Long term follow-up in canine leishmaniasis

Finding prognostic factors

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Contents

Abstract

Leishmaniasis is a zoonotic parasitic disease, with *Leishmania* infantum as the main etiological agent. It is transmitted by Phlebotominae sandflies, present in southern Europe, Africa, Asia and America. It causes many nonspecific clinical signs like lethargy, weight loss and skin lesions. Treatment, mainly with allopurinol, meglumine antimoniate and/or miltefosine, focusses on improving clinical signs and can lead to clinical cure. However, complete elimination of the parasite cannot be achieved.

The goal of this study was to find prognostic factors in the laboratory results of dogs at the time of their diagnosis.
This was done by performing a long-term follow-up retrospective cohort study on 49 dogs diagnosed with leishmaniasis, using Kaplan-Meier survival analysis. The effect of their Leishmania Working Group classification, their treatment and sex and blood, clinical chemistry and urine examination results on survival was determined.

Factors with a significantly negative influence on the survival rate turned out to be monocytes and MCV for blood hematology, creatinine and urea for clinical chemistry and glucose, granular casts, hemoglobin, protein/creatinine ratio and specific gravity for urine. Most of these results can be explained by the fact that most of these factors, when elevated, can be a sign of renal dysfunction which has a negative influence on survival.

It can be concluded that these factors could be used as prognostic factors when diagnosing dogs with leishmaniasis.
As most of them are an indicator of kidney function, kidney function should be seen as an important part of classification of severity of clinical Leishmaniasis.

1 Introduction

1.1 Life cycle

Leishmaniasis is a zoonotic parasitic disease caused by *Leishmania* spp., whereof *Leishmania* infantum has proven to be the main etiologic agent of the disease in Europe, Asia and Africa. It affects mostly dogs, making them the main reservoir for human leishmaniasis.[1,2]

The parasite is transmitted by Phlebotominae sandflies, which are present in the for leishmanial endemic areas southern Europe, Africa, Asia and America. For non-endemic countries like the Netherlands, Leishmania is becoming an important concern as imported dogs can be infected.[1–3]

When female Phlebotominae sandflies have a blood meal on an infected host, they ingest amastigotes, the nonflagellated form. Within the sandfly, these amastigotes multiply and transform into infective flagellated promastigotes. At the next blood meal these promastigotes are injected into the next host, a land mammal.
In this next host, the parasite is phagocytosed by macrophages and survives and replicates in these cells, because of its resistance to phagolysosomal digestion.[4,5]

Besides sandflies, other proven routes of transmission have been described. These include transplacental and venereal transmission and transmission by blood transfusion.[4]

1.2 Susceptibility and clinical signs

Not every dog infected with Leishmania develops clinical disease. Susceptibility to the development of clinical disease depends on several predisposing factors like breed, age and the balance between Th1 (cell mediated) and Th2 (humoral) immune response.
Boxers, Cocker Spaniels, Rottweilers and German Shepherds, dogs younger than 3 years and older than 8 years and dogs with a predominant Th2 response seem to be more susceptible to developing Leishmaniasis. The latter can be explained by the fact that dogs with a predominant Th1 response may be able to control the parasite and stay clinically healthy, while dogs with a predominant Th2 response are more susceptible to parasite dissemination to different tissues and are therefore more likely to display clinical signs.[4,5]

Leishmaniasis is a systemic disease potentially affecting every tissue of the body. Therefore many nonspecific clinical signs can be present, including skin lesions, intestinal symptoms, ocular problems and general symptoms like lethargy and weight loss. Blood and urine values are often abnormal. These can include azotemia, polyclonal gammopathy, decreased urine specific gravity and proteinurea.
The probable diagnosis is based on clinical signs and confirmed by the detection of Leishmania antibodies in serum or the detection of the parasite itself in tissues.[3–5]

Most of these clinical signs and laboratory values can be explained by the type of immune response Leishmania causes. The parasite causes an intense inflammatory response and production of significant amounts of antibodies, also known as a type III immune response. The deposition of immune complexes activates the complement system, which damages capillaries and its surrounding tissues. That way, clinical signs like skin lesions and kidney damage can occur.

