Acta vet. scand. 1986, 27, 369—377.

From the Departments of Clinical Pathology and Medicine, School of Veterinary Medicine, University of California, Davis, and Department of Veterinary Pathology, Royal Veterinary and Agricultural University, Denmark.

NATURALLY OCCURRING CANINE NEPHROTIC SYNDROME IS A POTENTIALLY HYPERCOAGULABLE STATE

By

A. Rasedee, B. F. Feldman and *R. Washabau*

RASEDEE, A., B. F. FELDMAN and R. WASHABAU: *Naturally occurring canine nephrotic syndrome is a potentially hypercoagulable state.* Acta vet. scand. 1986, 27, 369—377. — Fourteen dogs with naturally occurring nephrotic syndrome were evaluated for abnormalities in the hemostatic system. Histopathologic diagnoses included 8 dogs with membraneous glomerulonephritis, 1 dog with acute glomerulonephritis, 2 dogs with idiopathic glomerulopathy, and 2 dogs with amyloidosis. The coagulation protein assays performed included concentrations of factors V, VII, VIII: C, IX, X, fibrinogen (I), antithrombin III, and plasminogen. Thrombocyte counts were also performed. All of these analytes were significantly elevated ($P < 0.05$) with the exception of ATIII which was significantly decreased ($P < 0.05$). Five of the dogs had histologic evidence and 1 dog had angiographic evidence of thrombosis and thromboembolism. Naturally occurring canine nephrotic syndrome thus represents a potentially hypercoagulable state and may serve as a valuable model in the study of certain components of the human disease.

dog; hemostasis; hypercoagulation; thromboembolism; thrombosis; antithrombin III.

Hypercoagulability has been proposed to explain the increased incidence of thrombosis encountered in certain clinical states associated with thrombotic diatheses. Hypercoagulability has been defined as "an altered state of circulating blood that requires a smaller quantity of clot-promoting substances to induce intravascular coagulation than is required to produce comparable thrombosis in a normal subject" (*Wall & Harker* 1980). Human patients with nephrotic syndrome are at risk for thrombotic disease. The exact cause of thrombosis in these patients has yet to be determined although a variety of laboratory abnormalities

have been described. Nephrotic syndrome is generally regarded as hypercoagulable state (*Kendall et al.* 1971) and has been associated with thrombocytosis, elevated concentrations of procoagulants and decreased fibrinolytic activity (*Kendall et al.* 1971, *Vaziri et al.* 1983). It has been additionally established that decreased concentrations of antithrombin III (ATIII) are associated with thrombotic diatheses (*Cosgriff et al.* 1983). In nephrotic syndrome plasma, ATIII concentrations are decreased due to selective renal losses (*Kauffman et al.* 1978, *Jørgensen & Stoffersen* 1979, *Girot et al.* 1983). It should be emphasized that thrombosis in neprotic syndrome is dependent on the interaction of these factors rather than any single factor. Observations in previous studies of naturally occurring canine nephrotic syndrome documented similar hemostatic abnormalities associated with thrombosis (*DiBartola & Meuten* 1980, *Green & Kabel* 1982). Thus the availability of a relevant naturally occurring disease model would be useful in the investigation of thrombotic complications in nephrotic syndrome. The object of this study was to examine canine nephrotic syndrome for possible parallels with the human disease.

MATERIALS AND METHODS

Canine patients

Fourteen cases of canine nephrotic syndrome were examined. The criteria for diagnosis were hypoalbuminemia, proteinuria averaging more than 2 g in 24 h, hypercholesterolemia, and serum urea nitrogen less than 15.0 mmol/l. The group of dogs included 8 males and 6 females, aged from 1½ years to 11 years, and represented 14 different breeds.

Renal function was determined by serial serum urea nitrogen and/or creatinine measurements, endogenous creatinine clearance, and quantitative determinations of urinary protein loss. Cholesterol determinations and renal biopsies were performed. Glomerular changes were assessed by light microscopy and, less frequently, by electron microscopy. The histologic features of nephrotic syndrome in these dogs were then summarized.

