J Vet Intern Med 2010;24:1263–1270

Escherichia coli Pyometra Induces Transient Glomerular and Tubular Dysfunction in Dogs

B. Maddens, S. Daminet, P. Smets, and E. Meyer

Background: Pyometra in dogs has been associated with renal injury.

Hypothesis: Examine pyometra-related nephropathy by evaluating novel renal biomarkers.

Animals: Twenty-five dogs with *Escherichia coli* pyometra. Fourteen clinically healthy bitches of comparable age.

Methods: Prospective study. Urinary biomarkers determined by immunoassays (uIgG, uCRP, uAlb, uRBP, $uTXB_2$) or colorimetric test (uNAG) with results normalized to urine creatinine concentration. Nonparametric Mann-Whitney *U*-test and Wilcoxon's signed-rank test used to compare healthy dogs and dogs with pyometra, and dogs with pyometra at initial and follow-up examination.

Results: Urinary biomarkers (median, range) significantly increased in dogs with pyometra (uIgG/Cr: 169.7 mg/g, 4.8–1052.9; uCRP/Cr: 0.260 mg/g, 0.006–3.030; uAlb/Cr: 89.5 mg/g, 8.8–832.7; uRBP/Cr: 1.66 mg/g, 0.05–21.44; uNAG/Cr: 5.8 U/g, 1.6–27.7; $uTXB_2$/Cr: 15.3 μg/g, 3.2–139.6) compared with healthy bitches (uIgG/Cr: 3.4 mg/g, 0.6–8.9; uCRP/Cr: below detection limit; uAlb/Cr: 17.5 mg/g, 1.3–166.3; uRBP/Cr: 0.13 mg/g, 0.02–0.44; uNAG/Cr: 2.4 U/g, 1.4–7.4; $uTXB_2$/Cr: 2.4 μg/g, 1.2–4.7) ($P < .001$). Six months after ovariohysterectomy, urinary biomarkers in pyometra group (uIgG/Cr: 4.7 mg/g, 1.5–99.8; uCRP/Cr: below detection limit; uAlb/Cr: 13.9 mg/g, 2.1–471.2; uRBP/Cr: 0.05 mg/g, 0.02–0.32; uNAG/Cr: 1.6 U/g, 0.9–3.3; $uTXB_2$/Cr: 3.3 μg/g, 1.0–6.9) were significantly lower than before surgery ($P < .01$), and not significantly different to those of healthy dogs ($P > .05$).

Conclusion and Clinical Importance: Pyometra-related renal dysfunction affects the nephron both at glomerular and proximal tubular level and is a transient process in most dogs with *E. coli* pyometra.

Key words: Dog; Endometritis; Renal marker.

Renal dysfunction complicating the course of bacterial infections is of major importance in both human and veterinary medicine.[1–3] Pyometra is a frequently diagnosed bacterial infection of the uterus in intact, sexually mature bitches, leading to the accumulation of purulent material in the uterine lumen. Moreover, pyometra has been widely associated with postinfection renal dysfunction in dogs.[4–9]

Chronic stimulation of the immune system by the *Escherichia coli* antigen excess from the infected uterus might induce formation of circulating immune complexes, predisposed for precipitation in the glomerulus.[4,10,11] Historically, an immune complex-mediated glomerulonephritis has been assumed as the origin of the renal damage secondary to pyometra in dogs.[4,8,12,13] However, the nature of this acute glomerular dysfunction still needs to be defined.[6] There are no increased levels of circulating immune complexes in dogs with pyometra compared with healthy ones.[14] Other studies reported only tubulointerstitial lesions in dogs with pyometra[6,7] or even found no histological differences between healthy dogs and dogs with pyometra.[9]

In the current study, a different approach is used to gain better insight into this renal dysfunction by applying urinary biomarkers such as albumin (Alb), immunoglobulin G (IgG), retinol-binding protein (RBP), and *N*-acetyl-β-D-glucosaminidase (NAG), which have already shown their added value in the diagnosis of several human nephropathies.[15–17] Increases in urinary marker concentration parallel the localization of the structural damage. In addition, compared with routinely used renal markers, these biomarkers are early indicators of renal dysfunction and inform on the extent of the disease process.

Abbreviations:

CEH	cystic endometrial hyperplasia
E. coli	*Escherichia coli*
MW	molecular weight
sCr	serum creatinine
SD	standard deviation
sUN	serum urea nitrogen
uAlb	urinary albumin
uCr	urinary creatinine
uCRP	urinary C-reactive protein
uIgG	urinary immunoglobulin G
uNAG	urinary *N*-acetyl-β-D-glucosaminidase
UPC	urinary protein to creatinine ratio
uRBP	urinary retinol-binding protein
uSG	urine specific gravity
$uTXB_2$	urinary thromboxane B_2

From the Department of Pharmacology, Toxicology and Biochemistry (Maddens, Meyer) and the Department of Medicine and Clinical Biology of Small Animals (Daminet, Smets), Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium. This work was performed at the Department of Pharmacology, Toxicology and Biochemistry and the Department of Medicine and Clinical Biology of Small Animals, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium. Part of this work was presented as an abstract at the annual ECVIM-CA congress in Ghent, Belgium, 4–8 September 2008.

Corresponding author: Bert Maddens, DVM, Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium; e-mail: bert.maddens@ugent.be.

Submitted October 12, 2009; Revised July 28, 2010; Accepted August 9, 2010.

