Haemostatic abnormalities in cats with naturally occurring liver diseases

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Alterations in the haemostatic system were characterized in cats with different naturally occurring liver diseases. The study looked at 44 healthy cats and 45 cats with different liver diseases confirmed histologically or cytologically (neoplasia, n = 9; inflammation, n = 12; hepatic lipidosis, n = 13; other degenerative liver diseases, n = 11). The following parameters were evaluated: platelet count; prothrombin time; activated partial thromboplastin time; thrombin time; factor (F) II, FV, FVII, FX, and FXIII activities; fibrinogen concentration; activities of antithrombin, protein C, plasminogen, and α2-plasmin inhibitor, and D-dimer concentration.

In cats with liver diseases, 44/45 (98%) had one or more abnormalities of the coagulation parameters measured. In cats with inflammatory liver diseases, increased D-dimer concentrations and decreased FXIII activity were the most consistent abnormalities and were found in 83% and 75% of cats, respectively. The most common abnormality in cats with neoplastic liver disease was FXIII deficiency (78%). The most consistent abnormalities in cats with hepatic lipidosis were increased FV activity and D-dimer concentration with 54% of cats having values above the reference range for both parameters. Cats with miscellaneous degenerative liver disease most frequently showed FXIII deficiency (64%). The results of this study show that alterations of single haemostatic components are a frequent finding in cats with liver disease. Activation of haemostasis with subsequent consumptive coagulopathy (rather than decreased synthesis) seems to be responsible for these alterations. Increased blood levels of different haemostatic components in cats with inflammatory lesions may be related to an acute phase reaction.

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Introduction

Liver disease is associated with coagulation abnormalities and can result in an increased risk of bleeding. The liver is not only the major site of production of clotting factors, inhibitors and proteins of the fibrinolytic system, but is also responsible for their clearance and for the vitamin K-dependent carboxylation of clotting factors. Therefore, liver disease is associated with synthesis deficits and increased consumption of coagulation factors due to disseminated intravascular coagulation (DIC) (Amitrano et al., 2002; Lisman and Leebeek, 2007).

Coagulation abnormalities are reported to be a common finding in cats with different liver diseases. Of 22 cats with various naturally occurring liver diseases, prolongation of prothrombin time (PT) was the most common abnormality found in 77% of cats, whereas factor (F) VII activity was reduced in 68% and activated partial thromboplastin time (APTT) was prolonged in 55% (Liscian-dro et al., 1998). Furthermore, liver diseases have been reported to be associated with alterations in antithrombin activity and in concentration of fibrin degradation products (Thomas and Green, 1998). In a study by Center et al. (2000), only 4% of cats with hepatic lipidosis had a prolonged PT, 25% had a prolonged APTT, and 75% had increased proteins invoked by vitamin K absence (PIVKA) clotting times. This indicates that the coagulation screening tests PT and APTT do not adequately mimic the whole clotting process as it occurs in vivo. These tests do not detect alterations of primary haemostasis or of the inhibitory and fibrinolytic system (Thachil, 2008) so may not reflect the entire degree of impairment of haemostasis in liver disease.

Haemostatic abnormalities associated with different liver diseases have been investigated more extensively in dogs. As in humans, activities of all coagulation factors and inhibitors except FVIII, as well as of plasminogen and α2-plasmin inhibitor were reduced in eight dogs suffering from liver cirrhosis. Reduction was most severe in vitamin K-dependent FVII, FX, and protein C (Mischke et al., 1998). In a recent study Prins et al. (2010) measured activities of individual coagulation factors, D-dimers, and antithrombin and protein C activities in dogs with liver disease and 57% had at least one coagulation abnormality, while APTT was prolonged in chronic hepatitis with and without cirrhosis. Furthermore, dogs with liver cirrhosis had decreased platelet counts and reduced activities of antithrombin and FIX. Whereas coagulation abnormalities and the fibrinolytic system have been studied in
detail for dogs with liver disease, previous studies in cats have mainly addressed the global haemostatic tests.

In the present study, various individual components of different parts of the entire haemostatic system were measured in cats with histologically or cytologically confirmed liver disease. The hypothesis was that, as in dogs and humans (Mammen, 1994), cats with liver disease have frequently detectable abnormalities of single factors and inhibitors of the coagulation system and of proteins of the fibrinolytic system.

