy-Thollot *et al* 2006, Vandermeulen *et al* 2008, Heiene *et al* 2009, Vandermeulen *et al* 2010, Finch *et al* 2011, Katayama *et al* 2012, Katayama *et al* 2013). Other limitations are that the reference GFR, which is used to design LSS, is often computed using a clearance technique with less than 5 blood samples (Heiene *et al* 2009, Katayama *et al* 2012, Katayama *et al* 2013) or total duration of 5 or less hours (Goy-Thollot *et al* 2006, Vandermeulen *et al* 2008, Heiene *et al* 2009, Miyagawa *et al* 2010, Vandermeulen *et al* 2010, Katayama *et al* 2012, Katayama *et al* 2013).

Some authors report that feline GFR can be reliably estimated based on 1 or 2 blood samples (Barthez *et al* 2001, Goy-Thollot *et al* 2006, Vandermeulen *et al* 2008, Heiene *et al* 2009, Miyagawa *et al* 2010, Vandermeulen *et al* 2010, Katayama *et al* 2012, Finch *et al* 2013, Katayama *et al* 2013) which contrasts markedly with our finding that for clinic situations at least 3 blood samples and for research purpose at least 5 blood samples after injection of the clearance marker are required to estimate GFR with an acceptable margin of error. This might be explained by different methodologies to estimate the reference GFR and to define LSS based on this reference. Other authors calculated the reference GFR using a one- or two-compartmental model with or without a human- or dog-based correction formula to adjust for errors caused by the chosen model (Barthez *et al* 2001, Goy-Thollot *et al* 2006, Vandermeulen *et al* 2008, Heiene *et al* 2009, Miyagawa *et al* 2010, Vandermeulen *et al* 2010, Katayama *et al* 2012, Katayama *et al* 2013). However, because different pharmacokinetic approaches to estimate GFR are scarcely compared in cats and because human- or dog-specific correction formulae are not properly evaluated for use in cats, the reliability of these reference GFR values is difficult to judge. In contrast, we compared LSS with a GFR estimate that was calculated using a noncompartmental approach, which is assumption free and thus avoids these downsides (Heiene and Moe 1998, Von Hendy-Willson and Pressler 2011). One

group recently developed a 3-sample iohexol clearance method using the slope-intercept method and a feline correction formula (Finch *et al* 2011). This was followed by development of a single-sample method starting from their 3-sample method, using a modified Jacobsson technique (Finch *et al* 2013). As a first step, a 3-sample clearance method was created in 20 healthy cats using a one-compartmental model and compared to a multisampling method with blood samples taken over 6 hours after marker injection and using a two-compartmental model to calculate the reference GFR. These reference GFR values were shown to be in excellent agreement with GFR values reached by noncompartmental analysis of the same multisampling method (Finch *et al* 2011). A limitation was that these authors only used healthy cats to compare their two-compartmental model to noncompartmental analysis and the question remains whether the same level of agreement would have been reached in renal-impaired cats. For accurate GFR estimation using a noncompartmental approach, the extrapolated area of the area under the plasma concentration-versus-time curve (AUC) should never exceed 20% of the total area (Watson *et al* 2002). Six hours after injection, most of the marker will probably be excreted by the kidneys in healthy cats, but not in renal-impaired cats. This was clearly shown in our study, as some of our cats with CKD had an extrapolated area of the AUC larger than 20% for exo-iohexol clearance, even though we sampled until 10 hours after marker injection. Results were even more striking for creatinine clearance as the extrapolated area of the AUC exceeded 20% in most of our cats with CKD. The need for more prolonged sampling, between 10 and 24 hours after creatinine injection, for patients with renal dysfunction has previously been shown in dogs (Watson *et al* 2002). In a second step, a single-sample method was created using a group of nonazotemic (n = 73) and azotemic (n = 16) cats and compared to the previously developed 3-sample method (Finch *et al* 2013). To do so, the authors used a modification of the Jacobsson method that was originally designed for human patients. Other authors also used the Jacobsson formula to create single-sample methods to estimate feline GFR (Katayama *et al* 2012, Katayama *et al* 2013). This Jacobsson method is based on a one-compartmental model and assumes immediate and uniform admixture of the clearance marker in the distribution volume after injection. Also, the timing of the blood sample should be based on renal function and distribution volumes (Jacobsson 1983). In the studies of Finch *et al* (2011, 2013) the time points of the blood samples for the 3-sample clearance were randomly chosen and not based on clearance values or distribution volumes. Whether the other assumptions inherent to the Jacobsson method are applicable to cats is currently unknown. In addition, the Jacobsson method requires multiple calculations which hampers its use in daily practice.