10.1111/j.1939-1676.2010.0603.x

Glomerular dysfunction allows increased filtration of intermediate molecular weight (MW) serum proteins such as Alb (69 kDa).[18] In more advanced stages, it is characterized by the presence of high MW proteins in the ultrafiltrate such as C-reactive protein (CRP, 115 kDa) and IgG (160 kDa).[19,20] In contrast, tubular dysfunction is reflected by the urinary presence of RBP and of NAG.[21,22] In physiologic conditions, the low MW protein RBP (21 kDa) is reabsorbed by the proximal tubular cells after filtration by the renal glomeruli. *N*-acetyl-β-D-glucosaminidase is a lysosomal enzyme present in the proximal tubular cells, appearing in urine after disruption of cell integrity. Finally, the urinary presence of unmetabolized TXB_2 accurately reflects its renal synthesis and is a marker for intrarenal hemodynamics and altered glomerular filtration.[23,24]

The renal lesions associated with pyometra are thought to be of either an acute or subacute nature.[6,7,9] In addition, the combination of the renal histology and functional test results from different studies suggests that pyometra affects all segments of the nephron.[4–8] Consequently, this infectious disease provides major opportunities for the evaluation of a set of early and site-specific urinary biomarkers in the dog.[20] Previous studies by our group describe the validation of the assays for the detection of these markers, which is a necessary solid basis for future research.[20,25] The present study aims at evaluating these biomarkers in healthy dogs and dogs with uterine *E. coli* infection to reveal new functional aspects of postpyometra renal disease.

Materials and Methods

Dogs

Twenty-five dogs with pyometra and a positive intrauterine bacterial culture of *E. coli* and without concurrent diseases were included. Fourteen clinically healthy bitches served as controls. Dogs were excluded when medication with a possible influence on renal function had recently been administered. All animals were privately owned and presented at the Companion Animal Clinic of the Faculty of Veterinary Medicine, Ghent University, between September 2007 and March 2009. The owners signed an informed consent form to allow participation of their dog in the study.

Physical examination and routine hematological and biochemical tests were performed on all dogs. The diagnosis of pyometra was based on typical clinical signs with or without vaginal discharge, routine laboratory tests (CBC, serum biochemical analysis), an abnormally enlarged uterus on ultrasound examination with a hypoechoic luminal content and a uterine wall structure with presence of many and large cysts, an irregular surface and hypertrophic or atrophic endometrium, typical for pyometra,[26] and was confirmed during ovariohysterectomy and bacteriological culture of uterine fluid. Urine was collected by cystocentesis on ultrasound examination or at the start of the surgery. Twelve dogs with pyometra already received antibiotics during 2–7 days before their initial presentation at the Companion Animal Clinic of the university. The referring veterinarian had also administered a single injection of a nonsteroidal anti-inflammatory drug (NSAID) (carprofen)[a] to three of these dogs, on average 5–7 days before ovariohysterectomy. Upon their arrival at the Companion Animal Clinic of the University, all dogs were immediately administered fluid therapy adapted to the dogs' needs and received intravenous (IV) antibiotic treatment (amoxicillin-clavulanic acid). Sedation was performed with an IV injection combination of acepromazine[b] and morphine in 6 dogs, while the others received methadone IV. Anesthesia was induced by combination of diazepam[c] and propofol[d] (23 dogs) or sodium thiopental[e] (2 dogs) and maintained with isoflurane.[f] Ovariohysterectomy was performed by standard procedures and the abdominal incision was closed routinely. Appropriate analgesia with methadone or morphine was provided the day after surgery and further on with NSAIDS (carprofen). Intravenous administration of fluid was continued for 2 days. Dogs were discharged from the university clinic on average 3 days after the ovariohysterectomy. Owners continued the treatment with antibiotics (amoxicillin-clavulanic acid) and NSAIDS (carprofen) for 7 and 4 days, respectively.

Control dogs were considered healthy when no clinically relevant abnormalities were found on physical examination, routine laboratory tests on blood and urine, and abdominal ultrasound examination. Six of the 14 healthy dogs were presented for routine surgical ovariohysterectomy. Urine was sampled by cystocentesis during ultrasound examination or at onset of surgery in case of ovariohysterectomy.

Follow-up examination with blood and urine collection was performed 6 months after ovariohysterectomy in 13 of the 25 dogs originally presented with pyometra. The other 12 dogs were excluded on the basis of mortality (n = 5: 2 dogs developed acute renal failure after the ovariohysterectomy and were euthanized because the owners declined further treatment, 1 dog died by poisoning, 1 was hit by car, and 1 dog died in anesthesia during bite wound surgery), noncooperation of owners (n = 3) or concurrent diseases and interfering medication (n = 4: 2 dogs had pyoderma and were treated with antibiotics and prednisolone, 1 dog was meanwhile diagnosed with a dilatory cardiomyopathy and received ACE-inhibitors, furosemide, and pimobendan, and another dog suffered from epilepsy and was treated with phenobarbital).

Laboratory Methods

Routine Urinalysis. Urine specific gravity (uSG) was measured with a refractometer. Routine dipstick analysis,[g] urine culture and light microscopic sediment analysis were performed. Urine was centrifuged ($447 \times g$, 3 minutes), aliquoted and stored at $-80^{\circ}C$ until analysis of urinary IgG (uIgG), uCRP, uAlb, uRBP, uNAG, and $uTXB_2$. Urinary protein was determined with a turbidimetric method with benzethonium chloride.[h]

Immunoassays and Colorimetric Assay. All assays were validated for application in urine of dogs and urinary concentrations of the 6 biomarkers were determined as described previously by our group.[20,25] Because of the use of spot urine samples, urinary protein and biomarker concentrations were related to the urinary creatinine concentration (uCr) and expressed as ratios.[27] Urinary creatinine was measured by the modified Jaffé reaction.[28]

Briefly, frozen samples were thawed and used in commercially available canine-specific sandwich ELISA to determine the uIgG, uCRP, and uAlb concentrations.[i,j,k] A human RBP ELISA kit[l] quantified the relative amount of canine uRBP. Cross-reactivity between the chicken anti-human RBP antibody and canine RBP was demonstrated in a previous study from our group.[20] Urinary NAG activity was calculated by a standard formula after analysis with a colorimetric assay.[m] Urinary concentrations of TXB_2 were measured by a competitive enzyme immunoassay.[n] For each immunoassay, the absorbance was measured at the indicated wavelength by a plate reader.[o] The concentration of the urinary biomarker in the test sample was calculated from the standard curve by utilizing a 4 parameter logistic curve fitting program and corrected for the used urine dilution.[p]

