

A Retrospective Study Of The Causes Of Moderate To Severe Leukocytosis In Dogs

by

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LIST OF ABBREVIATIONS

BAB	Babesiosis group
C5a	Complement fraction 5a
CFU-E	Colony forming unit-eosinophil
CFU-G	Colony forming unit-granulocyte
CFU-GM	Colony forming unit-granulocyte-monocyte

CG	Control group
DIC	Disseminated intravascular coagulation
GALT	Gut-associated lymphoid tissue
G-CSF	Granulocyte Colony Stimulating Factor
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
Ht	Haematocrit
IFN γ	Interferon gamma
IMD	Immune-mediated disease group
INF	Infection group
IL-1	Interleukin 1
IL-3	Interleukin 3
IL-6	Interleukin 6
IL-8	Interleukin 8
LCG	Leukocytosis group
LTB₄	Leukotriene B ₄
MatNP	Post-mitotic bone marrow neutrophil pool
M-CSF	Monocyte Colony Stimulating Factor
NEC	Necrosis group
NEO	Neoplasia group
OT	Other disease group
OVAH	Onderstepoort Veterinary Academic Hospital
PAF	Platelet activating factor
PLT	Platelet count
Pro-NP	Proliferation bone marrow neutrophil pool
RBC	Total red blood cell count
SCF	Stem cell factor
TNF-α	Tumour necrosis factor alpha
TNF-β	Tumour necrosis factor beta
WBC	Total white blood cell count

SUMMARY

A RETROSPECTIVE STUDY OF THE CAUSES OF MODERATE TO SEVERE LEUKOCYTOSIS IN DOGS

by

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Background and objectives:

The aims of this study were to determine whether: i) diseases in hospitalised South African dogs with leukocyte counts $\geq 35 \times 10^9/l$ were different from, ii) hospitalisation time longer than and mortality rate higher than control dogs; iii) glucocorticoid treatment contributed to significant leukocytosis; iv) hypoalbuminaemia and thrombocytopaenia added prognostic value, v) high leukocyte counts predict complicated babesiosis.

Methods:

Records were examined from 182 hospitalized dogs with a WBC $\geq 35 \times 10^9/l$ (LCG) and 179 hospitalized dogs with $3.0 \leq WBC \leq 30 \times 10^9/l$ and immature neutrophil count $\leq 0.5 \times 10^9/l$ (CG). Diagnoses were assigned to groups Infection, Immune-mediated; Necrosis; Neoplasia; Babesiosis; Other.

Results:

WBC, neutrophil count, lymphocyte count and monocyte count were higher in LCG than CG ($p < 0.0001$) while eosinophil count was lower in LCG than CG ($p < 0.0001$). Hct, platelet count, and serum albumin concentration were lower in LCG than CG ($p < 0.0001$). There was no difference in neutrophil count, lymphocyte or monocyte count between glucocorticoid-treated and non-glucocorticoid-treated dogs in LCG. Disease frequencies differed significantly (LCG > CG) in Infection, Necrosis, Babesiosis and immune mediated haematological disease groups. The frequency of complicated babesiosis cases was higher in LCG than in CG ($p < 0.0001$). Time of hospitalization was significantly ($p < 0.0001$) longer for LCG than for CG. There was a significant relationship between total and immature neutrophil count and survival ($p = 0.01$)

Conclusions:

Leukocytosis is more likely to indicate infection, complicated babesiosis, immune mediated hematological disease or necrosis in the population of dogs examined. Hypoalbuminaemia and thrombocytopaenia in an animal with significant leukocytosis is not prognostically useful,

while the combination of total and immature neutrophil count is. In hospitalized animals with severe leukocytosis, glucocorticoid treatment does not increase the leukocyte count.

CHAPTER 1 LITERATURE REVIEW

1.1. LEUKOCYTOSIS

Leukocytosis is an increased leukocyte count in a sample collected from the circulating blood pool. It results from changes in production, distribution or utilization of neutrophils, lymphocytes, monocytes, eosinophils, basophils and mast cells. It is most commonly associated with neutrophilia and monocytosis⁹⁶. This discussion will focus on mechanism and dynamics of increases in neutrophils, lymphocytes and monocytes. The major conditions that cause leukocytosis are inflammation, glucocorticoid response, catecholamine response, neoplasia and hereditary conditions⁹⁶

1.2. LEUKOCYTES

1.2.1. Neutrophils

Neutrophils are produced in the bone marrow and are released into the blood on maturation. After a short period in circulation, they migrate to tissues and body cavities⁹⁵. In the bone marrow, pluripotential stem cells differentiate into committed stem cells (CFU-G). These are stimulated by IL-1, IL-3, IL-6, GM-CSF and G-CSF to differentiate into the neutrophilic line^{64,97}. Further maturation is as follows: Myeloblast \Rightarrow promyelocyte \Rightarrow neutrophilic myelocyte in the mitotic pool (proNP). Neutrophils remain in this pool for approximately 3 days in healthy mammals. Some myelocytes undergo apoptosis to limit neutropoiesis at this stage. If there is an excessive demand for neutrophils, more cells survive to the next phase, the post-mitotic pool (MatNP). In the MatNP, metamyelocytes mature to band neutrophils and mature neutrophils⁹⁷. They remain here for 2-3 days^{18,95,97}. Segmented neutrophils are released from the MatNP to marrow sinuses and into peripheral blood. Neutrophil releasing factors include chemo-attractants (C5a, IL-8, LTB₄, PAF) and cytokine leukocytosis factors (IL-1, IL-6, TNF- α , TNF- β , GM-CSF and G-CSF). Neutrophils circulate for 5-10 hours and are divided into a circulating and a marginated pool. The neutrophils in the circulating pool are free flowing in blood and are thus collected when blood is sampled. Neutrophils in the marginated pool temporarily adhere to endothelial cells in capillaries and venules. From there, they may re-enter the circulating pool or migrate into the tissues^{95,97}. Neutrophils are attracted to sites of inflammation or infection by directed migration or chemotaxis along gradients of mediators or chemoattractants⁹⁵. Inflammatory cytokines (including IL-1 and TNF from macrophages and IFN γ from lymphocytes) stimulate endothelial cells to produce and express adhesion proteins (selectins) that mediate migration and "rolling" along the endothelial surface. Endogenous chemical mediators then activate neutrophils leading to a conformational change in membrane integrins, which bind to endothelial cell receptors. These mediate the process of migration to tissues. This is followed by

phagocytosis and microbicidal activity.^{95,97} In dogs, the ratio of neutrophils in the circulating to marginated pools is 1:1⁹⁷. Because the marginated pool can be mobilized into circulation under the influence of catecholamines or corticosteroids, a 2- fold increase can be expected in the neutrophil count if that were the only factor causing an increase in dogs^{95,97}. However, in cats the marginated pool is three times the size of the circulating pool, so the increase may be as much as fourfold.^{95,97}

Neutrophils leave circulation randomly and migrate into tissues where they survive 1-4 days and then undergo apoptosis or are destroyed by macrophages in spleen, liver and bone marrow^{95,97}.

The absolute neutrophil count is a measure only of the circulating pool^{95,97}. This is dependent on i) stem cell proliferation and differentiation and effectiveness of maturation in the myelocyte stage; ii) release from bone marrow: the oldest almost mature neutrophils preferentially leave the marrow first; iii) the exchange between circulating and marginated pools iv) lifespan in circulation and iv) rate of migration into the tissues Once they have left the blood stream and entered the tissues, they do not return to the blood^{95,97}.

1.2.2. Lymphocytes

Lymphocytes are produced in bone marrow and lymphoid organs (lymph nodes, thymus, spleen, gut associated lymphoid tissue (GALT). Pluripotential stem cells in the bone marrow differentiate into committed lymphoid stem cells¹⁰⁴. These give rise to B-lymphocyte progenitor cells and T/NK progenitor cells which in turn give rise to T progenitor cells and natural killer (NK) progenitor cells³². In dogs B-lymphocyte progenitor cells produce B-lymphocytes in the bone marrow which then migrate to the cortex in lymph nodes, follicles in Peyer's patches and follicles of the spleen. T-lymphocyte progenitor cells migrate to the thymus and develop into T cells. After maturation, T-lymphocytes accumulate in the paracortical areas of lymph nodes, periarteriolar lymphoid sheaths of the spleen and interfollicular areas of Peyer's patches. NK cells are mostly produced and mature in the bone marrow.³²

Blood lymphocytes originate primarily from the lymph nodes^{32,97}. Like neutrophils, they are distributed into a circulating and a marginated pool. Most lymphocytes in blood are T lymphocytes⁹⁷. Lymphocytes migrate from blood to tissues and lymph nodes and back. In the lymph nodes they migrate to the cortex via specialised post-capillary venules called high endothelial venules because they have unique tall endothelial cells and receptors. They exit via efferent lymphatics and return to the blood^{97,104}. Approximately 25% of blood lymphocytes enter lymph nodes each day. Lymphocytes migrate to other tissues to perform their specialised immunological functions. There they may undergo blastogenesis, enter lymphatics to return to blood or die. Like neutrophils, migration to tissues involves lymphocyte chemotaxis and binding to endothelial cell receptors⁹⁷. Blood lymphocyte concentrations are influenced by: i) production factors-stem cell proliferation and differentiation and blastogenesis; ii) distribution of lymphocytes between the circulating and marginated pool; iii) migration from the lymph nodes

and other tissues; iv) migration from lymph nodes via efferent lymphatic vessels.⁹⁷ Lymphocyte life span varies from hours to years.

