Prognostic Markers in Acute *Babesia canis* Infections

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Background: Canine babesiosis, caused by *Babesia canis*, is a prevalent and clinically relevant disease in Europe. Severe acute babesiosis is characterized by a high mortality but prognosis is not always correlated with clinical signs nor with the level of parasitemia.

Objective: This study evaluated prognostic markers associated with poor outcomes in acute *Babesia canis* infections.

Animals and Methods: We compared the results of routine laboratory profiles, hand-held lactate and glucose analyzer, and the acute phase response in 2 groups of naturally infected dogs (7 survivors and 8 nonsurvivors). Samples were collected at the time of first admission and before any treatment. Subsequently, the course of prognostic markers was followed in 3 dogs experimentally inoculated with *B. canis*.

Results: Nonsurvivors showed significantly higher concentrations of lactate, triglycerides and phosphate and lower hematocrit, leukocyte counts, total serum protein concentrations, and thrombocyte counts when compared to survivors. All nonsurvivors (8/8) had hyperlactatemia, whereas most survivors (6/7) had values within the reference range. All survivors had leucocyte counts within the reference range, unlike the nonsurvivors, which showed leukopenia. During the course of acute babesiosis, the variables serum lactate, triglyceride, and phosphate concentrations, and thrombocyte count only exceeded a prognostic threshold during acute crisis.

Conclusions and clinical importance: Poor outcome in acute *B. canis* infection is indicated by changes in the laboratory profile. Intensive care should be considered for dogs presenting with moderate anemia, severe thrombocytopenia, mild to moderate leukopenia, hyperlactatemia, moderately increased serum phosphate, and triglyceride concentrations, and moderately decreased total serum protein concentrations.

Key words: Biomarker; Canine babesiosis; Dog; Outcome.

Canine babesiosis is a tick-borne disease caused by apicomplexan hemoprotozoan parasites. The 3 distinct large *Babesia* species, *B. canis*, *B. vogeli*, and *B. rossi*, and the small *B. gibsoni*, *B. conradae*, and *B. annae* have been characterized in dogs.[1–3] *Babesia canis* is the predominant and clinically relevant canine *Babesia* species in Europe[4] and infection typically is characterized by lethargy, apathy, and pale mucous membranes. The disorder can manifest as a mild or severe form. The clinical signs of the severe form are variable and often related to an excessive inflammatory response syndrome associated with multiple organ dysfunction, shock, and high mortality.[3,5]

Hematologic abnormalities in natural and experimental *B. canis* infections include anemia, thrombocytopenia, and inconsistent leukocyte abnormalities such as leukocytosis, leukopenia, neutrophilia, neutropenia, and eosinophilia. The most common abnormalities in the serum biochemical profile are increases in the activity of

Abbreviations:

ALT	alanine aminotransferase
AP	alkaline phosphatase
APP	acute phase protein
AST	aspartate aminotransferase
AUC	area under the curve
BW	body weight
CRP	C-reactive protein
DIC	disseminated intravascular coagulopathy
EDTA	ethylenediaminetetraacetic acid
hpi	hours postinfection
IFAT	immune fluorescence antibody test
IQR	interquartile range
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
PCR	polymerase chain reaction
RBC	red blood cell
ROC	receiver operation characteristic
SAA	serum amyloid A
WBC	white blood cell

aspartate aminotransferase (AST) and alanine aminotransferase (ALT), hyperbilirubinemia, hypoalbuminemia, and electrolyte and acid-base abnormalities.[6–10] Babesiosis in dogs affects primary and secondary blood coagulation and can induce disseminated intravascular coagulopathy (DIC).[11,12] Furthermore, a systemic inflammatory response syndrome (SIRS) has been described in acute *B. canis* infection characterized by an acute phase response.[12–14]

The clinical manifestations of acute babesiosis are not always proportional to the degree of anemia, and are not correlated with the level of parasitemia, which often remains below 1%.[1,6,15] Hence, besides mechanical ery-

throcyte damage, other pathophysiologic mechanisms have been proposed to contribute to hemolysis, such as toxic hemolytic factors and immune-mediated destruction of erythrocytes.[16] Furthermore, disease severity cannot be readily explained as a consequence of hemolysis alone, which often is mild to moderate in acute infections.[6] Severe complications of acute *Babesia* infections have been described such as hemolytic and septic shock, acute renal failure, multiple organ dysfunction syndrome, and other complications.[17,18]

The clinical outcome of a *B. canis* infection is influenced by many factors and the primary pathophysiologic mechanisms of babesiosis in dogs remain unclear.[19,20] In canine *B. rossi* infections, poor prognosis and mortality are associated with hyperlactatemia, hypoglycemia, clinically compromised circulation, high parasite load, increased serum cortisol concentrations, and signs of consumptive coagulopathy.[21–25] Accordingly in *B. canis* infections, an excessive inflammatory response with increased concentrations of fibrinogen, C-reactive protein (CRP) and secreted intracellular adhesion molecule-1 (sICAM-1) from erythrocytes, and thrombocytopenia have been associated with poor outcome.[19] Furthermore, an increase in lipid mediators has been shown to be associated with severe complications such as development of SIRS and multiple organ dysfunction.[5,26]

We aimed to evaluate routine laboratory and rapid in-clinic laboratory tests for their applicability as prognostic markers associated with poor outcome in acute *B. canis* infections in dogs.