The colonization of bone marrow by *Leishmania* spp. causes clinical and laboratory abnormalities as well. This can be a nonregenerative normocytic normochromic anemia and thrombocytopenia.[5,6]

1.3 Medicinal treatment

Medicinal treatment focuses on improving clinical signs and can lead to clinical cure. Unfortunately, complete elimination of the parasite is not achieved and patients will still be infectious to sand flies, but to a lesser extent than before treatment. The most common drugs used for treatment of Leishmaniasis are allopurinol (Allopurinol®), meglumine antimoniate (Glucantime®) and miltefosine (Milteforan®) . The parasitostatic effect of allopurinol may be combined with the parasiticidal effects of meglumine antimoniate or miltefosine.[1,3]

A few long-term cohort studies and clinical trials comparing different methods of treatment have been performed in endemic areas like Italy and Spain. These studies mostly focus on the efficacy of different kinds of medication and changes in laboratory results during treatment.[7–9]

For example, one of these, retrospective, studies suggests a better success rate in dogs treated with allopurinol plus meglumine antimoniate versus dogs treated with allopurinol plus miltefosine. In the first group, four out of nine dogs showed clinical relapse, whilst in the second group only one out of nine dogs showed clinical relapse. All dogs that relapsed had increased clinical signs, increased antibody titers and increased parasitic loads in their lymph nodes.[7]
Another, multicentric and controlled, study shows no difference in success rate between 72 dogs split into two groups that received either allopurinol plus meglumine antimoniate or allopurinol plus miltefosine. A significant clinical response was seen in both groups, with no statistical difference between the groups.[10]
The quality and results of studies available differ too much to draw an unambiguous conclusion about the preferred method of treatment.

The guidelines provided by the LeishVet working group and the Leishmania working group both suggest choosing a treatment based on a clinical classification that is based on disease severity.[4,11]
For the LeishVet working group, this classification is based on serology, clinical signs, laboratory findings, therapy and prognosis. Dogs can be classified as stage I (mild disease), stage II (moderate disease), stage III (severe disease) or stage IV (very severe disease). Stage I dogs should be treated with allopurinol, meglumine antimoniate, miltefosine/allopurinol + meglumine antimoniate, or allopurinol + miltefosine. Stage II dogs should be treated with allopurinol + meglumine antimoniate or allopurinol + miltefosine. Stage III dogs should be treated with allopurinol + meglumine antimoniate or allopurinol + miltefosine and have the IRIS guidelines for chronic kidney disease followed. Stage IV dogs should be treated with allopurinol only and have the IRIS guidelines for CKD followed as well.[4,12]

According to the Leishmania working group, dogs are classified as stage A (exposed), stage B (infected), stage C (sick), stage D (severely sick), stage E-a (sick and unresponsive to recommended treatment) or stage E-b (sick and relapsing soon after ceasing treatment).[11]

Up to now, therapy of first choice in the UKG (University Clinic for Companion Animals, located in Utrecht, The Netherlands) is allopurinol. Meglumine antimoniate and/or miltefosine are added if allopurinol does not show the desired effects.[13]

1.4 Goal of this study

The goal of this study was to find prognostic factors in the laboratory results of dogs diagnosed with Leishmaniasis.

2 Materials and methods

1.1 Materials

In this long-term follow-up retrospective cohort study 49 dogs, diagnosed with Leishmaniasis, were included. They were found by their positive serology results for *Leishmania* at the UKG and included because of their Leishmaniasis diagnosis.

Their category, treatment and sex and blood, clinical chemistry and urine results at time of diagnosis were used for further analysis. All blood and urine results were used in the statistical analysis, unless these data were available for <3 dogs or results were similar for all dogs.

Blood test results included: haematocrit, MCV, MCHC, MCH, reticulocytes, CHr, leucocytes, segments, band cells, blasts, lymphocytes, monocytes, eosinophils, basophils, normoblasts, thrombocytes, APTT, PT, fibrinogen, MPC, MPM and MPV.
Clinical chemistry results included: urea, creatinine, glucose, sodium, potassium, chloride, calcium, phosphate, AF, ALAT, ASAT, bile acids, total protein, albumin, lead, alpha 1, alpha 2, beta 1, beta 2 and gamma.
Urine results included: SG, pH, protein, creatinine, protein/creatinine, AF, AF/creatinine Hb, glucose, ketones, bilirubin, leukocytes, erythrocytes, triple phosphate crystals, squamous epithelium, transitional epithelium, kidney epithelium, granular casts, hyaline casts, bilirubin crystals and CaOx dihydrate crystals.

1.2 Methods

1.2.1 Collecting data

To find dogs with Leishmaniasis, the UVDL (University Veterinary Diagnostic Laboratory) was asked to provide the patient numbers of dogs with a positive serological titer against *Leishmania*.
Because of this search method, all dogs used in this study had been diagnosed and/or treated at the UKG at some point in their lives. As Leishmania is not endemic in the Netherlands, every dog's history included being adopted from or having visited another, endemic, country.[4]

The veterinary software program used at the UKG, Vetware, provided the data regarding breed, sex, age, treatment, origin, clinical signs and laboratory results.