Statistical Analysis

Data were analyzed using a commercial software package.[q] All variables were tested for normal distribution by the Kolmogorov-Smirnov test. Age, serum creatinine (sCr), serum urea nitrogen (sUN), uCr, and uSG were normally distributed in healthy dogs and in dogs with pyometra at the time of diagnosis, and the Student's *t*-test was used to compare these 5 parameters between both groups. The nonparametric Mann-Whitney *U*-test was applied to compare the urinary protein to creatinine ratio (UPC), uIgG/Cr, uCRP/Cr, uAlb/Cr, uRBP/Cr, uNAG/Cr, and $uTXB_2$/Cr ratios of both groups. Kendall's τ b was calculated to detect the level of correlation between the urinary biomarkers and between the biomarkers and routine renal markers (sCr, sUN, uSG, UPC) for nonparametric data, Pearson's correlation coefficient was used when data were normally distributed. The urinary concentrations of all 6 renal biomarkers, the UPC, uSG, sCr, and sUN on the day of initial presentation at the clinic of the dogs with pyometra and at the follow-up visit were compared using the Wilcoxon signed-rank test for paired samples. The level of significance was assigned with values of $P < .05$.

Results

Study Group Characteristics

No breed in the group of the healthy dogs and the dogs with pyometra was overrepresented. The mean age of the group of dogs with pyometra (mean age ± standard deviation [SD], 7.7 ± 2.7 years) was not significantly different from the mean age of the group of the healthy dogs (mean age ± SD, 5.4 ± 3.9 years) ($P = .097$).

History and clinical signs of pyometra, hematology, and biochemistry results before ovariohysterectomy are shown in Table 1. All dogs with pyometra had hypoalbuminemia (<20 g/L), but none was hypoproteinemic (reference range: 55–75 g/L). Nineteen of 25 dogs with pyometra (76%) had a mild-to-severe leucocytosis ($>16.10^9$ leucocytes/L) and 14 of 25 dogs with pyometra (56%) had a decreased packed cell volume (<43%). Ultrasound examination detected no abnormalities in the liver, spleen, gastrointestinal tract, adrenal glands, urinary tract, and both kidneys. The presence of a small amount of anechoic free abdominal fluid and enlarged medial iliac lymph nodes, indicative for peritonitis, was detected in 6 dogs with pyometra. In addition, 1 dog with pyometra had multiple cysts on its left ovary.

Routine Renal Tests in Healthy Dogs and Dogs with Pyometra

Descriptive statistics of routine renal markers are shown in Table 2. The serum parameters for renal dysfunction did not differ significantly between dogs with pyometra and healthy dogs (sCr, sUN; $P = .142$ and 0.551; reference ranges: sCr, < 125 μmol/L; sUN, 3.3–8.3 mmol/L), while UPC and uSG were significantly different between both groups ($P < .0001$). However, 8 dogs with pyometra were initially azotemic (sCr > 125 μmol/L) and 2 of these dogs developed acute renal failure after ovariohysterectomy. Eleven dogs with pyometra (44%) had a UPC < 0.5 and 8 dogs with pyometra (32%) had a UPC > 1.0. On light microscopic evaluation of the urine sediment, 4 dogs with pyometra (16%) had a combined microscopical hematuria (>25 erythrocytes/μL urine) and pyuria (>25 leucocytes/μL urine). Two dogs with pyometra (8%) only had a pyuria. Four of the dogs with pyometra and abnormalities on sediment analysis had a positive urine culture (*E. coli*).

Urinary Biomarkers in Healthy Dogs and Dogs with Pyometra

Dogs with pyometra had significantly increased ratios of uIgG/Cr ($P < .0001$), uCRP/Cr ($P < .0001$), uAlb/Cr ($P < .001$), uRBP/Cr ($P < .001$), uNAG/Cr ($P < .0001$), and $uTXB_2$/Cr ($P < .0001$) compared with healthy bitches (Table 3, Fig 1a). The ratios of uIgG/Cr, uCRP/Cr, uAlb/Cr, uRBP/Cr, and uNAG/Cr were all pairwise positively correlated at the 0.05 level, except for the ratio of $uTXB_2$/Cr (Table 4). Furthermore, a positive correlation was found between UPC and uIgG/Cr ($r = 0.65$), uCRP/Cr ($r = 0.76$), uAlb/Cr ($r = 0.71$), and uNAG/Cr ($r = 0.48$) ($P < .01$) and a weak correlation between UPC and uRBP/Cr ($r = 0.30$) ($P < .05$). The $uTXB_2$/Cr ratio was again not correlated with UPC. None of the biomarkers was correlated with uSG. Mean ± SD values of uCr for healthy dogs (1.36 ± 0.56 g/L) and dogs with pyometra (0.86 ± 0.66 g/L) were significantly different ($P < .01$). Exclusion of the 3 dogs with pyometra which received a single preceding NSAID injection, or of the 6 dogs with pyometra and urinary sediment abnormalities, had only minor effects on means and medians of urinary biomarker ratios, and no effects on ranges of UPC ratios or on the level of statistical significance in comparisons between healthy dogs and dogs with pyometra. Only the range of the uNAG/Cr changed when the 4 dogs with a positive urine culture were excluded (1.6–27.7 to 1.6–22.7 U/g). Median urinary biomarker and UPC ratios of the 6 dogs with pyuria, hematuria or bacteriuria did not significantly differ from the median value of the remainder of the group of dogs with pyometra ($P > .05$).

Table 1. History and clinical signs, hematology and biochemistry results (expressed as mean ± SD, n = 25) in dogs with *Escherichia coli* pyometra before ovariohysterectomy.

History and Clinical Signs		Hematology and Biochemistry Results (mean ± SD)		Reference Ranges
Vaginal discharge	19/25	Leucocytes (10^9/L)	25.5 ± 12.6	6–16
Polyuria-polydipsia	17/25	Packed cell volume (%)	41.9 ± 10.0	43–59
Vomiting	15/25	Platelets (10^9/L)	278.7 ± 140.7	164–510
Painful abdomen on palpation	14/25	Albumin (g/L)	17.3 ± 2.6	30–45
Fever	4/25	Total protein (g/L)	71.6 ± 5.2	55–75

Table 2. Routine parameters for renal function: laboratory results for healthy dogs (H, n = 14), dogs with pyometra (P, n = 25) and the 13 dogs with pyometra included both in the original (P′) and the follow-up study (P_{fup}) (sCr, sUN, and uSG are expressed as mean ± SD, UPC as median, range).

