

THE PROGNOSTIC VALUE OF MICROALBUMINURIA IN PUPPIES WITH CANINE PARVOVIRAL ENTERITIS

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Increased urine albumin concentration (UALB) or urine albumin-to-creatinine ratio (UACR) at admission has been associated with systemic disease and increased morbidity and mortality in critically ill canine patients. The objective of this study was to assess the prognostic value of UALB and UACR for the survival, as well as for the development and duration of systemic inflammatory response syndrome (SIRS) in puppies with canine parvoviral enteritis (CPVE). Unvaccinated puppies, aged 1-12 months with confirmed CPVE, hospitalized for ≥ 5 days were included. Urine was collected at admission via cystocentesis; albumin was measured immunoturbidimetrically and creatinine spectrophotometrically. The presence of SIRS was daily evaluated. Statistical analysis was conducted using R language. Twenty-six dogs were enrolled; 12/26 (46%) developed SIRS during hospitalization, while 5/26 (19%) died. A significant correlation was found between UALB and UACR ($\rho=0.868$, $p<0.001$). The dogs with SIRS had higher median UALB [0.5 (0-12.7) mg/dL] and UACR [4.2 (0-2,093) mg/g] compared to dogs without SIRS [UALB= 0.1 (0-0.8) mg/dL, UACR= 1.6 (0-5.6) mg/g], but the differences were non-significant ($p>0.05$). SIRS duration was significantly correlated with UACR ($\rho=0.427$, $p=0.030$), but not with UALB ($\rho=0.386$, $p=0.052$). The non-survivors had higher median UALB [0.6 (0.1-12.7) mg/dL] and UACR [19.6 (0.7-2,093) mg/g] compared to survivors [UALB= 0.2 (0-1.5) mg/dL, UACR= 2.3 (0-16.9) mg/g], but the differences were non-significant ($p>0.05$). UACR appears to be a prognostic indicator of SIRS duration in puppies with CPVE. However, a large-scale study is warranted to confirm the usefulness of UALB and UACR for clinical risk assessment in puppies with CPVE.

Key words: dog, inflammation, SIRS, systemic inflammatory response syndrome, urine albumin, urine creatinine

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INTRODUCTION

Canine parvovirus enteritis (CPVE) is one of the most common infectious disorders and a significant cause of morbidity and mortality in dogs younger than 6 months [1]. The etiological agent, canine parvovirus (CPV)-2, is a small, non-enveloped DNA virus with tropism to rapidly dividing cells, which can naturally infect almost all *Canidae* species [1].

Dogs with CPVE often develop systemic inflammatory response syndrome (SIRS), which can eventually lead to multiple organ dysfunction syndrome and death [2]. Studies in human medicine have shown that SIRS and sepsis are characterized by massive production of pro-inflammatory cytokines and activation of coagulation cascade, subsequently leading to increased vascular permeability [3,4]. The latter may be associated with increased urinary excretion of plasma proteins [5,6]. Increased urine albumin concentration (UALB) or urine albumin-to-creatinine ratio (UACR) at admission has been associated with systemic disease in dogs and cats [7,8] and increased morbidity and mortality in critically ill human and canine patients [9,10].

Our hypothesis was that UALB and UACR measured at admission, act as prognostic indicators of the outcome, SIRS development, and SIRS duration in puppies with CPVE. Therefore, the aim of this study was to assess the prognostic value of UALB and UACR for the survival, as well as the development and duration of SIRS in puppies with CPVE.

MATERIALS AND METHODS

The present study took place at the Clinic of Companion Animals, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece. The inclusion criteria were the following: a) age between 1 to 12 months; b) no previous treatment and no vaccination against CPV during the preceding 2 weeks of admission; c) clinical signs compatible with CPVE; d) absence of clinical or diagnostic imaging findings indicative of foreign body, intussusception, or other morphological abnormalities of the gastrointestinal tract; e) positive ELISA fecal test for CPV antigen (Canine Parvovirus Antigen Test Kit, IDEXX Laboratories, Westbrook, USA) and positive PCR for CPV using a previously described method [11]; f) negative ELISA fecal test for *Giardia* antigen (*Giardia* Antigen Test Kit, IDEXX Laboratories, Westbrook, USA); g) negative fecal parasitological examination (fecal zinc sulphate flotation) for intestinal parasites and protozoa performed at admission and every 48h; h) hospitalization for at least 5 days; i) owner's written consent.

