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Coagulation disorders in dogs with hepatic disease

M. Prins, C.J.M.M. Schellens, M.W. van Leeuwen, J. Rothuizen, E. Teske *

Department of Clinical Sciences of Companion Animals, Veterinary Faculty, Utrecht University, P.O. Box 80.154, 3508 TD Utrecht, The Netherlands

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ABSTRACT

Liver disease has been associated with abnormalities in haemostasis. In this study, coagulation times, platelet counts, platelet activity parameters, activities of individual coagulation factors, D-dimers, antithrombin (AT) and protein C activity were measured in 42 dogs with histologically confirmed liver disease. Outcome was correlated with histological diagnosis. One or more coagulation abnormalities were present in 57% of dogs with hepatic disease. Activated partial thromboplastin time was significantly prolonged in dogs with chronic hepatitis (CH), with or without cirrhosis. Mean platelet numbers, AT and factor IX activity were significantly lower in dogs with CH plus cirrhosis, compared to dogs with other hepatopathies. D-dimers were not significantly increased in any group. Only three dogs, all with different histological diagnoses, satisfied the criteria for disseminated intravascular coagulation (DIC). Haemostatic abnormalities were primarily seen in dogs with cirrhosis and this may be due to reduced synthesis rather than increased consumption of coagulation factors.

Introduction

The liver plays an important role in maintaining haemostasis. However, although producing pro-coagulant, anti-coagulant and fibrinolytic proteins, the liver also removes clotting factors from the circulation. Liver parenchymal cells synthesise most of the clotting factors, including fibrinogen and the factors II, V, VII, IX, X, XI, and XIII, whereas factor VIII is thought to be produced mainly within the liver vascular endothelium (Mischke and Nolte, 2000).

In humans with acute or chronic hepatocellular disease, levels of vitamin K-dependent clotting factors, particularly factor VII and protein C, may decrease (Mammen, 1992). However, increased activation may also occur in patients with liver diseases and a consumptive coagulopathy, leading to disseminated intravascular coagulation (DIC), has been reported in patients with end stage liver disease (Lettow, 1963). Hyperfibrinolysis may be observed in patients with advanced hepatocellular liver disease and cirrhosis due to decreased thrombin-activatable fibrinolysis inhibitor (TAFI) (van Thiel et al., 2001; Colucci et al., 2003). Any increase or decrease of a particular coagulation factor will depend on the type, severity and chronicity of the hepatic injury (Badylak, 1988; Mammen, 1992, 1994).

Liver-associated haemostatic abnormalities have also been reported in veterinary medicine. Ninety-three per cent of dogs with naturally occurring hepatic disease had at least one abnormal coagulation test result value, with an abnormal prothrombin time (PT) and activated partial thromboplastin time (APTT) in 50% and 75% of the cases, respectively (Badylak et al., 1983). Similarly, in dogs with congenital portosystemic shunts, dogs had lower platelet counts, lower activity of factors II, V, VII, and X, and increased factor VIII and activated partial thromboplastin time (APTT), compared to healthy dogs, although these factors returned to normal after surgical repair of the shunt (Kummeling et al., 2006). Furthermore, dogs with hepatobiliary disorders have been reported to have significantly lower concentrations of fibrinogen, protein C activity (Mischke et al., 1998; Toulza et al., 2006) and factors VII and X (Mischke et al., 1998), whilst cats with naturally occurring liver disease had prolonged PT and decreased factor VII (Lisciandro et al., 1998).

The type of histological abnormality of the liver may influence the haemostatic abnormality. In dogs, a decrease in coagulation factor XI was seen during hepatic degeneration, whereas a decrease in factors IX, X and XI and an increase in von Willebrand's factor was found in cases of hepatic cirrhosis, and a decreased factor VIII combined with increased von Willebrand's factor in dogs with hepatic neoplasia. An increase in von Willebrand's factor has been shown to be associated with hepatic inflammation (Badylak et al., 1983). Finally, dogs experimentally infected with infectious canine hepatitis, as a model for DIC in humans, developed clinical and laboratory signs of coagulopathy, including a decreased platelet count and function, prolonged clotting times, decreased coagulation factors VIII, IX, XI and increased fibrinogen degradation products (Wigton et al., 1976).

The objective of the current study was to enhance our understanding of the disturbances in haemostasis that may occur in dogs with hepatic disease. By evaluating the production of clotting factors, increased fibrinolysis and activation of platelets, the severity

* Corresponding author. Tel.: +31 302531592; fax: +31 30 2518126.
E-mail address: e.teske@uu.nl (E. Teske).

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of coagulopathy was correlated with the type of histological disease. In addition, the possible role of DIC in the process of these coagulopathies was investigated.