The follow-up period ranged from the time of diagnosis until time of death or the moment of last contact with the owner about the dog.

To obtain information about the dogs' current clinical health, the dogs' usual veterinarians were contacted. These veterinarians could provide further information about the dogs' follow-ups. As in some cases, treatment was continued by the dogs' usual veterinarian.

The following questions were asked:

- When was the last moment of contact with the owner about the dog?

- Was the dog still alive at that time?
    - If no: did the dog die because of the consequences of Leishmaniasis or because of another cause?
- Has the dog received any other medication than already provided by the UKG?

The data was entered in Excel and coded in case of categorical variables, enabling use of the file in SPSS for statistics.

Categorical variables included normoblasts for blood results and Hb, glucose, bilirubin, leukocytes, erythrocytes, squamous epithelium, transitional epithelium, granular casts, hyaline casts and bilirubin crystals for urine results. These variables were categorized as displayed in table 2.

1.2.2 Classification of dogs based on disease severity

Dogs were also classified according to the Leishmania Working Group classification. This classification includes 6 categories (A, B, C, D, Ea, Eb) and is based on clinical stage.

Stage A and B include exposed and infected dogs respectively. As dogs in these stages do not show clinical signs and will not visit a veterinarian for Leishmaniasis, they were not part of this study.

Stage C includes sick dogs with positive cytological results and/or high antibody titers against *Leishmania*. These dogs show at least one clinical sign common to Leishmaniasis.

Stage D includes severely sick dogs. These dogs show severe clinical signs like nephropathy, ocular disease or joint disease.

Stage Ea includes sick dogs unresponsive to recommended treatment, which consists of Allopurinol at the UKG.

Stage Eb includes sick dogs, relapsing soon after recommended treatment has stopped.[11,14]

1.2.3 Statistical analysis

Kaplan-Meier analysis was performed, including a log-rank test stratifying on categorical variables. Categorical variables included sex, treatment and category and some of the blood, clinical chemistry and urine laboratory results at the time of diagnosis. Dogs were censored if they died by other causes than (the consequences of) Leishmaniasis.

A Cox regression proportional hazards analysis was performed for all variables.

2 Results

2.1 Clinical information

Vetware provided the undermentioned information about the dogs. The signalment and disease classification of the dogs in this study is presented in Table 1.

Twenty dogs in this study were classified as stage C, fourteen dogs were classified as stage D, thirteen dogs were classified as stage Ea and two dogs as stage Eb.

Their breeds varied greatly. Most dogs, 25 of them, were cross breeds. Besides these cross breeds, the study included 4 Greyhounds (including 2 Galgo Español), 2 Siberian Husky's, 2 Shepherds (Belgian and German) and 2 Pointers (including 1 German Pointer). The remaining breeds included a Tibetan Terrier, Fox Terrier, Labrador, Labrador cross breed, Cane Corso, Leonberger, Welsh Springer Spaniel, Dobermann, Boxer, Poodle, Shih Tzu, Mountain Dog, Golden Retriever and Pug. The breed of one dog was unknown.

The dog's ages at the time of diagnosis ranged from 1 to 11 years old.
69% of these dogs were male (12 intact, 22 castrated) and 31% were female (1 intact, 14 castrated).

All dogs received allopurinol(A). If dogs did not show desirable results within a few months, therapy was extended with the use of meglumine antimoniate (G) and/or miltefosine (M). Out of 49 dogs, 30 dogs had only received allopurinol, 10 dogs were treated with allopurinol and meglumine antimoniate, 7 dogs were treated with allopurinol and miltefosine, and 2 dogs received treatment with allopurinol, meglumine antimoniate and miltefosine.

Follow-up time is given in months. In 5 dogs this period is described as 0, as their follow-up periods ranged from 1 to 4 days.

Of importance, but not mentioned in table 1, are origin and clinical signs.
65% of dogs were born in and imported from an endemic country, mostly Spain. 35% of dogs had visited an endemic country or their history was unknown.
Their clinical signs varied from skin lesions (mostly in category C), to ocular and kidney disease (mostly in category D).