	$P > .05$				
	sCr, sUN: $P > .05$ UPC, uSG: $P < .0001$		sCr, sUN: $P > .05$ UPC, uSG: $P < .05$		
Parameter	H	P	P′	P_{fup}	Reference Range
sCr (μmol/L)	100.3 ± 14.3	114.1 ± 34.2	109 ± 26.4	99.0 ± 12.2	< 125
sUN (mmol/L)	4.7 ± 1.0	5.1 ± 2.9	4.9 ± 2.2	4.7 ± 0.9	3.3–8.3
UPC	0.10, 0.02–0.47	0.54, 0.10–2.13	0.56, 0.12 – 2.13	0.11, 0.05 - 0.65	< 0.5
uSG	1.029 ± 0.009	1.014 ± 0.008	1.012 ± 0.006	1.022 ± 0.013	1.015–1.045

Routine Renal Tests and Urinary Biomarkers in Dogs with Pyometra at Follow-up Visit Compared with Initial Presentation

None of the dogs with pyometra were azotemic at the follow-up visit, as defined by the International Renal Interest Society (IRIS) guidelines.[29] Light microscopic examination of the urine sediment revealed no clinically relevant abnormalities in these dogs, except for 1 dog with a pyuria and positive urine culture (*E. coli*), indicative for a urinary tract infection. For the 13 dogs examined both at the follow-up visit and at initial presentation, UPC and all urinary biomarkers were significantly lower at follow-up than at their initial presentation ($P < .01$) (Tables 2 and 3, Fig 1b). uSG and uCr (mean ± SD) were significantly increased at follow-up (uSG 1.022 ± 0.013 g/L, uCr 1.37 ± 0.64 g/L) compared with their initial values (uSG 1.012 ± 0.006, uCr 0.79 ± 0.59 g/L) ($P < .05$). In fact, the concentrations of all urinary biomarkers, including the UPC, uSG, and uCr of the dogs with pyometra at the follow-up visit returned to values not significantly different from those of healthy dogs.

Discussion

In the present study, significant increases of the urinary biomarkers are found in dogs with pyometra upon initial admission compared with control bitches of comparable age. The increased concentrations of uIgG, uCRP, uAlb, and $uTXB_2$ clearly indicate that pyometra affects the nephron at the glomerular level. Furthermore, the increased uNAG activities are in agreement with the previously reported tubular lesions in dogs with pyometra,[6,7] and the increased uRBP concentrations are most likely the result of this tubular dysfunction. The suggested superiority of the applied biomarkers in comparison to routinely used renal parameters is manifested in their ability to detect renal impairment earlier than sUN and sCr, and to localize the dysfunction. The presence of 17 nonazotemic dogs with pyometra (68%) but with increased concentrations of urinary biomarkers compared with healthy dogs illustrates this aspect. According to the IRIS guidelines for proteinuria (ie UPC > 0.5),[29] UPC is only mildly increased in dogs with pyometra, and even below 0.5 in 11 dogs with pyometra (44%), yet the urinary biomarkers detect marked differences between healthy dogs and dogs with pyometra. Moreover, even if the UPCs are different between healthy dogs and dogs with pyometra, this routine renal parameter fails to provide further information on the origin of the urinary proteins.

The appearance of IgG in urine of dogs has been linked with postinfection glomerulonephritis.[30,31] The present study corroborates these gel-electrophoresis findings for pyometra by use of the elegant ELISA method, which allows the quantification of the uIgG

Table 3. Urinary concentrations of renal biomarkers: results for healthy bitches (H, n = 14), dogs with pyometra (P, n = 25) and for the 13 dogs included both in the original (P′) and follow-up study (P_{fup}) (expressed as median and range).

	$P > .05$			
	$P < .001$		$P < .001$	
Biomarker	H	P	P′	P_{fup}
uIgG/Cr (mg/g)	3.4(0.6–8.9)	169.7 (4.81–1052.9)	197.2(13.5–631.8)	4.7(1.5–99.8)
uCRP/Cr (mg/g)	BDL (n = 14)	0.260(0.006–3.030)	0.340(0.007–3.030)	BDL (n = 12),1 dog 0.013[a]
uAlb/Cr (mg/g)	17.5(1.3–166.3)	89.5(8.8–832.7)	155.5(8.8–832.7)	13.9(2.1–471.2)
uRBP/Cr (mg/g)	0.13(0.02–0.44) BDL (n = 3)	1.66(0.05–21.44)	3.65(0.05–21.44)	0.05(0.02–0.32) BDL (n = 3)
uNAG/Cr (U/g)	2.4(1.4–7.4)	5.8(1.6–27.7)	5.8(1.6–20.0)	1.6(0.9–3.3) BDL (n = 1)
$uTXB_2$/Cr (μg/g)	2.4(1.2–4.7)	15.3(3.2–139.6)	16.3(6.4–45.0)	3.3(1.0–6.9)

BDL: below detection limit (ie, uCRP: 5.28 ng/mL; uRBP: 14.11 ng/mL[20]; uNAG: 0.84 U/L [25]).

[a]Dog with urinary tract infection. Corresponding *p*-values are indicated for analyses between the different groups of dogs.

