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Plasma Antithrombin Activity as a Diagnostic and Prognostic Indicator in Dogs: A Retrospective Study of 149 Dogs

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Background: Antithrombin (AT) is the major inhibitor of coagulation. In people, hypoantithrombinemia is associated with hypercoagulability, thrombosis, and poor prognosis. Veterinary studies, however, have not demonstrated similar prognostic significance. Thus, AT activity (ATA) in dogs currently is interpreted based on human medicine guidelines.

Hypothesis: ATA can serve as a prognostic marker in dogs, as has been shown in people.

Objectives: (1) To describe the clinical and clinicopathologic findings, diagnoses, and outcome of dogs with decreased versus normal ATA, (2) to identify diseases and mechanisms associated with hypoantithrombinemia, and (3) to assess ATA as a prognostic indicator.

Animals and Methods: Retrospective study of 149 dogs with ATA measurement during their disease course.

Results: Hypoantithrombinemic dogs had a higher proportion of leukocytosis, hemostatic abnormalities, hypoalbuminemia, and hyperbilirubinemia versus dogs with normal ATA. Hypoantithrombinemia commonly was present in immune-mediated hemolytic anemia (IMHA), pancreatitis, hepatopathy, and neoplasia. It was associated with higher risk of mortality in the entire study population and for specific diseases (eg, IMHA, neoplasia). The odds ratio for mortality significantly and progressively increased when ATA was <60 and $<30\%$ (9.9, 14.7, respectively). A receiver operating characteristics analysis of ATA as a predictor of mortality showed an area under the curve of 0.7, and an optimal cutoff point of 60% yielded sensitivity and specificity of 58 and 85%, respectively.

Conclusions and Clinical Importance: In dogs, ATA $<60\%$ indicates increased mortality risk, similarly to human patients, but ATA has limited value as a single discriminating factor in the outcome.

Key words: Canine; Hypercoagulability; Outcome; Prognosis; Thrombosis.

Antithrombin (AT) is the most abundant and important physiologic inhibitor of coagulation, and accounts for 80% of the total inhibitory effect of plasma on coagulation. It is an α2-globulin, produced primarily by the liver and irreversibly binds and inactivates several serine proteases, including thrombin and factor X. Its activity is amplified 1,000-fold in vivo by interacting with endogenous heparin sulfate proteoglycans.[1,2]

AT can be measured by either a functional activity assay and compared with reference plasma, with results expressed as percent activity, or by an immunological assay (eg, immunoturbidometry), with results expressed as concentration. Functional assays are based on the sample plasma capacity to inhibit a standard excess amount of either thrombin or factor Xa. The residual protease concentration, which is inversely proportional to AT concentration, is measured by a clotting-based assay or with chromogenic peptide substrates. Immunological assays quantify the amount of AT present in plasma with an AT-specific labeled antibody.[3,4]

Decreased AT activity (ATA) is thought to deflect the hemostatic balance toward hypercoagulability, predisposing patients to thrombotic events, organ failure, and death.[5,6] As such, ATA is considered an important measure in assessing hypercoagulability. Additionally, studies in human medicine have demonstrated its usefulness as a prognostic marker and a therapeutic planning aid.[7–10] Based on these studies, specific therapeutic guidelines for different degrees of hypoantithrombinemia were suggested. When ATA was $<60\%$, heparin treatment alone did not suffice, and AT supplementation was warranted to achieve therapeutic goals.[7,10] Additionally, decreased ATA was significantly associated with increased risk of thrombosis and poor prognosis.[8,9]

Based on the aforementioned studies in human patients and baboons,[7,10] it has been suggested that the interpretation of ATA in dogs should be done similarly, and a critical and immediate thrombotic risk exists when ATA is $<30\%$, a high thrombotic risk at ATA $<60\%$, and potential hypercoagulability requiring no AT supplementation when ATA is $>60\%$.[5]

Hypoantithrombinemia is thought to occur by 3 major mechanisms, including AT loss (eg, protein-losing nephropathy [PLN], protein-losing enteropathy [PLE], bleeding), increased AT consumption (eg, hypercoagulable states), and decreased AT production (eg, liver diseases).[1,2] Hypoantithrombinemia has been reported in various canine diseases, such as hepatobiliary disease,[11–13] porto-systemic shunt (PSS),[13] sepsis,[14,15] disseminated intravascular coagulation (DIC),[16,17] PLN,[18–22] congestive heart failure,[23] trauma,[24] neoplasia,[25–27] and immune-mediated hemolytic anemia (IMHA),[28,29] and after surgery.[30,31] In most studies, ATA was determined to be a useful diagnostic tool, but it has not been demonstrated to be associated with mortality and prognosis.[3,13,17,29] The importance of ATA as part of the overall assessment of hemostasis and as a marker of disease severity has received

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growing attention in dogs. However, to date, the interpretation of ATA in dogs is based on studies in human patients and its real prognostic value is still unclear.

A study of dogs with sepsis demonstrated that hypoantithrombinemia was associated with a higher risk for mortality.[15] Another study of dogs with liver disease showed that a poor prognosis was associated with concurrently decreased ATA and protein C, and hyperbilirubinemia.[13] However, in both of these studies, ATA was evaluated as part of the overall diagnostic evaluation of these specific conditions and not as the single exposure variable.[13,15] To the best of our knowledge, ATA, as a single exposure parameter, has never been evaluated as a marker of disease severity or as a prognostic indicator in dogs in general. Consequently, the current therapeutic guidelines in dogs are extrapolated from human medicine, despite the well-known species differences in ATA reference intervals.[1] For example, an ATA of 90% in horses has been associated with a moderate risk for thrombosis, whereas this same activity level in dogs is considered within the reference interval.[1] The reference interval for ATA in human patients is $>75\%$, compared with $>85\%$ in dogs.[8] In addition, there is no published information regarding the main mechanisms and diseases responsible for hypoantithrombinemia in dogs in general.