Table 1: clinical stage, breed, sex, age, treatment and follow-up period of dogs included in this study

Number	Stage	Breed, sex, age	Treatment	Follow-up months
1	C	Tibetan Terrier, Mx, 8	A	86
2	C	Galgo Español, Fx, 6	A	41
3	C	Cross breed, Fx, 1	A	47
4	C	Unknown, Fx, 5	A	58
5	C	Cane Corso, M, 2	A	51
6	C	Cross breed, Fx, 2	A	7
7	C	Cross breed, Mx, 1	A + M	3
8	C	Greyhound, Fx, 5	A	6
9	C	Cross breed, Mx, 2	A + G	13

10	C	Cross breed, Mx, 2	A	14
11	C	Fox Terrier, Mx, 5	A	29
12	C	Cross breed, Mx, 3	A	4
13	C	Cross breed, Mx, 8	A	39
14	C	Cross breed, Fx, 6	A	1
15	C	Boxer, Mx, 5	A	36
16	C	Siberian Husky, Mx, 6	A	23
17	C	Cross breed, Mx, 5	A	72
18	C	Cross breed, Fx, 9	A	17
19	C	Golden Retriever, Fx, 1	A	0_1
20	C	Cross breed, Mx, 9	A	9
21	D	German Pointer, M, 5	A	0_2
22	D	Labrador cross breed, Fx, 3	A	49
23	D	Siberian Husky, M, 3	A + M	6
24	D	German Shepherd, F, 2	A	78
25	D	Galgo Español, Mx, 2	A	1
26	D	Leonberger, M, 11	A	13
27	D	Cross breed, Fx, 8	A + M	31
28	D	Dobermann, Fx, 2	A	0_3
29	D	Poodle, Fx, 2	A + M	20
30	D	Pointer, Mx, 10	A	17
31	D	Mountain Dog, Fx, 2	A	0_4
32	D	Cross breed, M, 1	A	65
33	D	Cross breed, M, 1	A + G	11
34	D	Cross breed, M, 3	A + G	0_5
35	Ea	Cross breed, Fx, 3	A + G	36
36	Ea	Cross breed, Mx, 3	A	3
37	Ea	Cross breed, Mx, 2	A + G	31
38	Ea	Cross breed, Mx, 2	A + G	67
39	Ea	Cross breed, Mx, 5	A + G	45
40	Ea	Welsh Springer Spaniel, M, 5	A + M	46
41	Ea	Cross breed, Mx, 1	A + G	51
42	Ea	Cross breed, Mx, 8	A + G	51
43	Ea	Belgian Shepherd, M, 9	M + A + G	37
44	Ea	Cross breed, M, 1	A + M + G	100
45	Ea	Shih Tzu, Mx, 5	A + G	3
46	Ea	Pug, M, 2	A + M + G	38
47	Ea	Cross breed, M, 3	A + M	35
48	Eb	Cross breed, Mx, 8	A	40

49	Eb	Greyhound, Mx, 5	A	32

1 4 days

2 1 day

3 1 day

4 4 days

5 3 days

2.2 Laboratory results

Dogs had laboratory work done at the time of diagnosis. The median, range, n and the references for their blood and urine results are given in table 2.

Table 2: laboratory results at time of diagnosis

Hematology				
	Median	**Range**	**n**	**Reference**
Haematocrit	0,36	0,11 - 0,58	46	0,42 - 0,61 L/L
MCV	62,7	56,0 - 80,5	40	63,5 – 72,9 fl
MCHC	21,2	17,9 - 24,6	40	20,5 – 22,4 mmol/L
MCH	1,38	1,19 – 1,54	40	1,37 – 1,57 fmol
Reticulocytes	0,6	0,1 - 7,5	25	<1,5 %
CHr	1,56	1,06 - 1,68	25	1,43 – 1,71 fmol
Leucocytes	7,4	2,0 - 31,9	46	4,5 – 14,6 $x10^9$/L
Segments	4,6	0,2 - 27,4	46	2,9 – 11,0 $x10^9$/L
Band cells	0,0	0,0 - 2,1	46	0,0 – 0,3 $x10^9$/L
Lymfocytes	1,6	0,1 - 4,2	46	0,8 – 4,7 $x10^9$/L
Monocytes	0,4	0,0 - 2,7	46	0,0 – 0,9 $x10^9$/L
Eosinophils	0,2	0,0 - 1,0	46	0,0 – 1,6 $x10^9$/L
Basophils	0,0	0,0 - 0,2	46	0,0 – 0,1 $x10^9$/L
Thrombocytes	195	24 - 495	43	144 – 603 $x10^9$/L
APTT	18,0	13,1 - 43,3	9	13,2 – 18,2 sec
PT	7,8	7,3 - 10,2	9	7,2 – 9,9 sec
Fibrinogen	3,1	1,7 – 4,2	9	1,0 – 2,7 g/L
MPC	225	185 - 272	22	162 - 261 g/L
MPM	2,36	1,71 – 4,48	22	1,42 – 2,46 pg
MPV	12,7	8,5 – 37,7	22	6,8 – 13,4 fl
	0	**+**	**++**	**Reference**
Normoblasts	42	2	2	0/100 leukocytes
Clinical chemistry				
	Median	**Range**	**n**	**Reference**