1.2.3. Monocytes

Monocytes are produced in the bone marrow. Pluripotential stem cells differentiate into bipotential precursor cells (CFU-GM) under the influence of SCF, IL-1, IL-6, and IL-3^{7,97}. These differentiate into monoblasts, promonocytes and monocytes. The cytokines promoting monocyte development and function are GM-CSF, M-CSF and IL-3^{7,64}. Monocytes are not stored in bone marrow. They appear in blood approximately 6 days after initiation of stem cell division⁷ They are distributed in a circulating and marginated pool⁹⁷. In the absence of an inflammatory stimulus, they exit from circulation in an apparently random manner. However, in response to inflammation, they accumulate specifically at the site, but in smaller numbers than neutrophils⁷. They differentiate in the tissues into macrophages, histiocytes or other specialised cells of the mononuclear phagocytic system⁹⁷. Tissue macrophages are capable of mitosis but fewer than 5% of the macrophages in sites of inflammation arise in this way⁷. Their functions include phagocytosis, regulation of the inflammatory response and haematopoiesis, antigen processing for presentation to lymphocytes, destruction of senescent cells and tissue debris and participation in regulation of body iron stores⁷.

1.2.4. Eosinophils

Like neutrophils and monocytes, eosinophils develop in the bone marrow from a distinct progenitor cell, CFU-E which originates from a pluripotential stem cell¹¹². Development is mediated by cytokines originating from mast cells, macrophages and T-lymphocytes. The most important of these is IL-5. Others include GM-CSF and IL-3^{97,112}. They become recognisable as eosinophils with the appearance of their specific secondary granules at the progranulocyte stage¹¹². They differentiate and mature in the bone marrow over 2-6 days. Migration into tissues occurs randomly and the circulating half- life in dogs is less than an hour¹¹². Eosinophils are capable of phagocytosis and bacterial killing. They inactivate mediators from mast cells and they attack the larval and adult stages of a few parasites⁹⁷.

1.2.5. Basophils

Basophils develop in the bone marrow under the influence of cytokines, particularly IL-3⁹⁷. Their bone marrow transit time is approximately 2.5 days and their circulating half life is about 6 hours. Their migration into tissues is promoted by IL-1, TNF α and endotoxin. They survive around 2 weeks in the tissues. They are activated by IL-3 and IgE binding⁹⁷. Basophil granules contain substances that promote allergic inflammatory reactions and attract eosinophils.

1.3 LEUKOCYTE KINETICS ASSOCIATED WITH LEUKOCYTOSIS

1.3.1. Catecholamines (Physiological leukocytosis)

Catecholamine release due to excitement, fright, pain, exercise, anxiety or catecholamine administration results in mobilisation of the marginated neutrophil pool, and thus a twofold increase in neutrophil count. Catecholamines may also cause a twofold increase in lymphocyte count^{90,96,97,106}. It is a transient effect which lasts less than an hour^{90,97}. It is more common in puppies than adult dogs⁹⁰.

1.3.2. Glucocorticoids

Endogenous or exogenous glucocorticoids result in an up to threefold increase in neutrophils as a result of mobilisation of the marginated pool, decreased migration to extravascular tissues pool due to down-regulation of the production of adhesion molecules^{96,97}, and mobilisation of mature neutrophils from bone marrow^{90,96,97}. With short-term use, there is a decrease in lymphocyte count due to decreased re-entry of lymphocytes from tissues and lymph nodes. With prolonged use, there is a lymphotoxic effect with decreased lymphopoiesis^{90,96,97}. Glucocorticoids result in an increase in monocyte count, probably due to a shift from the marginated to the circulating pool^{90,96,97}. Corticosteroid-associated leukogram changes occur within 4-8 hours of administration of glucocorticoids and resolve within 24 hours. After long-term exposure (>10 days), the leukogram only returns to normal 2-3 days after withdrawal of treatment⁹⁰.

1.3.3. Acute inflammation

Acute inflammation is associated with neutrophilia and (sometimes) a left shift. There may be lymphocytosis or lymphopaenia. There may or may not be monocytosis^{90,97,106}. Within hours of onset of inflammation, there is a release of neutrophils from the bone marrow storage pool. When that becomes depleted, band neutrophils are released from the maturing pool, resulting in a left shift. This is associated with increased production from the myelocyte stage. It takes 2-4 days before the effect of this is seen in peripheral blood. There is also increased production from the stem cell stage. It takes about 5 days before the effect of this is seen in peripheral blood⁹⁷.

1.3.4. Chronic inflammation

With chronic (established) inflammation, there expansion of the proliferation, maturation and storage pools of the bone marrow (granulocytic hyperplasia)^{90,97}. This is usually associated with a mature neutrophilia. There may or may not be a left shift. There may be lymphocytosis or lymphopaenia. There may or may not be monocytosis.^{90,97}

1.3.5. Neoplasia

1.3.5.1 Granulocytic (myeloid) leukaemia

Chronic granulocytic leukaemia usually presents with a marked neutrophilic leukocytosis ($>80.0 \times 10^9/l$) with a left shift which may be disordered with evidence of maturation arrest. Promyelocytes and myeloblasts may be seen on the blood smear⁹⁶.

1.3.5.2 Paraneoplastic neutrophilia

Paraneoplastic neutrophilia may be associated with necrosis or with secretion of G-CSF or GM-CSF by the tumour cells^{4-6,51,69,72,75,77,87,93,99,100}. This will be discussed in more detail later in this review.

1.3.6. Genetic disorders

Leukocyte adhesion deficiency of Irish Setters is caused by a $\beta 2$ integrin deficiency. Affected dogs have persistent neutrophilia and recurrent infections⁹⁷. Cyclic haematopoiesis (grey collie syndrome) is associated with cyclic fluctuations in myeloid cell products at 11-13 day intervals. Neutrophils are most affected with neutrophilia alternating with neutropaenia⁹⁰.

1.3.7 Lymphocytosis

Lymphocytosis may result from the following diseases and conditions^{90,96,97}:

- Chronic inflammation
- Infections
 - Bacterial or rickettsial
 - Fungal
 - Viral
 - Protozoal
- Physiological
 - Excitement, fright, pain, exercise, anxiety
 - Catecholamine administration
- Neoplasia
 - Leukaemic lymphoma
 - Lymphoid leukaemia
- Endocrine
 - Hypoadrenocorticism

1.3.8. Monocytosis

Monocytosis may result from the following diseases and conditions^{90,96,97}:

- Inflammation
 - Infections
 - Necrosis
 - Immune-mediated disease
- Glucocorticoid –induced.
 - Stress
 - Hyperadrenocorticism
 - Glucocorticoid administration
- Neoplasia
 - Monocytic or myelomonocytic leukaemia
 - Paraneoplastic

1.4. DISEASE CONDITIONS ASSOCIATED WITH EXTREME LEUKOCYTOSIS

Lucroy and Madewell performed retrospective studies on the clinical outcome and associated diseases in 118 dogs⁵⁷ and 104 cats⁵⁸ with a WCC $\geq 50 \times 10^9/l$ with > 50% neutrophils. In the dog study⁵⁷, they found that 34% of cases had immune mediated disease, 32% had infections, 20% had neoplasia, 12% had tissue necrosis and 2% had chronic myeloid leukaemia.

In the cat study⁵⁸, 37% of cats had infections, 22% had immune-mediated disease, 23% had neoplasia and 18% had tissue necrosis. Marked leukocytosis was associated with prolonged hospitalization and a high mortality rate (62% in dogs⁵⁷ and 61% in cats⁵⁸). A high mortality rate has also been observed in humans⁸¹. Other studies have also described severe leukocytosis associated with these disease categories and these are discussed in the paragraphs that follow.

1.4.1. IMMUNE MEDIATED DISEASE.

Moderate to marked leukocytosis has been described in IMHA in dogs^{63,65}, cats⁵⁸ and in humans⁷⁸. Moderate to marked leukocytosis associated with immune mediated haemolytic anaemia was shown by McManus and Craig⁶³ to be associated with moderate to severe post-mortem lesions in dogs. Ischaemic necrosis within liver, kidney, heart, lung, and spleen attributable to thromboembolic disease or anaemic hypoxia were the most common important lesions found at necropsy. Marked leukocytosis has also been described with immune mediated thrombocytopaenia^{58,111} polyarthritis³⁸, rheumatoid arthritis⁴⁹, juvenile cellulitis (puppy strangles)¹⁰⁹ and necrotising vasculitis in beagles⁹¹.

1.4.2. INFECTION

Most infections associated with leukocytosis are bacterial^{2,9,14,20,21,26,33,43,45,58,60,71,103,105,108}. A leukemoid response has also been described in babesiosis⁵⁴, malaria in humans⁸², haemobartonellosis⁵⁸, feline viral diseases⁵⁸ and Hepatozoonosis^{27,35}. The latter may be associated with a neutrophil myeloperoxidase deficiency in parasitised cells³⁵. In *Clostridium sordellii* infection in humans, bacterial neuraminidase enhanced the effect of GM-CSF on proliferation of promyelocytes in cell culture and modified vascular cell adhesion molecule 1, which controls the release of mature and immature granulocytes from bone marrow stromal cells². An eosinophilic leukemoid response has been associated with systemic toxocarosis in humans²²

1.4.3. BABESIOSIS

A leukemoid response has also previously been described in canine babesiosis⁵⁴. Apart from being an infection, babesiosis can be associated with a large spectrum of complications which are usually classified according to the organ(s) that are dysfunctional or failing³⁶. These include rhabdomyolysis, immune mediated haemolytic anemia, acute renal failure, pancreatitis³⁶, acute respiratory distress syndrome (ARDS), cerebral failure¹⁰⁷, and thrombocytopaenia⁴⁴.