Materials and methods

Animals

Forty-five cats, presented to the Small Animal Clinic, University of Veterinary Medicine Hanover with different liver diseases, were prospectively entered into the study. Cats were classified as having neoplastic (n = 9) or inflammatory disease (n = 12), hepatic lipodisosis (n = 13), or other degenerative liver disease (n = 11). Breed and sex distribution was heterogeneous among different liver disease groups. There was no significant difference in age between disease groups.

Neoplastic liver diseases included hepatic lymphoma (n = 5), cholangiocarcinoma (n = 3), and pancreatic carcinoma with liver metastasis (n = 1). Inflammatory liver diseases included neutrophilic cholangiohepatitis (n = 4), lymphatic portal hepatitis (n = 3), purulent portal hepatitis (n = 2), pyogranulomatous inflammation (n = 2), and lymphatic cholangiohepatitis (n = 1). Other degenerative liver diseases included non-fatty degeneration (n = 5), Ito cell hyperplasia (n = 3), and non-specific degenerative changes (n = 3). Liver disease was diagnosed by histology in 31 cats. In the remaining 14 cats, diagnosis was exclusively based on liver cytology and consisted of 9 cats with hepatic lipodisosis, 4 with neoplasia (hepatic lymphoma), and 1 cat with inflammation (pyogranulomatous inflammation).

A control group (for statistical comparison of each parameter and calculation of reference ranges) consisted of 94 healthy cats of different ages (median, 5 years; range, 1–14 years), sex, and breeds. Cats of the control group were significantly younger compared to cats with liver diseases (P < 0.045). These cats had no evidence of disease on clinical examination, complete blood count, and biochemistry profile. Healthy control cats were either client-, student- or staff-owned.

In accordance with the German animal welfare law, the experimental design was announced to and approved by the official Animal Health Care Officer of the university and the responsible national agency (Lower Saxony State Office for Consumer Protection and Food Safety; reference number 10A 035).

Sample collection

Blood was obtained from a jugular, cephalic or saphenous vein using sterile disposable needles (21 G) and only slight pressure was used to raise the vein. For measuring platelet counts, 1 mL of blood was collected into a tube containing EDTA. Samples were mixed by gentle inversion of tubes. Blood samples for measurement of platelet counts were stored at room temperature and measurements were performed within 30 min of collection. For coagulation testing, 2 mL of blood were collected into plastic tubes containing one part 0.11 mol/L sodium citrate solution for nine parts of blood, and immediately mixed by careful rocking. The samples were immediately centrifuged for 5 min at 10,000 g and then plasma was removed and frozen in aliquots at –70 °C.

Laboratory methods

Measurement of platelet counts were performed by use of a laser-based automated blood cell counter (Advia 120, Bayer Diagnostics). The group tests of the coagulation system and the individual FIB, FV, FVII, and FX activities were measured with a coagulation analyser (Axsym Destiny plus, Trinity Biotech GmbH) using the clotting (ball coagulometer) technique. PT was measured using a commercial reagent (Thromborel S, Siemens Healthcare Diagnostics) according to (1) the standard test procedure (i.e. manufacturer’s instructions; standard test, PTa) and (2) a modified test procedure (PTb): 25 μL of diluted platelet poor plasma (1:20-dilution with imidazole buffer [Siemens Healthcare Diagnostics]) was incubated with 25 μL fibrinogen solution (2 g/L human fibrinogen [Haemochrom Diagnostica] in isotonic sodium chloride solution) for 2 min at 37 °C and coagulation was induced by addition of 25 μL reagent. PTa was converted into percentage coagulation activity of a normal feline pool plasma (n = 50; activity = 100 %) using a calibration curve based on different dilutions of the feline pool plasma.

APTt was carried out because of a higher sensitivity for the detection of individual coagulation factor deficiencies in feline plasma compared to the PTa (Mischke et al., 1996). Furthermore, supplementation of fibrinogen in the PTa ensures adequate fibrin formation despite sample dilution and makes the test independent of fibrinogen concentration in the sample.

PTa was measured using a commercial reagent (PTT reagent, Roche Diagnostics) following the manufacturer’s instructions. Thrombin time (TT) was measured using bovine thrombin (Test thrombin reagent, Siemens Healthcare Diagnostics) following the recommendations of the manufacturer. Activities of individual coagulation factors (FII, FV, FVII, and FX) were measured with commercial human deficient plasmas (Siemens Healthcare Diagnostics) and Thromborel using a higher sample dilution (1:40 with imidazole buffer) than is recommended for the measurement of human samples so as to guarantee an adequate specificity (Mischke et al., 1994). The assay was calibrated with a standard curve based on different dilutions of feline pooled plasma.

