Hemorheology in spontaneous animal endocrinopathies

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Abstract. Plasma proteins and lipid profile influence whole blood fluidity through changes in plasma viscosity, red cell aggregability and deformation. In diseases like Cushing syndrome (CS) and diabetes mellitus (DM) plasma concentrations of fibrinogen, cholesterol, and triglycerides are increased, and, thus, blood rheology might be affected. Our aim was to determine parameters of blood fluidity in 26 dogs with spontaneous CS and DM. Ten dogs (CS) and 16 dogs (DM) showed typical signs of the respective disease, and their blood was tested for whole blood viscosity (WBV, at 37°C, LS30, Contraves, Zurich, Switzerland) at two shear rates (WBV0.7s–1; WBV94s–1), erythrocyte aggregation (AI, LS30), and plasma viscosity (PV, at 21°C, OCR-D, Paar, Graz, Austria). Plasma fibrinogen, cholesterol, and triglyceride concentration was increased in both groups of patients, plasma total protein was elevated only in dogs with DM. Plasma viscosity and erythrocyte aggregation was increased markedly in all dogs, however, WBV0.7s–1 was increased only in dogs with DM, whereas dogs with CS showed a decrease in WBV0.7s–1 despite the rise in fibrinogen and erythrocyte aggregation. Hypertension and microvascular complications have been demonstrated in patients with CS and DM. These effects are multifactorial, and it seems possible that changes in blood rheology contribute to these disturbances.

Keywords: Cushing syndrome, diabetes mellitus, dog, blood rheology, plasma viscosity

1. Introduction

Blood fluidity is determined by the quantitative and qualitative properties of the blood cells and the viscosity of the plasma. Diseases which affect the plasma protein and lipid profile influence the composition of plasma and, hence, elicit changes in suspending phase viscosity. Such changes were observed in patients suffering from Cushing syndrome (CS) and diabetes mellitus (DM) in man, as well as in dog [18,19,22,25,29,43,47,53]. The increase of the typical biochemical and hemostatic values during the course of these diseases [1,15,25,47] elevate cardiovascular risk and increase the incidence for thromboembolic events [15,19]. Both, CS and DM, are straightforward endocrinological disorders to diagnose in canine practice. Both are associated with severe cardiovascular complications [5,48,54].

In this study the hemorheologic status of 26 dogs with spontaneous CS or DM is described in order to evaluate a potential role of blood fluidity in the development of late complications. The dogs were patients at different stages of the disease.

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2. Material and methods

2.1. Patients

The study was undertaken on dogs of different breed and age and of unidentified type of the endocrinologic disorder. Each dog had spontaneous disease and was seen at a veterinary praxis for health maintenance or treatment. Ten dogs (5 male, 5 female; 10 ± 2 years) with CS and 16 dogs (4 male, 3 female, 9 female castrated; 11 ± 4 years) with DM were examined. Demographic data of the animals are shown in Tables 1a and 1b. The dogs were first examined clinically. In addition to the typical clinical signs (CS: polydipsia/polyuria, polyphagia, truncal obesity, muscle wasting, skin thinning; DM: polydipsia/polyuria, cataracta, retinopathy), a routine blood chemistry profile was made. CS was confirmed in the patients by the increase of the plasma concentration of alkaline phosphatase (AP) and cholesterol [46], and diagnosis of DM was estimated by the increase of the plasma concentration of glucose and fructosamin [30,42].

2.2. Withdrawal of blood

Blood was withdrawn by puncture of the cephalic vein. After sampling, the samples were put into an insulated bag and measurements started with a delay of 1–2 hours due to transportation from the veterinarian to the laboratory. The blood was then immediately rotated at room temperature and analyzed hematologically and rheologically. These measurements were finished within 4 hours following blood sampling.