1.4.4. NEOPLASIA

Many neoplastic conditions have been associated with severe leukocytosis or a leukemoid response⁶². In humans, it has been associated with renal transitional cell carcinoma⁷⁶, bladder transitional cell carcinoma^{1,10}, ovarian carcinoma¹⁶, gastric carcinoma^{24,73}, pancreatic carcinoma⁷⁹ liposarcoma⁶¹, lung sarcoma⁴¹, lymphoma²⁵, inflammatory malignant fibrous histiocytoma^{84,94,101}, metastatic carcinoma⁷⁰, epithelioid sarcoma⁸⁵, an undifferentiated nasal carcinoma⁸⁸, squamous cell carcinoma⁴⁶ and in response to treatment for cervical carcinoma⁷⁴. In dogs, it has been described in renal tubular carcinoma^{51,59}, renal transitional cell carcinoma⁷⁷, metastatic fibrosarcoma¹⁵, rectal adenoma^{47,102} and in squamous cell carcinoma^{23,58} and a number of other neoplasms in cats⁵⁸.

The direct induction of a leukemoid response to some tumours has been shown by implanting hepatoma, mammary carcinoma and rhabdomyosarcoma cell lines into mice¹². Production of colony-stimulating factors by tumours has been demonstrated in humans^{4-6,24,41,53,69,72,75,87,93,99,100} and dogs^{51,77}.

1.4.5. TISSUE NECROSIS

The most common condition in dogs associated with tissue necrosis and leukocytosis is pancreatitis³⁴. Leukocytosis has also been associated with pancreatic abscessation⁸⁶, and in

humans with alcoholic hepatitis⁶⁶. In cats, tissue trauma, pancreatitis and thromboembolism were the most common causes⁵⁸

1.4.6. OTHER CONDITIONS

Hereditary conditions affecting neutrophil function can result in severe leukocytosis^{28,39,40,80}. A drug induced leukemoid response has been described in humans in association with tetracyclines¹³, acetaminophen³⁰, carbamazepine⁵⁰ and methotrexate⁹⁸. A leukemoid response has also been described in humans in association with parenteral nutrition⁴², alcoholic hepatitis⁶⁸ and diabetic ketoacidosis⁸.

1.5. HAEMATOLOGICAL AND BIOCHEMICAL FACTORS ASSOCIATED WITH SEVERE LEUKOCYTOSIS.

1.5.1. ANAEMIA

Anaemia is directly associated with IMHA^{11,57,63,65} and babesiosis⁵⁴. It can also occur secondary to inflammation^{38,109} and as a paraneoplastic syndrome^{15,51,77}.

1.5.2. NEUTROPHIL TOXICITY

Toxic neutrophils have abnormalities in cell size, nuclear shape and consistency, and cytoplasmic content evident on examination of Romanowsky-stained blood smears³. These toxic changes occur during the development of neutrophils in the bone marrow. The most obvious changes are the presence of giant neutrophils, cytoplasmic basophilia, toxic granulation, cytoplasmic vacuolation, and presence of Dohle bodies³. Neutrophil toxicity may be associated with leukopaenia or leukocytosis³. The conditions found to be more prevalent in dogs with neutrophil toxicity in the study by Aroch et. al³ were pyometra, parvoviral infection, peritonitis, acute renal failure, immune mediated haemolytic anaemia, disseminated intravascular coagulation (DIC), pancreatitis and septicaemia. Neutrophil toxicity has also recently been studied in cats by Segev.et.al.⁹² They found a higher incidence of shock, sepsis, panleukopaenia, peritonitis, pneumonia, bacterial and viral infections and metabolic disorders in cats with toxic neutrophils

1.5.3. HYPOALBUMINAEMIA

Albumin is a negative acute phase protein⁹⁶, so it is not surprising that hypoalbuminemia occurs in many conditions in which there is marked leukocytosis^{11,20,34}. It was also associated with a paraneoplastic leukemoid response¹⁵.

1.5.4. THROMBOCYTOPAENIA

Thrombocytopaenia associated with a leukemoid response may be due to DIC^{11,20,33,63} or be a paraneoplastic syndrome⁷⁷

CHAPTER 2 STUDY OBJECTIVES

2.1. HYPOTHESES / PROBLEM STATEMENT

Most previous studies on moderate to severe leukocytosis have been in the form of case studies. Lucroy and Madewell's studies examined the clinical outcome and associated diseases in dogs⁵⁷ with leukocytosis and neutrophilia. They recorded the diseases, duration of hospitalisation and mortality rate of dogs with leukocyte counts $> 50 \times 10^9/l$ and with $>50\%$ neutrophils. However, significant leukocytosis in dogs, if one allows for doubling of the neutrophil and monocyte counts (upper reference intervals for our laboratory $11.9 \times 10^9/l$ and $1.3 \times 10^9/l$, respectively) due to mobilisation of the marginated pool and doubling of the lymphocyte count (upper reference interval $4.8 \times 10^9/l$) due to physiological lymphocytosis), is $35 \times 10^9/l$ and if that were taken as the cutoff in dogs, there may be a relationship between the leukocyte count and the disease category. Also, factors such as hypoalbuminaemia¹¹, and thrombocytopenia¹⁹ have been associated with increased risk of mortality so they may add to the prognostic value of the leukocyte count. Lastly, The studies of Lucroy and Madwell^{57,58} were performed in North America, and the distribution of diseases associated with leukocytosis is likely to be different in South Africa. Most importantly, their study was not case-controlled.

2.2. OBJECTIVES OF THIS STUDY

- 2.2.1. The first purpose of this study was to determine whether hospitalised dogs with moderate to severe leukocytosis (total leukocyte counts $\geq 35 \times 10^9/l$):
 - 2.2.1.1. fell into significantly different disease groups from
 - 2.2.1.2. were hospitalised for longer than and
 - 2.2.1.3. had a higher mortality rate than hospitalised control dogs with normal leukocyte counts.
- 2.2.2. Factors such as hypoalbuminaemia¹¹, and thrombocytopenia¹⁹ have been associated with increased risk of mortality, so the second aim was to determine whether these added to the prognostic value of the leukocyte count.
- 2.2.3. To determine whether high leukocyte counts could be used as a predictor for complicated babesiosis.



2.3. BENEFITS OF THIS STUDY

- Since it is an unfortunate fact that veterinary medicine is subject to weighing the cost of treatment against the chance of recovery, factors which assist in determining an accurate prognosis are of benefit to practitioners.
- Babesiosis is a common and important disease in South Africa and there is little warning on first presentation whether a particular case will develop complications. Any parameter that would alert a clinician to the possibility of a particular patient developing complications and thus requiring special care or hospitalisation for observation would be useful.
- The research conducted serves as partial fulfillment of the principal investigators' MMedVet (KDK) degree.

CHAPTER 3 MATERIALS AND METHODS

3.1. MODEL SYSTEM

This was a retrospective study based on hospital records from the Onderstepoort Veterinary Academic Hospital, University of Pretoria.

3.2. EXPERIMENTAL DESIGN

Medical records of dogs of any breed, age or sex that were admitted to Onderstepoort Veterinary Academic Hospital during February 2002-October 2005 of which the maximum recorded corrected white cell count equalled or exceeded $35 \times 10^9/l$ were included (Leukocytosis group, LCG), irrespective of the stage of the disease process, time point of hospitalisation or whether treatment had begun at the time the maximum count was recorded. The cutoff of $35 \times 10^9/l$ was chosen to ensure that the animals in this group had a true inflammatory leukogram and to exclude most of those with a stress leukogram only⁹⁶. Catecholamines may cause doubling of the neutrophil and lymphocyte counts. Glucocorticoids result in mobilization of granulocytes from the margined pool to the circulating pool, decrease migration of neutrophils out of circulation into tissues as well as mobilization of mature granulocytes from the bone marrow. The magnitude of increase is usually less than twice the upper limit of the reference range and not more than three times the upper limit of the reference range, and is accompanied by lymphopaenia and possible doubling of the monocyte count⁹⁶. The control group (CG) was selected from records of age-matched dogs of any breed or sex that were admitted to Onderstepoort Veterinary Academic Hospital during the same period for which the minimum absolute neutrophil count was not less than $3.0 \times 10^9/l$ (the lower end of the reference range), the absolute immature neutrophil count was not more than $0.5 \times 10^9/l$ (the

upper end of the reference range) and the maximum recorded corrected white cell count was not more than $30 \times 10^9/l$ (twice the upper reference range) at any stage of the disease process. Cases were excluded if: i) animals were euthanased on financial grounds if there was a reasonable chance of recovery; ii) animals for which there was no manual differential count, iii) animals for which the hospital records or laboratory records were deemed incomplete.

Haematological analyses were performed on the Cell-Dyn 3700 analyzer (Abbott Laboratories, Santa Clara, USA). Manual differential counts were performed on thin-film blood smears stained with a Romanowski-type stain (Rapidiff, Clinical Sciences Diagnostics, South Africa). Serum proteins were analysed on the Technicon RA-1000 analyser (Bayer (Pty) Ltd Isando, SA) using the biuret method for total serum protein and the bromocresyl green method for albumin.