Material & Methods

Animals

Naturally infected animals. The prognostic potential of different laboratory tests was evaluated in 15 naturally infected animals, of which 12 dogs were presented to the Clinic for Small Animal Internal Medicine at the Vetsuisse Faculty, University of Zurich, and 3 dogs to private veterinary practices in Switzerland in the years 2011 to 2013. Inclusion criteria were the presence of acute clinical signs consistent with canine babesiosis at admission and the identification of large *Babesia* species by microscopic evaluation of Giemsa-stained blood smears. In each dog, *B. canis* diagnosis was confirmed by PCR[27] and direct sequencing of the amplicons.[a] At time of admission, blood samples were collected, and all animals were treated with antibabesial therapy (a single dose of 3–6 mg/kg body weight [BW] imidocarb diproprionate IM or combined with 10 mg/kg BW doxycycline PO q12h for at least 10 days, and a second dose of imidocarb diproprionate after 14 days). Dogs were enrolled whenever inclusion criteria were met and adequate samples were available. The animals were categorized into 2 groups according to clinical outcome, which was defined as survival (survivor, n = 7 dogs) or death (nonsurvivor, n = 8 dogs). Six of the nonsurvivors died spontaneously within 24 hours of admission and 2 dogs had to be euthanized within 48 hours because of clinical deterioration within 48 hours. Survivors were considered to be cured based on the absence of parasites 14 days after first admission on evaluation of Giemsa-stained blood smears and PCR.

Experimentally-infected animals. The course of laboratory test results was evaluated in experimentally-infected animals. Three facility-housed adult beagles (of which 1 was 4 years and 2 were 6 years old) were inoculated IV with approximately 1×10^6 parasitized erythrocytes from an isolate stored in liquid nitrogen. The parasite isolate originated from a naturally infected Bernese mountain dog from Switzerland that had travelled to Hungary. The experiments were terminated at the very first signs of acute crisis (which was defined as weak pulse, shallow breathing, somnolence, and any clinical signs of acute shock or central nervous depression). Experiments with dogs were conducted according to Swiss animal rights and regulations standards and approved by the Cantonal Veterinary Office of Zurich (permission number 122/2012) before the study.

Samples

Venous blood samples from the naturally infected dogs were collected into tubes with and without ethylenediaminetetraacetic acid (EDTA) at the time of first admission and before any treatment. Serum and EDTA-preserved blood samples were collected through an indwelling catheter from the experimentally inoculated dogs at different times. In addition, citrated plasma samples were collected from these dogs at the end of the experiments.

Analysis of blood samples

Parasitemia was expressed as the percentage of infected erythrocytes in Giemsa-stained blood smears by manually scanning at least 5000 erythrocytes. Exposure to *Ehrlichia canis* and *Anaplasma phagocytophilum* was tested by an immuno-fluorescence antibody test (IFAT).[b,c] Complete blood cell counts were performed using EDTA-anticoagulated blood in an automated analyzer.[d] Hematologic analysis included total white blood cell (WBC), thrombocyte and red blood cell (RBC) counts and RBC indices. Serum biochemical profiles were performed using an automated analyzer.[e] Laboratory reference intervals are stated as 5% and 95% quantiles. Portable hand-held devices for rapid in-clinic testing were used to measure concentrations of lactate[f] and glucose[g] immediately in freshly collected EDTA samples.[28,29] Serum CRP concentration was determined using a canine-specific immunoturbidimetric assay[h] and serum amyloid A (SAA) concentration was measured using a latex agglutination turbimetric immunoassay on an automated analyzer.[e,i] In the citrated samples from the experimentally infected animals, fibrinogen concentrations were measured using the Clauss method and a semi-automated coagulometer.[j] D-dimer concentrations were measured on an automated analyzer.[e,k]

Statistical analysis

Results of the 2 groups (survivor and nonsurvivor) of naturally infected dogs were compared by the Mann–Whitney *U* test. The initially significant variables then were analyzed with receiver operator characteristic (ROC) curves for which the area under the curve (AUC) was calculated. The ROC analysis was used for determining a prognostic cut-off value for best differentiating between survivors and nonsurvivors with a maximal Youden's index.[30,31] If the cut-off value fell within the normal reference range, it was set at the corresponding border of the reference. Statistical analyses were performed using a statistical software package.[l] A *P*-value < .05 was considered statistically significant. The hematologic and serum biochemical profiles from the samples collected at private practices were excluded from the analysis because these variables were measured with other analytical instruments. Hence, for these variables 6 survivors and 6 nonsurvivors were included. For parasitemia, variables from hand-held devices, and the acute phase response, all of the naturally infected dogs were included in the analysis (7 survivors and 8 nonsurvivors). Graphs were generated using Graph Pad.[m]

