

Synovial fluid total protein concentration as a possible marker for canine idiopathic polyarthritis

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ABSTRACT. Idiopathic polyarthritis (IPA) is a very common inflammatory arthropathy in the dog. Canine IPA is diagnosed mainly by detecting increased number of leukocytes in the synovial fluid (SF), which is easily influenced by glucocorticoid therapy. We obtained 31 SF samples from 24 IPA dogs prior to ($n=19$) and/or after ($n=12$) 1 to 10 weeks of glucocorticoid therapy. The SF total protein concentrations of IPA dogs were significantly higher than those of dogs with non-arthritis diseases ($n=34$) and healthy controls ($n=10$). Our data revealed that the SF total protein concentrations are not influenced by several weeks of glucocorticoid therapy. Hence, the SF total protein concentration is applicable as a diagnostic marker of canine IPA even when the patients are receiving glucocorticoid therapy.

KEY WORDS: canine, idiopathic polyarthritis, synovial fluid protein concentration

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Canine idiopathic polyarthritis (IPA) is the most common type of polyarthritis in dogs [5]. The clinical signs of IPA include persistent or episodic fever, anorexia, malaise and ataxia with varying severity [4, 9, 14]. Canine IPA is diagnosed, if a dog shows polyarthritis, but does not satisfy the criteria for rheumatoid arthritis [1], systemic lupus erythematosus (SLE) [2], the polyarthritis/polymyositis syndrome [3] or infective arthritis [7]. Most of the untreated IPA cases show increased numbers of polymorphonuclear or mononuclear leukocytes in the synovial fluids (SF). The increase in leukocytes in the SF is considered to be one of important diagnostic markers of canine IPA [6, 9, 10, 14, 15, 17]. However, use of glucocorticoids (GC) ameliorates clinical signs and reduces numbers of leukocytes in the SF in most cases [6], sometimes making the diagnosis of IPA difficult.

In humans and dogs, various arthropathies are known to increase synovial permeability and elevate SF total protein concentrations [11, 16]. However, as to polyarthritis in dogs, only two reports mentioned elevated SF proteins [8, 15]. Especially, no study described the effect of GC therapy on SF protein concentrations. This study describes increased total protein concentrations in the SF of IPA dogs prior to and after successful GC therapy.

Twenty-four dogs with IPA (0.8–13.9 years of age; median 9.4 years, 2.2–13.7 kg of body weight; median 4.9 kg, 4 males, 4 neutered males, 8 females and 8 neutered females) were included in the present study. These dogs were referred

to The University of Tokyo Veterinary Medical Center (UT-VMC) between 2012 and 2014. Definitive diagnosis of IPA was done, if 1) erosive polyarthritis was excluded by radiography, 2) infectious arthritis was excluded by the SF microbiological tests in at least 2 joints, 3) polyarthritis-polymyositis syndrome and SLE were excluded, and 4) more than 2 leukocytes were observed in the SF from at least 2 joints in a microscopic high-power field [10, 15, 17]. Among these 24 IPA dogs, 4 dogs received GC treatment within 1 week prior to the diagnostic tests, and the rest 20 dogs did not. SF samples obtained from these 20 dogs were labeled as pre-GC-IPA group. Since a SF sample from a dog was excluded by visible blood contamination, the rest 19 SF samples were used in the present study. After diagnosis of IPA, all dogs received GC therapy. Among 24 IPA dogs, we could follow up 12 dogs. From these 12 dogs, SF samples were collected 1 to 10 weeks (median: 2.5 weeks) after the start of GC therapy. At those time points, clinical signs were improved in all patients, and all of the SF samples showed normal leukocyte counts. These 12 SF samples were labeled as post-GC-IPA group. As disease controls, dogs with non-arthritis diseases were included (1.2–14.6 years of age; median 8.4 years, 2.5–33.5 kg of body weight; median 5.1 kg, 2 males, 10 neutered males, 5 females and 17 neutered females; $n=34$). These dogs presented with symptoms, such as pyrexia or lameness, and/or with increased levels of plasma C-reactive protein (CRP). These dogs were submitted to arthrocentesis for excluding IPA. These dogs were finally diagnosed as aplastic anemia ($n=4$), pyrexia of unknown origin ($n=4$), myelodysplastic syndrome ($n=2$), pancreatitis ($n=2$), SLE ($n=2$), diagnosis unknown ($n=2$), cholangiohepatitis ($n=1$), chronic enteritis ($n=1$), food allergy ($n=1$), protein losing nephropathy ($n=1$), pyelonephritis ($n=1$), bacterial cystitis ($n=1$), prostate abscess ($n=1$), pyoderma ($n=1$), spondylitis ($n=1$), lymphoma ($n=1$), transitional cell carcinoma ($n=1$), osteosarcoma ($n=1$), immune-mediated

