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Age-related change of the plasma fibrinogen concentration in dogs

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Abstract

The aim of the present study was to investigate whether canine plasma fibrinogen concentration changes with age. We also investigated sex differences in the plasma fibrinogen concentration. As a result, the plasma fibrinogen concentration was found to moderately increase with age (Spearman's $r = 0.55$, $P < 0.001$), whereas that of the male and female dogs did not differ to a statistically significant extent. This finding suggests the need to consider aging as a factor that affects the plasma fibrinogen concentration.

Key Words: Age; Dog; Fibrinogen

Fibrinogen is a plasma glycoprotein which is synthesized in the liver. It plays an essential role in the secondary hemostatic process. Decreased plasma fibrinogen concentrations are therefore associated with life threatening bleeding. The common causes of hypofibrinogenemia are production insufficiency due to liver dysfunction, increased consumption during local hemostasis or systemic disseminated intravascular coagulation, hemodilution, and genetic problems that affect fibrinogen production.[1] On the other hand, the plasma fibrinogen concentration is increased during the inflammatory response because fibrinogen is an acute-phase protein.[11]

Aside from pathological states, the mean plasma fibrinogen concentrations of healthy humans are reported to differ according to age, sex, body mass index, and smoking status.[8,9,12] At present, there is no evidence regarding such differences in dogs. However, we occasionally encounter healthy adolescent dogs with a plasma fibrinogen concentration that is below the lowest limit of the reference interval (RI) and have hypothesized that the plasma fibrinogen concentration is correlated with age in dogs. Thus, the aim of this study is to investigate the age-related change in the plasma fibrinogen concentration in dogs. Sex differences in plasma fibrinogen concentrations were also investigated.

The present study included 159 client-owned

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dogs that were undergoing neuter surgery or deciduous tooth extraction between January 2009 and May 2015 at a primary care veterinary clinic (Higashihashimoto Animal Hospital, Kanagawa, Japan). The research protocols were approved by the Animal Experiment Committee of Azabu University. Prior to the operations, the dogs were confirmed to be healthy based on interviews, physical examinations, and blood tests. The blood tests included complete blood counts, liver and kidney biochemistry, and blood coagulation tests, including assessments of the prothrombin time and the activated partial thromboplastin time, while also determining the plasma fibrinogen concentration.

Blood samples were obtained from the jugular or cephalic vein of dogs after overnight fasting, and transferred to a 3.2% sodium citrate tube for the blood coagulation tests. The blood samples in the sodium citrate tubes were centrifuged for 15 minutes at 1,000 $\times g$ to separate the plasma immediately after collection. The citrate plasma was visually checked for hemolysis.

The plasma fibrinogen concentrations were measured by the Clauss method[9] using a WAKO Coag 2V system (Wako Corp., Osaka, Japan). This machine assesses clot formation time in an oscillating magnetic field, and calculates fibrinogen concentrations using the reference laboratory method.[10] The assay validation in dogs were reported by Kitoh et al.[7]; the domestic marketing of the canine system is permitted by the Japanese Ministry of Agriculture, Forestry, and Fisheries.

According to the Clinical and Laboratory Standards Institute guidelines adopted for veterinary species, the sample size was sufficient to determine the RI of plasma fibrinogen concentration.[3] Normal distribution was confirmed with the Anderson-Darling test. Outliers were examined with Dixon's Q test. The Reference Value Advisor software program (version 2.1) was used to calculate the RI based on non-parametric methods.[5] The 90% confidence interval (CI) was determined using the bootstrap method for a subgroup (sex). The relationship between the plasma fibrinogen concentration and age was analyzed by Spearman's rank correlation coefficient. The difference in the plasma fibrinogen concentration between the subgroups was also analyzed using the unpaired Student's *t*-test. The Prism software program (version 5.0, GraphPad Software Inc., San Diego, CA) was used to perform the Spearman's rank correlation coefficient analyses and *t*-test.