<table>
<thead>
<tr>
<th>DM</th>
<th>Breed</th>
<th>Age</th>
<th>Gender</th>
<th>Medication</th>
<th>Daily insulin (lente except 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cocker spaniel</td>
<td>11</td>
<td>M</td>
<td>–</td>
<td>18 IE</td>
</tr>
<tr>
<td>2</td>
<td>Pointer</td>
<td>12</td>
<td>F</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>Mongrel dog</td>
<td>7</td>
<td>F</td>
<td>Meloxicam</td>
<td>120 IE</td>
</tr>
<tr>
<td>4</td>
<td>Cairn terrier</td>
<td>13</td>
<td>M</td>
<td>Levothyroxin</td>
<td>10 IE</td>
</tr>
<tr>
<td>5</td>
<td>Yorkshire terrier</td>
<td>12</td>
<td>F</td>
<td>–</td>
<td>3 IE</td>
</tr>
<tr>
<td>6</td>
<td>Poodle</td>
<td>11</td>
<td>F</td>
<td>Sulfadoxin-trimethoprim</td>
<td>15 IE</td>
</tr>
<tr>
<td>7</td>
<td>Poodle</td>
<td>15</td>
<td>F</td>
<td>–</td>
<td>10 IE</td>
</tr>
<tr>
<td>8</td>
<td>Cocker spaniel</td>
<td>12</td>
<td>F</td>
<td>Epinephrine</td>
<td>25 IE</td>
</tr>
<tr>
<td>9</td>
<td>Fox terrier</td>
<td>10</td>
<td>F</td>
<td>–</td>
<td>30 IE</td>
</tr>
<tr>
<td>10</td>
<td>German shepherd</td>
<td>12</td>
<td>F</td>
<td>–</td>
<td>14 IE</td>
</tr>
<tr>
<td>11</td>
<td>Cocker spaniel</td>
<td>9</td>
<td>F</td>
<td>–</td>
<td>35 IE</td>
</tr>
<tr>
<td>12</td>
<td>Schnauzer</td>
<td>14</td>
<td>F</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>Poodle</td>
<td>13</td>
<td>M</td>
<td>Captopril</td>
<td>16 IE</td>
</tr>
<tr>
<td>14</td>
<td>Rottweiler</td>
<td>6</td>
<td>M</td>
<td>–</td>
<td>35 IE</td>
</tr>
<tr>
<td>15</td>
<td>German shepherd</td>
<td>10</td>
<td>F</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>Beagle</td>
<td>9</td>
<td>F</td>
<td>–</td>
<td>24 IE</td>
</tr>
</tbody>
</table>
2.3. Hematology and blood chemistry

From each dog a routine hematologic and blood chemical profile was obtained. Hematology was done by Cell-Dyn 3500 (Abbott, Abbott Park, Illinois, USA) for the measurement of red blood cell (RBC) count (RBC count, in cells \( \times 10^6/\text{ml} \)), mean corpuscular volume (MCV, in fl), mean corpuscular hemoglobin concentration (MCHC, in g/dl), white blood cell count (WBC count, in cells \( \times 10^3/\text{ml} \)), and platelet count (PLT count, in cells \( \times 10^5/\text{ml} \)). Hematocrit (HCT, in %) was measured by centrifugation (Hettich, Tuttlingen, Germany).

The routine blood chemistry profile including plasma cholesterol (in mg/dl), triglyceride (in mg/dl), glucose (in mg/dl), creatinine (in mg/dl), total protein (in g/dl), AP, ALT, LDH, \( \chi \)-GT, and AST (all: in U/l), was performed by Hitachi 904 (Hitachi, Tokyo, Japan).

2.4. Hemorheology

Hemorheology was carried out by LS30 viscometer (Contraves AG, Zürich, Switzerland) at two different shear rates (0.7 s\(^{-1}\) and 94 s\(^{-1}\)) at 37°C, to obtain whole blood viscosity (WBV, in mPa·s). Erythrocyte aggregation was estimated by means of an aggregation index (AI) done with the LS30 during the measurement of whole blood viscosity at low shear rate (0.7 s\(^{-1}\)) by the following procedure: after measure of the WBV at 0.7 s\(^{-1}\) the cup was furthermore rotated at the same speed for 45 seconds. After 45 seconds the WBV was read again. Aggregation index was determined by the formula: 
\[
AI = \frac{\text{peak WBV} - \text{WBV after 45 seconds}}{\text{peak WBV}} \times 100
\]
Calculation of AI by this formula is based upon the hypothesis that the peak value of WBV represents the characteristic viscosity of the blood sample at the given shear rate, whereas the secondary decline is thought to be affected by axial migration of red cell aggregates from the wall of the viscometer which is a measure for erythrocyte aggregation [31].