Results

At admission, all of the naturally infected dogs had diverse clinical signs consistent with canine babesiosis, including lethargy (all 15 dogs), pale mucous membranes (all 15 dogs), pigmenturia (10 of 15), icterus (6 of 15), pyrexia (5 of 15), anorexia (4 of 15), vomiting (4 of 15), "water hammer" pulse (4 of 15), and epistaxis (3 of 15). Although *Babesia* infection was assumed and antibabesial treatment initiated shortly after admission, 8 of the 15 dogs died or had to be euthanized within 2 days of admission. All of the dogs were positive for *B. canis* in Giemsa-stained blood smears and by PCR, and none of these dogs reacted serologically to *E. canis* or *A. phagocytophilum* on IFAT. Data on characteristics of the individual dogs (animal description, travel history, and clinical signs) are summarized in supplemental file 1. No statistical difference in age, sex, and clinical signs was identified between survivors and nonsurvivors.

The parasitemia ranged between 0.5 and 3.1% (median, 1.2%; interquartile range [IQR], 0.83–1.63), but no statistical difference was identified in the level of parasitemia between the survivors and nonsurvivors. Results of laboratory findings as well as comparison between outcome groups are summarized in Table 1. In

both groups of dogs, mild to moderate normochromic normocytic nonregenerative anemia, mild to severe hyperbilirubinemia, mild to moderate azotemia, mild to moderate hypoalbuminemia, mildly increased alkaline phosphatase (AP) activity, moderate to severe hyponatremia, moderate hypocalcaemia, and a mild to moderate increase in CRP concentration were observed commonly. Nonsurvivors had significantly higher concentrations of lactate ($P < .001$), triglycerides ($P < .01$), and phosphate ($P < .05$), and significantly lower hematocrit ($P < .05$), WBC counts ($P < .01$), total serum protein concentrations ($P < .05$), and thrombocyte counts ($P < .05$) than survivors.

These 7 initially identified prognostic factors were further analyzed by ROC analysis (Table 2). Of all variables studied, lactate concentrations and WBC counts showed the best prognostic sensitivity and specificity (both 100%) to differentiate between survivors and nonsurvivors. Fig 1 illustrates the significant prognostic variables for dogs in the 2 outcome groups. All nonsurvivors (8 of 8) had moderate to severe hyperlactatemia (median, 8.35 mmol/L; IQR, 7.18–9.13), whereas most survivors (6 of 7) had concentrations within the reference range (median, 1.6 mmol/L; IQR, 1.05–2.3). The WBC counts for all of the survivors (6 of 6) were within

Table 1. Median values of various variables (minimum–maximum value) in dogs with naturally acute *Babesia canis* infections: a comparison between survivors and nonsurvivors.

Variable (unit)	Reference range	Survivors	Nonsurvivors	*P*-value
Parasitemia (%)		1.2 (0.5–3.1)	1.25 (0.5–1.9)	1
Fast in-clinic variables				
Lactate (mmol/L)	<2.5	1.6 (0.5–3.6)	8.35 (4.3–11.8)	<0.001
Glucose (mmol/L)	3.9–6.7	4.8 (4.3–7.6)	5.2 (3–11.9)	1
Hematologic variables				
Hematocrit (%)	42–55	30 (25–40)	25.5 (17–28)	0.041
Hemoglobin (g/dL)	14.4–19.1	11.45 (9.2–14.2)	9.05 (7–18.2)	0.240
RBC ($\times 10^6/\mu L$)	6.1–8.1	4.52 (3.95–6.2)	3.95 (3.05–5.39)	0.240
MCH (pg)	23–26	24.5 (22–29)	23 (22–24)	0.180
MCHC (g/dL)	34–36	35.5 (34–36)	35 (35–39)	0.937
MCV (fL)	64–73	67.5 (64–74)	65 (58–67)	0.093
WBC ($\times 10^3/\mu L$)	4.7–11.3	6.85 (5.1–9.2)	2.65 (1.59–4.5)	0.002
Thrombocytes ($\times 10^3/\mu L$)	130–394	45 (17–190)	14.5 (6–41)	0.026
Reticulocytes (%)		0.31 (0–0.81)	0.53 (0.29–1.17)	0.132
Biochemical variables				
Total bilirubin (μmol/L)	<3.5	21.25 (4.7–85.3)	54.7 (19.7–221.8)	0.240
Urea (mmol/L)	3.8–9.4	12.65 (4.5–25.7)	30.45 (6.2–79.4)	0.065
Creatinine (μmol/L)	50–119	88 (75–133)	84 (54–651)	1
Total protein (g/L)	56–71	57 (40–64)	44 (34–50)	0.026
Albumin (g/L)	29–37	26.5 (19–38)	24 (13–26)	0.240
Cholesterol (mmol/L)	3.5–8.6	6.25 (4.9–7.7)	6.05 (2.7–9.3)	0.589
Triglycerides (mmol/L)	0.4–1.5	0.85 (0.8–1.1)	1.95 (0.9–3.5)	0.009
Alkaline phosphatase (U/L)	20–98	113.5 (74–184)	253.5 (61–358)	0.132
ALT (U/L)	20–93	47.5 (26–72)	72.5 (30–96)	0.132
Sodium (mmol/L)	152–159	144.5 (132–154)	142.5 (140–151)	0.485
Potassium (mmol/L)	4.3–5.3	4.2 (3.6–4.5)	4.4 (3.6–5)	0.394
Chloride (mmol/L)	113–124	113.5 (97–115)	109 (94–121)	0.485
Calcium (mmol/L)	2.4–2.8	2.43 (2.11–2.52)	2.225 (2.1–2.55)	0.180
Phosphate (mmol/L)	1.0–1.6	1.36 (1.05–1.68)	2.54 (1.39–3.08)	0.015
Acute phase response				
Canine CRP (mg/L)	<5	84.7 (3.3–169.8)	155.55 (22.5–232.8)	0.189
SAA (mg/L)	<2.19	0 (0–2.5)	0 (0–1.1)	0.536

