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Pilot study on serum C-reactive protein in pet rabbits: clinical usefulness

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ABSTRACT

Objectives The present study was performed to evaluate the usefulness of serum C-reactive protein (CRP) as an acute phase reactive protein in pet rabbits in clinical practice.

Methods The CRP level using a rabbit CRP ELISA and white blood cell (WBC) count in pet rabbits (30 healthy controls and 62 with various diseases) were measured in the clinical practice setting. The CRP level and WBC count were measured before and after ovariohysterectomy of a healthy rabbit and a rabbit with uterine adenocarcinoma. The association between the CRP level and mortality in rabbits with various diseases was assessed.

Results The CRP level in healthy controls was 0.52±0.82 mg/dl (mean±SD). No age and sex-related differences in neither the CRP level nor WBC count were observed in the healthy control rabbits. The CRP levels in rabbits with gastrointestinal disease (n=22, 11.74±22.89 mg/dl), reproductive and urinary system disease (n=20, 21.19±49.68 mg/dl), dental disease (n=6, 4.87±5.47 mg/dl) and musculoskeletal disease (n=4, 85.66±107.28 mg/dl) were significantly higher than those in healthy controls. The CRP levels in rabbits with neurological disease (n=7, 2.55±1.79 mg/dl) and dermatological disease (n=3, 8.84±7.71 mg/dl) were higher than those in healthy controls, but no significant difference was observed. The WBC counts were not significantly different between rabbits with diseases and healthy controls. Serum samples were collected from two rabbits before and after ovariohysterectomy. In both rabbits, the CRP peaked on postoperative day 1, but no obvious WBC peak was observed. The mortality rate increased as the CRP level increased; the mortality rate was significantly higher in rabbits with a CRP level of ≥100 mg/dl than of <10 mg/dl.

Conclusions This study indicates that the serum CRP level is useful to determine the disease status, monitor the treatment course and evaluate the prognosis in pet rabbits in clinical practice.

INTRODUCTION

C-reactive protein (CRP) is a major acute phase reactive protein that is largely regulated by circulating interleukin-6 and produced by hepatocytes after an inflammatory stimulus.[1 2] The serum CRP level is reportedly proportional to the severity of disease and prognosis, although it is non-specific for disease.[3 4] Measurement of the serum CRP level is frequently performed in humans and dogs in the clinical practice setting. In humans and dogs, the serum CRP level increases within 1–2 days after an inflammatory stimulus.[5–7] In rabbits, the peak serum response occurs 38 hours after turpentine injection.[1] The half-life of serum CRP in both normal rabbits and rabbits that have received inflammatory stimuli is 4–6 hours.[8] In an experimental study involving a rabbit mandibular bone infection model, the serum CRP level immediately increased and the peak level was reached within 3 days after inoculation.[9] Thus, serum CRP is thought to be useful for pathological examination of rabbits in the clinical setting. However, no reports have described the use of serum CRP in pet rabbits.

The purpose of the present study was to evaluate the usefulness of CRP in pet rabbits in clinical practice.

MATERIALS AND METHODS

Ninety-two rabbits were enrolled in this study. All rabbits were presented to the Akashiya Animal Clinic and participation in the study was approved by the rabbit owners before enrolment. The bloods used in this study were used as part of routine clinical blood tests.

Healthy control rabbits

Thirty clinically healthy control rabbits (19 females, 11 males; age 5 months–13 years; weight 1.0–3.2 kg) were enrolled based on a normal history and physical examination in combination with normal results of a complete blood cell count and serum biochemistry profile. The rabbit breeds were as follows: cross-breed (n=18), Holland Lop (n=3), Miniature Rex (n=3), Netherland Dwarf (n=3), Lionhead (n=2) and Standard Rex (n=1).