The aims of the present study were (1) to describe and characterize the history, clinical signs, clinicopathologic findings, diseases, and outcome of dogs presenting with hypoantithrombinemia, compared with dogs presenting with normal ATA; (2) to identify the main diseases and disease mechanisms associated with hypoantithrombinemia; and (3) to assess the association between ATA and the outcome.

Materials and Methods

Selection of Cases and Collection of Data

In this retrospective study, dogs were recruited consecutively from the canine population treated at the Hebrew University Teaching Hospital between 2004 and 2008. The only inclusion criterion was ATA measurement as part of their diagnostic workup. Dogs treated with blood products, heparin derivatives, or other anticoagulants before ATA measurement, and those with incomplete medical records were excluded.

Data retrieved from the patients' medical records included signalment, chief complaint, clinical signs, physical examination findings, clinicopathologic test results (eg, hematology, serum biochemistry, urinalysis, urine protein-to-creatinine [UPC] ratio), and coagulation tests (eg, prothrombin time [PT], activated partial thromboplastin time [aPTT], fibrinogen, D-Dimer, and ATA), diagnoses, complications, hospitalization time, and outcome. Dogs alive at 28 days from admission were defined as survivors.

Laboratory Methods

Blood samples for CBC were collected in potassium-EDTA tubes upon admission, and analyzed within 15 minutes of collection with automatic impedance cell analyzers[a] calibrated for canine blood. Blood samples for biochemical analysis, when performed, were collected in tubes containing no anticoagulant, and sera were separated within 1 hour and stored at 4°C pending analysis, which was performed within 24 hours of collection, with a wet chemistry autoanalyzer,[b] at 37°C. Electrolyte analysis was performed with an ion-selective electrode electrolyte analyzer.[c]

Blood samples for coagulation tests, including ATA, were collected in 3.2% trisodium-citrate tubes, and centrifuged within 15 minutes of collection. Plasma was harvested and either analyzed immediately or stored at 4°C for up to 24 hours pending analysis. PT and aPTT were determined with an automatic coagulometric analyzer[d] or a manual analyzer[e] within 15 minutes of separation of plasma. Fibrinogen concentration was determined by the Clauss method by the automatic autoanalyzer[d] within 30 minutes of plasma collection.

ATA was measured with the above-mentioned automated coagulometric analyzer[d] utilizing a chromogenic substrate[f] after incubation of factor Xa reagent[g] with the patient's own plasma in the presence of heparin excess. The results were expressed as percent activity compared with reference plasma. The reference interval (RI) for ATA (87–140%) was established based on the mean $\pm$ 2 SD ATA results of a reference population of 30 healthy dogs (based on history, physical examination, and CBC). These samples were pooled and used as the reference plasma for ATA analysis.

D-Dimer concentrations were measured quantitatively in 3.2% citrated plasma samples with a latex agglutination kit[h] and the results were interpreted either as negative or positive, with a cut-off value of 250 ng/mL.

Definition of Pathophysiologic Mechanisms, Diagnoses, and Secondary Complications

Dogs with hypoantithrombinemia were divided into 3 main categories based on the suspected mechanisms that were retrospectively hypothesized to be responsible for decreased ATA (eg, increased consumption, decreased production, and loss [urinary, gastrointestinal, bleeding]). Dogs were assigned several mechanisms if more than a single mechanism was considered responsible for hypoantithrombinemia. Assignment to such a mechanism was based on the established pathophysiology of the specific disease and its potential effect on coagulation in general, and on ATA in particular. For example, when PLN or PLE were present, loss was considered the main mechanism. In cases of liver failure, when consumption was excluded as the main mechanism of hypoantithrombinemia, decreased production was considered the major mechanism.

A diagnosis of DIC, which was always considered a secondary syndrome, was made if at least 3 of the following laboratory abnormalities were present concurrently: thrombocytopenia (platelets $<150 \times 10^9$/L), prolonged PT and aPTT ($>25\%$ of the upper RI), hypofibrinogenemia, hypoantithrombinemia, and a positive D-Dimer test. All of the dogs that were diagnosed with DIC in this study had at least 3 additional hemostatic abnormalities in addition to hypoantithrombinemia. The diagnosis of IMHA was based on presence of anemia and spherocytosis, and positive in-saline slide agglutination or Coombs' tests. Pancreatitis was diagnosed based on compatible history, clinical signs, and serum biochemistry abnormalities including amylase and lipase activities, canine pancreatic lipase-like immunoreactivity,[i] abdominal ultrasonography, and histopathology of biopsies obtained by exploratory laparotomy (the latter was performed in 1 dog).

Protein-losing nephropathy was diagnosed when the UPC ratio was >0.5 and pre- and postrenal causes of proteinuria were eliminated. Protein-losing enteropathy was diagnosed based on a history of chronic small intestinal diarrhea, panhypoproteinemia, and histopathology (eg, inflammatory bowel disease, lymphangiectasia, lymphoma).

Liver disease was diagnosed based on compatible clinical and laboratory test abnormalities (eg, icterus, increased serum activities of alanine aminotransferase and aspartate aminotransferase

[AST], hyperbilirubinemia, evidence of decreased liver function). Liver failure was diagnosed based on evidence of at least 2 decreased liver test results (eg, hypoalbuminemia, hyperbilirubinemia, hypoglycemia, hypocholesterolemia, decreased urea concentration, hyperammonemia, increased serum bile acids). Specific diagnoses of liver disease (eg, PSS, chronic hepatitis) were based on appropriate ultrasonographic and histopathologic findings.