Table 2. Results of the ROC analysis with prognostic cut-off values of significantly altered variables and respective sensitivity, specificity, area under the curve (AUC), and standard error (SE) associated with the outcome in *Babesia canis* infected dogs.

Parameter (unit)	Prognostic cut-off value	Sensitivity (%)	Specificity (%)	AUC	SE
Lactate (mmol/L)	3.95	100	100	1.00	0.00
Hematocrit (%)	28.5	66.7	100	0.86	0.11
WBC ($\times 10^3/\mu$L)	4.7[a]	100	100	1.00	0.00
Thrombocytes ($\times 10^3/\mu$L)	27.5	83.3	83.3	0.89	0.10
Total protein (g/L)	50.5	83.3	100	0.88	0.12
Triglycerides (mmol/L)	1.5	83.3	100	0.94	0.07
Phosphate (mmol/L)	1.72	83.3	100	0.92	0.09

[a]Set at the border of the reference range (calculated cut-off at $4.8 \times 10^3/\mu$L).
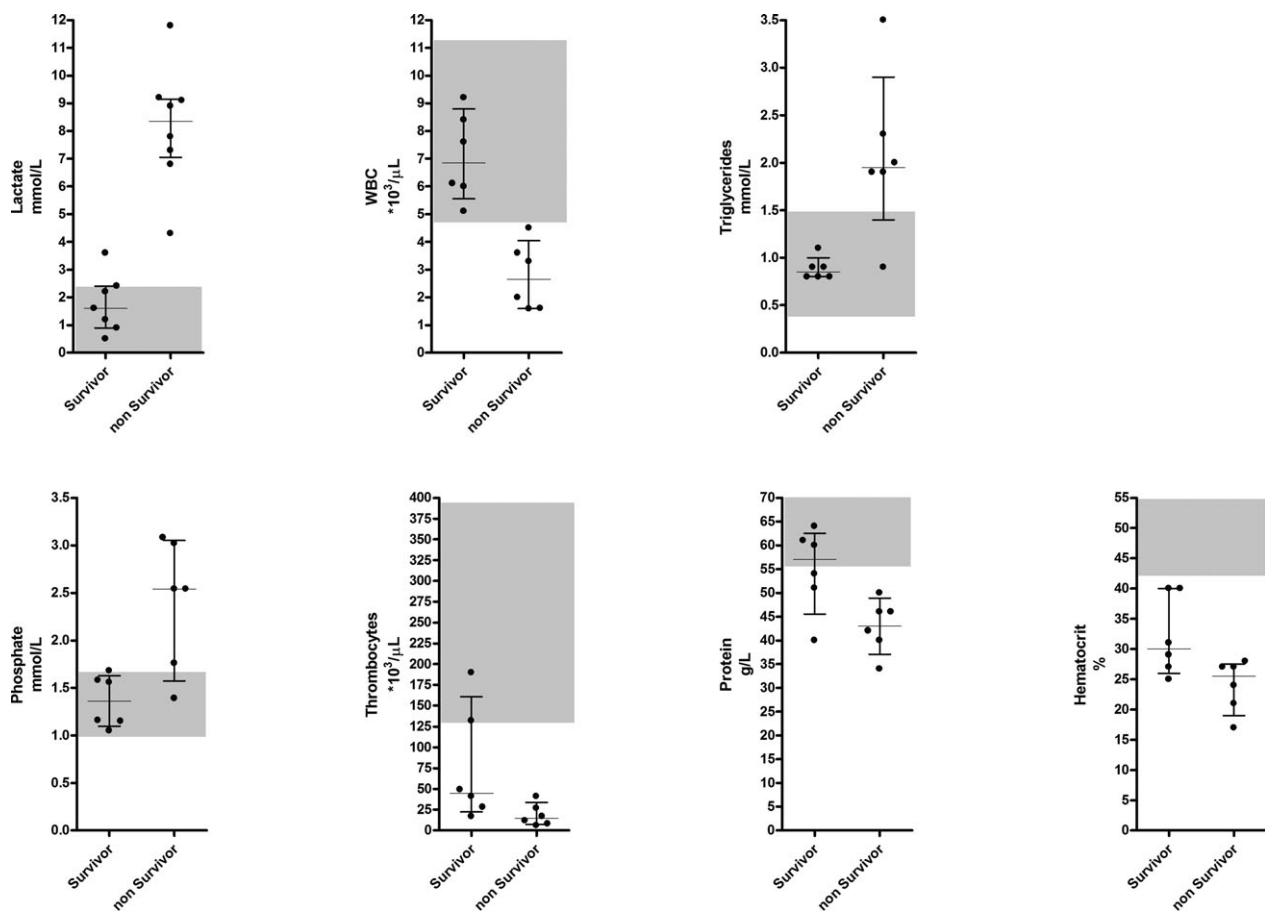
Fig 1. Prognostic markers in acute *Babesia canis* infected dogs. Median and interquartile range for significant prognostic markers (*P* < .05) recorded at admission in naturally infected dogs that did or did not survive an acute *B. canis* infection. Dots correspond to the data from individual dogs; the shaded grey areas represent the reference intervals. WBC: white blood cells.