Rabbits with disease

Sixty-two rabbits with disease (33 females, 29 males; age 11 months–10 years; weight 1.0–3.6 kg) were classified into six groups (gastrointestinal n=22; reproductive and urinary system n=20; neurological n=7; dental n=6; musculoskeletal n=4 and dermatological disease, n=3). The rabbits diagnosed with

gastrointestinal disease comprised 21 with gastrointestinal stasis syndrome and one with gastric rupture. The rabbits with reproductive and urinary system disease comprised six with uterine adenocarcinoma, six with acute renal failure, four with mammary tumours, two with cystitis, one with a uterine leiomyoma and one with a uterine leiomyosarcoma. All rabbits with neurological disease were diagnosed with encephalitozoonosis. The rabbits with dental disease comprised three with molar malocclusion, two with apical periodontitis and one with incisor malocclusion. The rabbits with musculoskeletal disease comprised three with fracture and one with a malignant fibrous histiocytoma with bone invasion. Finally, rabbits with dermatological disease comprised one with cutaneous lymphoma, one with melanoma and one with a basal cell tumour. When more than one disease was present, the disease showing the chief symptom was used for analysis. The rabbit breeds were as follows: crossbreed (n=45), Holland Lop (n=9), Netherland Dwarf (n=4), Miniature Rex (n=2) and Lionhead (n=2).

Blood sampling

Blood sampling was performed via venipuncture of the saphenous vein. The serum was separated after measurement of the total white blood cell (WBC) count and stored at −30° until CRP measurement. Each measurement was performed in duplicate. The serum CRP level was analysed with a commercial ELISA kit validated for rabbit CRP (RABBIT C-REACTIVE PROTEIN ELISA TEST KIT; Life Diagnostics, West Chester, Pennsylvania, USA).

Assessed items

The mean serum CRP level and WBC count were compared between the clinically healthy rabbits and rabbits in the six disease groups. The CRP level and WBC count were measured before and after ovariohysterectomy of a healthy rabbit for the purpose of neutralisation and a rabbit with uterine adenocarcinoma. The association between the CRP level and mortality in rabbits with various diseases was assessed. The all deaths in this study were natural cases, and there were no cases of euthanasia. The CRP level was compared in the three groups: <10 mg/dl, 10– 99 mg/dl and ≥100 mg/dl.

Statistical analysis

The Steel-Dwass multiple-comparison test was used to evaluate differences in mean values and Fisher's exact test and the Z-test were used to assess differences in proportions. A p value of <0.05 was considered statistically significant.

RESULTS

Healthy control rabbits

The results obtained from the clinically healthy control rabbits are summarised in tables 1 and 2. The mean CRP and WBC count showed no significant differences among the ages of <1 year, 1–3 years and ≥4 years. The mean CRP and WBC count showed no significant differences between female and male.

Table 1 Serum CRP level and WBC count by age in healthy control rabbits

Age	n	CRP (mg/dl)	WBC (10^9/l)
<1 year	9	0.32±0.43	5.367±1.340
1–3 years	12	0.73±0.12	6.583±1.889
≥4 years	9	0.44±0.52	5.750±3.200

Data are presented as mean±SD.
CRP, C-reactive protein; WBC, white blood cell.

Rabbits with disease

The results obtained from the clinically healthy control group and six disease groups are summarised in table 3.

The mean CRP level in all six disease groups was higher than that in the clinically healthy control group. The mean CRP levels of the rabbits with gastrointestinal, reproductive and urinary system, dental and musculoskeletal disease were significantly higher than those of the clinically healthy control rabbits (p<0.01, p<0.01, p<0.05 and p<0.05, respectively). In contrast, the WBC count showed no significant differences.

Changes in CRP level and WBC count before and after surgery

The results obtained from a healthy rabbit before and after spaying are shown in figure 1A. The CRP level peaked on postoperative day 1 and decreased rapidly thereafter. However, no obvious peak was observed in the WBC count. The results obtained from a rabbit with uterine adenocarcinoma before and after ovariohysterectomy are shown in figure 1B. Like the healthy rabbit that was spayed, the CRP level peaked on postoperative day 1, but no obvious peak was observed in the WBC count.