Urea	33	2,4 – 34,6	33	3,0 – 12,5 mmol/L
Creatinine	87	32 - 491	41	50 - 129 µmol/L
Glucose	5,7	4,9 – 9,6	13	4,2 – 5,8 mmol/L
Sodium	142	136 - 149	22	141 - 150 mmol/L
Potassium	3,9	3,3 – 5,2	22	3,6 – 5,6 mmol/L
Calcium	2,51	1,82 – 2,96	19	1,98 – 2,97 mmol/L
Phosphate	1,55	0,29 – 2,57	19	0,65 – 2,12 mmol/L
AF	49	13 - 604	26	<73 U/L
ALAT	38	13 - 513	15	<70 U/L
Bile acids	3	1 – 33	30	<10 µmol/L
Total protein	75	27 - 136	47	55 - 72 g/L
Albumin	24	7 - 39	47	26 - 37 g/L
Alpha 1	3	1 - 8	44	5 - 10 g/L
Alpha 2	10	5 – 16	44	4 - 13 g/L
Beta 1	4	2 – 9	44	3 - 10 g/L
Beta 2	11	3 - 54	44	4 - 10 g/L
Gamma	23	5 – 79	44	3 - 9 g/L
Urine				
SG	1,027	1,008 – 1,051	34	
pH	6,5	5,5 – 9,0	33	
Protein	1,4	0,0 – 67,0	39	<0,56 g/L
Creatinine	12776	1913 – 59995	36	- µmol/L
Protein/creatinine	0,87	0 – 14,14	31	<1,00
AF	17	11 – 43	5	- U/L

	+	-	n	Reference
Hb	15	18	33	-
Glucose	1	32	33	-
Bilirubin	4	1	5	-

	0-5	5-10	15-30	>30	n	Reference
Leukocytes	22	4	0	0	26	0 /HPF
Erytrocytes	15	1	3	2	21	0 /HPF
Squamous epithelium	17	3	0	0	20	0 /HPF
Transitional epithelium	10	1	1	0	12	0 /HPF

	0-1	1-3	>3	n	Reference
Granular casts	9	3	1	13	0 /LPF
Hyaline casts	3	0	1	4	0 /LPF
Bilirubin crystals	5	1	1	7	0 /LPF