Diagnosis of babesiosis was based on visualization of the parasite on blood smear examination. Diagnosis of ehrlichiosis was based on polymerase chain reaction, of parvoviral infection with electron microscopy of a fecal sample and of canine distemper infection with indirect fluorescent antibody testing for IgM antibodies in blood or IgG titers in the cerebrospinal fluid and/ or electron microscopy of a fecal sample. Bacterial and fungal infection infections were confirmed on culture. Spirocercosis was confirmed on thoracic radiography, fiber optic oesophagoscopy and ultrasonography. Immune mediated haemolytic anaemia was diagnosed if one or more of the following were present: more than 20% spherocytes on the blood smear, a positive Coombs test or a positive In Saline Agglutination Test. Immune mediated thrombocytopenia was diagnosed by exclusion of other causes of acute-onset thrombocytopenia, particularly Babesiosis and Ehrlichiosis. Demodicosis was diagnosed on skin scraping. The data were captured into a Microsoft Excel[®] (Microsoft Corporation), spreadsheet and analysed with the Analyze-It[®] (Analyze-It Software, Ltd, Leeds, United Kingdom) and NCSS[®] (Hintze, J. (2007), NCSS, GUESS and PASS, NCSS, Kaysville, Utah) statistical packages. The breed, age and gender were recorded as well as the following data for the date on which the highest leukocyte count was recorded: total serum protein concentration (g/l), serum albumin concentration (g/l) (albumin), serum globulin concentration (g/l), Haematocrit (%), platelet count ($\times 10^9/l$), WBC count ($\times 10^9/l$), segmented neutrophil count ($\times 10^9/l$), immature neutrophil count ($\times 10^9/l$), lymphocyte count ($\times 10^9/l$), monocyte count ($\times 10^9/l$), eosinophil count ($\times 10^9/l$), basophil count

($\times 10^9/l$), diagnosis, duration of hospitalisation (days), clinical outcome (mortality or survival). If the animal did not survive, time from first day of hospitalization to euthanasia or death was recorded. The total neutrophil count was calculated as segmented neutrophil count + immature neutrophil count. To determine whether glucocorticoid treatment contributed to the differences in leukocyte counts, WBC, segmented neutrophil count, immature neutrophil count, lymphocyte count, monocyte count and eosinophil count were compared between dogs that had received glucocorticoid treatment before the maximal WCC was recorded and those that had not.

Based on the diagnosis, dogs were classified into the following groups: Immune-mediated disease, Infection (other than babesiosis), Babesiosis, Necrosis (if not associated with infection or neoplasia), Neoplasia, and Other. If immune mediated disease was secondary to infection, the disease was classified as immune mediated if it did not resolve after the infectious agent was eliminated. Specifically in the case of babesiosis, because of the numerous syndromes which are associated with complicated cases (as described above), the disease was classified as uncomplicated if it resolved after babesiacidal chemotherapy to eliminate the parasite and complicated if it did not, irrespective of the syndrome. The time of hospitalization was taken as the time from admission to death or discharge. An animal was deemed to have survived if it was discharged alive.

3.3. STATISTICAL CONSIDERATIONS

All data were analysed descriptively using the Kolmogorov-Smirnov test and found not to be normally distributed. Between-group differences were analysed with the Mann-Whitney test and frequencies were compared with the Chi-squared and Fisher exact tests. Survival data were analysed with the Kaplan-Meier Product-Limit Estimator and Logrank test or Cox regression tests.

CHAPTER 4 RESULTS

A total of 6608 dogs were admitted to OVAH during Feb 2002-Oct 2005. Records from 200 dogs with leukocytosis were examined. Fourteen dogs were excluded for incomplete records and four because they were euthanased for financial reasons, leaving 182 dogs in LCG. One-hundred and seventy-nine cases were included in CG. There were no significant differences between groups in age, gender or breed distribution.

4.1. DISEASES ASSOCIATED WITH LEUKOCYTOSIS

There were 56 cases (31%) in INF in LCG and these included 7 (13%) cases each of canine parvoviral infection and pyometra, 5 (9%) cases of peritonitis, 4 cases (7%) each of *Spirocerca lupi* infection and pyelonephritis, 3 (5%) of pyothorax, 2 each (4%) of canine distemper virus, systemic mycoses, abscessation (1 prostatic, 1 disseminated), discospondylitis, cellulitis, septicemia, pneumonia, endocarditis, and lymphadenitis. There was 1 case each (2%) of Infectious Canine Hepatitis, intestinal roundworm infestation, ehrlichiosis, 1 each of cholecystitis, septic arthritis, prostatic abscessation, post-surgical infection and rhinitis in LCG. In CG, there were 31 cases in INF and these included 5 cases (16%) of *Spirocerca lupi* infection, 4 each (13%) of ehrlichiosis, distemper and deep pyoderma, 2 each (6%) of parvovirus and pyelonephritis and vaginitis and 1 case each (3%) of intestinal roundworm infestation, discospondylitis, otitis externa, pyometra, otitis media, rhinitis, osteomyelitis, and demodectic mange. There were 50 cases of babesiosis in LCG and 9 in CG. These included 12 cases (24%) of uncomplicated and 38 cases (76%) of complicated babesiosis in LCG, and 7 cases (78%) of uncomplicated and 2 cases (22%) of complicated babesiosis in CG.

There were 20 cases (11%) of IMD in LCG. Ten (50%) of these were cases of immune-mediated haemolytic anemia and 4 (20%) were immune-mediated thrombocytopenia (with no identified underlying cause). The remainder comprised 3 cases (15%) of immune-mediated polyarthritis, 2 (10%) of inflammatory bowel disease, and 1 (5%) of eosinophilic bronchopneumopathy. In CG, there were 12 dogs with IMD and these included 3 cases (25%) of granulomatous meningoencephalitis and 1 case each (8%) of systemic lupus erythematosus,

immune-mediated polyarthritis, perianal fistulas, erythema multiforme, bullous pemphigoid, pemphigus foliaceus, masticatory muscle myositis, lymphoplasmacytic rhinitis, and discoid lupus erythematosus.

There were 23 dogs with NEC in the LCG group which included 5 cases (22%) of acute pancreatitis, 4 (17%) of gastric ulceration, 3 each (13%) of toxic hepatopathy, cytotoxic snake (puff adder) bite (*Bitis arietans*) and intestinal foreign bodies, 2 cases each (9%) of hernia and trauma and 1 case (4%) of oesophageal perforation.. In CG, NEC (7 cases) included 2 cases each (29%) of pancreatitis and myelomalacia and 1 case each (14%) of puff adder bite, trauma, and spider bite.

In NEO, of 26 dogs in LCG, there were 7 cases of haemangiosarcoma (2 of liver, 3 of spleen, 1 of lung, 1 disseminated metastatic) 6 of (23%) of lymphoma, 4 (15%) of mast cell tumour (3 systemic, 1 mediastinal), and 1 each (4%) of acute lymphoblastic leukemia, chronic lymphocytic leukemia, leukaemic lymphoma, ovarian carcinoma, renal carcinoma, mesothelioma, thymoma, adenocarcinoma of the lung and Leydig cell tumor. In CG, 33 cases of NEO included 5 (15%) squamous cell carcinomas (4 of skin and 1 metastatic), 4 (12%) mammary tumours, and 3 cases (9%) each of mast cell tumours (2 skin, 1 metastatic) and benign hepatic tumours. There were 2 (6%) each of thyroid carcinoma, transitional cell carcinoma and osteosarcoma, and 1 case each of cutaneous haemangiopericytoma, neurofibroma of the jaw, adenocarcinoma of the lung, cerebral mass, histiocytic sarcoma of the spleen, synovial sarcoma, mesothelioma, renal carcinoma, invasive lipoma, nasal sarcoma and cutaneous haemangiosarcoma.

In LCG, 7 cases in OT included 2 cases (29%) of organophosphate toxicity and 1 case (14%) each of gastroenteritis, bee-sting evenomation, megaesophagus, cardiomyopathy and uncontrolled diabetes mellitus. In CG, there were 87 dogs in OT. These comprised 16 cases of (18%) non-inflammatory neurological disease, 13 (15%) of gastrointestinal disease, 11 (13%) renal disease, 8 (9%) skeletal disease, 7 (8%) of toxicity, 5 each (6%) of dermatological disease, endocrine disease and cardiovascular disease. There were 3 (3%) of upper respiratory disease, 2 each (2%) of lower respiratory disease, non-inflammatory gynecological cases,

behavioral disorders, hepatic disease, ocular disease, esophagitis and 1 each (1%) of benign prostatic hyperplasia and heat stroke.

Differences in disease frequencies for the major groupings are shown in Figure 4.1. Significant differences in frequencies between groups, analysed with the Fisher exact test were found in INF, NEC, BAB and OT.

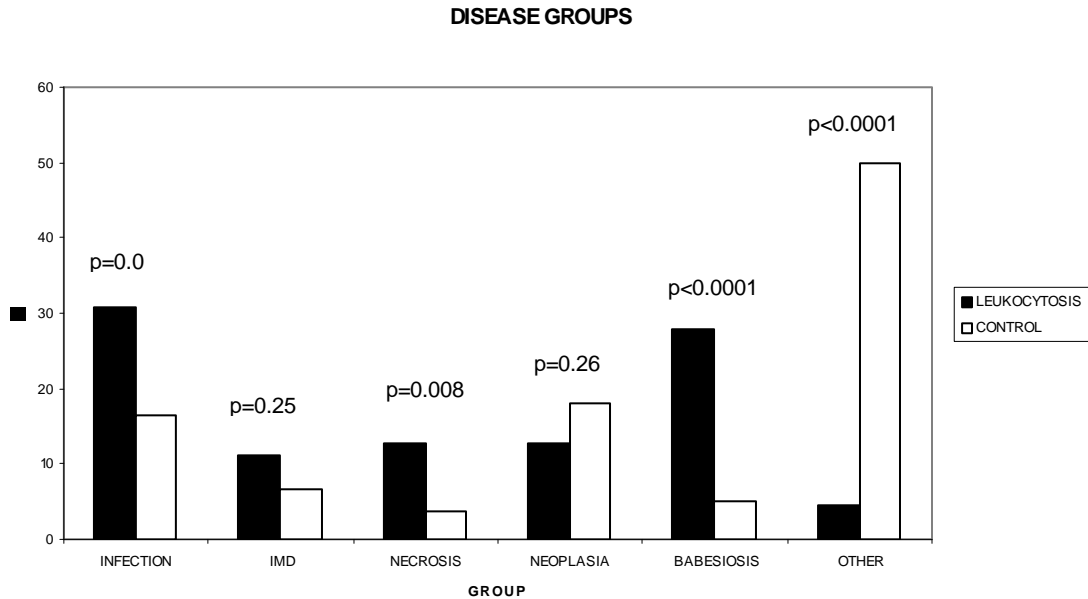


Figure 4.1

Percentage of LCG (Leukocytosis) and CG (Control) dogs falling into each of the disease categories: Infection; IMD: immune-mediated disease; Necrosis; Neoplasia; Babesiosis; Other.