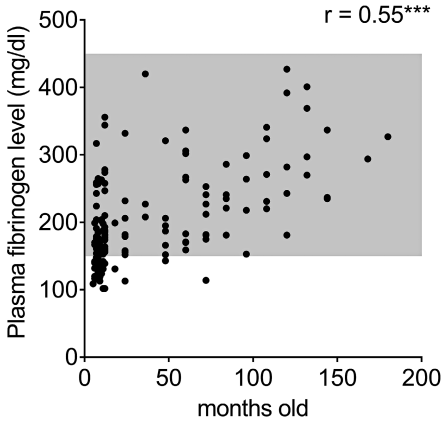

Fig. 1. The relationships between plasma fibrinogen concentration and age in all dogs. The shaded area indicated the manufacturer provided RI of the plasma fibrinogen concentration (150–450 mg/dl). ***: The plasma fibrinogen concentration was significantly correlated with age ($P < 0.001$).

A single outlier (590 mg/dl), which was detected among the adult male dogs was excluded from the analysis. As a result, there were 81 males and 77 females.

The breeds included Toy Poodle (n = 32), Chihuahua (n = 31), Miniature Dachshund (n = 17) and others (n=78; mixed breed dogs [n = 21], Maltese [n = 5], Welsh Corgi [n = 5], and purebred dogs from 21 other breeds [n = 47]). The median body weight of all dogs was 4.2 kg (range: 1.7–45.0 kg).

The overall RI of plasma fibrinogen concentration was 113–402 mg/dl (90% CI for the lower limit: 102–117; 90% CI for the upper limit: 344–590). The plasma fibrinogen concentration was moderately correlated with age (Fig. 1; Spearman's r = 0.55, $P < 0.001$). The data according to the major breeds and other breeds were also indicated in Fig. 2. The plasma

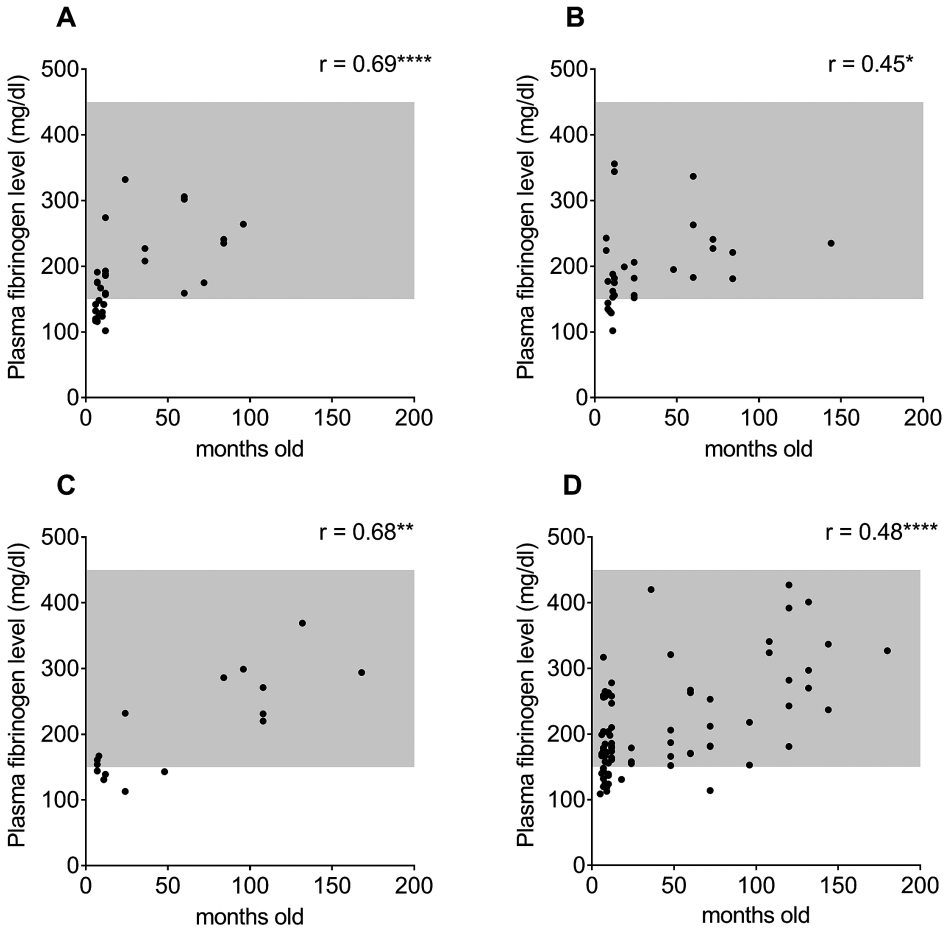

Fig. 2. The relationships between plasma fibrinogen concentration and age in Toy Poodle (A), Chihuahua (B), Miniature Dachshund (C) and others (D). The shaded area indicated the manufacturer provided RI of the plasma fibrinogen concentration (150–450 mg/dl). *, **, ****: The plasma fibrinogen concentrations were significantly correlated with age ($P < 0.05$, 0.01, 0.0001).