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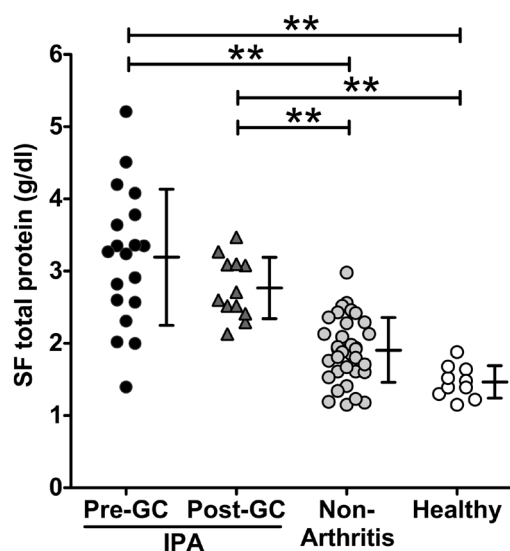


Fig. 1. Synovial fluid (SF) total protein concentrations of dogs with idiopathic polyarthritis (IPA) received no glucocorticoid (pre-GC-IPA: $n=19$) and treated with glucocorticoid (post-GC-IPA: $n=12$), dogs with non-arthritis disorders ($n=34$) and healthy controls ($n=10$). Each bar represents mean \pm standard deviation. $**P<0.01$, Scheffe's test.

neutropenia ($n=1$), immune-mediated hemolytic anemia ($n=1$), non-regenerative immune-mediated anemia ($n=1$), polymyositis ($n=1$), anemia of chronic disorders ($n=1$) or intervertebral disc disease ($n=1$). As healthy controls, ten adult beagle dogs (0.8–8.0 years of age; median 4.0 years, 4 males and 6 females) that had no evidence of disease were included.

All SF samples were stained with Wright Giemsa solution for cytological analysis. The rest of the SF was centrifuged (4°C , $5,000 \times g$, 10 min), and the supernatants were stored at -30°C before use. The stored aliquots of SF were 80-fold diluted with distilled water and were submitted to total protein determination by using a Bradford protein assay kit (Protein Assay, Bio-Rad Laboratories, Hercules, CA, U.S.A.).

Statistical analysis was performed with commercial software packages (Prism 5, GraphPad Software, Inc., La Jolla, CA, U.S.A.; Statcel 3, OMS publishing, Inc., Tokorozawa, Japan). SF protein concentrations among the groups were compared using multiple comparison analysis (Scheffe's test). The efficacy of IPA diagnosis was assessed by calculating the area under the receiver operating characteristic (ROC) curves (AUC_{ROC}) including healthy, non-arthritis and the post-GC-IPA groups. The cut-off for the variable to divide between dogs with and without IPA was determined by the positive/negative likelihood ratios based on the ROC curve. Statistical significance was accepted when $P<0.05$.