All these limitations indicate that there are many uncertainties to the published LSS. Although we did not primarily intend to compare our LSS with published LSS, we can summarize the advantages of our methodology as follows: our reference GFR was based on multiple blood samples over a 10 hour period, both in the distribution and elimination phases of the clearance marker, and computed using an assumption free noncompartmental approach. Additionally, LSS were designed for 3 different clearance markers in a cat population with wide range of GFR. So, based on current knowledge on feline GFR, our LSS are as reliable as possible.

An important further step in veterinary medicine is to evaluate whether GFR can be reliably estimated based on serum creatinine and demographic variables. In humans, GFR is estimated from prediction equations that take into account the serum creatinine concentration and some or all of the following variables: age, gender, race, and body size. These equations are more reliable than estimates of GFR from measurement of serum creatinine alone, mainly because they compensate for the substantial variation in creatinine production across sex, age and ethnicity (NKF 2002). Similary, the endogenous creatinine production rate has a high inter-individual variability in cats (Le Garreres *et al* 2007), but which factors (e.g. age, breed, sex) are responsible for this variation is currently unknown.

The methods to estimate GFR and identify cats with decreased GFR and the diagnostic algorithm (Fig 6.5) proposed in Chapter 6 can support veterinarians in the work-up of cats for early kidney disease. The methods to identify cats with GFR below borderline or low GFR thresholds are described for the first time. Remaining limitations are that iohexol assays are expensive and not widely available, that injectable creatinine is not yet commercialized and that the accuracy of these new methods was not evaluated in a separate cat population than the one they were designed in. However, these methods can give important additional information to the routine variables to evaluate kidney function in cats. Because these methods are easy to do, inexpensive and do not require difficult calculations, they are very suitable for daily use in practice. Main indications to apply these methods are cats with one or more risk factor(s) for CKD (e.g. age, breed, related condition, drug therapy), particularly if the routine blood and urine variables give doubtful results. These methods represent an important progress for detecting mild feline kidney dysfunction and these new tools can be employed in the screening for feline CKD. An improved opportunity to diagnose early feline CKD can lead to timely therapeutic intervention and possibly an improved prognosis.

7.7 GENERAL CONCLUSION

In this thesis, commonly applied screening techniques for CKD were assessed in two at-risk populations, namely aged and Ragdoll cats. Health screening of middle-aged and old cats did lead to multiple diagnoses such as FIV infection, CKD, hyperthyroidism and urinary tract infection. In addition, many cats had a suboptimal SBP or BCS, gingivitis, heart murmur or laboratory abnormalities for which further diagnostic intervention, treatment or follow-up were indicated. This clearly indicates the need and value of regular health checks in aged cats to improve early disease detection – including CKD – and to allow timely therapeutic intervention. We found that Ragdoll cats are predisposed for segmental cortical lesions, but an increased susceptibility of Ragdoll cats for CKD could neither be confirmed nor excluded. Although further studies are required, the ultrasonographic suspicion for CKD in 5 – 9% Ragdoll cats versus none in the control group may support continued pre-breeding screening of Ragdoll cats for kidney disease.

Because DM is an important risk factor for CKD in humans, we also evaluated if cats with DM were susceptible to develop DKD. By comparing diabetic cats with healthy and CKD cats, we could not demonstrate a major influence of feline DM on kidney function. Therefore, there is currently no rationale for more intense screening of diabetic cats for kidney disease than non-diabetic cats of similar age.

Throughout this thesis, several limitations of the currently used screening tests for CKD were noticed and solutions were proposed. For some restrictions, no clear solution could be given and further study is needed. An overview is provided in Table 7.1.

An important solution is the design of convenient and cost-effective methods and a diagnostic algorithm (Fig 6.5) that can be employed in the diagnosis of early feline CKD. Limited sampling strategies were developed allowing GFR estimation with a margin of error that is acceptable both for clinical and for research situations. Reliable new methods were created to identify cats with a GFR value below certain thresholds, using routine kidney variables or clearance marker concentrations at three time points after marker injection. These methods can be applied for screening of cats for CKD, particularly in cats with doubtful routine blood and urine variables, and will facilitate the diagnosis of early CKD. Future studies will need to elucidate whether early detection of kidney dysfunction and timely therapeutic intervention leads to an improved long-term outcome.

Table 7.1. Limitations of screening tests for chronic kidney disease and possible solutions.