Since frequency of cases appeared higher in LCG than CG for bacterial infection and fungal infections than other infections in the infection group, these were analysed separately from other infections. These results are presented in Figure 4.2.

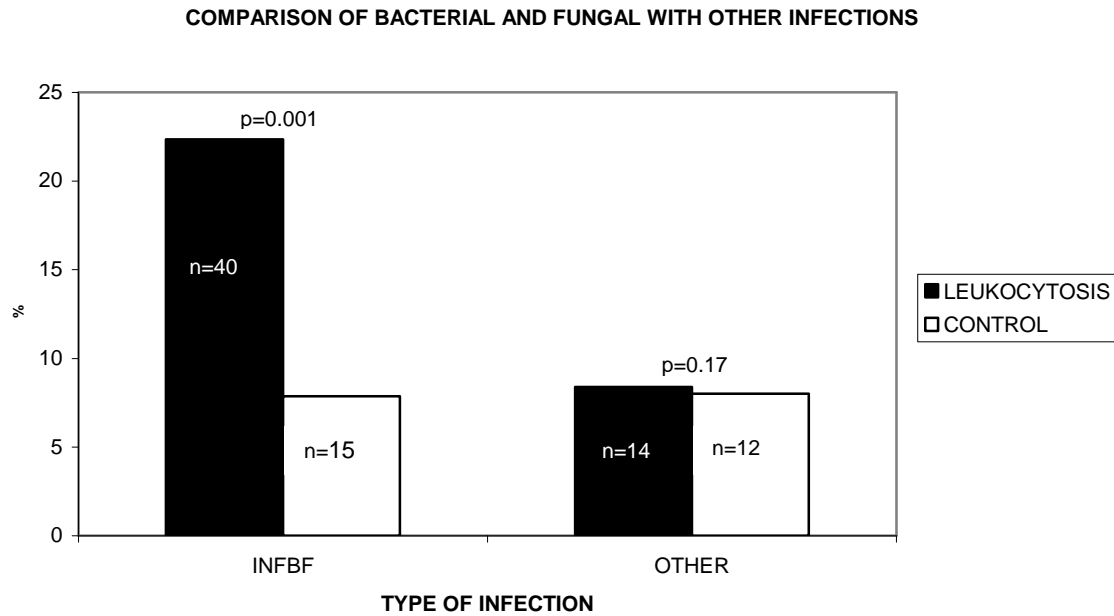


Figure 4.2

Percentage of LCG (Leukocytosis) and CG (Control dogs) with bacterial and fungal infections (INFBF) compared with other infections (OTHER). Other infections include viral, helminth and rickettsial infections, but exclude babesiosis.

It also appeared that the frequency of cases was significantly higher in LCG than CG for haematological immune-mediated diseases than other immune-mediated diseases, and thus these diseases were analysed separately. The results are presented in Figure 4.3.

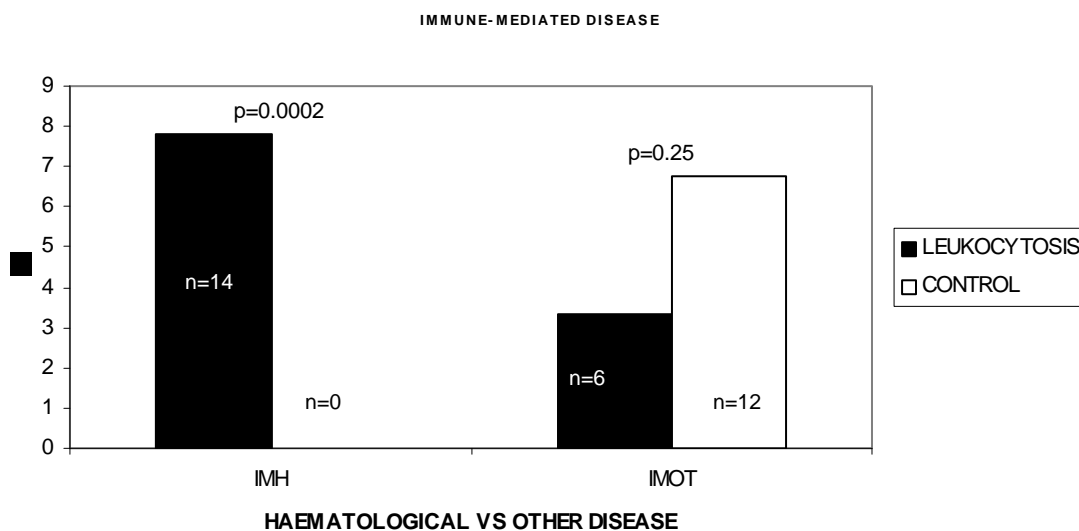


Figure 4.3

Percentage of LCG (Leukocytosis) and CG (Control) dogs with immune-mediated haematological disease (IMH) compared with other immune-mediated diseases (IMOT).

The frequency of complicated babesiosis ($p < 0.0001$), but not uncomplicated babesiosis ($p=0.41$) cases, was significantly higher in LCG than in CG. The results are presented in Figure 4.4.

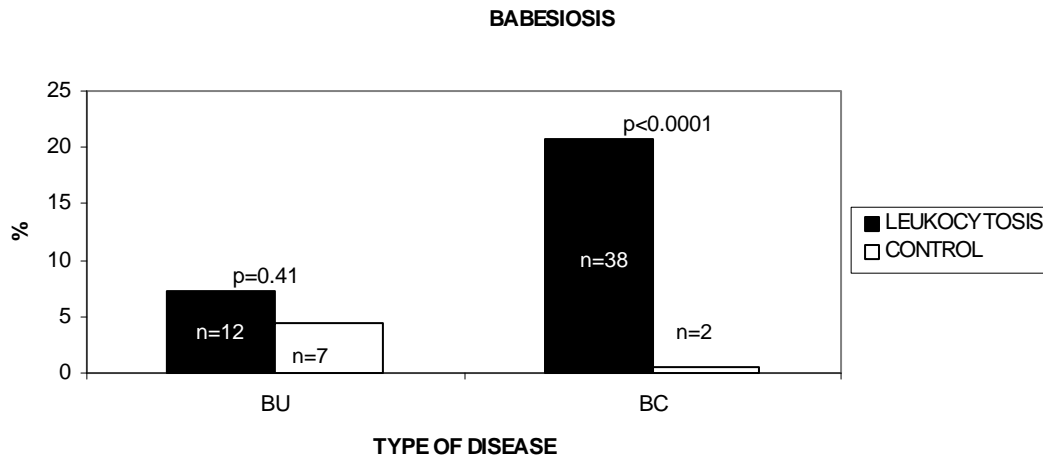


Figure 4.4

Percentage of LCG (Leukocytosis) and CG (Control) dogs with complicated compared with uncomplicated babesiosis: BU: uncomplicated babesiosis; BC: complicated babesiosis.

Results were also analysed separately for malignant and benign neoplasia, but no differences between groups were found.

4.2. DIFFERENCES IN HAEMATOLOGICAL AND SERUM PROTEIN PARAMETERS BETWEEN DOGS WITH LEUKOCYTOSIS AND CONTROLS

Haematological and serum protein data are presented in Table 4.1.

PARAMETER	GROUP		Ref Range	P
	LCG Median (Range)	CG Median (Range)		
Total Protein (g/l) (n=104)	59.2 (28.2-101.0)	65.5 (35.0-105.0)	53.0-75.0	p=0.04
Albumin (g/l) (n=102)	22.0 (13.5-39.2)	28.3 (15.4-43.6)	27.0-35.0	p<0.0001
Globulin (g/l) (n=101)	35.0 (12.4-81.0)	35.0 (15.8-84.1)	20.0-37.0	p=0.63
WBC ($\times 10^9/l$)	45.6 (35.0-150.0)	12.0 (4.3-29.9)	6.0-15.0	p<0.0001
Segmented neutrophil count ($\times 10^9/l$)	35.23 (7.43-108.00)	8.42 (3.23-29.00)	3.0-11.5	p<0.0001
Immature neutrophil count ($\times 10^9/l$)	4.31 (0.00-41.85)	0.01 (0.00-0.30)	0.0-0.5	p<0.0001
Lymphocyte count ($\times 10^9/l$)	2.06 (0.00-32.86)	1.60 (0.00-5.00)	1.0-4.8	p<0.0001
Monocyte count ($\times 10^9/l$)	3.06 (0.00-19.50)	0.59 (0.00-3.54)	0.15-1.35	p<0.0001
Eosinophil count ($\times 10^9/l$)	0.01 (0.00-19.40)	0.36 (0.00-3.40)	0.10-1.25	p<0.0001

Basophil count ($\times 10^9/l$)	0.00 (0.00-0.70)	0.00 (0.00-2.74)	0.00-0.10	P=0.0019
Haematocrit (%)	26 (9-56)	41 (11-64)	37-55	p<0.0001
Platelet count ($\times 10^9/l$)	154 (2-1075)	318 (2-1034)	200-500	p<0.0001

Table 4.1

Haematological and serum protein data for LCG and CG. WBC: white cell count. These data exclude the dogs with leukemia.