2.3 Kaplan-Meier

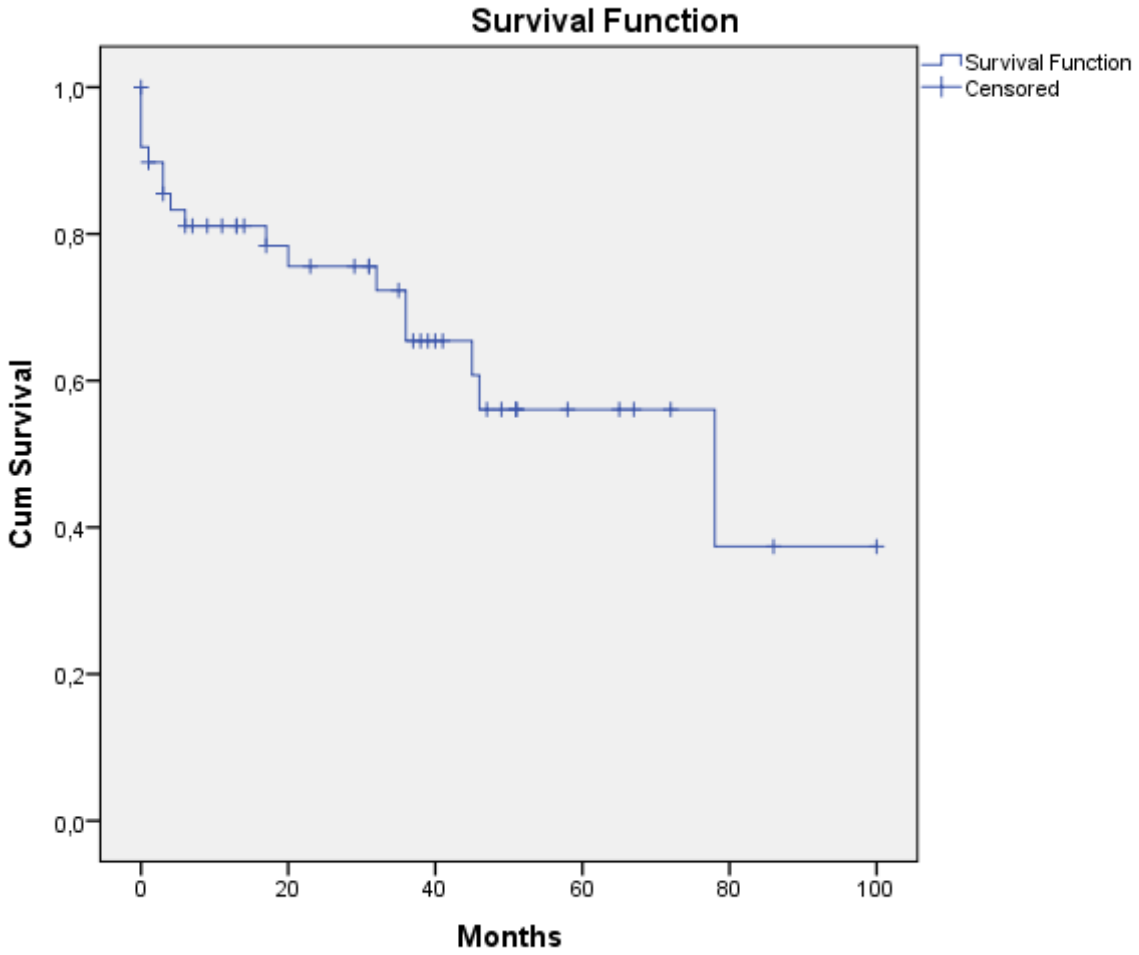

Figure 1: Kaplan-Meier survival curve for dogs with Leishmaniasis (n = 49)

Figure 1 shows the Kaplan-Meier survival curve for all dogs in the study. The curve shows a 24-month (2 year) survival rate of 75%, a 48-month (4 year) survival rate of 55% and a 96-month (8 year) survival rate of 35%.

2.4 Log-rank test

Kaplan-Meier survival analysis was continued with a log-rank test with categorical factors: category, treatment, sex and the categorical laboratory results at the time of diagnosis. Tables 3 shows the P-values of the log-rank test when adding these factors.

Table 3: P-values categorical factors

	P
General factors	
Category	0,091
Treatment	0,353
Sex	0,973
Hematology	

Normoblasts	0,230
Urine	
Glucose	**0,024**
Granular casts	**0,034**
Hb	**0,049**
Squamous epithelium	0,289
Leukocytes	0,551
Hyaline casts	0,564
Transitional epithelium	0,607
Bilirubin crystals	0,717
Erytrocytes	0,784

As shown in table 3, significant results (P <0,05) in the log-rank test include glucose (P = 0,024), granular casts (P = 0,034) and Hb (P = 0,049) for urine.

2.5 Cox regression

Table 4 shows the results of the Cox regression survival analysis, including all factors.

Table 4: Cox regression survival analysis results

Variable	Hazard ratio	n	95% CI	P
General factors				
Category				
C		20		0,130
D	0,443	14	0,049 – 4,022	0,469
Ea	1,567	13	0,191 – 12,880	0,676
Eb	0,509	2	0,056 – 4,627	0,548
Treatment				
A		30		0,668
A + G	25436,269	10	0,000-4,632e121	0,941
A + M	33973,271	7	0,000-6,196e121	0,940
A + G + M	54793,104	2	0,00-9,994e121	0,937
Sex	0,982	49	0,346-2,791	0,973
Hematology				
Monocytes	3,342	46	1,366 – 8,174	**0,008**
MCV	1,118	40	1,004 – 1,245	**0,043**
MPC	0,961	22	0,916 – 1,007	0,097
MCH	110,822	40	0,292 – 42045,405	0,120
MPV	1,066	22	0,979 – 1,162	0,141
Normoblasts				