Statistical Analysis

The distribution of all continuous variables (normal versus non-normal) was assessed using the Shapiro-Wilk's test. Normally and non-normally distributed continuous variables were compared between the ATA groups by Student's t or Mann-Whitney tests, respectively. One-way ANOVA was used to compare ATA among the different mechanisms among dogs with decreased ATA. Fisher's exact test was used to compare the categorical variables and proportion of diagnoses among ATA groups. Logistic regression analysis was performed to assess the relationship of ATA with outcome.

Dogs were divided into 2 groups: group 1, hypoantithrombinemic (ATA $<$ 87%) and group 2, ATA within or above RI. Hypoantithrombinemic dogs were further divided to 3 ATA level groups based on 2 previously published definitions for further analyses. The first was based on the scale suggested by Feldman, dividing ATA to 3 groups: $<$30%, $\geq$30 to $<$60%, and $\geq$60 to $\leq$90%.[5] The second was based on studies in humans with division of ATA into $<$50%, $\geq$50 to $\leq$75%, and $>$75%.[8] Logistic regression analyses were then performed, based on these divisions, using ATA as a categorical variable and dogs with normal ATA as the reference category, against the outcome. The association of ATA with the outcome also was assessed by the ROC procedure to select cut-off points with their corresponding sensitivity and specificity for prediction of the outcome. The optimal cut-off point was selected as the point that was associated with the least number of misclassifications. For all tests applied, $P \leq .05$ was considered statistically significant. All calculations were performed by statistical software.[j]

Results

Signalment and Clinical Signs

This study included 149 dogs with a median age of 8 years (range, 0.3–16), of which 61 had normal ATA (41%) and 88 were hypoantithrombinemic (59%) with no age, sex, or breed differences among groups. There were 73 females (60 spayed) and 76 males (22 castrated) of various breeds, including mixed (62), Labrador Retriever (16), Golden Retriever (7), Cocker Spaniel (6), German Shepherd Dog (6), Weimaraner (6), Boxer (5), standard Poodle (3), and American Pit Bull Terrier (3). Other breeds were represented by 2 dogs or fewer.

Clinicopathologic Data

Hypoantithrombinemic dogs had significantly ($P <$ .05) higher WBC count, serum AST activity, bilirubin concentration, aPTT, and significantly lower platelet count, serum albumin and total calcium concentrations as compared with normoantithrombinemic dogs (Table 1). There was no difference in ionized calcium concentration among groups, suggesting that total calcium concentration was decreased because of hypoalbuminemia in the hypoantithrombinemic dogs as compared with the normoantithrombinemic dogs. Hypoantithrombinemic dogs had a significantly ($P <$ .05) higher proportion of leukocytosis, thrombocytopenia, prolonged PT and aPTT, increased AST, hyperfibrinogenemia, hypercholesterolemia, hypoglycemia, hypoalbuminemia and hyperbilirubinemia, and lower proportion of hyperalbuminemia as compared with normoantithrombinemic dogs (Table 1).

Table 1. Median, range, and proportion of abnormalities of selected[a] hematologic, serum biochemistry, and coagulation measures of normo- and hypoantithrombinemic dogs.

	n_1^1, Median (range)				n_2^2 (n_2/n_1, %) above RI		n_3^3 (n_3/n_1, %) below RI	
Measure	ATA $<$ 87	ATA $>$ 87	RI	P-Value	ATA $<$ 87%	ATA $>$ 87%	ATA $<$ 87%	ATA $>$ 87%
WBC ($10^3/\mu L$)	88, 17.9 (0.16–90.7)[b]	61, 13.1 (5.9–113)	6.0–16	.013	50 (50/88, 57)[b]	17 (17/61, 28)	6 (6/88, 7)	2 (2/61, 3)
Platelets ($10^3/\mu L$)	88, 130 (0–858)[b]	58, 243 (0–778)	150–700	$<$ .001	1 (1/88, 1)	3(3/58, 5)	50 (50/88, 57)[b]	13 (13/58, 22)
Albumin (g/dL)	86, 2.8 (0.5–5.3)[b]	51, 3.2 (1.6–5.9)	2.8–3.8	.009	7 (7/86, 8)[b]	9 (9/51, 18)	44 (44/86, 51)[b]	19 (19/51, 37)
AST (U/L)	78, 86 (13–3,185)[b]	51, 48 (19–580)	9–47	.005	54 (54/78, 69)[b]	26 (26/51, 51)	0 (0/78, 0)	0 (0/51, 0)
Bilirubin (mg/dL)	82, 0.46 (0–58.77)[b]	51, 0.27 (0.04–10.2)	0.02–0.5	.005	35 (35/82, 43)[b]	11 (11/51, 22)	5 (5/82, 6)	2 (2/51, 4)
Calcium (mg/dL)	79, 9.5 (5.4–14.39)[b]	49, 10.3 (5.09–13.68)	8.5–11	.016	13 (13, 79, 16)	11 (11/49, 22)	18 (18/79, 23)	9 (9/49, 18)
aPTT (s)	79, 19.3 (6.45–82)[b]	42, 16.2 (6.3–60)	11–17.4	.002	50 (50/79, 63)[b]	15 (15/42, 36)	6 (6/79, 8)	7 (7/42, 17)
PT (s)	80, 8.1 (6–60)	42, 7.4 (6–60)	6–8.4	NS	37 (37/80, 46)[b]	5 (5/42, 12)	0 (0/80, 0)	0 (0/42, 0)
Fibrinogen (mg/dL)	46, 438 (40–929)	24, 296 (100–787)	200–400	NS	26 (26/46, 56)[b]	8 (8/24, 33)	7 (7/46, 15)	2 (2/24, 8)
Cholesterol (mg/dL)	81, 261 (6–1,114)	48, 241 (63–514)	118–309	NS	31 (31/81, 38)[b]	9 (9/48, 19)	8 (8/81, 10)	5 (5/48, 10)
Glucose (mg/dL)	77, 102 (9–624)	49, 102 (54–293)	65–103	NS	36 (36/77, 47)	21 (21/49, 43)	14 (14/77, 18)[b]	1 (1/49, 2)

[a]The measures included are those in which there were significant ($P \leq .05$) differences between antithrombin activity groups either in their median or in proportions of abnormalities.