Blood sampling

Thrombocyte poor plasma from clinically normal dogs was prepared from citrated whole blood (1 part of 3.8 % trisodium

citrate to 9 parts of whole blood) by centrifugation (2500 × G) for 15 min at 4°C. All samples were immediately frozen and kept at —30°C. All samples were thawed immediately before use. A canine plasma standard was pooled from 26 of these dogs. There were equal numbers of males and females in the pool. Patient blood was similarly handled.

Assay methods

Plasma ATIII and plasminogen concentrations were determined spectrophotometrically using Kabi chromogenic substrates S-2238 and S-2251 (Helena Laboratories, Beaumont, Texas, USA), respectively. Platelet counts were performed on a platelet counter (Thrombocounter, Coulter Electronics, Hialeah, Florida, USA).

Fibrinogen concentrations were measured by a thrombin clotting time (*Owen et al.* 1975). Factor deficient human plasma (Dade Division, American Hospital Supply Corp., Miami, Florida, USA) was used to assay concentrations of specific factors V, VII, and X, employing methods previously described (*Dodds et al.* 1975). Factor deficient canine plasma (courtesy of W. J. Dodds, Albany, New York, USA) was used for specific assays of factors VIII:C and IX. The factor assays were performed on a Fibrometer (Fibrosystem, Cockeysville, Maryland, USA). Pooled canine plasma was utilized to generate the standard curve.

Each sample was assayed for ATIII, plasminogen, and the coagulation factors. Values were expressed as a percentage of normal with the assumption that the pooled sample represented an arbitrary average of 100 %.

Statistical analysis

The statistical analysis involved a comparison of mean platelet counts, ATIII and plasminogen concentrations, and coagulation factor activity in the diseased dogs with the mean obtained from repetitions performed on the pooled plasma. A similar comparison was made between membranous and nonmembranous glomerulopathies. These comparisons were made for each variable using simple t-tests. Statistical significance was considered at the 0.05 level.

RESULTS

The results of renal biopsies are listed in Table 1. The patients were then divided into 2 groups, membranous and nonmembranous glomerulonephropathy, for comparison purposes. However, no significant differences ($P > 0.05$) were observed between the 2 groups for any of the analytes assayed.

Table 1. Summary of diagnoses in 14 cases of canine nephrotic syndrome.

Diagnosis	Number of cases
Idiopathic glomerulopathy	2
Membranous glomerulonephritis	8
Subacute glomerulonephritis	1
Acute glomerulonephritis	1
Amyloidosis	2

The mean values (± SE) of serum urea nitrogen, creatinine, cholesterol, endogenous creatinine clarance and, 24-h urine protein are summarized in Table 2. The mean (± SE) serum cholesterol concentration and 24-h urine protein were significantly elevated ($P < 0.05$) while endogenous creatinine clearance was significantly decreased ($P < 0.05$) below the mean value of the reference interval.

All animals demonstrated variable increases in coagulation protein concentrations in both the membranous and nonmembranous groups. Factors V, VII, VIII:C, IX and X were modestly

Table 2. The mean values (± SE) for serum urea nitrogen, creatinine, cholesterol, endogenous creatinine clearance and 24 h urine protein in 14 canine patients with nephrotic syndrome.

Analyte	Reference interval	Glomerulopathy	
		Membranous (n = 8)	Nonmembranous (n = 6)
Serum urea nitrogen (mmol/l)	3.3—9.4	7.9 ± 0.8	7.3 ± 0.65
Serum creatinine (mmol/l)	40.0—130.0	109.0 ± 4.5	102.2 ± 3.7
Endogenous creatinine clearance (ml/min/m)	50—70	44 ± 4.2	47 ± 3.7
Cholesterol (mmol/l)	3.0—5.0	8.7 ± 0.88	7.9 ± 1.1
Urine protein (g/24 h)	0.0—0.3	3.2 ± 1.7	3.6 ± 1.9

Table 3. Hemostatic analytes in 14 canine nephrotic syndrome patients.