None		42		0,290
Few	0,254	2	0,031 – 2,099	0,203
Multiple	0,771	2	0,047 – 12,716	0,855
Haematocrit	0,056	46	0,000 – 8,952	0,266
Eosinophils	0,307	46	0,033 – 2,862	0,300
CHr	26,918	25	0,052 – 14011,529	0,302
MPM	1,648	22	0,547 – 4,968	0,375
APTT	0,897	9	0,681 – 1,182	0,439
PT	0,550	9	0,119 – 2,541	0,444
Reticulocytes	1,124	25	0,729 – 1,732	0,598
Thrombocytes	1,001	43	0,996 – 1,006	0,639
Band cells	1,296	46	0,432 – 3,887	0,643
MCHC	0,906	40	0,580 – 1,415	0,665
Leucocytes	1,017	46	0,939 – 1,102	0,673
Segments	1,016	46	0,928 – 1,113	0,728
Lymphocytes	1,083	46	0,639 – 1,836	0,766
Fibrinogen	1,224	9	0,320 – 4,683	0,767
Basophils	1,850	46	0,000 – 11971,401	0,891
Clinical chemistry				
Creatinine	1,007	41	1,003 – 1,012	**0,002**
Urea	1,069	33	1,019 – 1,121	**0,006**
Bile acids	1,065	30	0,998 – 1,136	0,058
Phosphate	4,370	19	0,940 – 20,319	0,060
Albumin	0,958	47	0,901 – 1,017	0,161
ALAT	1,003	15	0,999 – 1,008	0,177
Beta 2	0,913	44	0,800 – 1,043	0,181
Total protein	0,984	47	0,956 – 1,012	0,260
Alpha 2	1,150	44	0,897 – 1,475	0,271
Alpha 1	0,880	44	0,651 – 1,188	0,403
Glucose	0,660	13	0,207 – 2,104	0,483
Sodium	0,948	22	0,805 – 1,115	0,517
Gamma	0,993	44	0,966 – 1,021	0,620
Potassium	1,293	22	0,443 – 3,769	0,638
AF	0,998	24	0,989 – 1,007	0,680
Calcium	0,562	19	0,034 – 9,163	0,686
Beta 1	1,005	44	0,711- 1,420	0,977
Urine				

Protein/creatinine ratio	1,222	31	1,056 – 1,413	**0,007**
SG	0,000	34	0,000 – 0,736	**0,048**
Hb	0,346	33	0,112 – 1,071	0,066
Glucose	0,128	33	0,014 – 1,150	0,067
Protein	1,071	35	0,995 – 1,152	0,069
Creatinine	1,000	36	1,000 – 1,000	0,082
Granular casts				
0-1		9		0,205
1-3	0,111	3	0,007 – 1,783	0,121
>3	0,006	1	0,000 – 10,659	0,181
pH	1,002	33	0,999 – 1,006	0,254
Squamous epithelium	25,295	20	0,002 – 319.123,220	0,503
Leukocytes	0,666	26	0,170 – 2,612	0,560
Erytrocytes				
0-5		15		0,933
5-15	0,532	1	0,062 – 4,600	0,567
15-30	0,000	3		0,989
>30	0,757	2	0,065 – 8,850	0,842
Transitional epithelium				
0-5		10		0,826
5-10	26,154	1	0,000 – 8.234.712,185	0,613
>10	0,961	1	0,000 – 3.484.916.626	0,997
Hyaline casts	31,526	4	0,000 – 7.314.677.857	0,725
Bilirubin crystals				
0-1		5		0,902
1-3	46,221	1	0,000 – 6,259e11	0,747
>3	1,000	1	0,000 – 1,892e14	1,000
AF	1,007	5	0,908 – 1,116	0,895

As shown in table 4, significant results (P <0,05) in the Cox regression survival analysis include monocytes (P = 0,008) and MCV (P = 0,043) for blood, creatinine (P = 0,002) and urea (P = 0,006) for blood chemistry and the protein/creatinine ratio (P = 0,007) and SG (P = 0,048) for urine.

3 Discussion

The survival curve of the UKG states there is a 4 year survival rate of 75%. This is less negative than the 4 year survival rate of this study, being 55%. However, the writer of the first mentioned article has excluded dogs with a blood creatinine above reference values.[13] If these kind of dogs are excluded in this research as well, the 4 year survival rate becomes 58%, which is quite similar to the before mentioned 55% and still a notable difference in survival rate.
It is not clear why these survival rates differ.

With the log-rank test, glucose, granular casts and hemoglobin in urine turned out to be factors with a significant influence on survival rate.
With Cox regression these factors were specific gravity and protein/creatinine ratio in urine, MCV and monocytes in hematology and creatinine and urea for blood chemistry.

Glucose appears in urine when the renal threshold for glucose reabsorption is exceeded. This can be caused by diabetes mellitus, pheochromocytoma and proximal renal tubular diseases.[15,16]
It is well known that the formation of immune-complexes in canine Leishmaniasis can cause damage to glomeruli and subsequently glomerulonephritis.[11]
Research has shown that the disease can also cause damage to the interstitium and tubuli of the kidneys. This would explain why being positive for urinary glucose increases the risk of death, as having glucosuria can be a sign of proximal renal tubular damage and thus indicates a more severe stage of disease.[17,18]

Granular casts in urine consist of degenerating epithelial cells, proteins and other substances. They are associated with diseases causing degeneration and necrosis of renal tubular epithelium.[15]
The presence of granular casts causing an increased risk of death can therefore be explained by the renal tubular damage Leishmaniasis can cause.