[b]Significant ($P \leq .05$) difference between ATA groups; 1, the total number of dogs in each group in which the parameters were measured; 2, the number of dogs presenting values greater than reference interval in each group; 3, the number of dogs presenting values $<$ reference interval in each group.

RI, reference interval; ATA, antithrombin activity; WBC, white blood cells; AST, aspartate aminotransferase; aPTT, activated partial thromboplastin time; PT, prothrombin time; NS, not significant.

There were significant ($P < .001$) correlations between ATA and albumin concentration, PT, and aPTT ($r = 0.3$, -0.35, and -0.33, respectively).

Diagnoses and Proposed Mechanisms of Hypoantithrombinemia

The most common diagnoses in dogs with hypoantithrombinemia were secondary DIC (21 dogs), liver disease (18 dogs), IMHA (16 dogs), neoplasia (16 dogs), and pancreatitis (13 dogs) (Table 2). When the proportions of diseases and diagnoses were compared between hypo- and normoantithrombinemic dogs groups, the proportions of dogs diagnosed with DIC, liver disease, and pancreatitis were significantly ($P < .001$, $P = .015$, .048) higher in the hypoantithrombinemic group as compared with the normoantithrombinemic group (Table 2).

Fourteen of the 88 hypoantithrombinemic dogs had more than a single proposed mechanism for the hypoantithrombinemia (12 and 2 dogs with 2 and 3 mechanisms, respectively). Increased consumption was the most common hypothesized mechanism of hypoantithrombinemia (69 dogs), followed by loss (19 dogs) and decreased production (16 dogs). Among the 18 dogs included in the liver disease group, 16 had liver failure and 2 had hepatopathy that was not deemed to contribute to development of hypoantithrombinemia (1 had concurrent sepsis whereas the other had concurrent PSS and pancreatitis). In both of these dogs, hypoantithrombinemia was considered to arise mainly from consumption rather than from decreased production, because there was no evidence of liver failure. In 10 dogs, decreased hepatic AT production was the sole hypothesized mechanism for hypoantithrombinemia, whereas in 6 additional dogs liver failure was diagnosed with other concurrent processes (eg, DIC, pancreatitis) that were considered to have contributed to hypoantithrombinemia. In the consumption group, the most common diagnoses and secondary complications were DIC, IMHA, neoplasia, and pancreatitis.

Hypoantithrombinemia (median ATA, 53%; range, 25–81%) was present in 16 of 21 dogs (76%) with a primary diagnosis of IMHA. This proportion was not significantly different compared with that of the rest of the dogs in the study (56%, 72 of 128 dogs). Twenty-nine dogs had neoplasia as their primary diagnosis, of which 14 (48%) had hypoantithrombinemia (median ATA, 48%; range, 19–80%). Two other hypoantithrombinemic dogs had neoplasia (Sertoli cell tumor and undiagnosed intracranial tumor, Table 2). These tumors were considered not to be responsible for the decreased ATA in these dogs (these dogs had concurrent lymphoma and IMHA, respectively). The proportion of hypoantithrombinemia in dogs with a primary diagnosis of neoplasia (14/29, 48%) was not significantly different compared with that of the dogs that did not have neoplasia (74 of 120 dogs, 62%). The proportion of hypoantithrombinemia among dogs with a primary diagnosis of pancreatitis (13 of 15 dogs, 87%; median ATA, 52%; range, 14–79%) was significantly higher ($P = .048$) compared with that of dogs without pancreatitis (75 of 134 dogs, 56%; median ATA, 78%; range, 17–149%) (OR = 4.8, CI_{95} = 1.04–22.17).

Loss of AT among hypoantithrombinemic dogs was because of severe bleeding (10 dogs), urinary loss (7 dogs), and gastrointestinal loss (2 dogs). The median UPC in the 7 proteinuric dogs was 3.7 (range, 0.6–11) and their median ATA was 47% (range, 28–69%). Dogs with hypoantithrombinemia because of bleeding had a median ATA of 65% (range, 40–83%).

Decreased AT production because of liver failure was presumed in 16 dogs (median ATA, 56%; range, 19–80%). The proportion of hypoantithrombinemia in dogs with liver disease (18 of 21 dogs, 86%) was significantly higher ($P = .014$) than the proportion of hypoantithrombinemia in the dogs without liver disease (70 of 128 dogs, 55%; OR = 4.7; CI_{95}, 1.33–16.87).

There was no significant difference in mean ATA among the different hypothesized mechanisms of hypoantithrombinemia (Fig 1). Of 56 dogs with clear evidence of consumption (eg, presence of thrombosis based on Doppler ultrasound, angio-computed tomography, or presence of DIC), 46 dogs (82%) had hypoantithrombinemia (median ATA, 51%; range, 14–86%).

Hospitalization Period and Mortality

Hypoantithrombinemic dogs had a significantly ($P = .016$) longer hospitalization period (median, 3 days; range, 0–14) as compared with dogs with normal ATA (median, 2 days; range, 0–9). Of the 149 dogs, 80 dogs (54%) died, of which 44 were euthanized at the owner's request within 28 days from admission because of poor prognosis, whereas 69 (46%) survived. The mortality rate of hypoantithrombinemic dogs was significantly ($P = .001$) higher as compared with dogs with normal ATA (67 versus 37%, respectively; OR, 3.5; CI_{95}, 1.73–6.69).