the reference range (median, $6.85 \times 10^3/\mu$L; IQR, 6.03–8.2) unlike the group of nonsurvivors, which had mild to moderate leukopenia (6 of 6; median, $2.65 \times 10^3/\mu$L; IQR, 1.7–3.53).

The course of the prognostic variable, parasitemia, and the acute phase response was followed in the 3 dogs experimentally inoculated with *B. canis*. The 3 infected dogs became lethargic and showed signs of hemolysis (pale mucous membranes and pigmenturia)

105, 120, and 119 hours postinoculation (on days 4–5), respectively. They had a low grade parasitemia with a maximum of 1.75% of the erythrocytes infected at the end of the experiment, and during the course 2 of the 3 dogs had episodes of pyrexia (Fig 2A). An acute phase response could be observed with a moderate increase in CRP concentration and a moderate decrease in serum albumin concentration (Fig 2B), whereas SAA concentrations remained below the diagnostic
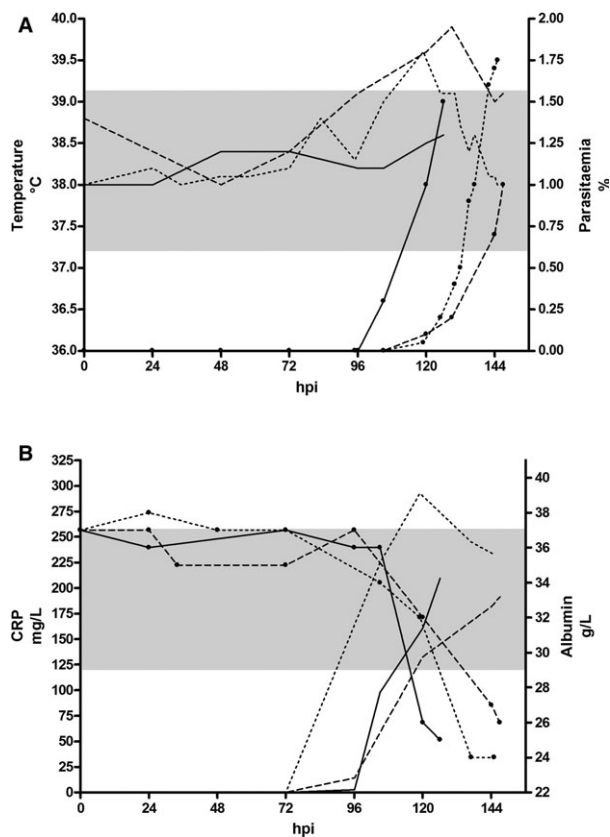
Fig 2. Course of selected variables in 3 experimentally infected dogs. Dog 1: solid line; dog 2: broken line; dog 3: dotted line. **(A)** Body temperature (left *y*-axis) and parasitemia (right *y*-axis; lines with dots). The shaded grey area represents the reference interval for the body temperature. **(B)** CRP (left *y*-axis) as a marker for positive acute phase response and albumin (right *y*-axis; lines with dots) as a marker for negative acute phase response. The shaded grey area represents the reference interval for albumin. hpi: hours postinfection, CRP: C-reactive protein.