Association between CRP level and mortality

The association between the CRP level and mortality in rabbits with various diseases is shown in figure 2. Mortality increased as the CRP level increased, and the mortality rate in rabbits with a CRP level of more than ≥100 mg/dl was significantly higher than that in rabbits with a CRP level of <10 mg/dl (p<0.01). Diseases in which the CRP level reached ≥100 mg/dl were gastric rupture (n=1), acute renal failure with acute gastrointestinal dilation (n=2), malignant fibrous histiocytoma (n=1) and tibial fracture (n=1). Of these five rabbits, one with acute renal failure, one with a malignant fibrous histiocytoma and one with a fracture died.

Table 2 Serum CRP level and WBC count by sex in healthy control rabbits

Sex	n	CRP (mg/dl)	WBC (10^9/l)
Female	19	0.61±0.91	5.689±1.991
Male	11	0.37±0.66	6.164±2.702

Data are presented as mean±SD.
CRP, C-reactive protein; WBC, white blood cell

Table 3 Serum CRP level and WBC count between clinically healthy rabbits and rabbits in the six disease groups and rabbits in the six disease groups

Rabbits	n	CRP (mg/dl)	WBC (10^9/l)
Healthy	30	0.52±0.82*†	5.863±2.243
Disease	62	17.71±43.43	6.995±3.815
Gastrointestinal	22	11.74±22.89*	6.355±3.109
Reproductive and urinary system	20	21.19±49.68*	7.790±5.027
Neurological	7	2.55±1.79	6.471±4.036
Dental	6	4.87±5.47†	8.267±2.493
Musculoskeletal	4	85.66±107.28†	7.000±2.134
Dermatological	3	8.84±7.71	4.633±2.479

Data are presented as mean±SD.
*The CRP levels in rabbits with gastrointestinal, reproductive and urinary system diseases were significantly higher than those in clinically healthy control rabbits (p<0.01).
†The CRP levels in rabbits with dental and musculoskeletal diseases were significantly higher than those in clinically healthy control rabbits (p<0.05).
CRP, C-reactive protein; WBC, white blood cell.

DISCUSSION

No age-related and sex-related differences in the serum CRP level was observed in the healthy control rabbits. Previous reports have also failed to show age-related and sex-related differences in dogs,[10] but horses and cattle have exhibited slight differences associated with age and the perinatal period.[11 12] The number of healthy controls was small in the present study; an increase in the number of controls might allow for detection of a small difference.

The CRP level is commonly increased in humans and dogs with neoplastic diseases, immune-mediated diseases, infection or trauma.[3–6 13 14] In the present study, we evaluated the CRP level in rabbits with various diseases. The CRP level was increased in association with many diseases, and this increase was remarkable in rabbits with musculoskeletal, reproductive and urinary system, gastrointestinal or dental disease. This finding is considered to have been caused by the fact that most diseases involving the reproductive and urinary systems were neoplasms and that the tissue damage was severe

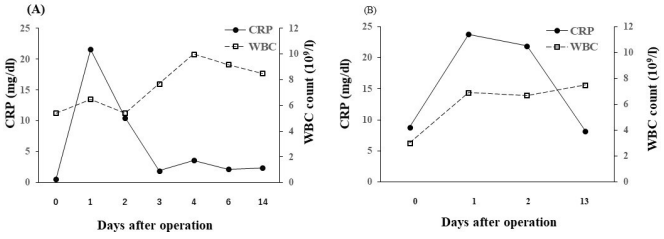

Figure 1 (A) Changes in serum CRP level and WBC count after spaying in a healthy rabbit. (B) Changes in serum CRP level and WBC count after ovariohysterectomy in a rabbit with uterine adenocarcinoma. CRP, C-reactive protein; WBC, white blood cell.