Haematological and serum protein data are presented in Table 4.1. Segmented neutrophil count, immature neutrophil count, lymphocyte count and monocyte count were significantly higher and eosinophil count, platelet count, serum protein and albumin concentrations and haematocrit were significantly lower in LCG than in CG. Three of the dogs in LCG had lymphocytic leukemia and even when they were excluded, lymphocyte counts were still significantly higher in LCG than in CG.

4.3. THE EFFECT OF GLUCOCORTICOIDS

The results of the comparison of dogs that had and had not received glucocorticoid treatment are presented in Table 4.2.

CELL ($\times 10^9/l$)	LCG		CG		p	Reference range
	Median (range)	Median (range)	Median (range)	Median (range)		
	Gluco- corticoids	No gluco- corticoids	Gluco- corticoids	No gluco- corticoids		
WBC	48.4 (36.1-148)	45.3 (35.0-182.0)	11.8 (4.5-15.4)	11.8 (4.3-29.9)	LCG: p=0.48 CG: p=0.48	6.0-15.0
SN	36.3 (13.4-76.6)	34.5 (7.4-63.1)	9.0 (3.3-12.7)	8.4 (3.2-29.0)	LCG: p=0.87 CG: p=0.66	3.0-11.5
IN	4.8 (0.0-39.3)	4.01 (0.0-41.9)	0.01 (0.0-0.3)	0.06 (0.0-0.03)	LCG: p=0.39 CG: p=0.76	0.0-0.5
L	1.8 (0-32.9)	2.2 (0-20.8)	1.52 (0.01-5.0)	1.52 (0.1-4.8)	LCG: p=0.38 CG: p=0.95	1.0-4.8
M	3.6 (0.0-15.5)	2.9 0.0-13.5	0.01 (0.01-1.6)	0.6 (0-3.6)	LCG: p=0.44 CG: p=0.95	0.15-1.35
E	0.0 (0.0-1.7)	0.02 (0.0-19.4)	0.01 (0.05-1.55)	0.37 (0.0-3.4)	LCG: p=0.41 CG: p=0.0003	0.10-1.25

Table 4.2

Effect of glucocorticoid treatment on haematological parameters for LCG and CG . WBC:

white cell count; SN: segmented neutrophil count; IN: immature neutrophil count; L:

lymphocyte count; M: monocyte count; EC: eosinophil count.

There was no difference in WBC, segmented or immature neutrophil count, lymphocyte or monocyte count between dogs that were treated with glucocorticoids and those that were not, but the eosinophil count was significantly higher in CG than in LCG (p=0.0003).

4.4. DIFFERENCES IN THE DIFFERENTIAL COUNT BETWEEN DISEASE GROUPS IN DOGS WITH HIGH LEUKOCYTE COUNTS

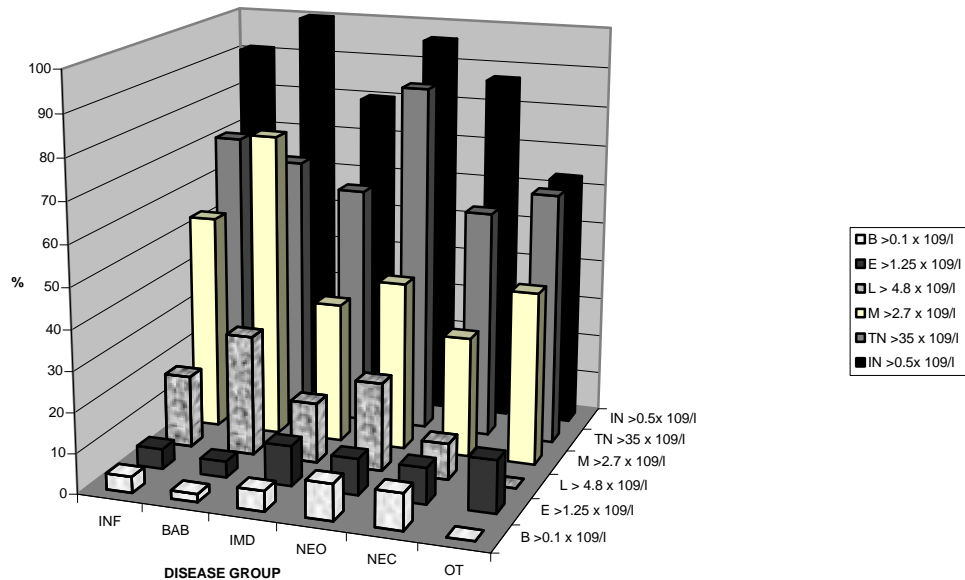


Figure 4.5

Percentage of dogs in LCG with immature neutrophils $>0.5 \times 10^9/l$, total neutrophils $>35 \times 10^9/l$, monocytes $>2.7 \times 10^9/l$, lymphocytes $>4.8 \times 10^9/l$, eosinophils $>1.25 \times 10^9/l$ and basophils $>0.1 \times 10^9/l$ falling into each disease group. INF infection; BAB babesiosis; IMD immune mediated disease; NEO neoplasia; NEC necrosis; OT other.

Ninety four dogs had total neutrophil counts greater than $35 \times 10^9/l$ (Figure 4.5). The highest WBC, total neutrophil count and monocyte count was in a case of systemic mycosis. Apart from this case, the dogs with the 20 highest total neutrophil counts included 3 cases of parvoviral disease, 6 cases of complicated babesiosis, 1 of uncomplicated babesiosis, 1 of immune-mediated haemolytic anemia, 1 case of pyometra, 1 of pyothorax, 1 of peritonitis, 1 with intestinal foreign body and four of neoplasia (two haemangiosarcoma, 1 adenocarcinoma and 1 lymphoma). A hundred and forty one dogs had monocyte counts greater than $1.35 \times 10^9/l$, which is the upper reference limit. Ninety-seven dogs had monocyte counts greater than $2.7 \times 10^9/l$ (Figure 4.5), which is twice the upper limit of the reference range. The highest monocyte

count was in the case of systemic mycosis, which also had the highest total neutrophil count. Thirty-five dogs had lymphocyte counts greater than the upper limit of reference range ($4.8 \times 10^9/l$) (Figure 4.5). Three of these had lymphocytic leukaemia. One had acute lymphocytic leukaemia (WCC $182 \times 10^9/l$, lymphocyte count $170.0 \times 10^9/l$), one had chronic lymphocytic leukaemia (WCC $148 \times 10^9/l$, lymphocyte count $121.36 \times 10^9/l$) and one had leukaemic lymphoma (WCC $135 \times 10^9/l$, lymphocyte count $133.65 \times 10^9/l$). Two of the non-leukaemic cases with the highest lymphocyte counts (20.79 and $10.88 \times 10^9/l$) had uncomplicated babesiosis. They also had high globulin concentrations (80 and 51 g/l, respectively). Two dogs in CG also lymphocyte counts in this range. One had hypoadrenocorticism and one had a gastrointestinal foreign body. Twelve dogs had eosinophil counts greater than $1.25 \times 10^9/l$, the upper limit of the reference range (Figure 4.5).

To further explore the differences in leukocyte counts between complicated and uncomplicated babesiosis, LCG and CG were combined and WBC, neutrophil, band neutrophil, lymphocyte and monocyte counts were compared between complicated and uncomplicated babesiosis cases. WBC (complicated babesiosis (n=39); median $51.8 \times 10^9/l$ ($12.3-96.3 \times 10^9/l$) vs uncomplicated babesiosis (n=21); median $36.1 \times 10^9/l$ ($8.2-74.50 \times 10^9/l$) (p=0.001), total neutrophil count (complicated babesiosis (n=39); median $43.1 \times 10^9/l$ ($5.9-89.6 \times 10^9/l$) vs uncomplicated babesiosis (n=21); median $27.5 \times 10^9/l$ ($6.2-64.4 \times 10^9/l$) (p=0.001), band neutrophil count (complicated babesiosis (n=39); median $6.4 \times 10^9/l$ ($0.1-39.3 \times 10^9/l$) vs uncomplicated babesiosis (n=21); median $4.08 \times 10^9/l$ ($0.0-17.1 \times 10^9/l$) (p=0.01) and monocyte count (complicated babesiosis (n=39); median $5.43 \times 10^9/l$ ($0.02-15.5 \times 10^9/l$) vs uncomplicated babesiosis (n=21); median $1.88 \times 10^9/l$ ($0.2-9.9 \times 10^9/l$) (p=0.01) were significantly higher in complicated babesiosis cases than in uncomplicated babesiosis cases. There were no significant differences in lymphocyte counts (complicated babesiosis (n=39); median $3.6 \times 10^9/l$ (0.4 to $9.7 \times 10^9/l$) vs uncomplicated babesiosis (n=21); median $2.93 \times 10^9/l$ (0.1 to $20.8 \times 10^9/l$) (p=0.7)

4.5. INVESTIGATION OF FACTORS INFLUENCING PROGNOSIS AND SURVIVAL

Of the animals that died or were euthanased, 51% had a platelet count below $150 \times 10^9/l$.