In all dogs included in the present study, a complete physical examination was performed 3 times a day. Blood samples were collected daily via jugular venipuncture into K₃EDTA tubes (Potassium - EDTA, Vet Collect tubes, IDEXX, UK) and a complete blood count was performed on the automated hematology analyzer Advia 120

(Siemens Healthcare Diagnostics, Deerfield, USA). Urine was collected at admission via cystocentesis; albumin concentration was measured using a previously validated method [12], while creatinine concentration was measured spectrophotometrically. Systemic inflammatory response syndrome was defined as the presence of at least three of the following criteria: heart rate >140 beats/min, respiratory rate >30 breaths/min, body temperature >39.2°C or <37.8°C, and total white blood cell count >17,000 cells/ μ L or <6,000/ μ L. The aforementioned criteria were daily evaluated for each dog during the hospitalization period.

The data distribution was assessed using the Shapiro-Wilk test. The exact Wilcoxon rank-sum test was used for the median comparison between two independent groups, while the Spearman's rank correlation coefficient was employed for the determination of the correlation between two variables. Receiver operator characteristics (ROC) analysis was carried out to assess the performance of UACR in predicting survival. The statistical analyses were conducted using R statistical language (R Foundation for Statistical Computing, Vienna, Austria).

Informed consent: informed consent has been obtained for client-owned animals included in this study.

RESULTS

In total, 26 dogs (12 males, 14 females) were enrolled in this study. The mean (\pm SD) age of the canine population was 3.9 ± 1.9 months. Forty-six percent (12/26) of the puppies developed SIRS during hospitalization, while 19% (5/26) of them died. A statistically significant and very good positive correlation was found between UALB and UACR ($\rho=0.868$, $p<0.001$). The median (range) UALB was higher in dogs that developed SIRS during hospitalization [0.5 (0-12.7) mg/dL] compared to dogs without SIRS [0.1 (0-0.8) mg/dL]. Similarly, the median UACR was also higher in dogs that developed SIRS [4.2 (0-2,093) mg/g] compared to dogs without SIRS [1.6 (0-5.6) mg/g]. However, the observed differences for both UALB and UACR were statistically non-significant ($p=0.113$ and $p=0.107$, respectively). No statistically significant correlation was observed between UALB and SIRS duration ($\rho=0.386$, $p=0.052$), whereas a statistically significant, moderate positive correlation was detected between UACR and SIRS duration ($\rho=0.427$, $p=0.030$). The median UALB was higher in non-survivors [0.6 (0.1-12.7) mg/dL] compared to survivors [0.2 (0-1.5) mg/dL], but the difference was proven to be statistically non-significant ($p=0.123$). The median UACR was also higher in dogs with negative outcome [19.6 (0.7-2,093) mg/g] compared to dogs with positive outcome [2.3 (0-16.9) mg/g], with the difference being statistically non-significant though ($p=0.087$). The area under the ROC curve (AUC) for UACR was 0.752 (95% confidence intervals: 0.443-1.000) for survival prediction (Figure 1). The optimal cut-off value was 18.3 mg/g (sensitivity: 60%, specificity: 100%).