limit (data not shown). Follow-up of prognostic markers is shown in Fig 3. A decrease in the hematologic variables leukocytes, thrombocytes, and hematocrit was found before the identification of parasites in stained blood smears, and resulted in moderate leukopenia, severe thrombocytopenia, and decreased hematocrit. In general, mild to moderate anemia was observed. Changes in lactate, triglyceride, and phosphate concentrations corresponded to the first appearance of parasites, and they only exceeded the prognostic threshold at the first observation of acute crisis. In addition, thrombocytopenia was a common finding and platelet counts exceeded the prognostic threshold toward the end of the experiment. Total serum protein concentrations also decreased over time but passed the threshold only in 2 of the 3 dogs before first signs of an acute breakdown. At the end of the experiment, the 3 dogs showed mildly increased levels of fibrinogen of 2.6 g/L, 3.8 g/L, and 3.2 g/L (reference range, 1.0–2.5 g/L), and D-dimer concentrations of 0.26 mg/L, 0.44 mg/L, and 0.92 mg/L (reference range, <0.4 mg/L), respectively.

Discussion

In this study, several variables were shown to be associated with poor outcome in acute *Babesia canis* infections. By including 2 rapid in-clinic tests, standard hematologi and biochemical variables, and acute phase proteins, we found the variables lactate, WBC, triglycerides, phosphate, thrombocytes, total serum protein, and hematocrit to be significant prognostic markers. Thus, nonsurvivors at admission had more severe anemia, leukopenia, and thrombocytopenia in addition to alterations in their serum biochemical profile results.

Lactate concentrations were significantly lower in survivors and showed a clear difference from the nonsurvivors. This finding is similar to what is observed in dogs infected with *Babesia rossi*, the agent of severe canine babesiosis in South Africa, where serum lactate concentration is used for post-treatment monitoring,[21] and high blood lactate concentrations correlate with poor outcome.[32–34] Nevertheless, the pathogenesis of hyperlactatemia in dogs with acute babesiosis is not well established, and it might not be caused by hypoxia as a consequence of anemia, which remains mild to moderate in most *B. canis* infected animals.[34] Hence, hypoxia in canine babesiosis may be the consequence of alterations in the macro- and micro-cirulation triggered by protozoal sepsis, hypotension, DIC, and SIRS, all of which are well known in *B. canis* infections[18,35]. Indeed, increased lactate concentrations have prognostic value in SIRS caused by various conditions.[36,37]

The second variable that clearly differentiates between the 2 studied groups was WBC count. Nonsurvivors had mild to moderate leukopenia in contrast to the survivors with WBC counts in the reference range. Although the WBC count was a significant marker for outcome in our study, leukopenia was reported in 60% of mild cases of acute canine babesiosis.[17] Indeed, the WBC count fell below the prognostic cut-off before any clinical signs were observed in the experimentally inoculated dogs. Severely affected dogs had mild to moderate neutropenia, with an overall degenerative tendency and lacking a left shift (see supplemental file 2). Furthermore, lymphopenia seems to be a hallmark of acute canine babesiosis.[17,19] A markedly increased serum cortisol concentration was found in dogs with lethal *B. rossi* infections, indicating a potential immunosuppressed state in these animals, which also is indicated by an unexpected mild to moderate regenerative response of lymphocytes in dogs that survived.[24,38] Furthermore, studies in humans with acute malaria infections with *Plasmodium falciparum* and *P. vivax*, which are related to *Babesia* spp., identified mechanisms that could explain a depletion of lymphocytes from the peripheral blood by acute sequestration of the cells in the lymph nodes or other parts of the body or by immune cell exhaustion and abnormal cell death through parasite-induced apoptosis.[39,40] Similarly, toxic parasitic factors have been shown to be involved in canine *B. gibsoni* infection.[41]