Serum protein concentrations were only available for 102 CG and 108 LCG dogs. Dogs in LCG had significantly lower serum total protein ($p < 0.04$) and albumin ($p < 0.0001$) than did dogs in CG. Dogs with a serum albumin concentration less than 25 g/l, platelet count less than $200 \times 10^9/l$ and without hypoglobulinaemia (serum globulin > 20 g/l) were investigated. Thirty-seven fell into LCG and 7 into CG (Figure 4.6).

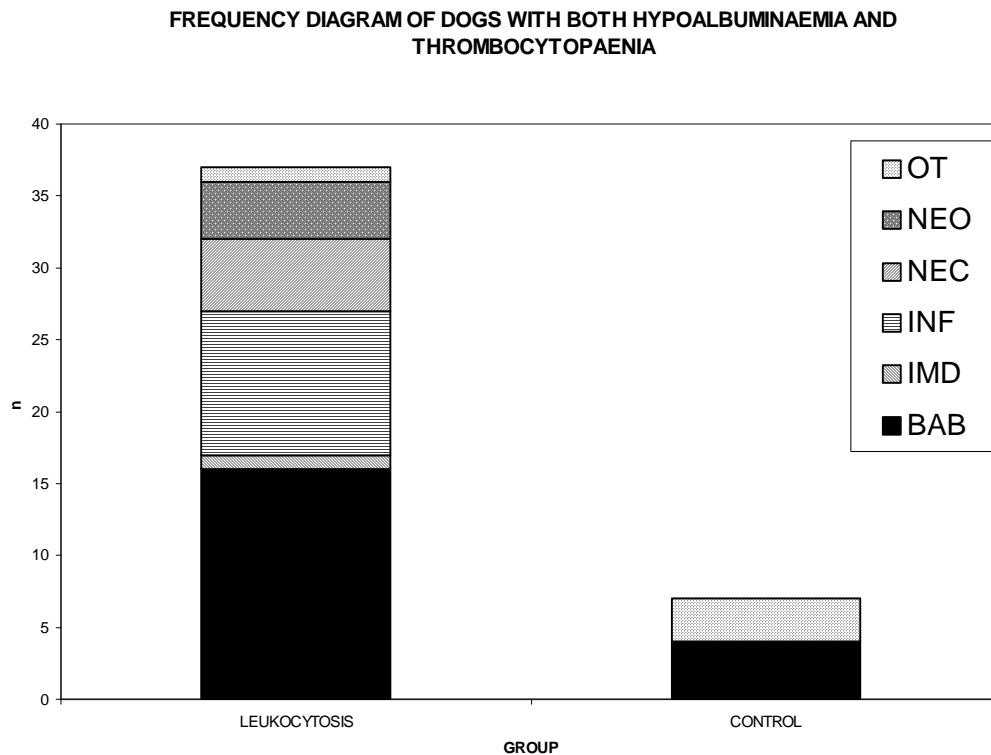


Figure 4.6

Frequency diagram for dogs with serum albumin concentration < 25 g/l, globulin concentration > 20 g/l and platelet count $< 200 \times 10^9/l$. There was a significant difference between groups ($p < 0.0001$) Legend: OT: Other; NEO: Neoplasia; NEC: Necrosis; INF: Infection; IMD: immune mediated Disease; BAB: Babesiosis.

Of these, 16 fell into Babesiosis and 10 into Infection in LCG and 4 into Babesiosis in CG. Of the dogs with babesiosis, hypoalbuminaemia and thrombocytopenia in LCG, 11 had complicated babesiosis. Twenty-eight of these 37 dogs with hypoalbuminaemia and thrombocytopenia had total neutrophil counts greater than or equal to $35 \times 10^9/l$. Of these, 12 fell into Babesiosis (of which 10 had complicated babesiosis), 8 into Infection, 3 each into Neoplasia and Necrosis and 1 each into Immune-mediated Disease and Other diseases. Of the 37 dogs in LCG, there were 16 non-survivors and 21 survivors. Cox regression showed no relationship between dogs having both thrombocytopenia and hypoalbuminaemia and mortality.

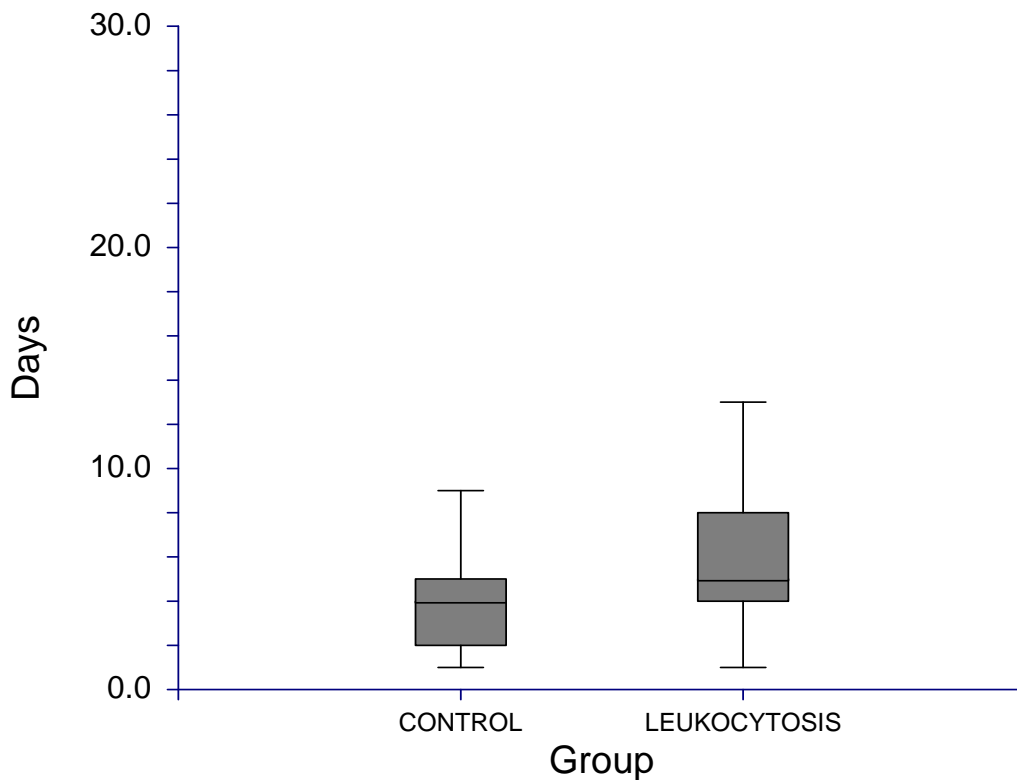


Figure 4.7

Box and whisker plot of time of hospitalisation for groups LCG (Leukocytosis) and CG (Control). Box = interquartile range (IRQ); whiskers = Upper: the largest observation that is less than or equal to the 75th percentile plus 1.5 times IQR. Lower: the smallest observation that is greater than or equal to the 25th percentile minus 1.5 times IQR; line in box = median

Comparisons of time of hospitalization are shown in Figure 4.7 and were significantly shorter for CG than for LCG ($p < 0.0001$). Thirty-five dogs died in hospital in LCG compared with 18 in CG ($p=0.01$), but there was no difference in survival between groups when the results were subjected to survival analysis. When dogs with extreme leukocytosis ($WCC > 50 \times 10^9/l$) were compared with controls, there was still no difference in survival. Survival data were analysed for total and band neutrophil count. There was no significant relationship between the immature neutrophil count and mortality. However, on Cox regression analysis, there was a significant relationship between survival and total neutrophil combined with immature neutrophil count ($p=0.01$).

CHAPTER 5 DISCUSSION

If cases with babesiosis are included, then 106 out of 182 cases (58%) had infectious disease in LCG (Figure 4.1). This is higher than in the study of Lucroy and Madewell (1999)⁵⁷. Previous studies have shown that most infections associated with leukocytosis are bacterial^{20,33,58}. In this study, 23% of cases in Infection in LCG were associated with bacterial or fungal infection. It is interesting that 7 (13%) of cases in Infection in LCG had parvoviral disease, and three of these were among the 20 highest neutrophil counts. Parvoviral enteritis is usually associated with severe granulocytopenia^{17,29,83}. On examination of the records, these animals had in fact been through a granulocytopenic stage of the disease and then developed rebound leukocytosis during the recovery stage of the viral infection. This has been previously described⁵².

The frequency of neoplasia in LCG and CG (Figure 4.1) was similar to the frequency in Lucroy and Madewell's study (1999)⁵⁷ and not significantly different between groups. The absence of a significant difference between groups does not alter the fact that many neoplastic conditions have been associated with severe leukocytosis or a leukemoid response⁶². In humans, it has been associated with, among others, renal transitional cell carcinoma⁷⁶, bladder transitional cell carcinoma¹, ovarian carcinoma¹⁶, metastatic carcinoma⁷⁰. In dogs, it has been described in renal tubular carcinoma^{51,59}, renal transitional cell carcinoma⁷⁷ and metastatic fibrosarcoma¹⁵.

These are similar to the tumour types found in the Neoplasia group in LCG. This may partly be due to the well-known phenomenon of tumours outgrowing their blood supply and undergoing necrosis, but direct induction of a leukemoid response to some tumours has been shown by implanting hepatoma, mammary carcinoma and rhabdomyosarcoma cell lines into mice¹². Production of colony-stimulating factors by tumours has been demonstrated in humans^{6,100} and dogs^{51,77}.