Haemoglobin can be found in urine during systemic haemolytic diseases and is an indicator of intravascular haemolysis. It can also be caused by lysis of previously intact erythrocytes within urine that is either dilute or very alkaline. When systemic haemolytic disease is not present, occult urinary tract haemorrhage can be the cause of the haemoglobinuria.[19]
Haematuria is seen in some dogs affected by Leishmaniasis. As inflammation in the kidneys can cause haematuria, and haematuria can be the cause of haemoglobinuria, the presence of haemoglobinuria can increase the risk of death.[20–22]

The urine specific gravity (USG) is an indicator of the ability of the renal tubules to concentrate the glomerular filtrate.[23]
Leishmania can cause tubular damage of the kidneys, decreasing their ability to concentrate urine. Therefore a lower USG can be a sign of kidney damage which increases the risk of death.

The MCV, or mean corpuscular volume, shows the average size of red blood cells in a blood sample. Reticulocytosis is the most frequent cause of elevated MCV but it can also be caused by bone marrow disease and artifacts. Increased MCV is considered a sign of regenerative anaemia.
Anaemia is the most common haematological change found in canine leishmaniasis. In this study, 67% of dogs was found to be anaemic when diagnosed. The pathogenesis of anaemia

in leishmaniotic dogs is thought to be a reduced synthesis of erythropoietin because of renal failure. Also, a hemolytic component is suspected.[5,16,24,25]
Therefore, the presence of elevated MCV can be a consequence of renal damage and thus increase the risk of death.

Creatinine is a degradation product of muscle and is formed by an irreversible non-enzymatic process. It is almost completely eliminated by the glomeruli. In case of renal failure, creatinine cannot be filtered properly and the plasma concentration will rise.[26]
Therefore, creatinine is a well-known biomarker for monitoring renal function. This biomarker is recommended by IRIS (International Renal Interest Society) and is used in their method for clinical classification of dogs with leishmaniasis.[27]
It makes sense that elevation of this indicator of renal dysfunction also has a negative influence on survival rate.

Urea is the main form in which nitrogen is eliminated. Like creatinine, urea is almost completely eliminated by the glomeruli and therefore often used for diagnosing and monitoring renal failure. Creatinine is used more frequently for this purpose, as many extrarenal factors may cause increased urea concentration, like liver insufficiency, dehydration and diet.[26]
Even though urea is less specific for renal dysfunction, the lower life expectancy of dogs with elevated urea in this study is probably caused by the renal damage leishmaniasis can cause.

The urine protein to creatinine ratio is one of the most commonly used tests to quantify and monitor proteinuria in dogs. Proteinuric nephropathy is caused by the deposition of circulating immune complexes at the glomeruli. This induces inflammatory changes that lead to chronic kidney disease.[5,28]

All of the results mentioned above can be explained by possible renal damage caused by leishmaniasis. The one result that cannot be explained that way, is the elevation of monocytes causing an increased risk of death.

Monocytes come from bone marrow precursors. After maturation in the bone marrow they circulate in the bloodstream for a few days. Then monocytes migrate into tissue where they develop into and function as macrophages.[29,30]
Monocytes and macrophages are part of the mononuclear phagocyte system, which belongs to innate immunity. In case of infection, monocytes will phagocytose and present antigens, secrete chemokines and proliferate.[31,32]
An elevation of monocytes could be a sign of a more active or severe infection and therefore increases the risk of death.

A study like this had not yet been performed, but there is a study available which has looked into the relationship between clinical condition and blood parameters.
It has shown that disease progression is associated with anemia, which corresponds to this study. What does not match is their outcome of disease progression being associated with a lower number of monocytes. This may be explained by the fact that intense parasitism can cause bone marrow redirection.[33]

To confirm the outcome of this research, it should be repeated with a higher number of other dogs.

4 Conclusion

This research shows that glucose, granular casts, hemoglobin, specific gravity and protein/creatinine ratio in urine, creatinine and urea in blood chemistry and MCV and monocytes in hematology, could be used as a prognostic factors when diagnosing dogs with leishmaniasis.
The fact that most of these factors are an indicator of kidney function, shows that kidney function is a very important part in classifying dogs into different disease categories.

However, more research is needed to confirm these factors and decide how exactly they could be used as prognostic.

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