Hemolytic anemia and thrombocytopenia are the most frequent abnormalities associated with a diagnosis of *B. canis* in naturally infected dogs and thrombocy-
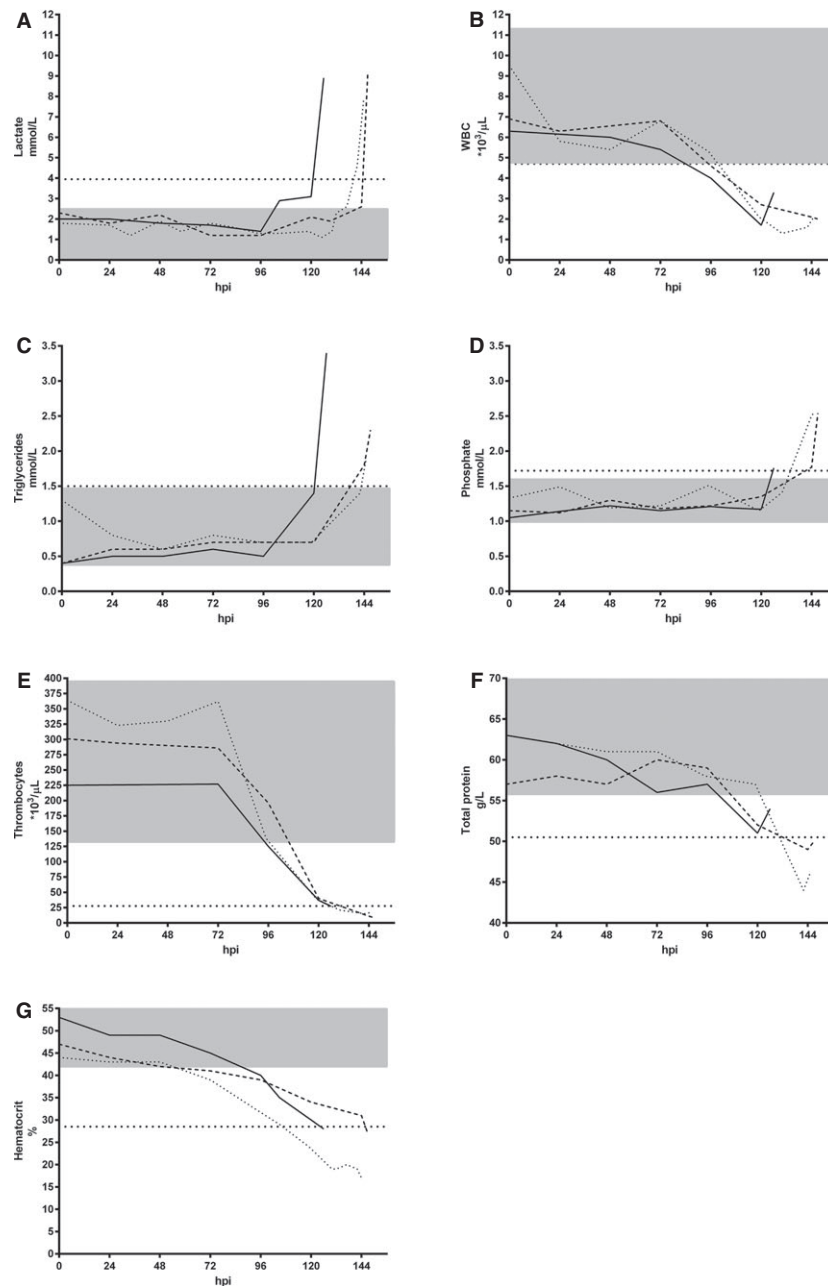
Fig 3. Course of significant prognostic markers in 3 experimentally-infected dogs. Dog 1: solid line; dog 2: broken line; dog 3: dotted line. The shaded grey areas represent the reference intervals. The horizontal broken lines represent the corresponding prognostic cut-off. **(A)** blood lactate, **(B)** WBC, **(C)** triglycerides, **(D)** phosphate, **(E)** thrombocyte count, **(F)** total protein, **(G)** hematocrit. hpi: hours postinfection, WBC: white blood cells.

topenia usually is the most dramatic hematologic abnormality in the course of babesiosis.[12,42–44] Our data indicate that severe thrombocytopenia is associated with poor outcome by a prognostic cut-off of 27,500 thrombocytes per μL, although a sensitivity and specificity of 83.3% for each indicates limited prognostic value. Presumably, several factors are involved in the origin of thrombocytopenia in canine babesiosis including increased platelet activation and consumption by a SIRS (hypercoagulable state), increased platelet sequestration and aggregation, and a decreased platelet pro-

duction.[19,45,46] Comparable in *B. rossi* infections, poor outcome was associated with a consumptive coagulopathy, although even severe thrombocytopenia was not accompanied by apparent bleeding diathesis and hemorrhage.[25,47,48]

Increased phosphate concentrations often are associated with metabolic acidosis characterized by tissue hypoxia and high blood lactate concentrations, although the underlying mechanisms have not been completely explained.[49] Hemorrhage, hypovolemia, and shock as cause or consequence of tissue hypoperfusion

could further explain changes in altered variables, also including azotemia and potential protein-losing nephropathy caused by hypoxic renal damage.[35] Complications related to hemolytic anemia, coagulation disorders and hypotension, SIRS, and secondary impaired renal function likely account for the severe outcome of the infection.[6,9,10,12] Furthermore, in other studies, acute respiratory distress syndrome, renal failure, immune-mediated hemolytic anemia, cerebral syndrome, and DIC were associated with increased mortality in acute *B. canis* infections.[17,50]