Plasma viscosity (PV, in mPa·s) was carried out by OCR-D (Paar, Graz, Austria) at 21°C. The viscosity at the shear rate of 10 s\(^{-1}\) was determined for plasma viscosity.

Hemorheology was done with native blood without standardisation to a constant hematocrit.

### Table 1b

Demographic data of 10 dogs suffering from Cushing’s disease. Medication = at time of blood sampling

<table>
<thead>
<tr>
<th>CS</th>
<th>Breed</th>
<th>Age</th>
<th>Gender</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Toy-poodle</td>
<td>10</td>
<td>F</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Yorkshire terrier</td>
<td>12</td>
<td>M</td>
<td>( \beta )-Methyl-digoxin, Captopril</td>
</tr>
<tr>
<td>3</td>
<td>Bloodhound</td>
<td>9</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>West-highland-white terrier</td>
<td>9</td>
<td>F</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Mongrel dog</td>
<td>5</td>
<td>F</td>
<td>Ampicillin, Ubretid, Ulsal</td>
</tr>
<tr>
<td>6</td>
<td>Mongrel dog</td>
<td>7</td>
<td>M</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Labrador retriever</td>
<td>6</td>
<td>F</td>
<td>Captopril</td>
</tr>
<tr>
<td>8</td>
<td>Yorkshire terrier</td>
<td>14</td>
<td>M</td>
<td>Captopril, ( \beta )-Methyl-digoxin</td>
</tr>
<tr>
<td>9</td>
<td>Dalmatian</td>
<td>7</td>
<td>F</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>German shepherd</td>
<td>4</td>
<td>M</td>
<td>–</td>
</tr>
</tbody>
</table>
2.5. Additional measurements

Fibrinogen was measured by the method of Clauss [14]. Fructosamine was measured in DM patients by Hitachi 717 (Hitachi, Tokyo, Japan).

2.6. Statistic

Statistical evaluation was performed with the analysis of variance, corrected with the Bonferroni–Holm procedure [28] for each group of patients. The data of the patients were compared to reference data obtained from our laboratory recently [3,55]. Fructosamine was compared with data from literature [30,42].

Data are represented as median and 25th and 75th percentile. \( P \)-values <0.05 were considered as indicating statistical significance.

3. Results

Table 2 shows the hematologic and blood chemistry profile of patients with CS and DM. Table 3 shows the hemorheologic data of these patients.