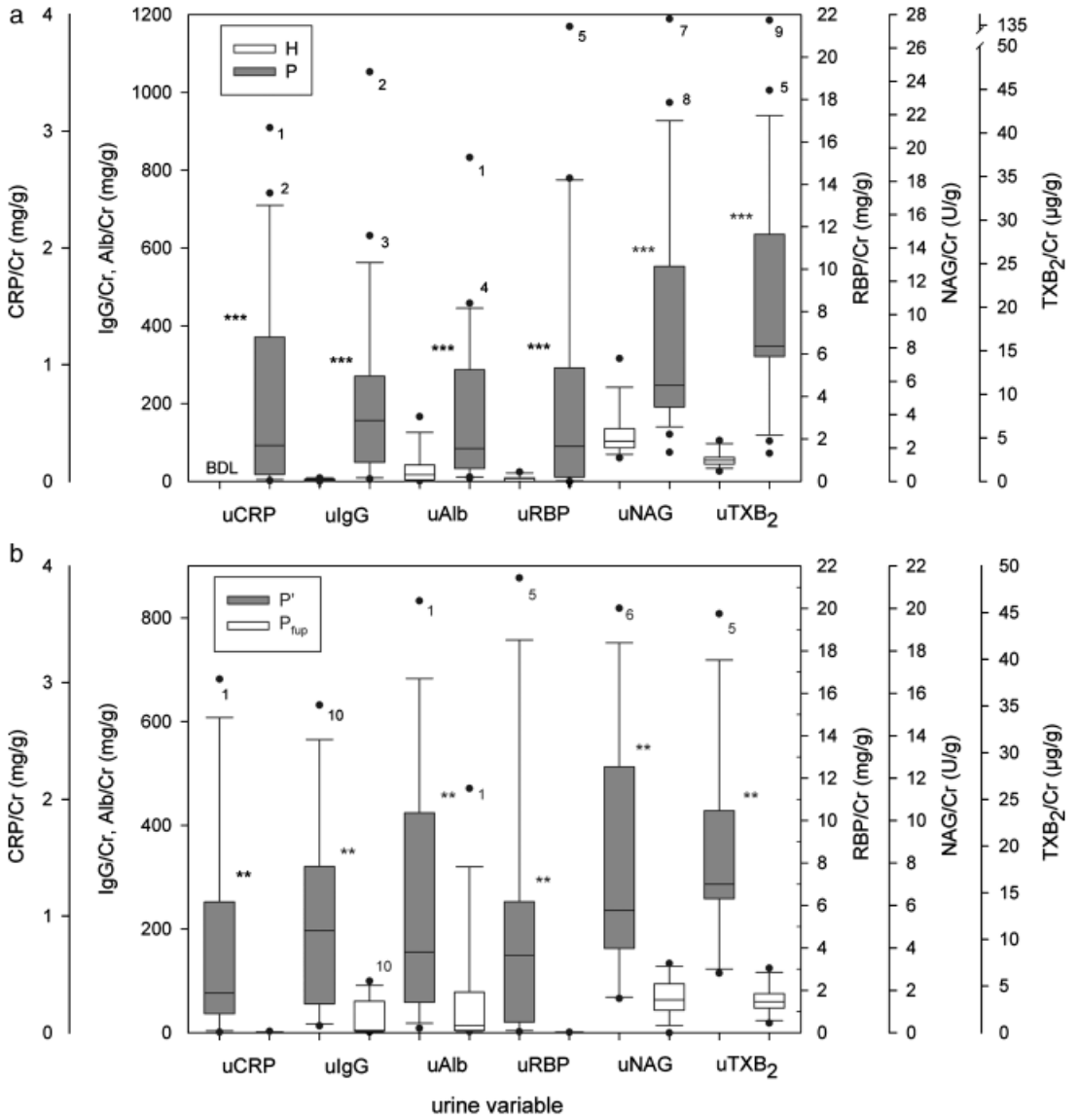

Fig 1. (**a**) Concentrations of the renal biomarkers uIgG, uCRP, uAlb, uRBP, uNAG, and $uTXB_2$ in urine of healthy dogs (H, n = 14) and dogs with pyometra (P, n = 25). (**b**) Concentrations of the renal biomarkers uIgG, uCRP, uAlb, uRBP, uNAG, and $uTXB_2$ in urine of dogs with pyometra included both in the original (P′) and follow-up (P_{fup}) study (n = 13). Concentrations are related to urinary creatinine (uCr), expressed as ratios and visualized as box plots. The box represents the interquartile range from the 25th to the 75th percentile. The horizontal bar through the box is the median. The "whiskers" represent the main body of data (10th and 90th percentiles), and all outliers are represented by dots. Numbered outliers refer to individual dogs. BDL, below detection limit (ie, uCRP: 5.28 ng/mL [20]); $^{***}P < .001$; $^{**}P < .01$.

Table 4. Correlations (*r*) between the urinary biomarkers in dogs with pyometra.

	uCRP/Cr	uAlb/Cr	uRBP/Cr	uNAG/Cr	$uTXB_2$/Cr
uIgG/Cr	0.68**	0.58**	0.31*	0.47**	0.19
	uCRP/Cr	0.64**	0.29*	0.49**	0.18
		uAlb/Cr	0.27*	0.37**	0.09
			uRBP/Cr	0.28*	0.16
				uNAG/Cr	0.15

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

concentrations. These uIgG concentrations are an indication of the severity of the altered glomerular permselectivity secondary to *E. coli* pyometra. In dogs, CRP is the major acute phase protein and increased but highly variable serum CRP concentrations have been reported in bitches with pyometra.[32] Independently of the circulating levels, the substantial urinary loss of CRP is the result of a glomerular dysfunction. Therefore, uCRP may be of added value in diagnosing initial postinfection glomerular dysfunction given the clear-cut difference between healthy dogs and dogs with pyometra.