LIMITATION	POSSIBLE SOLUTION
Early clinical signs of CKD, such as poor body condition or polyuria/polydipsia, may be overlooked by cat owners.	Veterinarians should educate cat owners to recognize early clinical signs of CKD. Routine health screening of cat populations at-risk for CKD is warranted.
Many healthy cats have values outside the RI for certain laboratory variables (e.g. serum creatinine concentration, serum phosphorus concentration), questioning the validity of the RI. Secondly, an age-effect was found for certain laboratory variables.	Veterinary laboratories should follow published guidelines to determine RIs. For age-dependent laboratory variables, partitioning the RIs for age will improve health screening of aged cats. Laboratories should calculate reference change values for laboratory variables with high individuality to facilitate detection of clinically relevant changes on serial laboratory results. Standardization of laboratory measurements is warranted in veterinary medicine.
Many cats have doubtful routine kidney variables, namely increased serum urea or creatinine concentration or poorly concentrated urine as single abnormality. Routine blood and urine examinations cannot distinguish whether these abnormalities are irrelevant or might indicate early CKD.	Simplified methods to estimate GFR (LSS) or to identify cats with decreased GFR (Chapter 6) can be applied to cats with doubtful routine kidney and urine variables.
Approximately ¼ of healthy cats of various ages have borderline proteinuria.	Longitudinal studies are needed to reveal the relevance of borderline proteinuria in healthy cats of different ages in order to ameliorate the recommendations for the management of borderline proteinuria in non-azotemic cats. Efforts should be taken to standardize UPC measurements in veterinary laboratories.

(CIN = Chronic interstitial nephritis; CKD = Chronic kidney disease; GFR = Glomerular filtration rate; LSS = Limited sampling strategy; PKD = Polycystic kidney disease; RI = Reference interval; UPC = Urinary protein: creatinine ratio)

Table 7.1. Continued.

LIMITATION	POSSIBLE SOLUTION
Chronic interstitial nephritis cannot be diagnosed using the current screening tests for Ragdoll cats, namely serum creatinine and urea concentrations, liver and kidney ultrasonography and genetic testing for PKD.	Using these screening tests, Ragdoll cats can only be evaluated for CKD and PKD. Kidney histology is needed to determine if CIN is present in Ragdoll cats suspected of CKD or diagnosed with CKD.
Interpretation of the current screening tests for Ragdoll cats is hampered by the lack of urinalysis and lack of knowledge of the frequency of renal ultrasonographic abnormalities in non-Ragdoll cats. Additional limitations revealed by the retrospective study were incomplete screening tests in many Ragdoll cats and many incomplete ultrasonography reports.	We described the frequency and type of renal ultrasonographic abnormalities in young healthy non-Ragdoll cats. Urinalysis should be added to the currently applied screening tests. Secondly, a standardized protocol for abdominal ultrasonography should be followed and was proposed in this thesis.
The retrospective study questioned whether a breed-specific RI was needed to interpret serum creatinine in Ragdoll cats.	The prospective study rejected the need for a breed-specific serum creatinine RI for Ragdoll cats.
As B-mode renal ultrasonography does not correlate with renal function and is not a useful tool to predict which cats will develop azotemic kidney disease, confirmation of CKD is not possible based on ultrasonographic findings.	Simplified methods to estimate GFR (LSS) or to identify cats with decreased GFR (Chapter 6) can be applied to non-azotemic Ragdoll cats with an ultrasonographic suspicion of CKD. Long-term follow-up of kidney function is needed in these cats to help determine the clinical relevance of renal ultrasonographic changes.

(CIN = Chronic interstitial nephritis; CKD = Chronic kidney disease; GFR = Glomerular filtration rate; LSS = Limited sampling strategy; PKD = Polycystic kidney disease; RI = Reference interval)

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SUMMARY

Chronic kidney disease (CKD) is a common condition in feline patients. The current knowledge on diagnosis, staging according to the *International Renal Interest Society* guidelines, and importance of screening for CKD is summarized in **Chapter 1**. This literature review underlines that CKD is mostly diagnosed late in the disease course, based on compatible clinical signs and renal azotemia. Detection of mild kidney dysfunction is difficult because azotemia and impaired urine concentrating ability can be absent. However, early detection of CKD is important to initiate appropriate therapy on time, aiming to slow down declining kidney function and to postpone disease complications. Determination of glomerular filtration rate (GFR) allows diagnosis of early CKD, but is too labor-intensive and time-consuming to perform in daily practice. Since early diagnosis of feline CKD is challenging, screening of at-risk populations is recommended.

Because of their increased susceptibility for chronic diseases – including CKD – old cats are the first cat population for which health screening was already performed before the start of this thesis. Guidelines for routine health screening of senior and geriatric cats were created by the *Feline Advisory Bureau*, *American Association of Feline Practitioners* and *American Animal Hospital Association*. Unfortunately, scientific data on clinical and laboratory abnormalities of aging cats were scarce which limited the interpretation of this health screening.

On the recommendation of breed clubs, Ragdoll cats are screened for years now in some countries for polycystic kidney disease (PKD) and chronic interstitial nephritis (CIN). For this screening, breed clubs advise measuring serum creatinine and urea concentrations and performing ultrasonography of liver and kidneys and a genetic test for PKD. However, scientific studies on the prevalence of Ragdoll cats for PKD and the predisposition of Ragdoll cats for CIN or CKD were not available prior to this thesis.