In the dogs with IMHA, hypoantithrombinemic dogs had a significantly ($P = .03$) higher risk for mortality as compared with dogs with normal ATA (16 of 21; OR, 10.5; CI_{95}, 1.03–107.66). Hypoantithrombinemic dogs with neoplasia also had a significantly ($P = .05$) higher risk for mortality as compared with normoantithrombinemic dogs (14 of 29; OR, 6.60; CI_{95}, 1.23–35.44). In dogs with pancreatitis and liver disease, there were no significant mortality differences between dogs with low or normal ATA. Such comparison was not applicable in the DIC group, because all of the dogs in this group had hypoantithrombinemia. The mortality of dogs with DIC in this study was 91%. ATA was significantly ($P < .05$) lower in nonsurvivors as compared with survivors in dogs with IMHA, liver disease and neoplasia, but not in those with pancreatitis (Fig 2).

Logistic regression analysis, using Feldman's previously suggested categories of hypoantithrombinemia,[5] with normoantithrombinemic dogs as the control category, showed that the OR for mortality significantly increased when ATA was <60%, and further increased when ATA was <30% (Table 3). There was no statistically significant mortality difference between the control category (normal ATA) and the 60–87% ATA level category. A similar analysis, based on a different division

Table 2. Specific diagnoses of hypoantithrombinemic dogs and selected diagnoses of normoantithrombinemic dogs.[a]

Diagnosis	Number of Diagnoses in Dogs with Decreased ATA	Number of Diagnoses in Dogs with ATA WRI
Cardiac disease	5	7
Cardiac temponade and CHF	2	2
CHF	1	2
Myocardial infarction	1	0
CHF and left atrial thrombus	0	3
Ventricular septal defect and atrial thrombus	1	0
Neoplasia	16	15
Hemangiosarcoma	4	2
Renal carcinoma	2	1
Mammary adenocarcinoma	2	0
Multiple myeloma	1	2
Heart base tumor	1	2
Ovarian tumor	1	0
Histiocytic sarcoma	1	3
Lymphoma	1	0
Acute lymphoblastic leukemia	1	0
Sertoli cell tumor	1	0
Intracranial tumor (undiagnosed)	1	0
IMHA	16	5
DIC	21	0
Pancreatitis	13	2
Snakebite[1]	4	0
Infectious disease	10	5
Sepsis	3	0
Bacterial endocarditis	2	1
Babesiosis (*Babesia canis vogeli*)	1	0
Bronchopneumonia	1	0
Pyelonephritis	1	1
Aspiration pneumonia	1	0
Meningitis	1	0
Spirocercosis[2]	3	1
Heatstroke	2	0
Major trauma	2	2
CNS disease	1	4
Vascular accident	1	1
FCI		1
Cerebellar hernia		1
Geriatric vestibular syndrome		1
Miscellaneous	4[3]	13[4]
Severe bleeding	10[5]	4[6]
PLN	7[7]	2[8]
PLE	2[9]	1[10]
Liver diseases	18[11]	3[12]

[a]The number of diagnoses exceeds the number of dogs as a single dog could have more than a single diagnosis; 1, *Vipera palaestinae*; 2, *Spirocerca lupi* and secondary aortic thromboembolism; 3, major trauma (2 dogs), CNS vascular accident, ATE of unknown cause; 4, CNS (4 dogs) vascular accident, fibrocartilaginous infarct, cerebellar hernia, geriatric vestibular syndrome, major trauma (2 dogs), lung lobe torsion, gastric dilatation and volvulus, splenic torsion, hiatal hernia, chronic kidney disease (CKD) (2 dogs), acute renal failure; 5, *S. lupi* and aortic aneurism rupture, Post op bleeding due to ehrlichiosis and thrombocytopenia, idiopathic renal hematuria, gastrointestinal (GI) bleeding secondary to liver failure (3 dogs), immune mediated thrombocytopenia (IMT), (2 dogs), thrombocytopenia due to hyperestrognism, ehrlichiosis; 6, anticoagulant poisoning, idiopathic renal hematuria, GI ulcers secondary to nonsteroidal anti-inflammatory drugs and steroids, GI bleeding secondary to IMT; 7, amyloidosis, glomerulonephritis, CKD, glomerulonephritis secondary to ehrlichiosis (2 dogs), diskospondylitis and multiple myeloma (MM); 8, CKD, glomerulonephritis secondary to MM; 9, inflammatory bowel disease (2); 10, lymphangiectasia; 11, severe hepatotoxicity (3 dogs), liver failure (6 dogs), chronic hepatitis and fibrosis (3 dogs), congenital PSS, congenital PSS and pancreatitis, acute lymphoblastic leukemia with liver infiltration, histiocytic sarcoma, hypoxia, and hepatic necrosis (1 dog each), sepsis with liver microabscessation. Liver failure was present in 16 dogs with liver disease; 12, chronic hepatitis (2 dogs), liver failure (1 dog).

ATA, antithrombin activity; WRI, within reference interval; CHF, congestive heart failure; IMHA, immune mediated hemolytic anemia; DIC, disseminated intravascular coagulation, which was a secondary complication in all cases; PLN, protein losing nephropathy; PLE, protein losing enteropathy.