The severe infection of the uterus and the subsequent inflammatory response affect albumin and RBP, which are both negative acute phase proteins in dogs.[33] Our data are the first to elaborate on the degree of albuminuria and retinol-binding proteinuria in dogs with pyometra. Approximately 20–30 fold higher median uAlb/Cr and uRBP/Cr ratios than those of the dogs with pyometra in the current study have been reported previously in chronic kidney disease (CKD) dogs predominantly in IRIS stages III and IV.[25] This suggests a milder glomerular and tubular dysfunction in dogs with pyometra, when compared with these CKD dogs. Surprisingly, median uNAG/Cr ratios are comparable between the dogs with pyometra and CKD dogs of the former study.[25] Although this observation suggests a more severe proximal tubular damage than assumed on the basis of uRBP/Cr, both tubular markers appear in urine by a different mechanism. From our results, it is not clear to what extent the acute phase response plays a part in the amount of uAlb and uRBP loss. In dogs like in other species, RBP is a negative acute phase protein. It is therefore highly likely that serum RBP concentrations decrease in dogs with pyometra, which is accompanied by an acute phase response. One should keep in mind that an underlying hypoalbuminemia or hyporetinol-binding proteinemia possibly influences the respective urinary biomarker concentration. Our results of uNAG parallel those of previous studies,[7,22] where the correlation between high uNAG values and severe morphological tubular lesions in dogs with pyometra has clearly been demonstrated.[7] In humans, it has been shown that the increased uNAG/Cr in renal disease does not originate from filtration because of glomerular damage but from the tubular epithelial cells.[34] Furthermore, based on the mildly increased UPC and uAlb/Cr ratios in dogs with pyometra, only a mild glomerular dysfunction can be concluded. Enzymuria originating from increased glomerular filtration of serum enzymes is therefore unlikely.[35]

Interestingly, $uTXB_2$ is not correlated with the other 5 urinary biomarkers in dogs with pyometra, suggesting a different underlying mechanism responsible for its urinary appearance. Indeed, TXB_2 is an eicosanoid while the other renal biomarkers are proteins. Thromboxane B_2 is only produced in small quantities in healthy kidneys,[23] where it mainly functions as a potent vasoconstrictor. In human pathological conditions, TXB_2 is associated with reduced glomerular filtration rate (GFR) and increased proteinuria.[36] However, a previous study in dogs with pyometra detected no general reduction in GFR.[7] Different sources of TXB_2, such as platelets and renal cells, and a diversity of potential functions including a role in the pathogenesis of proteinuria in dogs with immune complex-mediated glomerulonephritis have been assumed for this metabolite.[36–38] Although this complicates the interpretation of this marker, the observed different $uTXB_2$/Cr ratios between healthy dogs and dogs with pyometra must encourage further research to elucidate its exact contribution in various protein-losing nephropathies in dogs.

In this study, spot urinary biomarker to creatinine ratios were used. Urinary NAG/Cr has been shown to correlate to 24-hour excretion in dogs[27] and although previous studies have reported the use of uAlb/Cr and uRBP/Cr in dogs,[21,39] there is still lack of evidence that supports the correlation between the urinary biomarkers and urinary creatinine in dogs. The use of these ratios and the fluctuation in excretion or filtration of the urinary biomarkers throughout the day remains an important goal for future research.

With the exception of medications with known nephrotoxic or other renal effects (eg, aminoglycosides, corticosteroids, ACE-inhibitors, furosemide), we have no information on how the antibiotics or NSAIDS administered to some dogs before ovariohysterectomy may affect urinary biomarkers. Therefore, this might be a confounding factor in interpreting urinary biomarkers in these dogs with pyometra and requires further research.

It should be remarked that 2 dogs with pyometra developed acute renal failure after the ovariohysterectomy. Values for the routine renal parameters and the urinary biomarkers in both dogs were among the highest within the group of dogs with pyometra. Treatment of these dogs before urine sampling was similar as in the other dogs with pyometra. Advanced age, anesthesia, dehydration, and sepsis probably are risk factors for developing acute renal failure after pyometra. Therefore, supportive therapy including adequate fluid administration remains important in such dogs.

Six dogs with pyometra had abnormalities on light microscopic evaluation of the urine sediment and four of them had a positive urine culture. A previous study reported that cystitis can cause proteinuria,[40] whereas another study concluded that the majority of pyuric urine samples were not albuminuric and had normal UPC ratios.[41] However, 33 and 19% of urine samples with pyuria had increased urinary albumin concentrations and UPC ratios, respectively. Furthermore, the combination of hematuria, bacteriuria, and pyuria affected urinary albumin concentrations in a larger amount of samples.[41] Recent work from our group suggested no effect of ex vivo addition of white blood cells, red blood cells, and bacteria to urine samples regarding uAlb/Cr, uRBP/Cr, and uNAG/Cr.[42] However, the latter is not equivalent to tissue infection in vivo. Although the urinary biomarker concentrations of the mentioned 6 dogs with pyometra did not significantly differ from the median value of the remainder of the pyometra group, the effect of pyuria, hematuria, and bacteriuria on the urinary biomarkers needs to be addressed in future research. In the current study, a positive urine culture might still be the result of a cystitis or a pyelonephritis. Indeed, urinary tract infection is a common complication of pyometra and may have affected urinary biomarker levels.[6] Nevertheless, although no clinical signs of lower urinary tract infection were noted in the dogs with pyometra, probably because of the lethargic state of the dogs or the coincidence of the polyuria-polydipsia, the presence of pyelonephritis is less likely in the dogs with the positive urine culture because of the absence of fever and ultrasonographic findings typical for pyelonephritis.[43]

Finally, a high variance for the renal biomarkers is observed in the group of dogs with pyometra. Infection-

related renal manifestations, such as glomerulonephritis, acute tubular necrosis, tubulointerstitial nephritis, or vasculitis, are also reported to be variable in humans and to depend on both host- and organism-related factors.[2] However, all dogs with pyometra suffered from infection with the same bacterial species. Therefore, we suggest that individual host-related responses to the kidneys' insult are likely the main cause for this observed variation of renal biomarkers within each group of dogs.

At the 6-month postovariohysterectomy follow-up visit for the dogs with pyometra, all urinary biomarkers had decreased significantly and were not different from those of healthy dogs, indicating a transient renal dysfunction secondary to the *E. coli* pyometra. This temporary nature is in agreement with previous studies where the role of the uterine infection in the origin of the renal dysfunction and the subsequent recovery after hysterectomy was demonstrated.[6–8] IgG is an everpresent part of the humoral immunity and consequently, it is preferable to uCRP for monitoring altered glomerular permselectivity in dogs without systemic inflammatory response. At the follow-up visit, uCRP concentrations were not detectable. It has been reported that acute phase protein concentrations in dogs decrease rapidly after appropriate treatment.[32,33] None of the dogs had an inflammatory disease at the follow-up visit, except 1 dog with a cystitis. Interestingly, this latter dog had a detectable albeit strongly decreased uCRP concentration compared with initial admission.