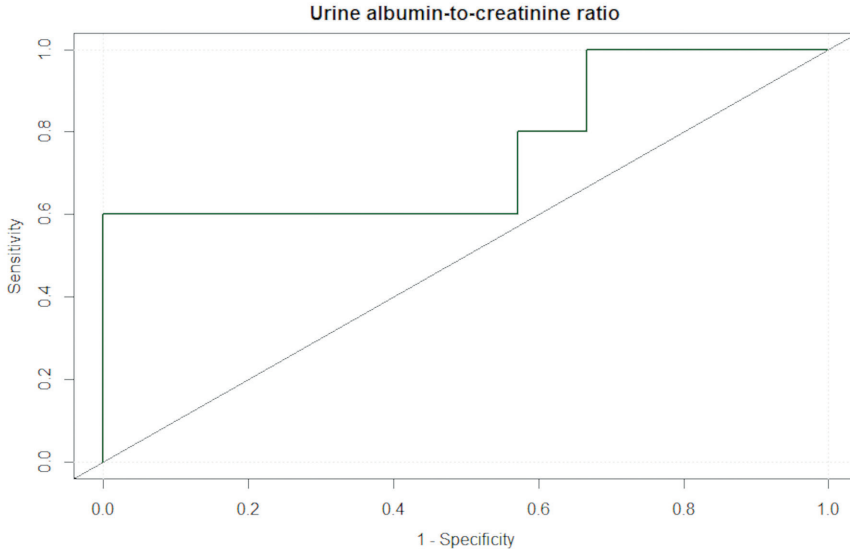


Figure 1. Outcome prediction receiver operating characteristics (ROC) curve for urine albumin-to-creatinine ratio (UACR) for 26 puppies naturally infected with canine parvoviral enteritis. The green line is the ROC curve, while the black diagonal represents the null hypothesis. The area under the ROC curve is 0.752 (95% confidence interval: 0.443-1.000).

DISCUSSION

In the present study, the prognostic value of UALB and UACR, measured at admission, for survival, SIRS development, and SIRS duration was investigated. Increased UALB or UACR at admission has been previously documented in dogs and cats with systemic disease [7,8] and have been related to increased morbidity and mortality in critically ill human and canine patients [9,10]. To the best of our knowledge, the present study is the first attempt to investigate the prognostic value of UALB and UACR for the survival, as well as the development and duration of SIRS in puppies naturally infected with CPVE.

Canine parvoviral enteritis is one of the most important infectious diseases of puppies due to its high prevalence and high mortality rate [1]. The intestinal tract damage by the CPV-2 and the compromised immune system increase the risk of SIRS due to the hematogenous spread of intestinal bacterial toxins and pro-inflammatory cytokines, such as tumour necrosis factor [13]. In fact, SIRS is commonly documented in dogs with CPVE and can lead to multiple organ dysfunction syndrome and death [2].

Urine albumin was very well correlated with UACR; however, our results suggest that the latter may be a better prognostic indicator in puppies with CPVE. Urine albumin was devoid of any prognostic value in puppies naturally infected with CPVE. On the other hand, a moderate positive correlation between UACR at admission and

SIRS duration was demonstrated, indicating that UACR might be useful in predicting the duration of SIRS in hospitalized puppies with CPVE. Altered urinary protein excretion has been previously documented in dogs with SIRS [14]; however, to our knowledge, this is the first study reporting an association between UACR at admission with the duration of SIRS in the hospitalization period.

The early phase of acute inflammation is characterized by increased vascular permeability, which allows the transmigration of neutrophils and the leakage of plasma proteins, including albumin. In human medicine, there are several experimental and clinical studies suggesting the increased vascular permeability as the mechanism that lies behind the association between microalbuminuria and SIRS. Specifically, increased transcapillary leakage rate of radiolabelled albumin has been found in human patients with sepsis, neoplasia, or after cardiac surgery [5]. Systemic endothelial dysfunction has also been associated with albumin transcapillary leakage rate and urine albumin in patients with diabetes or hypertension [15]. Furthermore, in a clinical setting, it has been documented that UALB was associated with the severity of inflammatory events [16].