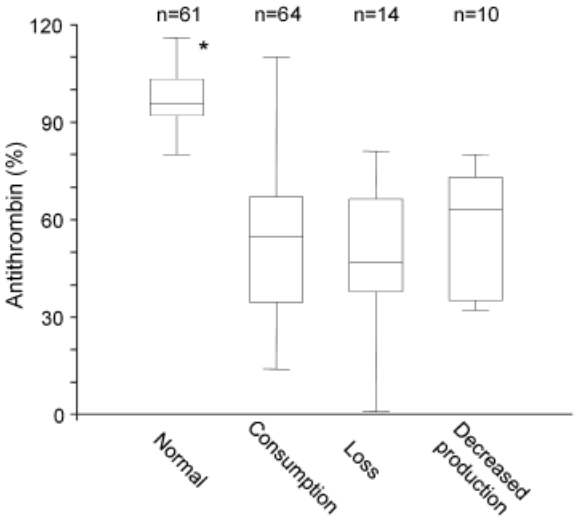

Fig 1. Antithrombin activity distribution in hypoantithrombinemic dogs by the 3 hypothesized mechanisms responsible for its decrease and compared with normoantithrombinemic dogs. Each hypoantithrombinemic dog was assigned a single major mechanism responsible for hypoantithrombinemia. Data are presented as boxes and whiskers plots. Each box includes the interquartile range, whereas the line within a box represents the median, and the whiskers represent the range, extending to a maximum of 1.5 times the interquartile range. *Significant ($P = .001$) difference from the other mechanisms of hypoantithrombinemia.

from human medicine showed that the OR for mortality increased progressively with the decline in ATA (Table 3).[8] Additionally, there was no mortality difference between dogs in the intermediate ATA level category (60–75%, ie, the ATA level considered not to be associated with increased risk by Feldman et al[5] but below normal ATA as suggested by human medicine guidelines[8]) and dogs with normal ATA.

ROC analysis of ATA as a discriminating marker of outcome (ie, mortality) showed an area under the curve of 70%. The optimal cutoff point was an ATA of 60%, corresponding to 58% sensitivity and 85% specificity.

Discussion

In order to assess the usefulness of ATA as a diagnostic and prognostic indicator, the present study investigated a population of ill dogs, for which the only inclusion criterion was an available ATA measurement. This was done to achieve a wide case selection, make a general assessment of the clinical usefulness of ATA, and provide a basis of its interpretation in dogs.

Several laboratory variables were significantly different between hypoantithrombinemic dogs and those with normal ATA. The significantly higher WBC and higher proportion of leukocytosis in hypoantithrombinemic dogs suggest that hypoantithrombinemia is associated with inflammation. There also were significant differ-

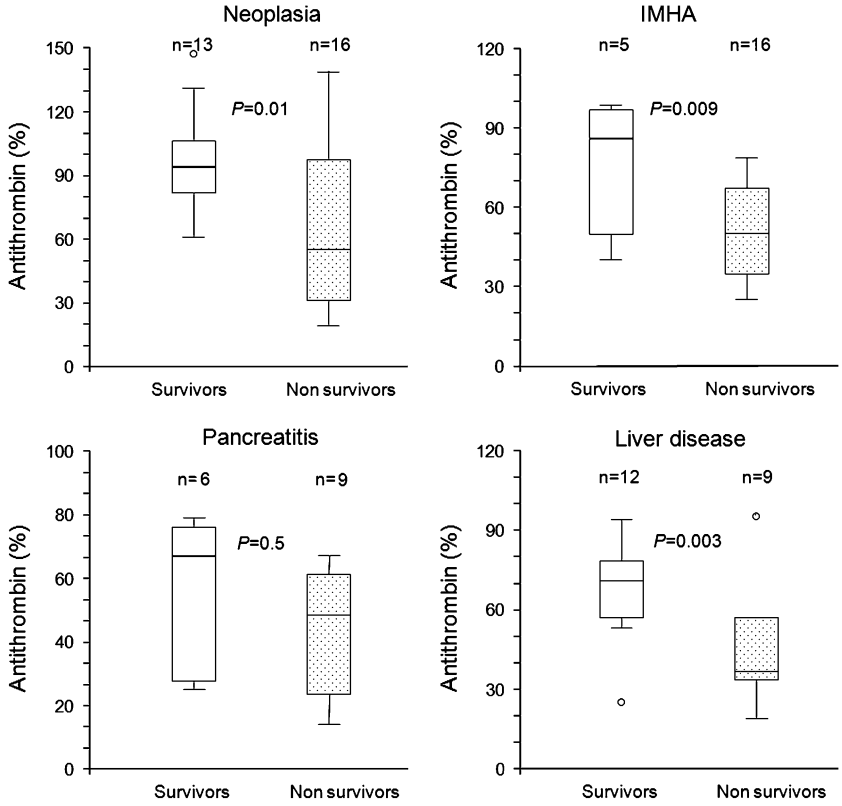

Fig 2. Comparison of antithrombin activity between survivors and nonsurvivors in diseases commonly observed in the study. Data are presented as boxes and whiskers. Each box includes the interquartile range, whereas the line within a box represents the median, and the whiskers represent the range, and extend to a maximum of 1.5 times the interquartile range. Outliers are depicted by circles.

Table 3. Odds ratio for mortality, its 95% confidence interval, and *P*-value at different degrees of hypoantithrombinemia compared with a reference category.

ATA (%)	OR for Mortality	CI 95% of the OR	*P*-Value
	Based on Feldman et al[5]		
0–30	14.7	2.8–70.7	.001
30–60	9.9	3.8–26.1	< .001
60–87	1.5	0.6–3.6	.32
	Based on McGann et al[9]		
0–50	7.2	2.8–18.5	< .001
50–75	2.9	1.3–6.4	.007

ATA, antithrombin activity; OR, odds ratio; CI 95%, 95% confidence interval of the odds ratio.

ences in several hemostatic variables between the 2 ATA groups, including lower mean platelet count, longer mean aPTT and higher proportion of thrombocytopenia and prolonged mean PT in hypoantithrombinemic dogs. This is probably because decreased ATA reflects derangement of hemostasis, and thus is often associated with additional hemostatic abnormalities.[1,2,6,32] The significant, albeit weak, inverse correlations between ATA and PT and aPTT may support the assumption that these changes are linked to common disease processes.