The studied urinary proteins are markers associated with different nephron segments. Although our data cannot elucidate the stepwise mechanism of glomerular and tubular dysfunction, the significant correlation between uIgG, uCRP, uAlb, uRBP, and uNAG is suggestive for a close underlying interaction in the processes involved in their urinary appearance. In this study, uSG remains valuable for detecting renal dysfunction. Moreover, an other marker for distal tubular dysfunction, such as Tamm-Horsfall protein, was not included in the selected panel of biomarkers.[21] Inclusion of a higher number of dogs might have prevented one of the limitations of this study, namely the high variance in the pyometra group. However, it should be highlighted that the existence of an individual kidney function response remains a challenge in infection-related research studies.[44,45]

Pyometra can be discriminated from cystic endometrial hyperplasia (CEH) and other uterine pathologies based on a combination of clinical signs, ultrasonography, and bacterial culture of uterine fluid.[26] Because these findings matched the diagnosis of pyometra as a clinical entity in the included dogs, histological examination of the uterine wall was not performed in the current study. Nevertheless, histological evidence of uterine inflammation could have definitively diagnosed the presence of pyometra in the studied dogs. Future studies should include dogs with CEH alone and dogs with the CEH-pyometra complex to investigate if the origin of the renal dysfunction and of the increased urinary biomarker concentrations is bacteria- or CEH-related.

Results of this study suggest that postpyometra nephropathy is transient in most dogs, and often resolves with adequate supportive care. However, 2 of 25 dogs with pyometra (8%) developed acute renal failure and were euthanized. Future research might therefore focus on defining the subset of dogs with pyometra and clinically relevant kidney injury. Furthermore, it would be helpful to evaluate the correlation of selected urinary biomarkers with histological lesions in dogs with postpyometra nephropathy. The observed clear but apparently reversible lesions in most of the affected dogs makes pyometra a good candidate for a model of nephropathy that would allow prospective evaluation of other early renal biomarkers.

Footnotes

[a] Rimadyl, Pfizer A.H., London, UK
[b] Placivet, Codifar, Wommelgem, Belgium
[c] Valium, Roche, Anderlecht, Belgium
[d] Propovet 10 mg/mL, Abbott Lab, Maidenhead, UK
[e] Nembutal, CEVA Santé Animale, Haren, Belgium
[f] Isoflo, Abbott Lab
[g] Multistix 8 SG, Bayer Diagnostics Mfg., Ltd, Bridgend, UK
[h] Roche Diagnostics Urinary/CSF Protein 911 analyser, Basel, Switzerland
[i] Dog IgG ELISA-kit, Immunology Consultants Laboratory, Newberg, OR
[j] Dog CRP ELISA-kit, Immunology Consultants Laboratory
[k] Dog albumin ELISA kit, Immunology Consultants Laboratory
[l] Immunology Consultants Laboratory
[m] Sigma-Aldrich, St Louis, MO
[n] Assay designs, Ann Arbor, MI
[o] Multiskan MS, Labsystems Thermo Fisher Scientific, Waltham, MA
[p] Deltasoft JV, BioMetallics Incorporated, Princeton, NJ
[q] SPSS 15, SPSS Inc, Chicago, IL

Acknowledgments

This research was funded by the Bijzonder Onderzoeksfonds of the University of Ghent (BOF, grant to B.E.J. Maddens). The authors are grateful to K. Demeyere for her excellent technical assistance.

References

1. Grauer GF. Canine glomerulonephritis: New thoughts on proteinuria and treatment. J Small Anim Pract 2005;46:469–478.

2. Naicker S, Fabian J, Naidoo S, et al. Infection and glomerulonephritis. Semin Immunopathol 2007;29:397–414.

3. Rodriguez-Iturbe B, Musser JM. The current state of poststreptococcal glomerulonephritis. J Am Soc Nephrol 2008;19:1855–1864.

4. Asheim A. Pathogenesis of renal damage and polydipsia in dogs with pyometra. J Am Vet Med Assoc 1965;147:736–745.

5. de Schepper J, van der Stock J, Capiau E, et al. Renal injury in dogs with pyometra. Tijdschr Diergeneeskd 1987;112(Suppl 1):124S–126S.

6. Heiene R, Kristiansen V, Teige J, et al. Renal histomorphology in dogs with pyometra and control dogs, and long term clinical outcome with respect to signs of kidney disease. Acta Vet Scand 2007;49:13–22.

7. Heiene R, Moe L, Molmen G. Calculation of urinary enzyme excretion, with renal structure and function in dogs with pyometra. Res Vet Sci 2001;70:129–137.

8. Obel AL, Nicander L, Asheim A. Light and electron microscopic studies of the renal lesion in dogs with pyometra. Acta Vet Scand 1964;5:93–125.

9. Stone EA, Littman MP, Robertson JL, et al. Renal dysfunction in dogs with pyometra. J Am Vet Med Assoc 1988;193: 457–464.

10. Faldyna M, Laznicka A, Toman M. Immunosuppression in bitches with pyometra. J Small Anim Pract 2001;42:5–10.

11. Hagman R, Kindahl H, Fransson BA, et al. Differentiation between pyometra and cystic endometrial hyperplasia/mucometra in bitches by prostaglandin F-2 alpha metabolite analysis. Theriogenology 2006;66:198–206.

12. Sandholm M, Vasenius H, Kivisto AK. Pathogenesis of canine pyometra. J Am Vet Med Assoc 1975;167:1006–1010.

13. Newman SJ, Confer AW, Panciera RJ. Urinary system. In: McGavin MD, Zachary JF, eds. Pathologic Basis of Veterinary Disease, 4th ed. St Louis, MO: Mosby-Elsevier; 2006:613–652.