Fibrinogen concentration was measured according to Jacobsson (1955) by dissolving the fibrin clot received after thrombin addition in alkaline urea solution, measuring the extinction at 280 nm and using an extinction coefficient of E280nm/Ɛ 1% = 1.480. Each test was performed in duplicate.

Antithrombin, protein C (Antithrombin III BM/Hitachi, Protein C chromogen, Roche Diagnostics), plasminogen, and uPA-plasmin inhibitor activities (Coamatic Plasminogen, Coatest Antiplasmin, Haemochrom Diagnostica) as well as FXIII (fibrin stabilizing factor) activity (Berichrom Factor XIII; Siemens Healthcare Diagnostics) were determined with commercial test kits based on chromogenic substrates using an autoanalyser and based on instrument settings provided by the reagent manufacturers (which had partly to be adapted to feline plasma). Different dilutions of pooled feline plasma served as the standard.

D-dimers were measured with a latex turbidimetric assay (Tinaquant D-dimer, Roche Diagnostics) based on cross-reacting antibodies against human D-dimers. The test was calibrated with a set of human standards (D-dimer Calibrator, Roche Diagnostics). All tests conducted in the present study were validated in preliminary internal laboratory test series for the use in cats (data not shown).

Statistical analysis

All statistical analyses were performed using commercial software (SPSS for Windows 17.0.1). Due to the non-normally distributed data, Kruskal–Wallis test was used to test equality between groups. If significant differences were found, pair-wise comparison of groups was performed with the Mann–Whitney U test without further alpha adjustment. Reference values were defined on the basis of the 2.5% and 97.5% quantiles from the values of the healthy control cats. Data of each group are presented as median, minimum, and maximum. P < 0.05 was considered significant.

Results

Overall, 44/45 (98%) cats had one or more abnormalities of the coagulation parameters measured. Among the global coagulation tests (APTT, PT, TT), prolonged APTT was the most consistent abnormality (Table 1). In 40% of all cats with liver disease the APTT was above the reference range. Cats of all disease groups except those with neoplastic liver disease had a significantly prolonged APTT compared to the healthy control group (P < 0.01; Mann–Whitney U test) (Fig. 1).

The PT was prolonged in 18%, and shortened in 9% of all cats with liver diseases, while PTc values were above and below the reference range in each 18% of these cats. There was a significant difference in PTc between cats with inflammatory liver diseases and healthy control cats, whereas no significant difference was observed in PTc between any of the liver disease groups and the control group. The TT was prolonged in one-third of cats with liver diseases. Cats with neoplastic and inflammatory liver disease and with hepatic lipodisosis had significantly longer TT than control cats.

With regard to single clotting factors, decreased FXIII activity was the most common alteration: 78% of cats with neoplastic liver disease, 75% of cats with inflammatory liver disease, 31% of cats with hepatic lipodisosis, and 64% of cats with other degenerative liver disease had a FXIII activity below the reference range. Compared to the control group, all disease groups had significantly reduced FXIII activities (P < 0.001).

Thirty-six per cent of all cats with liver disease had fibrinogen concentrations exceeding the normal range and this abnormality was observed in 67% of cats with hepatic inflammation. Fibrinogen concentration was significantly increased in cats with hepatic neoplasia, inflammation, and lipodisosis compared to healthy controls. Increased FV activity was seen in 40% of all diseased cats and in 54% of cats with hepatic lipodisosis. Cats with hepatic lipodisosis and
inflammation had significantly increased FV activities compared to the control group. Among the vitamin K dependent clotting factors (FII, FVII, FX), there was no significant difference between disease groups and the control group.

Protein C activity was the most consistent abnormality for the inhibitors measured in this study: 44% of cats with various liver diseases had protein C activities below the reference range and 58% of cats with inflammatory liver disease had decreased values. All disease groups had significantly lower protein C activities in comparison to the control group (P < 0.005).

In cats with various liver diseases, 40% had increased α2-plasmin inhibitor activity. This was observed in about two-thirds of cats with inflammatory disease. The α2-plasmin inhibitor activity was significantly increased in all disease groups except in cats with various degenerative liver diseases.

Increased D-dimer concentration was present in about half of the patients and was the second most common alteration of all cats with liver diseases. Within the inflammatory liver disease group, an increased D-dimer concentration was observed in 83% of cats. Cats with neoplastic, and inflammatory liver diseases, and hepatic lipidosis had significantly increased D-dimer concentration compared to the control group (P < 0.05).