Materials and methods

Study population

Forty-seven dogs (26 male, 21 female), of 20 different breeds and six mixed-breed, were referred to the Department of Clinical Sciences of Companion Animals, Utrecht University, with suspected hepatic disease and were prospectively entered into the study. Ages at presentation varied from 1–14 years (median 6 years). Liver disease was suspected following either abnormal liver parameter results on clinical biochemistry and/or abnormal appearance of the liver during abdominal ultrasonography. Dogs were included into the study if a histological liver biopsy was indicated and if they had the fully informed consent of their owners.

In dogs with suspected focal liver disease on abdominal ultrasonography, coagulation was checked and control tests biopsy samples obtained under ultrasound guidance using an 14 G Tru-cut needle (Medicor), as described previously (Stockhaus et al., 2004). Blind percutaneous liver biopsies were performed under local anaesthesia with a Menghini needle (14 G), using a technique first described by Lettow (1963). Biopsy samples were fixed in 10% neutral buffered formalin and routinely embedded in paraffin, then 4 μm-thick sections were stained according to standard techniques with haematoxylin and eosin. Liver diagnoses were based on the classification of the World Small Animal Veterinary Association (WSAVA) (Van den Ingh et al., 2006).

At the first visit, blood samples were collected for clinical chemistry, coagulation tests and haematology. Liver biopsy was postponed if the dog had a severe coagulopathy indicated by either a fibrinogen value <1.0 g/L, or severe prolongation of the PT or APTT. In these dogs treatment with prednisone (1 mg/kg) was instituted for 7 days before re-sampling (Yamazaki et al., 1999; Pulitanò et al., 2007).

Histological diagnoses were grouped in eight categories: (1) Histology revealed no hepatic lesions (five dogs); (2) acute hepatitis (three dogs); (3) chronic hepatitis (CH) (eight dogs); (4) CH plus hepatic cirrhosis (five dogs); (5) congenital portosystemic shunt (four dogs) and portal vein hypoplasia (one dog); (6) steroid induced hepatopathy (four dogs); (7) non-specific reactive hepatitis (12 dogs), and (8) miscellaneous diseases (five dogs) comprising malignant lymphoma ($n = 1$), extra-hepatic bile duct obstruction ($n = 2$), hepatocellular neoplasia ($n = 1$) and destructive cholangitis ($n = 1$).

The non-specific reactive hepatitis cases could be subdivided according to additional findings into four groups: (a) biopsy performed because of increased liver enzymes and/or bile acids at blood examination, but further diagnostic work up did not reveal a primary cause ($n = 4$); (b) labrador retrievers suspected of having copper-associated hepatitis, in which non-specific inflammation coincided with grey zone quantitative copper concentration (400–1000 μg/g dry weight; $n = 3$); (c) remaining non-specific inflammatory lesions after prednisone treatment of idiopathic hepatitis ($n = 2$); (d) chronic small intestinal disease with diarrhoea, in which non-specific hepatic inflammation was most likely associated with increased exposure to endotoxins ($n = 3$).

Bile acids, alkaline phosphatase (AP), alanine transaminase (ALAT) were measured in heparin anti-coagulated blood. In EDTA anti-coagulated blood the following parameters were measured: packed cell volume (PCV), platelet number (PLT), platelet distribution width (PDW), mean platelet volume (MPV), mean platelet component (MPC), and mean platelet mass (MPM) using a haematology analyser (Advia 120, Siemens Medical Solutions Diagnostics) with high and low angle detection. PT, APTT, D-dimers; protein C, fibrinogen, antithrombin and the coagulation factors II, V, VII, VIII, IX, X, and XI were measured in citrated blood.

Haemostatic analysis

Plasma samples were collected from the jugular vein in 1.8 mL Vacutainers (Becton Dickinson) containing sodium citrate (0.129 M or 3.8%), diluted 9:1. Because determination of D-dimers and factors II, V, VII, VIII, IX, X, and XI were not performed immediately after blood collection in most cases, citrated plasma was stored at −70 °C until measurements were performed, with a median storage period of 3 months and a maximum of 6 months. Plasma storage at −70 °C for more than 6 months may significantly shorten APTT, implying an increase in factor activity (Bateman et al., 1999a,b). D-dimers in dogs will remain stable up to 11 months when stored at <−20 °C (Stokol et al., 2000).

Automated PT, APTT, fibrinogen, determination of coagulations factors activities, antithrombin, and protein C were performed with a coagulation analyser (Thrombolyzer Compact X, BioMérieux). PT was measured using a commercial reagent (Simplastin Excel S, BioMérieux), according to the manufacturer's instructions. Activated partial thromboplastin time was measured using a commercial reagent (Automated APTT, BioMérieux), according to the test instructions and added to 60 μL sample volume to activate at 37 °C for 3 min. Fibrinogen was quantitatively determined with a commercially available assay (Fibriquik, BioMérieux) using a modified test performed according to manufacturer's instructions using human standards. Twenty microlitres of kaolin 3 g/L suspension were added to 0.15 mL of 1:30 veronal buffer-diluted sample and incubated at 37 °C for 3 min. After separate incubation, 20 μL of thrombin were added to the sample and clot formation detected optically and recorded automatically. Fibrinogen concentration was interpolated from the calibration curve (Clauss, 1957). Laboratory specific reference intervals were used for PT, APTT and fibrinogen in dogs.