Although, UALB and UACR were higher in dogs with negative outcome compared to dogs with positive outcome, the observed differences were proven to be statistically non-significant. Nonetheless, it is worthy of mentioning that UACR was significantly higher in non-survivors at 0.10 significance level compared to survivors. Thus, the results of this pilot study should be interpreted cautiously, especially in the context of a small sample size. The substandard AUC for UACR was similar to that previously reported in critically ill dogs [10]. The calculated optimal cut-off value of 18.3 mg/g is characterized by excellent specificity (100%), but lacks a substantial sensitivity (60%).

In conclusion, our results are suggestive of the potentially adjunct role of UACR in predicting SIRS duration and survival in puppies with CPVE. Nevertheless, this study shares a limitation with other pilot studies, which is the small sample size. However, it could be a trigger for a large-scale prospective study, which is warranted to confidently determine the usefulness of microalbuminuria for clinical risk assessment in puppies with CPVE.

Authors' contributions:

OIL did the statistical analysis, wrote the manuscript, and was involved in the routine laboratory work (e.g. hematology analysis) of this study. SN was responsible for the study design and was involved as a clinician (clinical examinations, sampling and management of the patients) in the study. CJJ did the measurement of urine analysis of this study. TK and RT were involved as clinicians (clinical examinations, sampling and management of the patients) in the study. PZ was involved in the routine laboratory work (e.g. biochemical analysis) and was the coordinator of the study. All authors have approved the final version of the manuscript.

Declaration of conflicting interests:

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals could be published in this journal.

REFERENCES

1. Greene CE, Decaro N: Canine viral enteritis, in: Greene CE (Ed.), *Infectious diseases of the dog and cat*. Elsevier Saunders, St. Louis, Missouri, USA, 2012: 67-75.
2. Prittie J: Canine parvoviral enteritis: a review of diagnosis, management, and prevention. *J Vet Emerg Crit Care* 2004, 14:167-176.
3. Nyström PO: The systemic inflammatory response syndrome: definitions and aetiology. *J Antimicrob Chemother* 1998, 41:1-7.
4. Smith JW, Gamelli RL, Jones SB, Shankar R: Immunologic Responses to Critical Injury and Sepsis. *J Intensive Care Med* 2006, 21:160-172.
5. Fleck A, Hawker F, Wallace PI, Raines G, Trotter J, Ledingham IM, Calman KC: Increased vascular permeability: a major cause of hypoalbuminaemia in disease and injury. *Lancet* 1985, 325:781-784.
6. Gosling P, Brudney S, McGrath L, Riseboro S, Manji M: Mortality prediction at admission to intensive care: A comparison of microalbuminuria with acute physiology scores after 24 hours. *Crit Care Med* 2003, 31:98-103.
7. Whittemore JC, Gill VL, Jensen WA, Radecki SV, Lappin MR: Evaluation of the association between microalbuminuria and the urine albumin-creatinine ratio and systemic disease in dogs. *J Amer Vet Med Assoc* 2006, 229:958-963.
8. Whittemore JC, Miyoshi Z, Jensen WA, Radecki SV, Lappin MR: Association of microalbuminuria and the urine albumin-to-creatinine ratio with systemic disease in cats. *J Amer Vet Med Assoc* 2007, 230:1165-1169.
9. Gopal S, Carr B, Nelson P: Does microalbuminuria predict illness severity in critically ill patients on the intensive care unit? A systematic review. *Crit Care Med* 2006, 34:1805-1810.
10. Whittemore JC, Marcum BA, Mawby DI, Coleman MV, Hackett TB, Lappin MR: Associations among Albuminuria, C-Reactive Protein Concentrations, Survival Predictor Index Scores, and Survival in 78 Critically Ill Dogs. *J Vet Intern Med* 2011, 25:818-824.
11. Buonavoglia C, Martella V, Pratelli A, Tempesta M, Cavalli A, Buonavoglia D, Bozzo G, Elia G, Decaro N, Carmichael L: Evidence for evolution of canine parvovirus type 2 in Italy. *J Gen Virol* 2001, 82:3021-3025.
12. Gentilini F, Dondi F, Mastroianni C, Giunti M, Calzolari C, Gandini G, Mancini D, Famigli Bergamini D: Validation of a human immunoturbidimetric assay to measure canine albumin in urine and cerebrospinal fluid. *J Vet Diagn Invest* 2005, 17:179-183.