Discussion

In the present study, almost all cats with liver disease had at least one abnormal coagulation parameter. These data support our initial hypothesis that cats with liver disease, as in other species, have alterations in haemostasis with respect to single coagulation factors, inhibitors, and proteins of the fibrinolytic system. Haemostatic abnormalities are considered to be a consequence of decreased hepatic synthesis and increased consumption of coagulation factors, hyperfibrinolysis, thrombocytopenia, platelet functional disorders, and portal hypertension (Mammen, 1994; Amitrano et al., 2002).

The percentage of cats with abnormal parameters in the present study was higher when compared to a previous study in which coagulation abnormalities were reported in 82% of cats with liver disease (Lisciandro et al., 1998). However, comparability of percentages of cats with abnormal values is limited between studies due to a much wider range of parameters measured in the present study and the consequently increased sensitivity for detection of haemostatic abnormalities.

Reduced FXIII activity was the most consistent alteration among all cats with liver disease. This should be kept in mind when coagulation tests are performed in cats with liver disease, since the standard coagulation tests PT and APTT do not screen for FXIII deficiency. Severe FXIII deficiency is associated with bleeding diathesis and potential life-threatening bleeding complications in humans (Burchhardt et al., 1978; Gödde et al., 1998; Gerlach et al., 2000). FXIII plays an important role in the final stages of the clotting process by converting the loose fibrin polymer into a firm, highly organized, cross-linked structure (Francis and Marder, 1987, 1988). Furthermore, FXIII also regulates fibrinolysis. It mediates binding of α2-plasmin inhibitor to fibrin, which protects fibrin against early lysis by plasmin (Sakata and Aoki, 1980). Extremely low FXIII levels have been observed in seriously ill patients (Nussbaum and Morse, 1964; Egbring et al., 1996). Furthermore, FXIII has been suggested as a prognostic indicator in patients with liver disease and DIC (Song et al., 2006; Tacke et al., 2006).

Acquired FXIII deficiency is either caused by impaired synthesis or increased turnover and consumption, and has been reported in various diseases (Egbring et al., 1996). Milder alteration of other coagulation factors measured in the present study as well as significantly increased D-dimer values in most disease groups suggest that not only impaired synthesis but also increased consumption is responsible for decreased FXIII activity in cats. There is an equimolar consumption of FXIII with fibrinogen (Greenberg et al., 1985), but in contrast to equally sized fibrinogen it is only present in low concentrations in plasma (Hedner et al., 1975). Therefore, FXIII is more affected by consumption than fibrinogen. Additionally, fibrinogen is an acute phase protein in cats (Ceron et al., 2005). Elevated D-dimer concentrations in cats with hepatic diseases in our study are well in agreement with findings in humans. Increased D-dimer concentrations have been reported in patients with hepatic cirrhosis (Gürsoy et al., 2005), acute viral hepatitis, and hepatocellular carcinoma (Kruskal et al., 1992). In the latter study patients with hepatocellular carcinoma had markedly higher D-dimer values than those with hepatitis, whereas in the present study cats with inflammatory diseases had most consistent changes. D-dimers result from the degradation of cross-linked fibrin and their presence indicates generation of thrombin and plasmin (Carr et al., 1986).

In addition to increased intravascular formation of insoluble fibrin with subsequent fibrinolysis (Brazzell and Borjesson, 2007), D-dimers might have been elevated in the present study due to
Fig. 1. Coagulation parameters in control cats (C), cats with neoplastic (N) and inflammatory (I) liver diseases, hepatic lipidosis (L), and other degenerative liver diseases (D). Data for each group are presented as box-and-whisker plots; boxes represent the 25th–75th percentiles, the horizontal line within each box represents the median value, and the whiskers represent the 5th–95th percentiles. Dotted lines indicate the reference range, results of Kruskal–Wallis test are shown as P values in right top angles, and the same small letters above the boxes indicate significant difference between groups e.g. there is a significant difference in PT, between the control group and cats with inflammatory liver disease and between cats with inflammatory liver disease and cats with hepatic lipidosis.
decreased hepatic clearance. Systemic inflammatory diseases can trigger DIC (Estrin et al., 2006; Ogura et al., 2007) and are reported to cause elevated D-dimers in cats (Tholen et al., 2009). Inflammatory liver diseases may not only cause local activation of haemostasis, but also result in systemic inflammation with subsequent DIC (Miyake et al., 2007).