<table>
<thead>
<tr>
<th></th>
<th>Cushing syndrome (( n = 10 ))</th>
<th>Diabetes mellitus (( n = 16 ))</th>
<th>Healthy dogs*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT (%)</td>
<td>43 (40/53)</td>
<td>45 (42/51)</td>
<td>45 (40/52)</td>
</tr>
<tr>
<td>RBC (( \times 10^6/\text{ml} ))</td>
<td>6.47 (5.90/7.23)</td>
<td>6.53 (5.74/7.51)</td>
<td>6.89 (6.51/7.47)</td>
</tr>
<tr>
<td>MCV</td>
<td>60.8 (59.7/63)</td>
<td>61 (57/63)</td>
<td>61 (59/62)</td>
</tr>
<tr>
<td>MCHC</td>
<td>39.1 (38.4/40.0)</td>
<td>37.7 (37.0/38.7)</td>
<td>38.6 (38/39.4)</td>
</tr>
<tr>
<td>WBC (( \times 10^3/\text{ml} ))</td>
<td>9.28 (7.59/12.5)</td>
<td>9.08 (8.17/14.35)</td>
<td>8.19 (6.99/10.3)</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>428 (243/511)*</td>
<td>340 (266/450)*</td>
<td>182 (146/235)</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>7.0 (6.4/7.8)</td>
<td>8.2 (7.6/8.5)*</td>
<td>6.6 (6.2/6.9)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>262 (225/488)*</td>
<td>364 (285/417)*</td>
<td>199 (173/275)</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>118 (90/310)*</td>
<td>92 (64/125)*</td>
<td>42 (33/52)</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>94 (91/95)</td>
<td>261 (147/386)*</td>
<td>72 (49/89)</td>
</tr>
<tr>
<td>AP (U/ml)</td>
<td>1882 (975/3245)*</td>
<td>380 (133/447)*</td>
<td>59 (40/79)</td>
</tr>
<tr>
<td>ALT (U/ml)</td>
<td>74 (53/188)*</td>
<td>51 (27/85)*</td>
<td>21 (19/29)</td>
</tr>
<tr>
<td>AST (U/ml)</td>
<td>14 (12/19)</td>
<td>18 (14/25)*</td>
<td>12 (11/13)</td>
</tr>
<tr>
<td>LDH (U/ml)</td>
<td>58 (43/179)</td>
<td>170 (127/223)*</td>
<td>82 (61/113)</td>
</tr>
<tr>
<td>( \chi )-GT (U/ml)</td>
<td>18 (10/33)*</td>
<td>8 (7/14)*</td>
<td>5 (3/6)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.86 (0.75/0.95)</td>
<td>0.82 (0.73/0.94)</td>
<td>1.03 (0.96/1.14)</td>
</tr>
<tr>
<td>Fructosamine</td>
<td>–</td>
<td>363 (248/495)</td>
<td>–</td>
</tr>
</tbody>
</table>

*Statistically significant \( p \)-values (\( p < 0.05 \)). Data represent median values (25%/75% percentile).

*Data for the control group (healthy dogs) origin from recent studies [3,55].
3.1. Dogs with Cushing disease

3.1.1. Hematology and blood chemistry

Hematocrit, red and white blood cell count were not different to our control group [3] and were within a normal range [49]. However, fibrinogen was increased in the patients \( (p < 0.05) \). The dogs showed significant increases in the plasma AP-, cholesterol-, and triglyceride-concentration (all: \( p < 0.01 \)). Plasma levels of ALT and \( \chi^-GT \) were increased compared to normal dogs \( (p < 0.05) \). Total protein, glucose, creatinine, AST, and LDH were not different compared to our control group [3] and were in normal ranges [36] (see Table 2).

3.1.2. Hemorheology

Whole blood viscosity at low and high shear rate \( (WBV_{0.75\text{s}^{-1}}, WBV_{94\text{s}^{-1}}) \) remained unchanged compared to reference values [55]. Aggregation index (AI) and plasma viscosity (PV) were increased compared to normal dogs (both: \( p < 0.05 \)) (see Table 3).

3.2. Dogs with diabetes mellitus

3.2.1. Hematology and blood chemistry

Hematocrit, red and white cell count were not different to our control group [55] and were within normal ranges [49]. Dogs showed increased plasma concentrations of fibrinogen, total protein, glucose, AP, ALT, AST, \( \chi^-GT \), LDH, cholesterol, and triglyceride (all: \( p < 0.05 \)). Plasma-creatinine was not different compared to the control group [3] and reference values [36]. Fructosamine was above the physiological limits [30,42] (see Table 2).

3.2.2. Hemorheology

Whole blood viscosity at low shear rate \( (WBV_{0.75\text{s}^{-1}}) \), as well as aggregation index (AI) and plasma viscosity (PV) were increased (all \(<0.05\)) compared to reference values (see Table 2). At high shear rate \( (WBV_{94\text{s}^{-1}}) \) no difference between patients and the control group [3,55] was observed (see Table 3).