13. Otto CM, Drobatz KJ, Soter C: Endotoxemia and Tumor Necrosis Factor Activity in Dogs With Naturally Occurring Parvoviral Enteritis. *J Vet Intern Med* 1997, 11:65-70.
14. Schaefer H, Kohn B, Schweigert FJ, Raila J: Quantitative and Qualitative Urine Protein Excretion in Dogs with Severe Inflammatory Response Syndrome. *J Vet Intern Med* 2011, 25:1292-1297.
15. Pedrinelli R, Dell’Omo G, Penno G, Mariani M: Non-diabetic microalbuminuria, endothelial dysfunction and cardiovascular disease. *Vasc Med* 2001, 6:257-264.
16. De Gaudio AR, Spina R, Di Filippo A, Feri M: Glomerular permeability and trauma: A correlation between microalbuminuria and Injury Severity Score. *Critic Care Med* 1999, 27:2105-2108.

PROGNOSTIČKI ZNAČAJ MIKROALBUMINURIJE KOD ŠTENADI SA PARVOVIRUSNIM ENTERITISOM

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Povećane koncentracije albumina u mokraći (UALB) odnosno albumin-kreatin odnos u urinu (UARC) je povezan sa sistemskim oboljenjima i povećanim morbiditetom i mortalitetom kod kritično obolelih pasa. Cilj studije je bio da se proceni prognostički značaj UALB i UACR u odnosu na stepen preživljavanja, kao i razvoj sindroma sistemskog zapaljenskog odgovora (SIRS) i dužine trajanja kod štenadi obolelih od parvovirusnog enteritisa (CPVE). Posmatrani su nevakcinisani štenci, starosti 1-12 meseci, sa potvrđenim CPVE, koji su bili hospitalizovani ≥ 5 dana. Urin je sakupljan cistocentezom, prilikom prijema životinja u bolnicu; obavljano je imunoturbidimetrijsko merenje albumina uz spektrofotometrijsko određivanje kreatinina. Svakodnevno je određivano prisustvo SIRS. Statistička analiza je obavljena upotrebom R-jezika. Obrađeno je 26 pasa: 12/26 (46%) je razvilo SIRS tokom hospitalizacije, pri čemu je 5/26 (19%) uginulo. Značajna korelacija je uočena između UALB i UARC ($p=868$, $p<0,001$). Psi sa SIRS su imali veće prosečne vrednosti UALB [0,5 (0-12,7) mg/dL] i UACR [4,2 (0-2,093) mg/g] u poređenju sa psima bez SIRS [UALB= 0,1 (0-0,8) mg/dL, UACR= 1,6 (0-5,6) mg/g], ali je razlika bila statistički beznačajna ($p>0,05$). Trajanje SIRS je bilo u statistički značajnoj korelaciji sa UACR ($\rho=0,427$, $p=0,030$), ali ne i sa UALB ($\rho=0,386$, $p=0,052$). Životinje koje nisu preživele oboljenje imale su veće srednje vrednosti UALB [0,6 (0,1-12,7) mg/dL] and UACR [19,6 (0,7-2,093) mg/g] u poređenju sa psima koji su preživeli [UALB= 0,2 (0-1,5) mg/dL, UACR= 2,3 (0-16,9) mg/g], međutim razlike nisu bile statistički značajne ($p>0,05$). Na osnovu rezultata može da se zaključi da se UACR može da koristi kao prognostički indikator trajanja SIRS kod štenadi sa CPVE. Međutim, neophodno je da se obavi obuhvatnija studija, a radi potvrđivanja upotrebljivosti UALB i UACR u cilju procene kliničkog rizika kod štenadi sa CPVE.