Determined SF total protein concentrations are shown in Fig. 1. The pre-GC-IPA group exhibited higher protein concentrations (3.19 ± 0.94 g/dl: mean \pm SD) than those of non-arthritis group (1.91 ± 0.50 g/dl) and healthy group (1.46 ± 0.22 g/dl). The post-GC-IPA group also showed higher

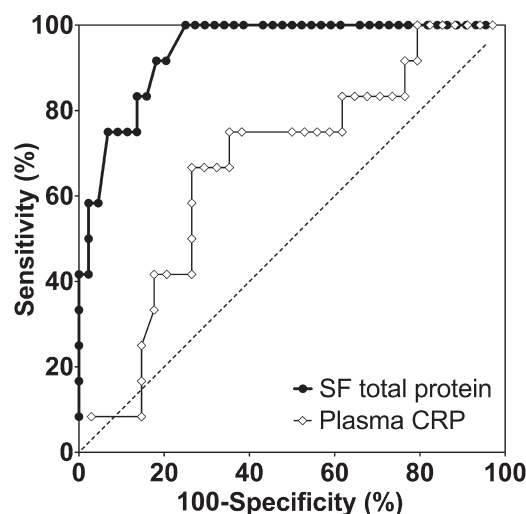


Fig. 2. Receiver operating characteristic curves of the concentrations of synovial fluid (SF) total protein and plasma C-reactive protein (CRP) for the prediction of idiopathic polyarthritis.

protein concentrations (2.76 ± 0.42 g/dl) than non-arthritis group and healthy group. Among post-GC-IPA group, four SF samples were obtained from IPA dogs received GC for 7 to 10 weeks. These dogs still exhibited higher SF protein concentrations (2.43 ± 0.10 g/dl) than the healthy controls ($P<0.01$). There was no statistical difference in the SF protein concentrations between pre- and post-GC-IPA groups.

To examine the ability of SF total protein concentrations for diagnosing IPA during GC therapy, ROC curves were plotted (Fig. 2). Then, the diagnostic significance of SF protein concentrations and plasma CRP was compared. SF total protein concentrations showed higher diagnostic accuracy (AUC_{ROC} : 0.94) than plasma CRP levels (AUC_{ROC} : 0.67). ROC curve analysis determined >2.54 g/dl for the SF total protein concentration as an optimal cut-off value to diagnose canine IPA (sensitivity 58.3% and specificity 95.5%). Additionally, the probability not having an IPA when the test on SF became below the cut-off value was 89%.

In the present study, we found the elevated SF total protein concentrations in dogs with IPA prior to GC treatment. GC treatment successfully reduced numbers of leukocytes in the SF of dogs in post-GC-IPA group. However, the SF total protein concentrations of post-GC-IPA group were still higher than those of healthy and non-arthritis groups. The concentration of SF total protein is considered to reflect the integrity of the synovial intercellular matrix [12], indicating that the barrier function of the synovial tissue was still lost after successful GC therapy. Although high plasma CRP concentration was reported to be a sensitive diagnostic marker for detection of IPA [9, 14], plasma CRP is increased in various inflammatory diseases as well [13]. In addition, GC treatment decreases plasma CRP concentration in parallel with a decrease of SF leukocyte count in IPA dogs [9]. Thus, the diagnosis of IPA is difficult when the dog is already treated with GC. Our data indicate that when a dog

received GC therapy, high SF total protein concentration is a better diagnostic marker of IPA than leukocyte count in the SF and plasma CRP.

This study demonstrates the valid diagnostic test for canine IPA. However, there are some attentions to use this test. First, because not all joints of the dog develop inflammation in IPA, it's necessary to choose an affected joint in order to distinguish IPA from other disorders by SF total protein concentration. Measuring the concentrations obtained from multiple joints will help us to avoid diagnosing incorrectly. Next, we need to exclude other arthropathies which can increase SF total protein concentration. Careful physical and X-ray examination and thorough clinicopathological assessment are required.

In conclusion, although the present study deals with small numbers of dogs, high levels of SF proteins would be a usable diagnostic marker for canine IPA, even if the dogs are receiving GC therapy.

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