4. Discussion

The present study shows variations in blood fluidity in dogs suffering from Cushing syndrome and diabetes mellitus, compared to a reference group of normal subjects [3,55]. Blood fluidity exerts its effect on vascular dynamics through its influence on peripheral resistance and through shear stress mediated mechanisms on the endothelial cell. Several metabolic diseases are manifested in both, the derangement of blood fluidity, and morphologic changes of the vessel wall. For instance, in diabetes mellitus (DM)
basement membrane thickening, increased endothelial permeability and vascular damage together with
deterioration of hemorheological parameters have been described in man [9,33,52] and dog [24,44].
Uptake of glycosylation products, homocystein, and plasma lipids together with oxidative damage of
endothelial cells [10,34,38,45] promote a clear shift toward atherogenic circumstances. Differently to
man, in dogs typical atherogenic lesions are unusual, although vascular changes in relevant tissues are
described [44]. In contrast to DM, vascular and hemorheological changes in Cushing syndrome (CS)
are not well determined, although CS is frequently diagnosed in the veterinary practice. Both diseases
are associated with a derangement in peripheral blood flow, documented as increase of capillary pres-
sure [37], decrease of erythrocyte capillary velocity [35], hypertension [9,12,48], changes of regional
perfusion and aggravation of the hemorheological profile.

Typical rheological changes in DM by using standard laboratory investigations are the increase of
plasma viscosity, and the decrease of filterability [8,10,27,32,34,35,37,53]. The increase in whole blood
viscosity and erythrocyte aggregation associated with differences in aggregate morphology has been
described as well. The question arises, whether hemorheological parameters are able to predict clinical
pathologies of DM and CS, suggesting them to be included into routine laboratory profiles. The animals
used in this study were patients at a veterinary praxis, being seen for health maintenance or treatment.
The diagnosis was made clinically and by laboratory tests. The type of the disease was not tested, neither
for CS nor for DM. However, it is known that 90% of canine CS has its origin in pituitary adenoma, and
90% of canine DM is type I [51]. 5–10% of dogs with Cushing’s syndrome also have diabetes mellitus
[19].

In the present study, both groups of patients showed a rise in plasma viscosity and red cell aggrega-
tion. In diabetic dogs, whole blood viscosity at low shear rate was increased as well, which fits well to
the increased red cell aggregation, measured in this group. However, in patients with CS, whole blood
viscosity remained unchanged, although slightly elevated, despite an increased erythrocyte aggregation.
Since hematocrit was essentially not different between the two groups, hemoconcentration did not con-
tribute to this effect. Several years ago, mechanical or geometric properties of the red cell [41] have
been discussed in this context, and it seems likely that pathologically enlarged or altered aggregates [21]
might influence blood viscosity in an unpredictable way.

Red cell aggregation was enhanced in both diseases. The impact of erythrocyte aggregation on pe-
ripheral perfusion is discussed [6,11,31], however, in pathological cases erythrocyte aggregation con-
tributes to disturbances in peripheral perfusion in those patients with DM [5] or CS [48,54]. In both
groups plasma fibrinogen was increased which is a major determinant of erythrocyte aggregation, irre-
spective of the mechanism by which red cell aggregation occurs [2,7,13,40]. Because of the impact of
erthrocyte aggregation on vascular damage [33,39], the possibility of an abrasion injury together with
biochemical alterations of the endothelium seems possible in dogs with metabolic diseases like DM
and CD.

All animals had an increased plasma viscosity, which was the result of the increased plasma
fibrinogen- and lipid concentration [26,27]. In dogs with CS, the plasma after overnight fasting was
sometimes even milky. Both, the increase of plasma cholesterol and triglyceride [50] combined with an
unfavourable lipid profile, and the increase of plasma fibrinogen is a common laboratory result in both
diseases in dog (CS: 1, 15, 19, 29; DM: 17, 20, 23, 26). Although the dog is a species which can deal
with unfavourable plasma lipid concentrations without expressing atherogenic alterations, the increase
in the plasma lipid and protein concentration elevates the viscosity, which raises capillary resistance and
wall shear stress and which might also modulate endocrine secretion [10,16].
In conclusion, blood fluidity is impaired in dogs suffering from CS or DM, potentially contributing to impaired blood flow and endothelial shear. However, this study is limited due to the small number of patients tested, in order to evaluate a role of hemorheological parameters as marker for these diseases. Therefore, a relation between the value of hemorheological deterioration and the progression of the diseases could not be ruled out. Plasma viscosity is extensively increased in some cases and a detailed study on a greater number of patients would provide helpful information.

References