Acute phase proteins were used as prognostic factors for different inflammatory processes,[51] and an acute phase response also was observed in acute *B. canis* infections.[12–14,20] We measured the acute phase proteins CRP and SAA, because they are considered major APP in dogs[52] and are not significantly affected by hyperbilirubinemia, which is commonly present in acute babesiosis.[51] We found an increase in CRP before parasite detection as previously observed,[12] without any significant difference between the outcome groups. This finding is in accordance with findings in *B. rossi* infections in which no prognostic value for CRP concentrations was observed.[53] Furthermore, the SAA concentrations did not increase significantly in naturally and experimentally infected animals. This finding is in contrast to other observations of increased SAA concentrations in dogs with babesiosis on the day of admission.[14] As another indicator, serum albumin concentration could serve as a negative APP.[51] With the onset of acute infection, we observed a moderate decrease in serum albumin concentration and it had no prognostic relevance. Although differences between survivors and nonsurvivors were absent for an acute phase response, APP (among other variables) could serve as important variables for monitoring response to therapy.[14,54]

In the course of validating prognostic markers in 3 experimentally inoculated dogs, we observed low grade parasitemia with a maximum of 1.75% of infected erythrocytes, which was comparable to the group of naturally infected animals. Even in infections with serious clinical signs, low parasitemia is a common finding in *B. canis* infections.[1,6,12] The course in the infected dogs highlights the prognostic value of lactate, triglycerides, and phosphate concentrations, and thrombocyte counts, because these factors only crossed the prognostic threshold in an acute crisis. Referring to the ROC analysis of these variables, only lactate showed optimal characteristics. Therefore, any prognosis based on individual variables should be interpreted with caution.

Missing data about the course of disease before admission and the time point of infection in the naturally infected dogs is a limitation of this study. Generally, practitioners inquire about the duration of illness and the appearance of the first clinical signs, and they can estimate the time of the infection in affected dogs. In this respect, the prognostic markers are helpful for guiding clinical decision making. To get an overall picture of individual cases, a systematic collection of clinical, laboratorial, and other individual factors must be emphasized. For example, in our cohort of infected dogs, circulatory disturbances were detected in 4 relatively young dogs (7 month to approximately 3 years), of which 3 dogs died (see supplemental file 1). Such clinical variables could affect outcome in the laboratory test results and the likely progression of a patient's infection.[55] In any case, outcome depends on a rapid diagnosis and early treatment.

Mortality in the investigated group of dogs was higher as compared to an endemic area.[5] This finding reflects a typical situation for nonendemic areas such as Switzerland, where dogs became infected from local *Babesia* outbreaks or have traveled to an endemic area. These dogs likely never have had contact with the parasite and therefore did not develop partial immunity.[46,56] Nonetheless, findings on mortality rates should not be over interpreted because of the small sample size. In our cohort, we included every possible case for which we could obtain comparable clinicopathologic data.

Unfortunately, we did not have precise data about infection rates in dogs in Switzerland. However, during the sampling period, 2 indigenous outbreaks were reported in 44 dogs, of which 10 died.[57,58] Most indigenous cases in our cohort originated from these areas (4 survivors and 1 nonsurvivor), whereas 1 dog originated from Geneva, a known endemic region in Switzerland.[59] The remaining 9 infected dogs had a positive travel history. Information about infection rate in dogs in Switzerland that have travelled is rare. For example, from 2011 to 2013, the diagnostic unit of the Institute of Parasitology in Zurich (which offers a travel screening panel) identified 2.1% of 804 samples as positive on blood smears for large *Babesia* species (F. Grimm, personal communication). This observation is in agreement with observed cases in dogs in Germany that have travelled, with 3.7% (19/508) of animals positive for large *Babesia* spp. in Giemsa-stained blood or buffy coat smears.[60] Hence, to compensate for the small sample size, prognostic markers were cross-validated in the course of experimental babesiosis.

Although a significant prognostic marker is not necessarily clinically relevant, the pathophysiologic reason for death would be of interest. With this in mind, additional studies should include postmortem examination, and more prognostic factor studies should be conducted including other nonroutine variables. This study focused on rapid in-practice tests (e.g. lactate and glucose determined by hand-held analyzers) and routine laboratory variables, and the associated findings summarize the prognostic value of these variables. Nevertheless, additional research is needed to evaluate what additional evaluation and intensive care is needed for dogs with a poor prognosis. In this context, several markers have been demonstrated as good variables for follow-up and post-treatment monitoring after antibabesial therapy, such as APP, lactate, thrombocytes, and leukocytes.[13,14,21,43,54]

Footnotes

Acknowledgments

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Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Supplemental File 1. Characteristics of the individual dogs (animal description, travel history, and clinical signs).

Supplemental File 2. Data on differential WBC count. Differential WBC count in the course of 3 experimentally inoculated dogs, and in dogs that did or did not survive a naturally acquired acute Babesia canis infection.