The significant (but weak) correlation between ATA and albumin and the higher proportion of hypoalbuminemia in hypoantithrombinemic dogs exemplify the similarity of changes in the concentrations of both proteins, which share comparable properties. Both are produced by the liver, and are of relatively low molecular weight, and thus are concurrently lost in PLN, PLE, and bleeding.[1,2,18,33] In dogs with glomerular disease, there is an association between severe hypoalbuminemia (albumin <2 g/dL) and ATA $< 75\%$, and this was interpreted as an increased risk factor for thrombosis.[19,21] In addition, the concentration of albumin, a negative acute phase protein, tends to decrease in inflammatory conditions, which also can be associated with decreased ATA.[34] Although AT is considered a positive acute phase protein,[35] the tendency for hypoantithrombinemia probably overwhelms the hepatic synthetic capacity when consumptive and inflammatory processes are advanced.[10]

The higher bilirubin concentrations in hypoantithrombinemic dogs likely was associated with hepatobiliary diseases that were significantly more common in this group as compared with normoantithrombinemic dogs, and because IMHA also was more common, although not significantly, in hypoantithrombinemic dogs. The higher proportion of hypercholesterolemia in hypoantithrombinemic dogs likely resulted from cholestasis, secondary to liver disease, pancreatitis and sepsis that were common diagnoses in this group. The higher proportion of hypoglycemia in hypoantithrombinemic dogs might have resulted from sepsis, liver failure, pancreatitis, and paraneoplastic processes.

Consumption was the most common hypothesized mechanism of hypoantithrombinemia in this study. IMHA, which was a common consumptive disorder in hypoantithrombinemic dogs, can lead to hypercoagulability because of increased concentrations of procoagulants, decreased anticoagulant (eg, AT) concentrations, vasculitis, and platelet activation leading to a hypercoagulable state, immune-mediated platelet destruction, and possibly DIC.[28,29,36] Hypoantithrombinemia and a hypercoagulable state were present in 10 of 20 dogs with IMHA,[29] however, in that study there were no differences in survival and prevalence of thrombotic events between hypo- and normoantithrombinemic dogs.[29] In the present study, hypoantithrombinemia was present in 76% of the 21 dogs with IMHA, and ATA was a negative prognostic marker in this disease. This difference between studies may be due to relatively small study populations and differences in case selection and thus in disease severity.

Recent studies using thromboelastography and other hemostatic measures in dogs with DIC have demonstrated the diagnostic importance of ATA measurement in DIC, although it was not a significant prognostic marker.[16,17] In the current study, hypoantithrombinemia was present in all dogs with DIC. The observed high mortality rate among dogs with DIC in the current study (91% versus 45 and 50% in the aforementioned studies) suggests that patients with DIC in our study were more severely affected, thus leading to a higher proportion of hypoantithrombinemia.

Hypercoagulability has been demonstrated in various neoplastic diseases in dogs, and increased thrombin-AT complexes were detected in several malignancies, especially in hemangiosarcoma and hematopoietic neoplasia.[25–27] In canine mammary carcinoma, ATA was significantly lower in dogs in stage IV as compared with stages I–III.[27] Our results demonstrate that hypoantithrombinemia is common in neoplasia in dogs, although this might have been influenced by bias toward selection of severe, systemically ill dogs. Nevertheless, hypoantithrombinemia was associated with a higher risk for mortality among dogs with neoplasia. It is difficult to assess whether neoplasia per se was responsible for the hypoantithrombinemia, because some of the dogs in this group had concurrent processes such as IMHA, bleeding, PLN, and liver failure that probably contributed to development of hypoantithrombinemia.

Pancreatitis also was a common diagnosis in the consumption group. Hemostatic impairment is a well known complication in pancreatitis, affecting morbidity and mortality and influencing therapy.[37,38] Activation of circulating plasma clotting factors by free pancreatic proteases that leak from the diseased pancreas to the vascular space and lead to AT consumption is one of the speculated mechanisms for hypercoagulability in pancreatitis.[39] In addition, AT has been shown to bind plasma pancreatic trypsin in humans. In fact, pancreatic trypsin inactivation by AT occurs faster compared with its inactivation by antitrypsin, its specific inhibitor.[40] Thus, pancreatitis, leading to increased plasma trypsin activity likely results in its binding to AT, which also may contribute to hypoantithrombinemia. To the best of our knowledge, there are no published data on ATA in naturally occurring pancreatitis in dogs. The observed high

proportion of hypoantithrombinemia in pancreatitis in this study suggests that ATA measurement should probably be included in the assessment of dogs with pancreatitis, because it is commonly decreased, and may affect therapy.

Dogs with clear evidence of consumption (eg, iliac thrombosis) comprised an interesting subgroup in the present study. Although hypoantithrombinemia was very common in these dogs, its proportion did not reach 100% and was only 82%. Thus, normal ATA cannot exclude presence of a consumptive or thrombotic process. In such conditions, ATA depends on the balance between hepatic AT production and AT consumption rates.

Loss, another hypothesized mechanism of hypoantithrombinemia, may occur through the kidneys, intestines, and because of severe bleeding.[20,33] Selective loss of low molecular weight plasma proteins such as albumin and AT was described in PLN and has been suggested to contribute to hypercoagulability in this syndrome.[18,19,21,22,41] Hypoantithrombinemia was commonly observed in our PLN patients (7 of 9 dogs), and because previous studies have suggested an association between the presence of PLN-associated hypoantithrombinemia and occurrence of thrombosis, it seems advisable to closely monitor ATA in such patients.[19,21] There were only 3 PLE cases in the present study, of which 2 had hypoantithrombinemia. Although PLE is a common disorder in dogs, and thromboembolic complications were reported in dogs with PLE, presumably due to AT loss,[33,42–45] investigation of ATA in this condition in dogs has yet to be conducted. Clinicians should be aware of potential hypercoagulability in PLE, especially when protein loss is severe. Severe bleeding was a common cause of hypoantithrombinemia in this study, but its clinical relevance as a potential cause of thrombosis is questionable, because bleeding results in concurrent loss of both plasma anticoagulants and clotting factors. Nevertheless, ATA probably should be monitored when bleeding is severe and clinicians should be aware of potential hypoantithrombinemia in such dogs.