Cats with diabetes mellitus (DM) are a third cat population for which screening for CKD might be recommended. Diabetic kidney disease (DKD) or diabetic nephropathy is a frequent and serious complication in human medicine, particularly in humans with type 2 DM. Because cats mainly suffer from type 2 DM, the question did exist whether feline diabetics are susceptible for DKD?

The final goal of this thesis **(Chapter 2)** was to demonstrate the limitations of currently performed screening practices for feline CKD and to develop possible solutions

to improve early CKD detection. Therefore, the screening of middle-aged and old cats and Ragdoll cats for CKD was evaluated and diabetic cats were evaluated for the occurrence of DKD. Finally, we aimed to develop convenient and cost-effective methods to estimate GFR and to identify cats with GFR values below certain thresholds.

In **Chapter 3** general health screening was executed in 100 apparently healthy middle-aged and old cats by fulfilling a complete history, systolic blood pressure (SBP) measurement, physical examination, ophthalmic examination, complete blood count, serum biochemistry profile and urinalysis. The cats were allocated to two groups, namely middle-aged (6 –10 years; n = 56) and old cats (> 10 years; n = 44). The mean SBP of our population was 133.6 ± 21.5 mmHg and SBP exceeded 160 mmHg in 8% of cats. Interesting findings on physical examination were gingivitis (72%), local lymphadenopathy (34%), palpable thyroid goiter (20%) and murmur (11%). The body condition score (BCS) was abnormal in 51% of cats: 40% were overweight and 11% underweight. Major findings on laboratory tests were increased creatinine concentration (29%), hypophosphatemia (40%), borderline proteinuria (25%) and crystalluria (41%). The tear production was similar for both eyes and ocular lesions secondary to hypertension were not detected. Cats over 10 years of age had significantly higher SBP, heart rate, murmur frequency, platelet count, serum urea concentration, serum bilirubin concentration and urinary protein: creatinine ratio (UPC) and a significantly lower BCS, hematocrit, serum albumin concentration and serum calcium concentration than middle-aged cats (6 – 10 years). Health screening allowed us to diagnose feline immunodeficiency virus infection in 14 cats, CKD in 2 cats and hyperthyroidism and urinary tract infection in one cat.

This study showed that physical and laboratory abnormalities are frequent in ‘apparently healthy’ old cats. Our findings confirmed the importance of regular health screening – including nutritional assessment – and individualized nutritional support for aging cats. Further, this study emphasized the significance of reliable reference intervals (RIs) to allow an accurate interpretation of health screening. Finally, age-specific RIs are required, at least for some laboratory parameters (e.g. serum creatinine, serum phosphor).

The occurrence of kidney diseases in Ragdoll cats was investigated in **Chapter 4**. At first, data of Ragdoll cats that were presented at our institution for PKD and CIN screening over an 8 years period were retrospectively evaluated **(Section §4.1)**. Renal

ultrasonography of 244 healthy Ragdoll cats revealed a PKD prevalence of 2.9%, suspicion for CKD in 8.6%, abnormalities with unclear significance in 3.3% and unilateral renal agenesis or severe hypoplasia in 2 cats (0.1%). All genetically tested cats (n = 125) were homozygous for the wild-type PKD-1 alleles indicating a PKD negative state. Almost 11% of the 141 Ragdoll cats for which serum creatinine was measured had an increased concentration, which raised the question whether a breed-specific creatinine RI for Ragdoll cats would be appropriate?

Given the limitations inherent to the retrospective study design, a prospective study was the logical next step **(Section §4.2)**. Serum creatinine and urea concentrations, routine urinalysis and ultrasonography of the urinary tract and liver according to a standard protocol were compared between healthy Ragdoll and age-matched control cats. Ragdoll cats also underwent a genetic test for the PKD-1 mutation. In total, 133 Ragdoll cats and 62 control cats were included. Renal ultrasonographic abnormalities were frequent, in Ragdoll cats (49.6%) as well as in control cats (40%). None of the control cats, but 5.3% of Ragdoll cats were suspected for CKD on ultrasonography, which approached significance. Ragdoll cats showed significantly more frequent segmental cortical lesions, abnormal renal capsule, echogenic urine, significantly lower serum urea concentration and higher urine specific gravity (USG) compared to the control cats. The other ultrasonographic and laboratory variables did not significantly differ between both groups. Blood and urine examinations confirmed CKD in only 1 of the Ragdoll cats and all Ragdoll cats were PKD negative based on genetic testing. Borderline proteinuria and crystalluria were frequent in both groups.