Hemostatic Analyte	Reference Interval	Glomerulopathy	
		Membranous (n = 8)	Nonmembranous (n = 6)
Thrombocytes ($\times 10^6/\mu l$)	200—400	565 ± 35	527 ± 67
Antithrombin III (%)	89—108	67 ± 35	58 ± 8
Plasminogen (%)	88—120	142 ± 7	135 ± 5
Fibrinogen (mg/dl)	200—400	635 ± 61	547 ± 47
Factor V (%)	80—120	135 ± 5	131 ± 8
Factor VII (%)	70—130	141 ± 7	145 ± 6
Factor VIII:C (%)	75—125	147 ± 8	134 ± 5
Factor IX (%)	50—150	153 ± 6	160 ± 8
Factor X (%)	60—135	166 ± 11	174 ± 8

All observed values are expressed as mean ± SE

increased ($P < 0.05$), whereas fibrinogen was markedly increased though not in a statistical context ($P > 0.05$) (Table 3).

Mean thrombocyte counts were significantly higher ($P < 0.05$) than the mean value of the reference interval (Table 3).

Mean plasminogen concentration was significantly elevated ($P < 0.05$) as compared to the mean value of the reference interval (Table 3).

The mean ATIII concentration was significantly decreased ($P < 0.05$) as compared to the mean value of the reference interval (Table 3).

Histologic evidence of renal vein thrombosis was noted in 3 of the 14 renal biopsies. Angiography demonstrated pulmonary embolism in 1 dog. At necropsy, 2 additional dogs had evidence of venous and/or arterial thrombosis. These dogs also had histologic evidence of thrombosis. There was no evidence of vasculitis in any of the dogs.

DISCUSSION

Nephrotic syndrome is caused by primary glomerular disease, by systemic diseases including diabetes mellitus and systemic lupus, by drugs, infections, malignancy and other miscellaneous conditions such as allergy. Nephrotic syndrome in man is generally accepted to be associated with increased risk for thrombotic disease. This tendency has been attributed to the presence of a hypercoagulable state (*Kendall et al.* 1971). Comparable

studies in dogs are few, but a similar hypercoagulable state has been documented (*DiBartola & Meuten* 1980, *Green & Kabel* 1982).

The present study revealed that of the 14 dogs diagnosed with nephrotic syndrome, 6 of the dogs had demonstrable evidence of thrombosis and/or thromboembolism. In people mild thrombocytosis, elevations in fibrinogen concentration and concentrations of factors V, VII, VIII:C, and X in nephrotic syndrome have been suggested to represent a hypercoagulable state (*Kendall et al.* 1971). Similar findings were observed in this canine study.

Various suggestions have been offered to explain the increase in concentrations of factors VIII:C and fibrinogen. Both of these proteins are acute phase inflammatory proteins. Elevations in concentrations of these proteins may be associated with renal inflammation. Furthermore, factor VIII:C may be released by damaged renal endothelium in nephrotic syndrome in the absence of inflammation (*Bloom et al.* 1973). Alternatively, these changes in factor concentrations may reflect a nonspecific increase in hepatic synthesis of fibrinogen and other coagulation proteins. The mechanisms responsible for the elevations in the concentrations of factors VIII:C and fibrinogen were not elucidated in this study. However, glomerular inflammation was not a prerequisite since 4 of the 14 dogs had noninflammatory glomerular disease.

Abnormalities in coagulation proteins are not as consistent in nephrotic syndrome as might be anticipated, regardless of etiologies. In contrast to our findings, concentrations of factors IX and X have been reported to be decreased in human renal amyloidosis (*McPherson et al.* 1977). Speculatively these differences could be attributed to species variation.