The coagulation factors FII, FVII, FIX, and FX and the inhibitors protein C and S are vitamin K dependent (Dunn, 2009). In the present study, FII, FVII, FX, and protein C were measured, and only the latter was significantly decreased in liver disease groups compared to healthy controls. This suggests that vitamin K deficiency only played a minor role in the liver diseases included in the present study. The finding is somewhat surprising since at least some of the cats suffered from cholestatic diseases and is in contrast to the conclusions of Center et al. (2000), who indicated that vitamin K deficiency is a frequent finding in feline hepatopathies. The conclusions were based on the findings of an abnormal PIVKA test, which is, however, a modified PT test and not specific for the presence of PIVKA. Lisciandro et al. (1998) also concluded that vitamin K deficiency is a common finding in cats with liver disease but FVII was the only factor measured in that study, and therefore interpretation regarding the mechanism can be only speculative.

It is noteworthy that cat populations were not uniform in the different studies and that differences in the incidence and severity of cholestatic liver disease may be partly responsible for the observed discrepancies. Furthermore, in all studies, the values represent only one point of dynamic haemostatic changes during disease progression.

Our results confirm the higher sensitivity of the PT\textsubscript{MT} to detect individual factor abnormalities, as already reported (Mischke et al., 1996). This was achieved by the higher predilution of the plasma sample.

Protein C deficiency was seen consistently in cats with inflammatory liver diseases. In humans, the protein C pathway is down-regulated during states of systemic inflammation and sepsis, which can promote microvascular thrombosis, increased leukocyte adhesion, and increased cytokine formation (Esmon, 2003; Liaw et al., 2004; Danese et al., 2010).

Despite suspected consumption due to activated coagulation and fibrinolysis, elevated fibrinogen concentrations as well as FV and a2-plasmin inhibitor activities were found in cats with different liver diseases. These findings may be explained by the possible role of these parameters as acute phase reactants in cats. Elevated fibrinogen levels in response to inflammation and infection has been shown in cats (Weiss et al., 1980; Boudreaux et al., 1989; Coron et al., 2005). Although the function as an acute phase protein is to our knowledge not yet reported for FV, the simultaneous elevations of fibrinogen and FV in cats with inflammatory disease and hepatic lipidosis in the present study support this assumption. The function of a2-plasmin inhibitor as an acute phase protein is well described in humans (Risberg et al., 1986; Ueyama et al., 1992) and has been suggested in dogs (Mischke, 2005). The high percentage of cats with inflammatory liver diseases having increased levels of a2-plasmin inhibitor in the present study is consistent with a role as an acute phase reactant in the feline species.

The observed increase in FV activity could also be a consequence of reduced protein C activity, which may have led to reduced inactivation of activated FV in cats with inflammatory liver disease and hepatic lipidosis. The importance of activated protein C (APC) for the “down-regulation” of FV activity has been well described in humans (Nicolaes et al., 1995).

An important limitation of our study is that for some cats (almost exclusively cats with hepatic lipidosis and lymphoma), the diagnosis of liver disease was solely based on cytology, which has limited specificity and sensitivity for the evaluation of liver disease, and an additional underlying liver disease might have been overlooked. However, hepatic lipidosis as well as lymphoma are both conditions for which cytology is thought to be adequate for diagnosis (Weiss and Moritz, 2002). Furthermore, this was a clinical study and liver biopsy sampling was only performed when necessary as part of the diagnostic work-up.

Another limitation is that the haemostatic abnormalities we observed may not have been caused by liver disease alone. The existence of a concurrent disease or systemic involvement was not an exclusion criterion and post-mortem examinations were not evaluated for the study as they were carried out only on a limited number of cats. Therefore, part of the observed changes may have been induced by concurrent inflammatory or neoplastic diseases.

Conclusions
Alterations of single haemostatic components are a common finding in cats with different liver diseases, and cats with inflammatory lesions seem to have most consistent alterations. Acute phase reactions might lead to the often elevated blood levels of different haemostatic components in these animals. Increased D-dimer concentrations suggest that activation of haemostasis with subsequent consumptive coagulopathy (rather than decreased synthesis of coagulation proteins) is the main cause of most haemostatic abnormalities in cats with liver disease. Vitamin K deficiency did not seem to play a major role in cats with hepatic disease in our study, since even the factor with the shortest half-life time (i.e. FVII) was not significantly decreased in any of the liver disease groups. Since FXIII deficiency is frequently found in cats with liver disease and has been shown to be of prognostic value in humans with liver cirrhosis, further studies are warranted to investigate its prognostic utility in cats.

Conflict of interest statement
None of the authors of this paper has a financial or personal relationship with other people or organisations that could appropriately influence or bias the content of this paper.

References