Coagulometric tests were used to determine the activity of specific coagulation factors in the collected plasma samples, as described previously (Kummeling et al., 2006) using human deficient plasma (Biopool, Trinity Biotech). The samples were diluted 1:3 for factor II, 1:10 for factor V, factor VII, factor VIII, factor IX, and factor XII, and 1:4 for factor X. The specific factor is supplied by the unknown sample; all other factors are supplied by the deficient sample. The test principle is based on modified screening tests for PT (factors II, V, VII and X) or APTT (factors VIII, IX, XI). The activity of a specific factor is expressed as a percentage of the pooled plasma samples of 15 control dogs (100% activity).

For determination of normal controls, 15 clinically healthy adult dogs of varying breeds and sexes were used for blood collection to prepare canine pooled plasma. The activities of several factors (V, VII, VIII, IX, and XI) in plasma from healthy dogs are approximately eight (factor VIII:C) to nine (factor V) times greater than activities in human plasma (Mischke and Nolte, 2000; Mischke, 2001). To enable measurements of individual coagulation factor activities in dogs using human deficient plasma, a series of dilutions of the canine pooled plasma was made to prepare accurate activity curves.

A commercially available chromogenic substrate kit (BioMérieux Antithrombin III) to determine antithrombin activity was used according to manufacturer's instructions using 1:30 diluted citrated plasma. Protein C activity was measured with a commercial available kit (CRYOcheck Clot C, Precision BioLogic) by use of an optical method. The assay was modified by using undiluted samples and by use of canine pooled plasma and veronal buffer to generate a standard curve. Test values were reported as the percentage antithrombin and protein C activity of the pooled plasma samples of 15 healthy dogs.

For semi-quantitative determination of D-dimers, a latex agglutination test (Minutex D-dimer Latex, Biopool) was used. Briefly, undiluted, 1:2, 1:4 and 1:8 diluted serum was mixed with a suspension of latex beads coated with antibody against human D-dimer and agglutination was evaluated after 180 s. The assay was validated for dogs and the minimum positive concentration was equivalent to a D-dimer concentration of 250 ng/mL (Lara-García et al., 2008). The results of this assay were expressed semi-quantitatively within four ranges: (1) <250 ng/mL; (2) 250–500 ng/mL; (3) 500–1000 ng/mL; (4) 1000–2000 ng/mL.

A diagnosis of DIC was made by fulfilling at least three of the following criteria: (1) abnormal aPTT or PT; (2) low plasma fibrinogen concentration; (3) low plasma AT activity; (4) high D-dimer concentration, or (5) low platelet count (Bateman et al., 1999a,b).

Statistical analysis

All statistical analyses were performed using commercial software (SPSS for Windows 15.0.1). One-Sample Kolmogorov–Smirnov tests were used to determine whether distribution of variables was normal. To test equality between groups, one-way ANOVA was used to analyse the parametric variables platelets, PDW, MPV, MPM, and fibrinogen; the Kruskal–Wallis test was used for the other, non-parametric, variables. If significant differences were found, pair-wise comparison of subgroups was performed with t tests and Mann–Whitney tests for parametric and non-parametric variables, respectively. A P value <0.02 was considered significant.

Results

Liver biopsy was postponed in five dogs which had a coagulopathy, indicated by decreased fibrinogen and/or prolonged PT and APTT times. In these dogs treatment with prednisone (1 mg/kg) was instituted for 7 days before a definitive biopsy. Two of these dogs appeared to have CH and three CH plus hepatic cirrhosis. In all of these dogs, fibrinogen, PT and APTT were normalised within reference values, although the initial blood samples were also used for the platelet and coagulation study.

Results of platelet and coagulation analysis are presented in Table 1. Fifty-seven per cent of all dogs with hepatic disease had at least one abnormal coagulation parameter. Mean platelet count (182.8×10^9 cells/L) was significantly lower in the group dogs with CH plus cirrhosis when compared to the group without histological changes ($P = 0.003$). No difference between groups was found for the other platelet parameters: PDW, MPV, MPC or MPM.

Mean fibrinogen (0.98 g/L) values were lower in dogs with CH plus cirrhosis compared to the other hepatopathies, but did not reach significance. Although both mean PT and APTT concentrations were above upper reference values in CH, CH plus cirrhosis