The number of cases of immune-mediated disease in LCG was lower than that found in Lucroy and Madewell's study(1999)⁵⁷. This is probably because many of the cases of immune-mediated disease were included in the complicated babesiosis group. Complicated babesiosis is regarded as babesiosis that does not resolve after parasitocidal treatment for the condition alone^{37,55}. Known complications include in saline agglutination (ISA) positive immune mediated haemolytic anemia³⁶, thrombocytopenia⁴⁸ (most likely immune mediated), and Acute Respiratory Distress Syndrome³⁶, all of which were represented in the dogs in this study. Complicated babesiosis has also been associated with systemic inflammatory response syndrome and multiple organ dysfunction syndrome¹⁰⁷ and has been likened to human malaria in that the pathophysiology and clinical presentation of the diseases appears to be similar³⁷. Moderate to marked leukocytosis associated with immune mediated haemolytic anemia was shown by McManus and Craig⁶³ to be associated with moderate to severe post-mortem lesions in dogs. Ischaemic necrosis within liver, kidney, heart, lung, and spleen attributable to thromboembolic disease or anaemic hypoxia were the most common important lesions found at necropsy. Complicated babesiosis is also associated with hypoxic organ damage⁵⁶ and thromboembolism⁶⁷ and these may thus contribute to the marked leukocytosis in this disease. The findings of McManus and Craig⁶³ probably also explain why higher leukocyte counts were found with immune-mediated haematological disease than other types of immune-mediated disease, for example autoimmune skin diseases.

A common condition in dogs associated with tissue necrosis and leukocytosis is pancreatitis^{34,57} and 5 cases (22%) in LCG associated with necrosis had pancreatitis. Snake bites from the cytotoxic puff adder (*Bitis arietans*) accounted for 3 (13%) cases with necrosis In LCG, but there

was also one case in CG which did not develop leukocytosis. Three dogs in NEC and LCG had toxic hepatopathy, and these were cases of aflatoxicosis. The pathophysiology of the leukocytosis in these cases could possibly be similar to that of the extreme leukocytosis associated with alcoholic hepatitis in humans⁶⁸.

Ninety-four (53%) dogs in LCG had total neutrophil counts greater than $35 \times 10^9/l$. It has been previously shown that most cases of leukocytosis are associated with neutrophilia and monocytosis⁹⁶ and the dog with the highest neutrophil count also had the highest monocyte count. This was a case of systemic mycosis. Approximately one third of the dogs with total neutrophil counts greater than $35 \times 10^9/l$ fell into the Infection group and another third into Babesiosis (Figure 4.5). Babesiosis also featured prominently as a cause of severe monocytosis, since 70% of dogs with babesiosis had monocyte counts greater than twice the upper limit of the reference range. That is not surprising since a prominent feature of canine babesiosis is activated monocytes phagocytosing parasitised erythrocytes in circulation⁵⁴. An interesting finding was that all dogs with babesiosis had immature neutrophil counts greater than $0.5 \times 10^9/l$. A recent study⁸⁹ on the haematological kinetics of canine babesiosis has shown that the majority of canine babesiosis cases have a degenerative left shift, defined in that study as an increased immature neutrophil count with normal or low mature neutrophil count. Over 90% of dogs with neoplasia and infections also had immature neutrophil counts above the upper limit of the reference range (Figure 4.5).

When dogs in leukocytosis and control groups were combined and grouped into complicated and uncomplicated babesiosis groups, the WCC ($p=0.001$), total neutrophil count ($p=0.001$), band neutrophil ($p=0.01$) count and monocyte count ($p=0.01$) were significantly higher in dogs with complicated babesiosis than with uncomplicated babesiosis. There was no significant difference between these groups in their lymphocyte counts. However, there was too much overlap between groups to be able to use any of these individual parameters as a definitive diagnostic marker for complicated babesiosis.

Thirty percent of dogs with babesiosis in the LCG group had lymphocyte counts above the upper limit of the reference range (Figure 4.5). Two of the cases with the highest lymphocyte counts had uncomplicated babesiosis. They also had high globulin concentrations. However, there were also cases of complicated babesiosis that had lymphocyte counts within the reference range but globulin concentrations above 50 g/l. High lymphocyte counts were also present in 22 % of dogs with neoplasia (Figure 4.5), but this did include the 3 dogs with leukaemia.

There was no significant difference in the WBC count between dogs within CG that received glucocorticoid treatment and those that did not, and the only difference within LCG was in the eosinophil count (Table 4.2). This was an unexpected finding as it is generally accepted that a large contribution to the leukocytosis seen in animals that undergo glucocorticoid treatment is due to the effect of the glucocorticoids themselves. Glucocorticoids result in mobilization of granulocytes from the marginated pool to the circulating pool, decrease migration of neutrophils out of circulation into tissues as well as mobilization of mature granulocytes from the bone marrow. The magnitude of increase is usually less than twice the upper limit of the reference range and not more than three times the upper limit of the reference range⁹⁶. The reason that the cut off of $35 \times 10^9/l$ for the white cell count was chosen for LCG was to ensure that the animals in this group had a true inflammatory leukogram and to exclude most of those with a stress leukogram only. However, it would be expected that if they did not have a stress leukogram and that the leukocytosis was purely due to inflammation that glucocorticoid treatment would cause a further increase in the leukocyte count consisting of mature neutrophils and monocytes. The most likely explanation for the lack of difference was that all the animals were hospitalized and severely ill and as such had significant disease and so most probably already had their disease leukogram superimposed on a stress leukogram. The fact that there was no effect in CG may also be due to the fact that they were hospitalized, but the statistical analysis may have been biased in that group since only 11 dogs received glucocorticoid treatment.

There was a significant difference in serum albumin concentrations between the LCG and CG groups. (Table 4.1) Of the animals that died or were euthanased, 35% had serum albumin concentrations below 20g/l. Many factors can affect albumin concentrations¹¹⁰. The decrease in albumin concentration in cases of sepsis has been ascribed to increased vascular permeability and a shift of hepatic synthetic pathways toward acute phase proteins³¹. Hypoalbuminaemia may also be due to loss (renal, gastrointestinal or blood loss) or decreased production (hepatic insufficiency) or reduced absorption. The low albumin concentrations in LCG were accompanied in some cases by hypoglobulinaemia, suggesting protein loss. However, most cases had concurrent hyperglobulinaemia suggesting that albumin was acting in those cases as a negative acute phase protein. Hypoalbuminaemia has been previously described in conditions in which there is marked leukocytosis^{11,20,34}.

Thrombocytopenia has been previously described associated with a leukemoid response and may be associated with disseminated intravascular coagulopathy^{11,20,33,63} or be a paraneoplastic syndrome⁷⁷. The lower platelet counts in LCG could also be partially explained by the fact that some dogs had immune mediated thrombocytopenia, but this was a small percentage of the group. A more significant contribution would be the fact that 27% of dogs in LCG were infected with *Babesia rossi*. Infection with this parasite is almost always associated with thrombocytopenia⁴⁴. Of the animals that died or were euthanased, 51% had a platelet count below $150 \times 10^9/l$. There were significantly more dogs with both thrombocytopenia and hypoalbuminaemia in LCG than in CG (Figure 4.6), but that did not appear to influence survival. This may be because the majority of animals in these groups had infections, including babesiosis, and therefore recovered with intensive treatment and metabolic support. Considering the number of cases of immune-mediated haemolytic anemia and complicated babesiosis, both of which are associated with anemia in LCG, the lower median HCT in this group compared with CG was to be expected. Other conditions which could have contributed to the lower median HCT of this group are uncomplicated babesiosis, gastrointestinal blood loss, tumour haemorrhage, paraneoplastic anemia^{15,51,77} and anemia associated with inflammation^{38,109}.

The longer time of hospitalization in LCG compared with CG (Figure 4.7) was in agreement with the findings of Lucroy and Madewell⁵⁷ and indicates that it is likely to cost more to treat a dog with any significant leukocytosis. However, in contrast with their study, there was no difference in mortality rate. The reason for this may be that there were equal numbers of malignant neoplasia in both groups and that most of the other causes of severe leukocytosis were treatable conditions. An additional factor may have been the choice of cutoff for LCG. Catecholamines or glucocorticoids can result in doubling of the neutrophil count due to mobilisation of the marginated pool⁹⁶. If one allows for doubling of the neutrophil and monocyte counts (upper reference intervals for our laboratory $11.9 \times 10^9/l$ and $1.3 \times 10^9/l$, respectively) and doubling of the lymphocyte count (upper reference interval $4.8 \times 10^9/l$) due to physiological lymphocytosis), a cutoff of $35 \times 10^9/l$ is appropriate. Glucocorticoids can, however, result in a three-fold increase in neutrophil count⁹⁶ but this is accompanied by lymphopaenia and a twofold increase in the monocyte count. The maximum possible leukocyte count in this case is likely to be approximately $40 \times 10^9/l$ so some of these cases could be included in LCG. However, the magnitude of the total neutrophil count taken together with the degree of left shift does have a significant impact on prognosis ($p=0.01$).

CHAPTER 6 CONCLUSIONS

It can be concluded from this study that the presence of significant neutrophilia with a severe left shift is more likely to indicate serious disease with prolonged hospitalisation times. In this population of dogs from Pretoria, South Africa, the most likely disease categories in a dog with a leukocyte count $>35 \times 10^9/l$ were bacterial or fungal infections, immune-mediated haematological disease, complicated babesiosis or necrosis. Neoplasia should also always be considered in any dog with severe leukocytosis. The presence of both hypoalbuminaemia and thrombocytopaenia in an animal with significant leukocytosis is not of prognostic significance. However, the magnitude of the total neutrophil count taken together with the degree of left shift does have prognostic significance. An unexpected finding was that in hospitalised animals with severe leukocytosis, glucocorticoid treatment is not likely to increase the leukocyte count any further.

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