14. Bartoskova A, Vitasek R, Leva L, et al. Hysterectomy leads to fast improvement of haematological and immunological parameters in bitches with pyometra. J Small Anim Pract 2007;48:564–568.

15. Price RG. The role of NAG (N-acetyl-beta-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. Clin Nephrol 1992;38(Suppl 1):S14–S19.

16. Sarasua SM, Mueller P, Kathman S, et al. Confirming the utility of four kidney biomarker tests in a longitudinal follow-up study. Ren Fail 2003;25:797–817.

17. Stuveling EM, Bakker SJ, Hillege HL, et al. Biochemical risk markers: A novel area for better prediction of renal risk? Nephrol Dial Transplant 2005;20:497–508.

18. Lees GE, Brown SA, Elliott J, et al. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM forum consensus statement (small animal). J Vet Intern Med 2005;19:377–385.

19. D'Amico G, Bazzi C. Pathophysiology of proteinuria. Kidney Int 2003;63:809–825.

20. Maddens B, Daminet S, Demeyere K, et al. Validation of immunoassays for renal biomarkers in canine urine. Vet Immunol Immunopathol 2010;13:259–264.

21. Raila J, Forterre S, Kohn B, et al. Effects of chronic renal disease on the transport of vitamin A in plasma and urine of dogs. Am J Vet Res 2003;64:874–879.

22. Sato R, Soeta S, Miyazaki M, et al. Clinical availability of urinary N-acetyl-beta-D-glucosaminidase index in dogs with urinary diseases. J Vet Med Sci 2002;64:361–365.

23. Imig JD. Eicosanoid regulation of the renal vasculature. Am J Physiol Renal Physiol 2000;279:F965–F981.

24. Nasrallah R, Clark J, Hebert RL. Prostaglandins in the kidney: Developments since Y2K. Clin Sci 2007;113:297–311.

25. Smets P, Meyer E, Maddens B, et al. Urinary markers in healthy young and aged dogs and dogs with chronic kidney disease. J Vet Intern Med 2010;24:65–72.

26. Bigliardi E, Parmigiani E, Cavirani S, et al. Ultrasonography and cystic hyperplasia-pyometra complex in the bitch. Reprod Domest Anim 2004;39:136–140.

27. Grauer GF, Greco DS, Behrend EN, et al. Estimation of quantitative enzymuria in dogs with gentamicin-induced nephrotoxicosis using urine enzyme creatinine ratios from spot urine samples. J Vet Intern Med 1995;9:324–327.

28. Bartels H, Bohmer M, Heierli C. Serum creatinine determination without protein precipitation. Clin Chim Acta 1972;37: 193–197.

29. Polzin DJ. Chronic kidney disease. In: Ettinger SJ, Feldman EC, eds. Textbook of Veterinary Internal Medicine. St Louis, MO: Saunders Elsevier; 2010:1990–2021.

30. Zaragoza C, Barrera R, Centeno F, et al. SDS-PAGE and Western blot of urinary proteins in dogs with leishmaniasis. Vet Res 2003;34:137–151.

31. Zaragoza C, Barrera R, Centeno F, et al. Canine pyometra: A study of the urinary proteins by SDS-PAGE and Western blot. Theriogenology 2004;61:1259–1272.

32. Dabrowski R, Wawron W, Kostro K. Changes in CRP, SAA and haptoglobin produced in response to ovariohysterectomy in healthy bitches and those with pyometra. Theriogenology 2007;67:321–327.

33. Paltrinieri S. Early biomarkers of inflammation in dogs and cats: The acute phase proteins. Vet Res Commun 2007;31:125–129.

34. Costigan MG, Rustom R, Bone JM, et al. Origin and significance of urinary N-acetyl-beta-D-glucosaminidase (NAG) in renal patients with proteinuria. Clin Chim Acta 1996;255:133–144.

35. Grauer GF. Early detection of renal damage and disease in dogs and cats. Vet Clin North Am Small Anim Pract 2005;35:581–596.

36. Wardle EN. Thromboxanes in glomerulonephritis: What about therapy? Am J Ther 1999;6:111–114.

37. Grauer GF, Frisbie DD, Longhofer SL, et al. Effects of a thromboxane synthetase inhibitor on established immune-complex glomerulonephritis in dogs. Am J Vet Res 1992;53:808–813.

38. Longhofer SL, Frisbie DD, Johnson HC, et al. Effects of thromboxane synthetase inhibition on immune-complex glomerulonephritis. Am J Vet Res 1991;52:480–487.

39. Schellenberg S, Mettler M, Gentilini F, et al. The effects of hydrocortisone on systemic arterial blood pressure and urinary protein excretion in dogs. J Vet Intern Med 2008;22:273–281.

40. Bagley RS, Center SA, Lewis RM, et al. The effect of experimental cystitis and iatrogenic blood contamination on the urine protein/creatine ratio in the dog. J Vet Intern Med 1991;5:66–70.

41. Vaden SL, Pressler BM, Lappin MR, et al. Effects of urinary tract inflammation and sample blood contamination on urine albumin and total protein concentrations in canine urine samples. Vet Clin Pathol 2004;33:14–19.

42. Smets P, Meyer E, Maddens B, et al. Effect of sampling method and storage conditions on albumin, retinol-binding protein and N-acetyl-β-D-glucosaminidase concentrations in canine urine samples. J Vet Diagn Invest 2010;22. In press.

43. Neuwirth L, Mahaffey M, Crowell W, et al. Comparison of excretory urography and ultrasonography for detection of experimentally induced pyelonephritis in dogs. Am J Vet Res 1993;54:660–669.

44. LaClair R, O'Neal K, Ofner S, et al. Precision of biomarkers to define chronic inflammation in CKD. Am J Nephrol 2008;28: 808–812.

45. Zeledon JI, McKelvey RL, Servilla KS, et al. Glomerulonephritis causing acute renal failure during the course of bacterial infections. Histological varieties, potential pathogenetic pathways and treatment. Int Urol Nephrol 2008;40:461–470.