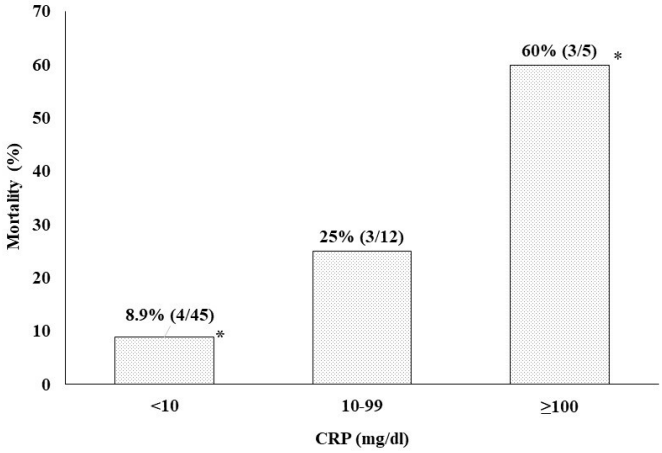

Figure 2 Association between C-reactive protein (CRP) level and mortality in rabbits with disease. *P<0.01.

in the rabbits with musculoskeletal disease. Rabbits are herbivores, and gastrointestinal stasis syndrome is a very common disease; affected rabbits often show anorexia, severe pain or a reduced level of consciousness. If left untreated, gastrointestinal stasis syndrome can rapidly become life-threatening.[15] The increased CRP level in the rabbits with gastrointestinal disease suggests that gastrointestinal disease in rabbits is accompanied by systemic inflammation. The CRP level was increased in human patients with periodontitis when compared with healthy controls.[16] Periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone loss. Dental disease in rabbits is common; the disease is associated with periapical infections, osteomyelitis and resorption of bony tissue.[17] We confirmed that the dental disease in rabbits was related to inflammation in the study.

In the present study, the CRP levels in rabbits with dermatological disease were not significantly increased. All three rabbits with dermatological disease had localised or benign tumours. In one study, the CRP levels in dogs with systemic or disseminated neoplastic lesions were higher than in dogs with localised or benign lesions.[18] In the three rabbits with dermatological disease in the present study, we considered that the CRP level did not substantially increase because the dermatological neoplasms were localised lesions and the inflammatory response was poor.

The above-mentioned study[18] showed that the CRP level was not increased in dogs with neurological diseases such as necrotising meningoencephalitis and intervertebral disk protrusion, and the authors considered that CRP was not useful for diagnosis of neurological disease.[18] In the present study, the CRP levels in rabbits with neurological disease were not also significantly increased. All seven rabbits with neurological disease in the study had encephalitozoonosis. Rabbit encephalitozoonosis is commonly observed worldwide, including in Japan.[19] Differential diagnosis is important because its clinical symptoms, such as head tilt, are similar to those

of otitis interna. Because the CRP level is increased in humans with otitis interna,[14] CRP may be effective for distinguishing between encephalitozoonosis and otitis interna in rabbits. However, further studies are needed on this topic.

The CRP level was much more sensitive than the WBC count before and after laparotomy in the two patients who underwent surgery in the present study. Similar results have been reported in dogs with local inflammation induced by intramuscular injection of turpentine oil.[18] The results of the present study also suggest good sensitivity of CRP to inflammation in rabbits.

The mortality rate increased as the CRP level increased in the present study. In humans, CRP is a long-term predictor of the risk of cardiovascular and non-cardiovascular mortality independent of known risk factors, the fibrinogen level and the WBC count.[3] In contrast, a study of the prognostic value of the baseline CRP, longitudinal CRP and its change over time on mortality in human patients undergoing continuous ambulatory peritoneal dialysis showed that a higher longitudinal CRP level and a trend of elevation over time, but not the baseline CRP level, were predictive of a worse prognosis.[4] We consider that investigation of the relationship between CRP and prognosis with respect to multiple factors such as disease type, age and breeding environment is necessary by accumulating more cases involving rabbits.

The results of this study indicate that measurement of the CRP level is useful to determine the disease status, monitor the treatment course and evaluate the prognosis of pet rabbits in clinical practice. We consider that the availability of CRP measurement in rabbits in clinical practice would be important for clinical examination of this species.

Contributors EO formulated an experimental plan for this study, collected samples, interpreted data and wrote the manuscript. KM measured the CRP using ELISA and interpreted. YK analysed the data. All authors co-written this paper and approved the final manuscript.

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