fibrinogen concentration of Toy Poodles and Miniature Dachshunds were strongly correlated with age (Toy Poodles: Spearman's $r = 0.69$, $P < 0.0001$, Chihuahuas: Spearman's $r = 0.45$, $P = 0.011$, Miniature Dachshunds: Spearman's $r = 0.68$, $P = 0.005$, Others: Spearman's $r = 0.48$, $P < 0.0001$).

The plasma fibrinogen concentrations of the male and female dogs did not differ to a statistically significant extent (Fig. 3). The RIs for plasma fibrinogen concentration in male and female dogs were 114–400 mg/dl (90% CI for the lower limit: 102–120; 90% CI for the upper limit: 341–420) and 109–394 mg/dl (90% CI for the lower limit: 102–119; 90% CI for the upper limit: 332–427), respectively.

In this report, although there were some differences between breeds, the plasma fibrinogen concentrations were relatively correlated with age. A similar correlation is also reported in human medicine, and the higher plasma fibrinogen concentration in older people is thought to be a risk factor for the development of ischemic cardiovascular disease.[8] We did not identify the reasons why older dogs tended to have higher plasma fibrinogen concentrations; however, occult acute phase reactions, subclinical activations of coagulation, and a lower turnover rate of fibrinogen have been reported as reasons in older people.[4,6]

Younger dogs tended to have lower plasma fibrinogen concentrations. If we used the

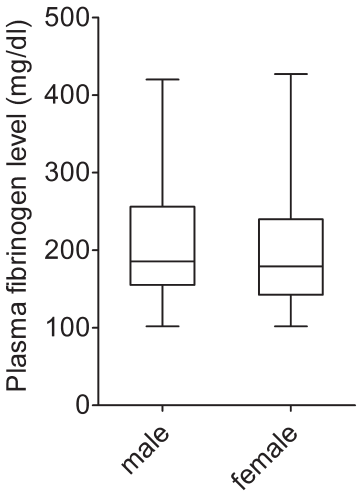

Fig. 3. The plasma fibrinogen concentrations of male and female dogs. For box graphs, the line within the box represents the median value, the limits of the box represent the 25th and 75th percentile values, and the whiskers represent the range. The single outlier (male, 590 mg/dl) is not included in these graphs.

manufacturer-provided RI of the plasma fibrinogen concentration (150–450 mg/dl), 37 dogs would have been below the RI; 33 of these dogs were 12 months of age or younger. However, the low plasma fibrinogen concentration of the younger dogs in this study was not associated with life threatening bleeding. Thus, if we encounter younger dogs with a plasma fibrinogen concentration below the lowest limit of the RI without bleeding events, a re-examination after an interval of several months might show that the concentration has increased into the reference range because of age-related change.

We did not observe a sex difference in the plasma fibrinogen concentration. In human medicine, the plasma fibrinogen concentrations of females are higher than those of males.[8,9,12] In particular, the plasma fibrinogen concentration in pregnant individuals would be higher than that in non-pregnant individuals, and pregnant females are at a high risk of developing thrombosis.[2] Although we did not include pregnant dogs in this study, we might need to investigate the plasma fibrinogen concentration of pregnant dogs and the risk of thrombosis.

The present study is associated with some limitations. The body condition score and the owner's smoking status, which have been reported as potentially modifiable determinants of the fibrinogen concentration in healthy humans, were not investigated in this study. Also, because small breeds are popular in Japan, this report only included a few large breeds. The correlation between plasma fibrinogen concentration and age in large breeds might need to be investigated separately.

The results of this study imply that the plasma fibrinogen concentrations increase significantly with age in dogs. The cause of this phenomenon remains to be studied.

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