Thombocytosis has been reported in association with many pathologic conditions including acute and chronic inflammatory diseases. The injection of foreign substances which produce inflammation in experimental animals has resulted in thrombocytosis associated with an increase in megakaryocyte colony-forming units in vitro. The process is blocked by inhibitors of T lymphocytes in vivo and in vitro. These results suggests a nonspecific release of a mitogen from T lymphocytes in the marrow mediates megakaryocyte hyperplasia. The combination of thrombocytosis and thrombocyte stimulation in nephrotic syndrome

results in hypercoagulable thrombocytosis (*Walter et al.* 1981). It would be intersting to speculate about a renal role in thrombopoiesis especially since the renal role in erythropoiesis has been well established.

The hypercoagulable state may be enhanced by a decrease in fibrinolytic activity. Diminished fibrinolytic activities could result from one or a combination of the followng factors: decreased plasminogen synthesis, increased clearance of plasminogen and plasmin, deficiency in plasminogen activators, decreased clearance of plasminogen and plasmin inactivators (antiplasmins). In this study with plasminogen concentrations increased, a lack of plasmin production or, more specifically, an impaired conversion of plasminogen to plasmin by endogenous activators is implied. This is supported by observations that deficiency in the release of plasminogen activators from the walls of superficial veins (*Isacson & Nilsson* 1972) and diminished concentrations of activators were observed after venous occlusion in patients with deep vein thrombosis (*Ratnoff* 1981, *Wiman et al.* 1985).

Antithrombin III is the main inhibitor of the coagulation system's serine proteases (*Rosenberg* 1975). Inherited or acquired ATIII deficiency is associated with thrombotic diatheses (*Thaler & Lechner* 1981, *Cosgriff et al.* 1983). The ATIII concentrations in this study, as previously observed in humans and dogs (*Kauffman et al.* 1978, *Jørgensen & Stoffersen* 1979, *Green & Kabel* 1982), were decreased. This decrease has been correlated with serum albumin concentrations (*Kauffman et al.* 1978), suggesting either lack of hepatic synthesis or, more likely, concommitant urinary losses of these proteins in the disease.

The hypercoagulable state in nephrotic syndrome cannot be attributed to abnormalities in the coagulation cascade alone since thrombosis is a multistep process involving abnormalities in coagulation proteins, the controls of their activation, platelets and the endothelial lining of blood vessel walls. Thus the etiopathogenesis of hypercoagulability involves all components of the thrombotic process.

Thromboembolism is often a sequela of naturally occurring canine nephrotic syndrome which closely parallels the human disease. This animal model may serve as a valuable tool in the investigation, control, and treatment of thrombosis in nephrotic syndrome.

ACKNOWLEDGEMENT

This work was supported, in part, through a National Institute of Health (USA), Public Health Service Biomedical Research Support Grant (84-15).

REFERENCES

Bloom, A. L., J. C. Giddings & C. J. Wilke: Factor VIII on the vascular intima: possible importance in hemostasis and thrombosis. Nature 1973, *241*, 217—219.

Cosgriff, T. M., D. T. Bishop, E. J. Hershgold, M. H. Skolnick, B. A. Martin, B. J. Baty & K. S. Carlson: Familial antithrombin III deficiency: its natural history, genetics, diagnosis and treatment. Medicine 1983, *62*, 209—220.

DiBartola, S. P. & D. J. Meuten: Renal amyloidosis in two dogs presented for thrombolic phenomena. J. Amer. Anim. Hosp. Assoc. 1980, *16*, 19—135.

Dodds, W. J., A. C. Moynihan, R. F. Benson & C. A. Hall: The value of age- and sex-matched controls for coagulation studies. Brit. J. Haematol. 1975, *29*, 305—317.

Girot, R., F. Jaubert, M. Leon, B. Bellon, M. Aiach, F. Josso, O. Lepelletier, S. Beguin & J.-P. Monnet: Albumin, fibrinogen, prothrombin and antithrombin III variations in blood, urines and liver in rat nephrotic syndrome (Heymann nephritis). Thrombosis and Haemostasis 1983, *49*, 13—17.