Based on both studies of Chapter 4 we concluded that young, healthy Ragdoll cats are uncommonly affected by PKD, are suspected for CKD on ultrasonography in 5 – 9% of cases and are predisposed for segmental cortical lesions. A breed-specific serum creatinine RI does not seem required for Ragdoll cats. A remarkable finding of the prospective study was the high frequency of renal ultrasonographic abnormalities in juvenile healthy cats. However, none of the control cats showed segmental cortical lesions or was ultrasonographically suspected for CKD. Therefore, it appears wise to continue pre-breeding screening of Ragdoll cats and to avoid breeding with Ragdoll cats with segmental cortical lesions and with an ultrasonographic suspicion of CKD.

In **Chapter 5** routine kidney variables (serum urea, serum creatinine, USG, UPC), SBP, GFR and urinary Cystatin C (uCysC) as tubular marker were prospectively compared between cats with DM, cats with CKD and healthy cats that were age-matched to the diabetic cats. The same variables were compared between recently (i.e. less than 1 month) and not-recently diagnosed (i.e. 1 month or longer) diabetic cats. Cats with DM only had significantly lower USG compared to healthy cats. In contrast, most variables significantly differed between cats with DM and cats with CKD: diabetic cats had significantly lower serum urea concentration, serum creatinine concentration and uCysC: creatinine ratio and significantly higher USG and GFR. Systolic blood pressure and UPC did not significantly differ between the 3 groups of cats. None of the variables evaluating kidney function or kidney damage differed significantly between recently and not-recently diagnosed diabetic cats.

Thus, based on the single evaluation of routine kidney variables, GFR and uCysC, a major impact of feline DM on kidney function could not be demonstrated. This indicates that there is currently no rationale to screen diabetic cats more frequently for CKD than non-diabetic cats of similar age.

Finally, we aimed to develop simple methods to identify cats with early kidney dysfunction in **Chapter 6**. Data of 73 cats that underwent a 9-sample combined plasma exogenous creatinine-iohexol clearance test at our institution were used. Regression formulae based on routine variables (serum urea and creatinine concentrations, USG, UPC) could not reliably estimate the actual GFR value, but were trustworthy to identify cats with GFR below low or borderline thresholds. For reliable GFR estimation, at least 3 blood samples for clinical purpose and 5 blood samples for research purpose are required and optimal timing for these samples was determined. Finally, cut-off marker concentrations at three time points after marker injection were defined and these were able to identify cats with GFR below low and borderline thresholds with high sensitivities, specificities, positive and negative predictive values. Based on these new methods, we proposed a diagnostic algorithm to identify cats with early CKD.

These convenient and cost-effective methods to estimate GFR and identify cats with decreased GFR can give crucial additional information to evaluate kidney function in cats. These methods represent an important progress for detecting mild kidney dysfunction and can be employed as part of the screening for feline CKD.

Throughout this thesis, we have gained important knowledge on the value and limitations of currently applied screening techniques for feline CKD and we have expanded the diagnostic tools for early detection of feline CKD. We can conclude that routine screening for CKD seems mandatory for aged cats and Ragdoll cats, but not for diabetic cats. To allow an accurate interpretation of this screening, a combination of blood and urine examinations is needed and well-designed reliable laboratory RIs are required. Additionally, a standardized protocol should be followed to screen Ragdoll cats using renal ultrasonography. In order to detect mild kidney dysfunction in case of doubtful routine laboratory results, methods to identify cats with GFR below certain thresholds were created. Also, limited sampling strategies were developed to estimate GFR in a convenient way. These techniques will facilitate the diagnosis of early feline CKD, aiming for more timely therapeutic intervention and possibly an improved prognosis.

SAMENVATTING

Chronische nierziekte (CNZ) is een frequent voorkomende aandoening bij katten. De huidige kennis over de diagnose, de stagering volgens richtlijnen van de *International Renal Interest Society* en het belang van screening voor CNZ wordt samengevat in **Hoofdstuk 1**. Dit literatuuroverzicht benadrukt dat CNZ jammer genoeg vaak in een vergevorderd stadium wordt gediagnosticeerd, op basis van typische klinische symptomen en renale azotemie. Een milde afname van de nierfunctie is moeilijk vast te stellen omdat azotemie vaak niet aanwezig is en de nieren nog in staat zijn de urine te concentreren. Vroegtijdige detectie van CNZ is echter belangrijk om tijdig een gepaste therapie op te starten om zo verdere achteruitgang van de nierfunctie af te remmen en complicaties uit te stellen. Door bepaling van de glomerulaire filtratiesnelheid (GFS) kan milde CNZ gediagnosticeerd worden, maar dit is te arbeidsintensief en tijdrovend voor dagelijks gebruik. Feliene CNZ vroegtijdig opsporen is een uitdaging, daarom wordt screening van risicopopulaties aangeraden.

Omdat oude katten vatbaar zijn voor verschillende chronische aandoeningen, waaronder CNZ, werd bij deze populatie gezondheidsscreening al toegepast vóór de start van deze thesis. Richtlijnen voor gezondheidscontroles van senior en geriatrische katten werden opgesteld door de *Feline Advisory Bureau*, *American Association of Feline Practitioners* en *American Animal Hospital Association*. Jammer genoeg was de interpretatie van dergelijke gezondheidscontroles moeilijk omdat wetenschappelijke informatie over klinische en laboratorium afwijkingen bij oude katten ontbrak.

In sommige landen worden Ragdolls op aanraden van rasverenigingen al vele jaren gescreend voor polycystische nierziekte (‘polycystic kidney disease’; PKD) of chronische interstitiële nefritis (CIN). Voor deze screening adviseren rasverenigingen de bepaling van serum creatinine en ureum concentraties, echografie van lever en nieren en een genetische test voor PKD. Wetenschappelijke literatuur over de prevalentie van PKD en predispositie van Ragdolls voor CIN of CNZ was echter niet beschikbaar bij aanvang van deze thesis.

Katten met diabetes mellitus (DM) zijn een derde kattenpopulatie waarvoor screenen van de nierfunctie belangrijk kan zijn. Diabetes nefropathie (= ‘Diabetic kidney disease’; DKD) is een frequente en ernstige complicatie in de humane geneeskunde, vooral bij mensen met type 2 DM. Aangezien katten meestal type 2 DM hebben, bestond de vraag of ze ook DKD kunnen ontwikkelen?

De algemene doelstelling van deze thesis **(Hoofdstuk 2)** was het aantonen van de beperkingen van huidige screeningmethodes voor feliene CNZ en mogelijke oplossingen te bieden om de detectie van vroegtijdige CNZ te verbeteren. Daarom werd de screening voor CNZ geëvalueerd bij katten van middelbare tot oude leeftijd en bij Ragdolls en werd onderzocht of DKD voorkomt bij suikerzieke katten. Tot slot wilden we gebruiksvriendelijke en betaalbare methodes ontwikkelen om de GFS in te schatten en om katten met een GFS onder een bepaalde grenswaarde te detecteren.

In **Hoofdstuk 3** ondergingen 100 schijnbaar gezonde katten van 6 jaar of ouder een algemene gezondheidscontrole door middel van een complete anamnese, bloeddrukmeting, grondig lichamelijk onderzoek, oogonderzoek, hematologisch en biochemisch bloedonderzoek en urineonderzoek. De katten werden verdeeld in 2 groepen, namelijk katten van middelbare leeftijd (6 – 10 jaar; n = 56) en oude katten (> 10 jaar; n = 44). De gemiddelde bloeddruk was 133,6 ± 21,5 mmHg en de systolische bloeddruk (SBD) was meer dan 160 mmHg bij 8% van onze katten. Interessante bevindingen op lichamelijk onderzoek waren gingivitis (72%), lokale lymfadenopathie (34%), een vergrote schildklier (20%) en een bijgeruis (11%). De lichaamsconditie score was abnormaal bij 51% van de katten: 40% had overgewicht en 11% ondergewicht. De belangrijkste bevindingen op laboratorium onderzoek waren gestegen creatinine concentratie (29%), hypofosfatemie (40%), borderline proteïnurie (25%) en kristallurie (41%). De traanproductie was gelijkaardig in beide ogen en geen enkele kat vertoonde oogletsels secundair aan hypertensie. Katten ouder dan 10 jaar hadden een significant hogere SBD, hartslag, frequentie van bijgeruis, aantal bloedplaatjes, serum ureum concentratie, serum bilirubine concentratie en urinaire eiwit/creatinine ratio (E/C) en een significant lagere lichaamsconditie score, hematocriet, serum albumine concentratie en serum calcium concentratie dan katten van middelbare leeftijd (6-10 jaar). De gezondheidsscreening leidde tot diagnoses zoals infectie met felien immunodeficiëntievirus (14%), CNZ (2%), hyperthyroïdie (1%) en urineweginfectie (1%).

Deze studie toonde aan dat afwijkingen op lichamelijk en laboratorium onderzoek frequent voorkomen bij ‘schijnbaar gezonde’ oude katten. Onze bevindingen bevestigden het nut van regelmatige gezondheidscontroles – inclusief beoordelen van de nutritionele status – en geïndividualiseerde nutritionele ondersteuning voor oudere katten. Verder benadrukte deze studie het belang van betrouwbare referentie intervallen om de resultaten van de gezondheidsscreening correct in te schatten. Tot slot konden we stellen dat

leeftijdspecifieke referentie intervallen noodzakelijk zijn voor sommige laboratorium parameters (bv. serum creatinine, serum fosfor).

Het voorkomen van nieraandoeningen bij Ragdolls werd in **Hoofdstuk 4** onderzocht. Eerst en vooral werden gegevens van Ragdolls die gedurende een periode van 8 jaar aan onze instelling werden aangeboden voor PKD en CIN screening retrospectief geëvalueerd **(Sectie §4.1)**. Renale echografie toonde een PKD prevalentie van 2,9%, een vermoeden van CNZ bij 8,6%, abnormaliteiten waarvan de betekenis onduidelijk was bij 3,3% en unilaterale renale agenesie of ernstige hypoplasie bij 2 katten (0,1%). Alle genetisch geteste Ragdolls (n = 125) waren homozygoot voor de wild-type allelen, wat wijst op een PKD-negatieve status. Bijna 11% van de 141 Ragdolls waarbij serum creatinine gemeten werd vertoonde een verhoogde waarde waardoor de vraag rees of een rasspecifiek referentie interval voor serum creatinine noodzakelijk is voor Ragdolls?

Omwille van de beperkingen van deze retrospectieve studie, was een prospectieve studie de logische volgende stap **(Sectie §4.2)**. Serum creatinine en ureum concentraties, routine urineonderzoek en echografie van urinair stelsel en lever volgens een standaard protocol werden vergeleken tussen gezonde Ragdolls en controlekatten van dezelfde leeftijd. De Ragdolls werden ook genetisch getest voor de PKD-1 mutatie. In totaal werden 133 Ragdolls en 62 controlekatten onderzocht. Echografische veranderingen ter hoogte van de nieren waren frequent, zowel bij Ragdolls (49,6%) als bij controlekatten (40%). Geen enkele controlekat, maar wel 5,3% van de Ragdolls was echografisch verdacht voor CNZ, wat significantie benaderde. Ragdolls vertoonden significant vaker segmentale corticale letsels, een abnormaal nierkapsel en echogene urine en hadden een significant lagere serum ureum concentratie en hoger urinair soortelijk gewicht (USG) dan de controlekatten. De andere echografische en laboratorium variabelen verschilden niet significant tussen beide groepen. Bloed- en urineonderzoek bevestigden CNZ bij slechts 1 van de Ragdolls en alle Ragdolls waren PKD negatief volgens de genetische test. Borderline proteïnurie en kristallurie waren frequent in beide groepen.

Beide studies van Hoofdstuk 4 toonden aan dat jonge, gezonde Ragdolls zelden PKD hebben, verdacht zijn van CNZ op echografie in 5 – 9% van de gevallen en een verhoogde vatbaarheid hebben voor segmentale corticale letsels. Een rasspecifiek serum creatinine referentie interval voor Ragdolls blijkt niet noodzakelijk. Een opmerkelijke bevinding van de prospectieve studie was de hoge frequentie van echografische

veranderingen ter hoogte van de nier bij jonge gezonde katten. Segmentale corticale letsels werden echter niet opgemerkt bij controlekatten en geen enkele controlekat was echografisch verdacht voor CNZ. Daarom lijkt het verstandig om verder Ragdolls te screenen vóór het fokken en niet te fokken met Ragdolls met segmentale corticale letsels en met een echografisch vermoeden van CNZ.

In **Hoofdstuk 5** werden routine renale variabelen (serum ureum, serum creatinine, USG, E/C), SBD, GFS en urinair Cystatine C als tubulaire merker prospectief vergeleken tussen katten met DM, katten met CNZ en gezonde katten van vergelijkbare leeftijd als de suikerzieke katten. Dezelfde variabelen werden vergeleken tussen recent (i.e. minder dan 1 maand) en niet-recent (i.e. 1 maand of langer) gediagnosticeerde katten met DM. Suikerzieke katten hadden enkel een lager USG in vergelijking met gezonde katten. In tegenstelling, de meeste variabelen verschilden tussen katten met DM en CNZ: suikerzieke katten hadden significant lagere serum ureum concentratie, serum creatinine concentratie en urinair Cystatine C/creatinine ratio en significant hoger USG en GFS. De SBD en E/C waren niet significant verschillend tussen de 3 groepen katten. Geen enkele renale variabele verschilde tussen recent en niet-recent gediagnosticeerde suikerzieke katten.

Met andere woorden, een duidelijke impact van feliene DM op de nierfunctie kon niet aangetoond worden op basis van een eenmalige beoordeling van routine renale variabelen, GFS en urinair Cystatine C. Daaruit volgt dat er momenteel geen wetenschappelijke basis is om katten met DM meer intensief te screenen op het voorkomen van CNZ in vergelijking met niet-suikerzieke katten van dezelfde leeftijd.

Tot slot wilden we in **Hoofdstuk 6** eenvoudige methodes ontwikkelen om katten met milde afname van de nierfunctie te onderkennen. We gebruikten gegevens van 73 katten die aan onze instelling een gecombineerde plasma exogene creatinine-iohexol klaringstest van 9 stalen ondergingen. Hieruit bleek dat regressie formules op basis van routine variabelen (serum ureum, serum creatinine, USG, E/C) de eigenlijke GFS van een kat niet betrouwbaar konden inschatten, maar wel konden bepalen of een kat een GFS had beneden lage en borderline grenswaardes. De GFS kon betrouwbaar geschat worden met behulp van ten minste 3 bloedstalen voor klinische doeleinden en 5 bloedstalen voor onderzoeksdoeleinden. Ook werden de optimale tijdstippen voor deze staalname bepaald. Als laatste stelden we vast dat bepaalde ‘cut-off’ concentraties op 3 tijdstippen na injectie van de klaringsmerker in staat zijn om katten met een GFS beneden een lage of borderline

grenswaarde te detecteren met hoge sensitiviteit, specificiteit, positief en negatief voorspellende waarde. Met behulp van deze nieuwe methodes stelden we een diagnostisch algoritme op om katten met vroegtijdige CNZ te detecteren.

Deze gebruiksvriendelijke en betaalbare methodes om GFS te schatten en katten met verminderde GFS te detecteren kunnen waardevolle bijkomende informatie geven om de nierfunctie van katten te beoordelen. Deze methodes betekenen een belangrijke vooruitgang om milde nierdysfunctie vast te stellen en kunnen gebruikt worden als onderdeel van de screening voor feliene CNZ.

Dankzij deze thesis hebben we belangrijke kennis bekomen over de waarde en beperkingen van huidige screeningsmethodes voor feliene CNZ en werden de diagnostische opties voor vroegtijdige detectie van feliene CNZ uitgebreid. We kunnen besluiten dat routinematig screenen van oudere katten en Ragdolls voor CNZ essentieel blijkt te zijn, in tegenstelling tot katten met DM. Om deze screening accuraat te interpreteren, is een combinatie van bloed- en urineonderzoeken noodzakelijk en zijn goed opgestelde, betrouwbare laboratorium referentiewaardes vereist. Bovendien moet een standaard protocol gevolgd worden om Ragdolls te screenen via renale echografie. Om milde nierdysfunctie vast te stellen in geval van twijfelachtige routine laboratorium resultaten werden methodes ontworpen om katten met een GFS beneden bepaalde grenswaarden te identificeren. Ook werden gebruiksvriendelijke methodes ontwikkeld om de GFS in te schatten op basis van een beperkt aantal bloedstalen. Deze technieken zullen de diagnose van beginnende feliene CNZ bevorderen, met als doel tijdig een therapie in te stellen en mogelijk de prognose te verbeteren.

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CURRICULUM VITAE

Dominique Paepe werd geboren op 25 november 1979 te Brugge. Na het behalen van het diploma secundair onderwijs in de richting Grieks-Wiskunde aan het Sint-Lodewijkscollege te Brugge startte ze in 1997 de studies Diergeneeskunde aan de Universiteit Gent. In 2003 behaalde ze, met grote onderscheiding en als primus over de volledige opleiding, het diploma van Dierenarts in de afstudeerrichting Kleine Huisdieren.

Onmiddellijk daarna volbracht ze een Roterend Internship aan de vakgroep Geneeskunde en Klinische Biologie van de Kleine Huisdieren van de faculteit Diergeneeskunde van de Universiteit Gent. Dit werd aan dezelfde vakgroep gevolgd door één jaar deeltijds werk als praktijkassistent en daarna door een intensieve opleiding tot Europees Specialist interne geneeskunde voor kleine huisdieren. Na het afleggen van de nodige examens werden deze zware inspanningen in oktober 2009 bekroond met de titel *"Diplomate of the European College of Veterinary Internal Medicine – Companion Animals"*.

Sinds februari 2009 werkt Dominique Paepe aan dezelfde vakgroep als voltijds assistent op de dienst interne geneeskunde bij Prof. Dr. Sylvie Daminet. Ze doet sinds oktober 2009 halftijds consultaties als specialist interne geneeskunde kleine huisdieren waarbij ze een belangrijke bijdrage levert aan de klinische opleiding van de laatstejaarsstudenten Diergeneeskunde. Daarnaast heeft ze sindsdien ook halftijds aan haar doctoraat gewerkt.

Dominique Paepe vervolledigde in 2013 het trainingsprogramma van de *Doctoral Schools of Life Sciences and Medicine* van de Universiteit Gent. Ze is auteur of mede-auteur van meer dan 40 wetenschappelijke publicaties in 'peer-reviewed' (inter)nationale tijdschriften. Ze nam actief deel aan talrijke internationale veterinaire congressen en presenteerde een 10-tal abstracts tijdens deze congressen. Ze is sinds enkele jaren lid van de *European Society of Veterinary Endocrinology* en van de *European Society of Veterinary Uronephrology*. Tot slot geeft ze jaarlijks verschillende bijscholingen aan Vlaamse of Nederlandse praktiserende dierenartsen.

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