Decreased AT production was the least common cause of hypoantithrombinemia in the present study, although hypoantithrombinemia was described previously in liver failure in dogs.[12] In a previous study in dogs, ATA was considered a relatively insensitive marker of liver disease, because it was decreased in only 43% of the cases.[13] However, concurrently decreased ATA, protein C activity, and hyperbilirubinemia were associated with a grave prognosis in liver disease in dogs.[13] In the present study, hypoantithrombinemia was detected in 86% of the dogs with evidence of liver disease. The proportion of concurrent liver failure however was higher in these dogs compared with the above-mentioned study, probably accounting for the higher proportion of hypoantithrombinemia. Half of the hypoantithrombinemic dogs with liver disease presumably had concurrent consumption and AT loss through bleeding. Thus, in dogs with liver disease (similarly to those with neoplasia) more than a single mechanism of hypoantithrombinemia may be present.

There was no difference in the severity of hypoantithrombinemia among the 3 hypothesized pathophysiologic mechanisms in this study. Thus, the specific disease, its pathophysiology and reported effect on coagulation seem to be of greater importance. The significantly longer hospitalization time observed in hypoantithrombinemic dogs as compared with those with normal ATA suggests that the former had more severe disease, and that in dogs with hypoantithrombinemia, higher morbidity and treatment costs should be expected. This observation is in agreement with studies in people, associating hypoantithrombinemia with prolonged hospitalization and greater costs due to thromboembolic complications.[9] The present study also has demonstrated a significantly higher risk for mortality in hypoantithrombinemic dogs, as has been observed in human patients, but ATA was not a strong discriminator of outcome, as reflected by the area under the ROC curve. Nevertheless, the present OR for mortality is in agreement with previously published guidelines based on experiments in baboons as well as in human patients. In our study, a significantly higher risk for mortality was documented when ATA was $<60\%$, and this risk increased even more with lower ATA. Many of the nonsurvivors were euthanized; however, the attending clinicians were unaware of the results of this retrospective study. Thus, ATA did not have a major deciding role in euthanasia, which rather was based on the overall assessment of the patient. It is currently unknown whether hypoantithrombinemia is the cause for this apparently increased risk for mortality, or if it is a general marker of disease severity, and as such, is indirectly associated with mortality. Based on the current results, we could not provide an ATA cut-off point below which the risk of thrombosis significantly increases, mainly because thrombosis was infrequently diagnosed. When thrombosis was diagnosed in our patients, it might have resulted from several pathophysiologic mechanisms, of which hypoantithrombinemia is only 1 factor.

This study had several limitations. First, there was bias in patient selection because ATA was not routinely measured, but was rather measured in severely affected patients with a high index of suspicion of hypoantithrombinemia. Thus, the true prevalence of hypoantithrombinemia in different diseases in dogs still is unknown. Second, the number of cases in each specific diagnosis and mechanism of hypoantithrombinemia was limited, reducing the statistical power of our analyses and increasing the likelihood of making a type II error. In addition, the decision as to which one of several mechanisms of hypoantithrombinemia present in a single dog was the main one was sometimes difficult, and based on authors' best assessment. These inherent limitations warrant future prospective, case-controlled, large-scale studies to assess the true prevalence of hypoantithrombinemia and the usefulness of ATA as a diagnostic and prognostic marker, as well as its impact on therapeutic intervention in dogs.

In conclusion, hypoantithrombinemia is significantly associated with a higher risk of mortality in various diseases and the latter occurs when ATA is $<60\%$ and

significantly increases further when ATA is $<30\%$. ATA is a potentially useful clinical variable and its measurement is becoming increasingly more available. Thus, it should be considered in diseases with a high index of suspicion of one or more of the pathophysiologic mechanisms of hypoantithrombinemia, particularly in IMHA, pancreatitis, neoplasia, DIC, and liver diseases.

Footnotes

[a] Automatic impedance cell analyzers, Abacus or Arcus, Diatron, Wien, Austria
[b] Wet chemistry autoanalyzer, Cobas-Mira, Roche, Rotkreutz, Switzerland
[c] Ion-selective electrode electrolyte analyzer OmniC, Roche, Mannheim, Germany
[d] Automatic coagulometric analyzer, ACL200, Reagents; APTT, HemosIL APTT-SP (liquid) 0020006300; PT and fibrinogen, HemosIL PT-Fibrinogen Recombinant 0020005000, Instrumentation Laboratories, Warrington, UK
[e] Manual coagulometric analyzer, KC1A, micro, Amelung, Germany. Reagents; APTT, Biopool APTT-P 50320; PT, Biopool, Thromboplastin-S 500221, Trinity Biotech plc Bray, Co. Wicklow, Ireland
[f] Chromogenic substrate, HemosIL 0020008910, Instrumentation Laboratory
[g] Factor Xa reagent, HemosIL 0020008920, Instrumentation Laboratory
[h] Minutex D-dimer, Trinity Biotech, Bray, Ireland
[i] Canine pancreatic lipase-like immunoreactivity, SNAP cPL, Idexx laboratories, Westbrook, ME
[j] SPSS 15.0 for Windows, SPSS Inc, Chicago, IL

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