Green, R. A. & A. L. Kabel: Hypercoagulable state in three dogs with nephrotic syndrome: role of antithrombin III. J. Amer. vet. med. Assoc. 1982, *181*, 914—917.

Isacson, S. & I. M. Nilsson: Defective fibrinolysis in blood and vein walls in recurrent "idiopathic" venous thrombosis. Acta chirurg. scand. 1972, *138*, 313—319.

Jørgensen, K. A. & E. Stoffersen: Antithrombin III and the nephrotic syndrome. Scand. J. Haematol. 1979, *22*, 442—448.

Kauffman, R. H., J. J. Veltkamp, N. H. Van Tilburg & L. A. Van Es: Acquired antithrombin III deficiency and thrombosis in nephrotic syndrome. Amer. J. Med. 1978, *65*, 607—613.

Kendall, A. G., R. C. Lohmann & J. B. Dosseter: Nephrotic syndrome, a hypercoagulable state. Arch. intern. Med. 1971, *127*, 1021—1027.

McPehrson, R. A., J. W. Onstad, R. J. Ugoretz & P. L. Wolf: Coagulopathy in amyloidosis: combined deficiency of factors IX and X. Amer. J. Haematol. 1977, *3*, 225—235.

Owen, C. A., E. J. W. Bowie & J. E. Thompson: The Diagnosis of Bleeding Disorders, Second Edition, Little, Brown and Co., Boston 1975, p. 134—135.

Ratnoff, O. D.: The role of haemostatic mechanisms. Clinics in Haematology 1981, *10*, 261—282.

Rosenberg, R. D.: Actions and interactions of antithombin and heparin. New Eng. J. Med. 1975, *292*, 146—150.

Thaler, E. & K. Lechner: Antithrombin III deficiency and thromboembolism. Clinics in Haematology 1981, *10*, 369—390.

Vaziri, N. O.: Nephrotic syndrome and coagulation and fibrinolytic abnormalities. Amer. J. Nephrol. 1983, *3*, 1—6.

Wall, R. T. & L. A. Harker: The endothelium and thrombosis. Annu. Rev. Med. 1980, *31*, 361—370.

Walter, E., K. Depperman & J. Andrassay: Platelet hypercoagulability as a consequence of the nephrotic syndrome. Thromb. Res. 1981, *23*, 473—479.

Wiman, B., B. Ljungberg, J. Chmielewska, G. Urden, M. Blomback & H. Johnsson: The role of the fibrinolytic system in deep vein thrombosis. J. Lab. clin. Med. 1985, *105*, 265—270.

SAMMENDRAG

Naturligt forekommende nefrotisk syndrom hos hund er en potentiel hyperkoagulationstilstand.

Fjorten hunde med naturligt forekommende nefrotisk syndrom blev undersøgt for abnormaliteter i hæmostasesystemet. Histopatologisk diagnosticeredes membranøs glomerulonefritis hos 8, idiopatisk glomerulopati hos 2, akut glomerulonefritis hos 1, subakut glomerulonefritis hos 1 og amyloidose hos 2 hunde. Koncentrationen af følgende koagulationsproteiner blev bestemt: faktorerne V, VII, VIII:C, IX, X, fibrinogen (1), antithrombin III og plasminogen. Trombocyttælling blev også foretaget. Alleparametre var signifikant forhøjede ($P < 0,05$) med undtagelse af ATIII, som var signifikant formindsket ($P < 0,05$). Hos 5 hunde fandtes histologiske og hos 1 hund angiografiske tegn på tromboembolisme. Naturligt forekommende nefrotisk syndrom hos hund repræsenterer således en potentiel hyperkoagulationstilstand og kan tjene som værdifuld model for studiet af visse elementer af den humane lidelse.

(Received May 23, 1986).

Reprints may be requested from: Bernard F. Feldman, the Department of Veterinary Pathology, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark.