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A study of the prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis

by

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We know that half of what we teach will be proved false in ten years; the hard part is that we do not know which half.

- Wise Medical Pedagogue

University of Pretoria etd – Goddard, A (2006)

TABLE OF CONTENTS

University of Pretoria etd – Goddard, A (2006)

University of Pretoria etd – Goddard, A (2006)

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University of Pretoria etd – Goddard, A (2006)

LIST OF FIGURES

University of Pretoria etd – Goddard, A (2006)

University of Pretoria etd – Goddard, A (2006)

LIST OF TABLES

University of Pretoria etd – Goddard, A (2006)

University of Pretoria etd – Goddard, A (2006)

LIST OF ABBREVIATIONS

ANCOVA	Analysis of Covariance
Bands	Band (immature) neutrophil
Baso	Basophil
CBC	Complete Blood Count
CDV	Canine distemper virus
CNS	Central nervous system
CPV	Canine parvovirus
DIC	Disseminated intravascular coagulation
EDTA	Ethylenediamene tetra-acetic acid (anti-coagulant)
EM	Electron microscopy
Eos	Eosinophil
FBC	Full Blood Count
FPV	Feline panleukopaenia virus
Hb	Haemoglobin
H&E	Haematoxylin & Eosin staining
Ht	Haematocrit
Lymph	Lymphocyte
MBC	Mean basal cortisol
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean cell volume
Mono	Monocyte
MPS	Mononuclear phagocytic system
Neut	Segmented (mature) neutrophil
OVAH	Onderstepoort Veterinary Academic Hospital
PCV	Packed cell volume
PLT	Platelet count
PM	Post mortem
RBC	Total red blood cell count
RCC	Red cell count
RDW	Red cell distribution width
WBC	Total white blood cell count

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SUMMARY

A study of the prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis

Goddard, A. University of Pretoria, 2006

Canine parvoviral enteritis is an economically important disease in South Africa and globally. Although treatment of dogs with parvoviral enteritis is often successful, many dogs die of complications related to septicaemia or are euthanized because of anticipated high costs. More effective prediction of the outcome of this disease will have an economic impact if a prognosis can be determined early in the course of the disease. Although leukocyte responses seldom are pathognomonic for a specific disease, they can provide clinical information to establish a fairly reliable prognosis.

A prospective study was performed on 62 puppies presented to the OVAH with typical clinical signs of canine parvoviral enteritis that subsequently was confirmed on electron microscopy. Full haematology was performed at admission as well as every consecutive day until death or discharge. Of the 11 puppies that died (18%), nine died due to complications of the disease and two were euthanized due to financial restrictions and a poor prognosis. The puppies that died due to the disease died within the first three days of hospitalization. All the puppies that died were sent for a full post mortem examination and histopathological evaluation.

Statistical analysis of the data showed that there was a definite difference between the puppies that died and those that survived in several of the leukocyte parameters. These parameters included the total leukocyte, lymphocyte, monocyte and eosinophil counts. In none of the puppies that died from the disease did the total leukocyte count rise above 2.0×10^9/l (normal reference range: $6.0\text{-}15.0 \times 10^9$/l). In the puppies that survived, the total leukocyte count started rising within 24 – 48 hours after admission and often resulted in a rebound leukocytosis. The puppies that died did not develop lymphocytosis to indicate an immune response, whereas the surviving puppies developed lymphocytosis within 24 – 48 hours after admission. The puppies that died also did not develop monocytosis and remained severely eosinopaenic during the course

of the disease. Evidence of impaired leukocyte production was found on histopathology. Most of the puppies that died from the disease showed marked to severe thymic and lymphoid atrophy and marked to severe bone marrow hypocellularity.

These results show that a reliable prognosis can be obtained at 24 and 48 hours after admission by evaluation of the leukocytes, specifically the total leukocyte, lymphocyte, monocyte and eosinophil counts.

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CHAPTER 1 LITERATURE REVIEW

1.1 CANINE PARVOVIRAL INFECTION

Proviruses (Parvoviridae) are small, non-enveloped viruses. They replicate only in cells synthesizing DNA and show a tropism for rapidly dividing cells (i.e. cells like enterocytes, precursor cells in the bone marrow, and myocardiocytes) and use the cell's machinery to produce viral rather than cellular proteins. This results in cell death and loss due to the failure of mitosis.[1] Parvoviral enteritis is a common infectious disease primarily affecting puppies between 6 weeks and 6 months of age.[1-3] Susceptibility of puppies to viral infection increases as the maternally-acquired antibody titre declines to non-protective levels. Inadequate immunization to parvovirus during the first year of life is an additional risk factor for disease.[1] In susceptible canine populations, parvovirus infection most often presents as a severe systemic and even life-threatening illness.[1] It is associated with a survival rate as low as 9.1% in the absence of treatment, and 64% with treatment.[2] During a 6 year period (1999-2004) an annual average of 522 dogs with clinical signs of parvovirus enteritis were hospitalized at the Onderstepoort Veterinary Academic Hospital (OVAH), Pretoria, South Africa (not all of these were however confirmed as being positive for parvovirus on electron microscopy). Of these cases, 444 (85%) survived and 78 (15%) died.

Canine parvovirus (CPV) (type 2a and 2b) has a predilection to infect rapidly dividing cells of the gastrointestinal tract, lymphoid tissue, and bone marrow leading to bloody diarrhoea, vomiting, profound leukopaenia, and immunosuppression.[2-6] The CPV-2 variant is believed to have emerged from the virus causing feline panleukopaenia, which also is a parvovirus. Neutropaenia and lymphopaenia occur in feline panleukopaenia as a result of haematopoietic precursor cell destruction.[7-8] Ten percent of parvovirus isolates from cats with naturally occurring disease are antigenically identical to CPV-2a or –2b.[1]

1.2 PATHOGENESIS OF CANINE PARVOVIRUS

Factors that predispose to parvovirus infection in puppies are lack of protective immunity[1], internal parasites, and overcrowded, unsanitary and stressful environmental conditions.[1,3,9] CPV-2 spreads rapidly among dogs via faecal-oral route (direct transmission) or oro-nasal exposure to fomites contaminated by faeces (indirect transmission).[1,3,10] Virus can be excreted for a maximum period of 3 to 4 weeks after clinical or subclinical disease.[10] Virus replication begins in the lymphoid tissue of the oropharynx, mesenteric lymph nodes, and thymus and is disseminated to the intestinal crypts of the small intestine by haematogenous spread (3 to 4 days after infection).[1,3,6,11] Marked plasma viraemia is observed 1 to 5 days after infection. Subsequent to the viraemia, CPV-2 localizes predominantly in the epithelium lining the tongue, oral cavity, and oesophagus; the small intestine; bone marrow; and lymphoid tissue, such as thymus and lymph nodes.[3] Parvoviral infection is a systemic disease, although it usually manifests as an enteric infection. The virus may also be isolated from the lungs, spleen, liver, kidneys, and myocardium.[1,3,11]

The rate of lymphoid and intestinal cell turnover appears to be the main factor determining the severity of the disease – higher rates of turnover are directly correlated with virus replication and cell destruction. Stress factors, in particular parasitic and non-specific (e.g. weaning) factors, may predispose dogs to clinical disease by increasing mucosal cell activity.[1,5,6,10,12] It has been hypothesized that co-pathogenic factors (stress, internal parasites, concurrent infections) may play an important role in the clinical expression of disease in both feline panleukopaenia virus (FPV) and canine parvovirus (CPV).[5,13]

In neonates, the cells infected are those in the bone marrow, lymphoid tissue, intestinal epithelium, and myocardium.[3] Normally, intestinal crypt epithelial cells mature in the small intestine and migrate from the germinal epithelium of the intestinal crypts to the tips of the villi. Upon reaching the villous tips, the intestinal epithelial cells acquire their absorptive capability and aid in assimilating nutrients. Parvovirus infects the germinal epithelium of the intestinal crypts, causing epithelial destruction and collapse. As a result, normal cell turnover (usually one to three days in the small intestine) is impaired, leading to the characteristic pathologic lesion of

shortened and atrophic villi.[1,3,6,14] During this period of villous atrophy the small intestine loses its absorptive capacity. The changes in the thymus are dramatic. The lesions are usually most obvious in the germinal centres and the thymic cortex, reflecting the tropism of CPV for mitotically active cell populations. The extensive lymphocytolysis in the thymic cortex, compared to other lymphoid tissues, further reflects the high mitotic rate found in this organ, and it is thus not surprising that infected dogs develop lymphopaenia.[1,15]

1.3 LEUKOCYTE KINETICS

Because blood leukocyte numbers and morphology are relatively stable in health, leukocyte responses can be useful clinically as they may change dramatically in disease. Although leukocyte responses are seldom pathognomonic for a specific disease, they can provide clinical information to establish a list of differential diagnoses, to assess the patient's response to treatment, or to suggest a prognosis.

All blood cells (leukocytes, erythrocytes, and platelets) are derived from pluripotential stem cells that are present in the bone marrow and blood, from which individual cell lines develop independently.[16] Because neutrophils are the most numerous leukocytes in dog and cat blood, a change in the neutrophil count will usually result in a change in the total leukocyte count.[16]

Neutrophils are produced in the bone marrow and can be divided into two compartments. The proliferation (mitotic) compartment, which includes immature neutrophils (myeloblasts, promyelocytes, myelocytes) that are capable of cell division, and the maturation and storage (post-mitotic) compartment, which includes neutrophil metamyelocytes, bands, and segmenters that are stored for a variable period of time while the cells undergo maturation. Mature neutrophils are released into the blood from the latter compartment, in an age-ordered fashion.[4,16-18] The entire generative process takes approximately 6 days.[4,16,18] A stable equilibrium exists between bone marrow neutrophil production and peripheral tissue use.[4,17] If tissue demand for neutrophils intensifies, the bone marrow storage pool of mature segmented cells becomes depleted, leading to less mature forms and more immature

forms appearing in circulation, constituting what is termed a left shift.[4,17,18] The functions of neutrophils include ingestion and killing of bacteria, as well as damaging fungi, yeast, algae, parasites and viruses; and induction of antibody-dependent cellular cytotoxicity to destroy infected or transformed cells.[16,17]

Neutrophils are distributed in one of two dynamic sub-pools in the blood: (1) the circulating pool consisting of cells in the mainstream of circulation, usually sampled by venipuncture, and (2) the marginal pool consisting of cells that move slowly along the endothelial surface of small capillaries and venules because of reduced blood flow and adhesion molecules on neutrophils and endothelial cells. The distribution of neutrophils between the circulating and marginal pools is approximately 1:1 in dogs.[4,16-18] The life span in circulating of neutrophils is approximately 7½ to 10 hours in the blood before they marginate and emigrate into the tissue in a random (non-age-ordered) and unidirectional fashion. Once in the tissue neutrophils are viable for 1 to 4 days, when they subsequently undergo apoptosis and are phagocytized by macrophages.[4,16-19] Once recruited to sites of inflammation and having exerted their activity, neutrophils die by programmed cell death or apoptosis.[17] All blood neutrophils are replaced about 2½ times each day.[18]

One or a combination of the following three variables can influence the neutrophil count: (1) the release rate of neutrophils from the marrow into the blood, (2) the cell distribution between the circulating and marginal neutrophil pools, and (3) the rate of neutrophil emigration from the blood into the tissue.[16,17,19] Increased rate of release from the storage compartment is the reason for the rapid increase in the neutrophil count (earlier than 2 days) that follows the initial tissue demand.[18]
The three most common mechanisms of a neutrophilia (increased peripheral blood neutrophil count) are a physiological response following fear, excitement, or strenuous exercise (pseudoneutrophilia mediated by epinephrine); corticosteroid-induced neutrophilia (endogenous release or exogenous administration); and with established inflammation or infection which may be accompanied by a left shift and/or toxic changes. The magnitude of the left shift is considered a direct indication of the severity of the disease. Localized purulent diseases such as pyometra or abscessation stimulate greater neutrophilic responses than generalized infections or septicaemias.[16]

Neutrophil toxicity, where the toxic neutrophils have abnormalities in cell size, nuclear shape and consistency, and cytoplasmic content, has been associated with systemic rather than localized processes. This phenomenon has been traditionally linked with bacterial infections, bacteraemia, abscesses and septicaemia, severe inflammatory processes, myeloproliferative disorders, and drug toxicity. Toxic changes occur in neutrophils during their development in the bone marrow, and are divided into 3 categories: nuclear, cytoplasmic, and giant neutrophils. The most common and most important changes seen are the cytoplasmic ones. These changes include diffuse basophilia, granulation, foamy vacuolation, and the presence of Döhle bodies.[20]

The most common causes for a neutropaenia are deficient neutrophil production in the bone marrow, a shift in neutrophils from the circulating to the marginal pool, and emigration of neutrophils from the blood into the tissues at a rate that exceeds neutrophil replacement into the blood from the bone marrow.[4,16-18,21] Viral infections, i.e. feline leukaemia virus, feline immunodeficiency virus, and parvoviral infections, commonly cause neutropaenia in many different species.[21] During gram-negative bacterial infections neutrophils are shunted from the circulating and bone marrow pools to the marginal pool, frequently causing severe neutropaenia. This effect is believed to be mediated through endotoxin.[18,21]

Lymphocytes are the second most common blood leukocytes in healthy dogs and are essential components of humoral and cell-mediated immune responses. Lymphopoiesis is ultimately dependent on pluripotential stem cells in the bone marrow, but in the adult the majority of lymphocytes arise from the peripheral lymphoid tissues (excluding the thymus and bone marrow).[16,19] In immature animals certain T-lymphocyte precursors migrate to the thymus, the central lymphoid organ, where they are educated and selected for self-tolerance.[22] T-lymphocytes are involved in cell-mediated immunity by modulating the activity of other cells; B-lymphocytes are involved in humoral immunity by producing antibodies. Blood lymphocytes are unique in that they are able to recirculate from lymphoid tissues, allowing lymphocytes an increased opportunity to perform immune surveillance.[16] Blood lymphocytes are mainly long-lived (months to years) T-lymphocyte memory cells that retain the ability to undergo mitosis under appropriate stimulation. Their

numbers in the blood may be altered dramatically by disease, physiological states, and drug administration.[16,19]

Persistent antigenic exposure, secondary to chronic infectious or inflammatory diseases, may be the most common cause of lymphocytosis. Physiologic lymphocytosis is a more frequent finding in young animals. Corticosteroid-associated lymphopaenia is seen in severe stress or following administration of glucocorticoids. Lymphopaenia of acute infection involves more than one mechanism: (1) severe stress may cause a redistribution of lymphocytes secondary to endogenous corticosteroid release; (2) recirculating lymphocytes may be trapped temporarily in draining lymph nodes following antigen exposure to promote antigen contact; and in addition (3) pathogens, like parvovirus, may cause atrophy or direct destruction of lymphoid tissue.[4,16,19]

Monocytes and tissue macrophages comprise the mononuclear phagocyte system (MPS) present in virtually all tissues and serosal cavities.[18,23] Monocytes share a common parental cell with neutrophils in the bone marrow, but unlike neutrophils, no bone marrow maturation and storage pools of monocytes exist and these cells are released from the bone marrow soon after generation. Monocytes spend approximately 1 to 2½ days in the bone marrow before entering the blood.[4,18,23] Monocytes have a short circulating half-life (8½ hours) and randomly leave the blood to enter tissue and turn into macrophages. Macrophages do not return to the circulation and can survive for days to months in tissues.[4,16,23] It appears that resident tissue macrophages (i.e. Kupffer cells, alveolar macrophages etc.) are long lived, whereas macrophages responding to inflammatory stimuli are short lived.[23] Monocytes/macrophages function in the phagocytosis and digestion of cellular debris, micro-organisms and particulate matter; secretion of inflammatory mediators and antigen presentation to lymphocytes. [4,18,23]

Monocytosis is a common leukogram finding in acute and chronic diseases of dogs, and usually occurs concurrently with neutrophilia, but because of a large variation in monocyte counts, monocytopaenia is difficult to document and rarely appreciated in domestic animals.[4,16,18,23] Monocytopaenia is of lesser importance compared to life-threatening neutropaenia, and recovery of monocyte numbers precedes that of neutrophils in the blood. This is especially true in panleukopaenia secondary to

various conditions i.e. parvoviral infection, oestrogen toxicity, or chemotherapy.[19,23] In these circumstances, monocytopaenia followed by recovering monocyte counts or monocytosis heralds the return of neutrophil production. Monocytes are produced in 3 days, whereas neutrophils take 6 days to be produced from a common precursor cell. Thus, monitoring the monocyte count in the blood may be beneficial in evaluating leukopaenic and neutropaenic states.[4,23]

The production of eosinophils in the bone marrow is controlled by T-lymphocytes, and newly formed eosinophils are stored in the bone marrow in a fashion similar to neutrophils.[16] Eosinophils are tissue-dwelling cells with their primary functions being to kill parasites and modulate immune reactions, but inflammatory and/or hypersensitivity reactions involving the gastrointestinal tract may also produce eosinophilia in response to mast cell degranulation.[16,24] Corticosteroid-associated eosinopaenia is observed frequently in clinical practice, and eosinopaenia of acute infection has been attributed to endogenous release of corticosteroids, but this hypothesis has never been verified. The underlying mechanism is thought to be vascular sequestration of eosinophils, probably in response to chemotactic factors generated during acute inflammation.[16]

Postnatal development of leukocyte subsets differs significantly from one week to another. The development of the canine immune system is not completed at birth. During the first few days after birth there is a predominance of circulating neutrophils. This change within the first week of life to a transient predominance of lymphocytes, but the initial state, which is common to older dogs, is restored at the age of approximately 1 month. Neutrophil counts reach values comparable with those in adults at 1 month of age, whereas lymphocyte counts are higher than those in adults during the first 3 months. [25]

1.4 LEUKOCYTE KINETICS IN CANINE PARVOVIRUS

Extensive destruction of mitotically active lymphoblasts in the lymphatic tissues and of myeloblasts in the bone marrow by CPV-2 leads to panleukopaenia (specifically lymphopaenia and neutropaenia) during the first four days after infection.[3,6,10,14,16]

Panleukopaenia could also be the result of viral depletion of lymphoid and haematopoietic tissues as well as intestinal loss of blood cells.[5] It has been reported that even in normal dogs there is considerable loss of neutrophils into the intestinal lumen and that this loss may be greatly increased in enteric disease.[15,26] The development of panleukopaenia may be gradual, peaking at the height of clinical disease, or may occur suddenly in association with peak clinical signs.[10] Profound leukopaenia, seen in acute parvoviral enteritis, is usually at its most severe 5 to 8 days post infection.[16]

Animals with CPV will often demonstrate a total white blood cell count (WBC) below 2.0×10^9/l (Normal: $6.0 – 15.0 \times 10^9$/l) without a significant left shift.[10] WBC of less than 0.1×10^9/l have been recorded.[27] However, available information suggests that counts of 0.5×10^9/l through 2.0×10^9/l are more commonly observed at the peak of illness.[27] Although not found in all dogs, the leukopaenia associated with CPV is usually proportional to the severity of illness and the disease stage at the time of sampling.[3] The mechanism of neutropaenia in canine parvoviral enteritis is multifaceted and may be the result of (1) direct destruction of haematopoietic cells by the virus, such as cells of the neutrophil marrow proliferation pool, leading to neutropaenia that is most severe approximately 5 to 8 days post-infection; (2) excessive tissue demand and depletion of marrow neutrophil stores, frequently seen in septicaemia or localized bacterial infections that involve body cavities like the gastrointestinal tract; (3) a neutrophil shift from the circulating to the marginal pool in response to endotoxaemia, with shortened neutrophil life span in the blood, increased predilection for margination, and facilitated emigration into the tissues; and/or (4) ineffective granulopoiesis associated with increased phagocytosis of neutrophils by marrow macrophages.[4,16,17,19] It is documented that with supportive care, haematological recovery begins within 1 to 6 days, and that the rapidly rising leukocyte count is characterized by a rebound leukocytosis.[4,16]

Secondary bacterial infection from gram-negative and anaerobic microflora causes additional complications leading to intestinal damage, bacteraemia and endotoxaemia, and disseminated intravascular coagulation (DIC).[3] Monocytosis reduces the risk of infection considerably even though the bactericidal capabilities of monocytes are inferior to those of neutrophils.[4,16]

Potgieter et. al.[28] reported that experimentally infected dogs that remained healthy developed lymphopaenia only, and that recovery from infection was associated with transient lymphocytosis, a rapid antibody response and cessation of virus excretion. A study by Woods et. al.[7], on several confirmed cases of parvovirus enteritis, suggested that the leukogram could be helpful in evaluating the patients' prognosis. An elevated WBC was found in one patient that recovered quickly. Conversely, leukopaenia (WBC $< 4.5 \times 10^9$/l) was associated with cases that required more aggressive therapy.[7] This hypothesis, that leukopaenia was indicative of a poor prognosis, was confirmed in a study by Mason et al.[29] The data from this latter study suggested a significant relationship between WBC and lymphocyte counts and survival.

According to a study by Macartney et. al.[30], parvoviral antigen (using immunocytochemical examination) was not consistently present in the bone marrow of infected dogs. This result, together with the very minor destructive changes observed on conventional microscopic examination, suggested that the bone marrow was not a major site of viral replication. The hypothesis that neutropaenia, found in severely ill dogs, was the result of a net loss of neutrophils through the damaged intestinal mucosa rather than a primary failure of granulopoiesis, was also supported by this finding.[30] The study by Potgieter et. al.[28] contradicted these findings. They stated that CPV resulted in marked depletion of granulocytes in the bone marrow suggesting that neutrophil precursors constituted one of the targets of the virus. They also found that the erythroid elements were slightly affected, although anaemia was not observed, presumably because of the long life span of circulating mature erythrocytes. No effect was observed on the other cellular elements of the bone marrow. During convalescence there was hyperplasia of the granulocytic and erythroid elements of the bone marrow in an apparent effort to replenish cells lost during the acute phase of the disease. Potgieter et. al. also stated that the dramatic drop in circulating neutrophil numbers appeared to be the result of interrupted production in the bone marrow. Although transient, decreased production was considered important since the lifespan of neutrophils is very short and extensive tissue damage creates a great demand for neutrophils.[28] The first specific report on bone marrow alterations in CPV was by Boosinger et al.[31] who found alterations in the myeloid, erythroid, and megakaryocytic cell lines. The myeloid storage pool of mature neutrophils was severely depleted and there were signs of toxicity and

degeneration evident. The erythroid series was the least affected with a predominantly normal maturation sequence, but the megakaryocytes' cytoplasm was often very vacuolated. Marrow macrophages were increased in number and erythrophagocytosis was commonly observed. Marrow lymphocytes, reactive lymphocytes and plasma cells were numerous. These marrow changes are non-specific and may also reflect extreme marrow toxicity and reactivity with increased turnover and destruction of normal elements. In another study by Evermann et al,[32] the bone marrow was consistently gelatinous and haemorrhagic macroscopically, and was hypocellular microscopically.

In the study by Macartney et. al.[30], rising circulating antibody titres were detected from day 5 after oral inoculation with canine parvovirus of faecal origin, indicating the development of a humoral response. This response almost certainly was responsible for termination of the plasma viraemia, as well as for the elimination of virus from the intestinal mucosa and cessation of faecal excretion. This rapid humoral response was an interesting finding considering the striking lymphocytolytic activity of this virus. It would be expected that the initial contact between the virus and lymphocyte would lead to division of the lymphocyte to produce clones of effector cells including antibody producing plasma cells (the clonal selection hypothesis for production of antibody). This cell division would render the lymphocyte more susceptible to viral replication with subsequent destruction. According to Macartney et. al.[30] there are at least three explanations for this paradoxically rapid humoral response: (1) presentation of antigens to lymphocytes may occur only after inactivation of the virions, possibly by macrophages, (2) since defective particles are produced, it is possible that some lymphocytes may be primed by these non-infectious virions, (3) the presence of specific receptors on the lymphocytes, while allowing stimulation, might prevent penetration and replication.

Erythroid elements can be slightly affected during the course of the disease, but severe anaemia is not a common finding in CPV. Circulating numbers of erythrocytes apparently are not affected since mature cells have a long half-life relative to the short period the virus suppresses production in the marrow.[28] A decreasing packed cell volume (PCV) through the course of the disease is probably due to a combination of intestinal haemorrhage and rehydration therapy.[3,5]

Although treatment of dogs with parvoviral enteritis is often successful, many dogs die of complications related to septicaemia or are euthanized because of anticipated high costs. Because of the high cost of treatment for dogs with canine parvoviral enteritis, indicators of prognosis for affected dogs would be clinically useful. Although dogs of certain breeds or age groups are known to be at risk for developing parvoviral enteritis,[9,33] definitive prognostic indicators have not yet been identified. A few attempts were made to find prognostic indicators in CPV, but the results were contradictory and inconclusive.[7,12,28,29,34]

Leukopaenia as an indicator of a fatal prognosis has been a controversial issue in the literature, but some authors have suggested in their studies that leukopaenia could be indicative of a poor prognosis. Woods et. al. (1980)[7] conducted a trial on 31 dogs diagnosed with CPV housed in 2 kennels. From their results they found the leukogram to be a helpful tool in evaluating the patients. They found an elevated WBC was associated with quick recovery, and leukopaenia (WBC $< 4.5 \times 10^9$/l) was associated with the need for more aggressive therapy and a poorer prognosis. Potgieter et. al. (1981)[28] followed the progress of the disease after inoculating eight, 8-week-old puppies with CPV. Their results showed that the dogs that became ill developed mild to severe neutropaenia and moderate lymphopaenia. The animals most severely affected had the most severe neutropaenia (2 – 6% of normal counts) whereas lymphocyte counts fell to only approximately 50% of normal counts. Recovery from infection was associated with transient lymphocytosis, which was sometimes seen as a transient leukocytosis. The findings of O'Sullivan et. al. (1984)[12] supported those of Woods et. al.[7] in that very low leukocyte counts indicated a poor prognosis. Their findings also indicated that leukopaenia may persist even after clinical improvement.

However, in a study over a six-year period on CPV in a closed Beagle colony, Mason et. al.(1987)[29] concluded that leukopaenia may indicate the presence of more severe disease, but should not be used as the sole criterion of prognosis in individual CPV cases. Furthermore, a retrospective study by McCaw et. al. (1996)[34] that was conducted on 89 cases of confirmed parvovirus infection showed that lower age, the absence of vomiting, and monocytopaenia were associated with a poorer chance of survival. This study also found that neutropaenia at the time of initial presentation, even when severe, was not a significant prognostic factor.

These studies have all been conducted on central venous blood and the question might arise whether the same will be seen on a peripheral blood smear (from a capillary stick), seeing that for many veterinary practitioners a peripheral blood smear would be the first and only diagnostic test that is immediately available. The need often arises to set a prognosis before embarking on costly laboratory test panels and treatment. Consequently, this issue should be taken into account.

University of Pretoria etd – Goddard, A (2006)

CHAPTER 2 STUDY OBJECTIVES

2.1 HYPOTHESES / PROBLEM STATEMENT

Leukopaenia is a significant problem in canine parvovirus (CPV) enteritis. We hypothesized that a predictive regression model based on leukocyte types could be derived to predict clinical outcome in canine parvoviral enteritis.

2.2 OBJECTIVES OF THIS STUDY

- To investigate whether a total WBC count that doesn't increase over the course of hospitalization or remains less than 4.5×10^9/l is strongly correlated with a poor outcome in a regression model.[7] [**Definition of a poor outcome: death, euthanasia or "days-until-readiness-for-discharge" longer than 7 days].**

- To investigate whether the kinetics of lymphocyte, neutrophil and monocyte numbers, over the time period of hospital stay, will differ in a regression model.

- To investigate whether the kinetics of the specific WBC types (segmented neutrophils, band neutrophils, lymphocytes, monocytes and eosinophils) are more strongly correlated with outcome than total WBC count in a regression model.

- To investigate whether the red blood cell (RBC) parameters (Hb, RCC, Ht, MCV, MCHC and RDW) are correlated with case outcome in a regression model.

- To investigate whether a low platelet (PLT) count, without evidence of regeneration or improvement over the days of hospitalization, is correlated with a poor outcome in a regression model.

- To investigate whether there is a statistically significant difference between a peripheral smear differential count and a central smear differential count in the same dog, in a regression model.

2.3 BENEFITS OF THIS STUDY

- Canine parvoviral enteritis is an economically important disease in South Africa and globally. More effective prediction of the outcome of this disease will have an economic impact if a prognosis can be determined early in the course of the disease.

- The research conducted serves as partial fulfilment of the principal investigators' MMedVet (KDK) degree.

University of Pretoria etd – Goddard, A (2006)

CHAPTER 3 MATERIALS AND METHODS

3.1 MODEL SYSTEM

This study was a prospective study on clinical cases diagnosed with canine parvovirus enteritis.

3.2 EXPERIMENTAL DESIGN

Sixty-two puppies, which were presented at the Outpatients clinic of the Onderstepoort Veterinary Academic Hospital (OVAH) and which were diagnosed with acute parvoviral gastroenteritis, were considered for the study. Owner's consent was necessary for inclusion of each puppy in the study **(Appendix A)**.

Inclusion criteria:

- Puppies that were between the ages of 6 and 24 weeks;
- that exhibited clinical signs of canine parvoviral enteritis (e.g., depression, anorexia, vomiting, haemorrhagic diarrhoea, dehydration, collapse);
- whose clinical condition warranted hospitalization in the opinion of the clinician on duty in the OVAH Outpatients clinic (i.e. severe depression/collapse, > 5% dehydration, severe vomiting and haemorrhagic diarrhoea, hypothermia, hypoglycaemia and hypokalaemia), unless the owner requested euthanasia;
- that were of any breed and either sex;
- that weighed more than 3kg;
- that were diagnosed, within 24 hours, as CPV positive on faecal electron microscopy (EM), with- or without corona virus (any animal found to be negative for CPV on EM, after already being included into the trial, was censored from the trial);

- that had no haemoprotozoal parasites identified on peripheral "CAMS-quick"-stained blood smear;
- without light-microscopically visible Giardia organisms on faecal "wet prep" slides at any time point during hospital stay;
- without any evidence of Canine Distemper Virus (CDV) infection: Clinical – muco-purulent ocular-nasal discharge, neurological signs unresponsive to intravenous glucose therapy, naso-digital hyperkeratosis, enamel hypoplasia; Specific diagnostic tests for CDV – cytological or electron microscopic evidence of CDV infection;

were included in the trial.

All owners of sick dogs that met the criteria, after being fully informed of the nature of the project **(Appendix B)**, were asked to sign a consent form **(Appendix A)** to include their dog in the trial.

3.3 EXPERIMENTAL PROCEDURES

All the patients admitted to the trial were managed according to the treatment guidelines for CPV enteritis set out by the OVAH as seen in **appendix C**. At admission, and every subsequent day until discharge or death, an EDTA sample (vacutainer tube / paediatric tube) was collected from the jugular vein of each patient. A faecal sample was also collected at the time of admission (a lubricated 1ml syringe inserted into the rectum was used to aspirate at least 1ml of faeces). At the time of admission a drop of blood from the ear of each patient was used to make a peripheral blood smear to allow for comparison with the central blood. The peripheral blood smear was identified as such (i.e. PS) and was given the same number as the smear made from the central blood (EDTA). Smears were then fixed and stained with "CAMS-quick" and stored together with the smear made from the central blood (EDTA) until analysis.

An EDTA sample and peripheral blood smear was submitted on a daily basis (including day of admission), for haematological analysis. A Full Blood Count (FBC) was performed by means of an automated cell counter on the EDTA sample (CELL-DYN®3700 System). The FBC included a manual differential leukocyte count

performed by an experienced veterinary haematology technologist. A manual differential leukocyte count was also performed on the peripheral blood smear to compare with that of the central blood. To avoid bias the primary investigator did not have access to the haematological data until after discharge or death of the patient.

The faecal sample was submitted to the Electron Microscopy (EM) unit of the Department of Anatomy and Physiology, Faculty of Veterinary Science, Onderstepoort for examination by direct transmission electron microscopy. The sample was refrigerated immediately after collection and submitted to the EM-unit within 12 hours. This sample was examined for the presence of parvo-, corona-, and distemper virus particles. In cases where samples were collected after hours the sample was kept in the refrigerator overnight. The EM-unit was closed over weekends; therefore new cases were admitted to the trial until 13h00 on Fridays and from 21h00 on Sundays.

The primary investigator was responsible for collecting data. At admission, each patient underwent a clinical examination and these data were recorded as per **Appendix H**. The primary investigator also allocated a clinical score to each patient (as laid out in **Appendix I)**. The clinical score was calculated on observations made over the first 24hrs following admission and was updated every 24hrs until discharge or death.

The primary investigator determined whether a specific patient was ready to be discharged from the hospital. Data regarding the outcome of hospitalization, i.e. death (natural or euthanasia requested by the owner or the duty clinician) or discharge were also recorded **(Appendix J)**.

Patients that died naturally or those that were euthanized were appropriately identified and submitted to the Pathology unit, Faculty of Veterinary Science, Onderstepoort for post mortem examination. The pathologist on duty was notified and the necropsy was carried out as soon as possible. In cases where the patient died after hours, the carcass was refrigerated as soon as possible after death.

During the necropsy lesions were described and the following tissues were collected in 10% formalin for routine haematoxylin and eosin (H&E) processing: small intestine (duodenum, proximal and distal ileum), spleen, lymph nodes (mesenteric and cervical), thymus, bone marrow from the proximal femur, liver and CNS. After fixation, the

tissues were processed, sectioned at 4 microns, stained with H&E and then examined. Lesions were described and graded where possible.

3.4 OBSERVATIONS

The blood samples collected were used to determine the following parameters:

Whole blood in EDTA (central):

Serial FBC were performed by means of an automated cell counter, the CELL-DYN®3700 **(Appendix K).** Manual leukocyte differential counts were performed by an experienced veterinary haematology technologist by counting 50 – 100 cells, depending on the severity of the leukopaenia.

Whole blood from ear pricks (peripheral):

For comparison with the findings on central blood, a manual differential leukocyte count was performed by an experienced veterinary haematology technologist by counting 50 – 100 cells, depending on the severity of the leukopaenia.

Collection times:

Day 0 – time of admission

Day 1 – usually the next morning, 12 – 24 hours after admission

Day 2 – 24 hours after last collection (day 1)

Day 3 – 24 hours after last collection (day 2)

Day 4 – 24 hours after last collection (day 3)

Day 5 – 24 hours after last collection (day 4)

3.5 DATA CAPTURE AND ANALYSIS

The data generated were captured into a spreadsheet database (Microsoft Excel 2003, Microsoft Corp. USA). Regular back-ups of this data were made on two 3.5mm discs

(one of which was made available to the statistician), the primary investigators' computer hard drive, and as printouts.

The data generated were statistically analyzed using a statistical analysis package (StataCorp. 2003. *Stata Statistical Software: Release 8*. College Station, TX: StataCorp LP.).

3.6 STATISTICAL CONSIDERATIONS

For variables total white blood cell (WBC) count, segmented neutrophils (Neut), band neutrophils (Bands), lymphocytes (Lymph), monocytes (Mono), eosinophils (Eos), various red blood cell (RBC) parameters and platelets (PLT), the following statistical analyses were performed (as all the non-surviving puppies died or were euthanized within the first 5 days, data were only analysed up to the fifth day):

Box plots (representing the interquartile range), of the pooled sample of WBC and the various specific leukocyte types were created to indicate the distribution of the various values by day for the first 5 days.

Graphs were generated to compare groups (survivors vs. non-survivors) with respect to the specific leukocyte types and PLT.

The Fisher's exact test was used to compare groups (survivors vs. non-survivors) on days 0 through 5, with respect to the WBC, specific leukocyte types, various RBC parameters and platelets.

Besides the absolute count of a particular leukocyte type on a specific day, it also was important to consider the change in number over time of that particular leukocyte type. We hypothesised that puppies with increases in certain leukocyte types over time, specifically total WBC and lymphocyte counts, (irrespective of the actual counts) would have a better outcome. Therefore for WBC, the various specific leukocyte types, RBC parameters and platelets, groups were compared with respect to change from baseline

(value at admission) in an analysis of covariance (ANCOVA), with baseline as covariant. Means were reported as adjusted to a given baseline value.

The reference intervals used as cut-off values were taken from the Clinical Pathology laboratory, Faculty of Veterinary Science, Onderstepoort as shown in table 3.1.

Table 3. 1 Haematology reference intervals for canines used by the Clinical Pathology laboratory, Faculty of Veterinary Science, Onderstepoort

Parameter	**Units**	**Canine reference interval**
Haemoglobin (Hb)	g/l	120 – 180
Red Cell Count (RCC)	X 10^{12}/l	5.5 – 8.5
Haematocrit (Ht)	l/l	0.37 – 0.55
Mean Corpuscular Volume (MCV)	fl	60 – 77
Mean Corpuscular Haemoglobin Concentration (MCHC)	g/dl rbc	32 – 36
White Blood Cell (WBC) count	x 10^9/l	6.0 – 15.0
Segmented neutrophils (Neut)	x 10^9/l	3.0 -11.5
Band neutrophils (Bands)	x 10^9/l	0.0 – 0.5
Lymphocytes (Lymph)	x 10^9/l	1.0 – 4.8
Monocytes (Mono)	x 10^9/l	0.15 – 1.35
Eosinophils (Eos)	x 10^9/l	0.10 – 1.25
Basophils (Baso)	x 10^9/l	0.0 – 0.1
Platelets (PLT)	x 10^9/l	200 - 500

CHAPTER 4 RESULTS

A complete data set is provided in Appendix L.

Sixty-two puppies that presented to the Onderstepoort Veterinary Academic Hospital (OVAH) during the period August 2004 to April 2005, showing typical signs of canine parvoviral enteritis, were sick enough to be admitted to the isolation ward. All 62 of the puppies with the exception of 2 met all inclusion criteria, (see Note below). No puppies were excluded from the study. Of the 62 puppies, 11 puppies died, indicating a survival rate of 82%. Of the 11 puppies that died (18%), nine died due to complications of the disease and two were euthanized due to a poor prognosis and financial restrictions. The puppies that did not survive died within the first five days of hospitalization. Those that died naturally (9) died within the first three days, and those that were euthanized (2) were euthanized on day 3 and day 5 (a blood sample was collected before the puppy was euthanized). See fig.4.1 for the Kaplan-Meier survival estimate curve.

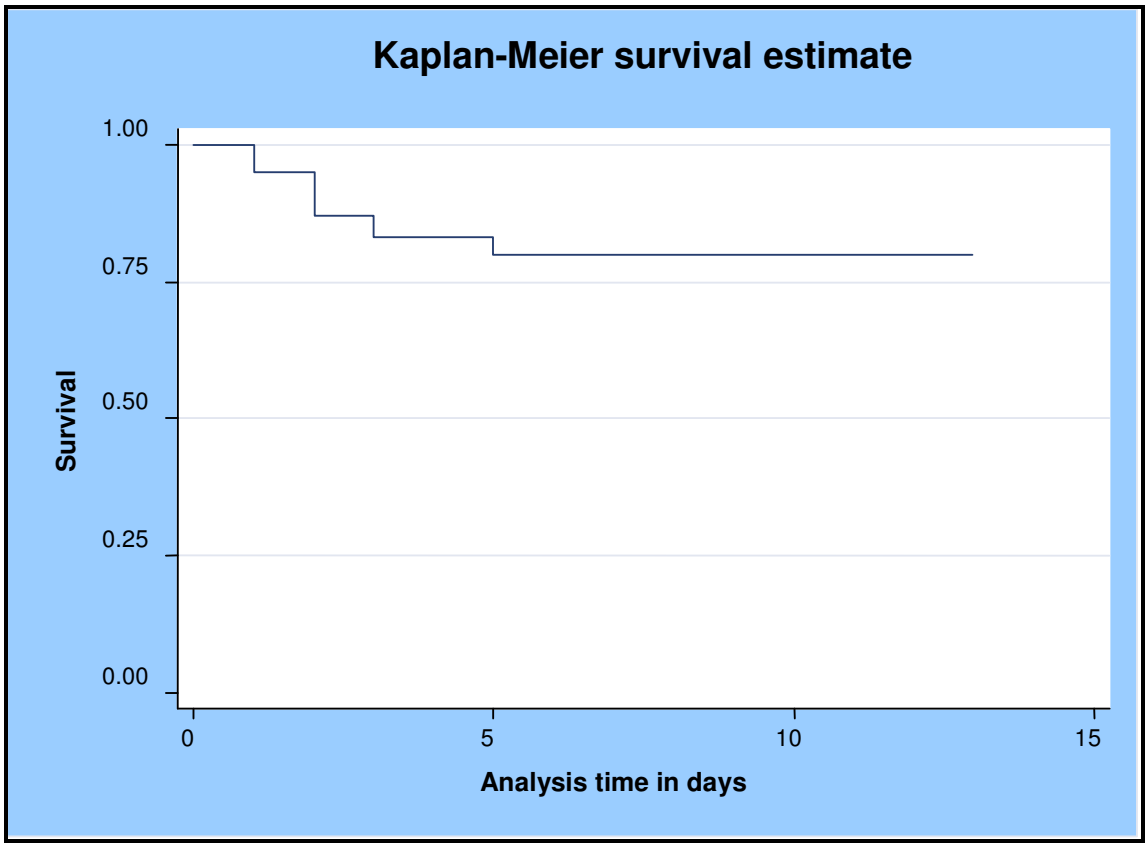

Figure 4.1 A Kaplan-Meier survival estimate in puppies with canine parvoviral enteritis (August 2004 – April 2005)

NOTE:

- Seeing as all the non-surviving puppies died or were euthanized within the first 5 days, data were only analysed up to the fifth day.
- Two of the patients that were included in the study (one from the group that died and one from the surviving group) did not have blood collected for full haematology at admission because they presented after-hours. They were included in the study anyway, because the author did not want to lose them and cases were very scarce at that point in time. However, because of the lack of baseline data, tables formulated for the Fisher's exact test include 60 rather than 62 total patients at admission (day 0). See table 4.1.

Table 4. 1 Number of survivors vs. non-survivors per day for the first 5 days analyzed.

Day	Survivors	Non-survivors	Total (n)
Day 0	50	10	60
Day 1	51	9	60
Day 2	51	8	59
Day 3	42	2	44
Day 4	32	1	33
Day 5	24	1	25

4.1 EVALUATION OF THE TOTAL WHITE BLOOD CELL COUNTS IN SURVIVORS AND NON-SURVIVORS WITH CANINE PARVOVIRAL ENTERITIS.

The box plot of total WBC count, displayed in fig.4.2 below, indicates the distribution of the various values on a specific day. Significant differences were found in the WBC between the puppies that died and those that survived (see fig.4.3 below). In none of the puppies that died from the disease did the WBC rise above 4.5×10^9/l, a cut-off value established by Woods et. al.[7] which is less than the lower limit of the reference interval of 6.0×10^9/l. In fact, the mean WBC of the puppies that did not survive remained less than 2.0×10^9/l over the first 3 days post admission.

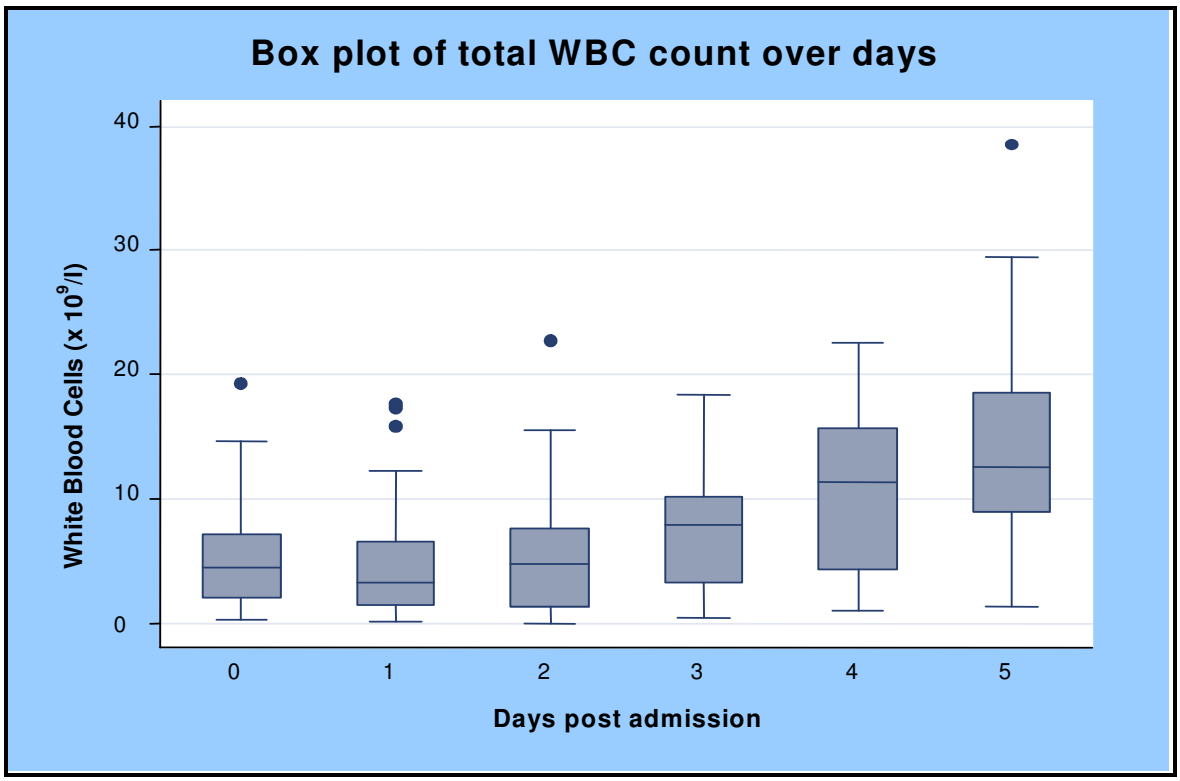

Figure 4.2 Box plot (representing the interquartile range) of the WBC over days in puppies (survivors and non-survivors) with canine parvoviral enteritis for the first 5 days post admission. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

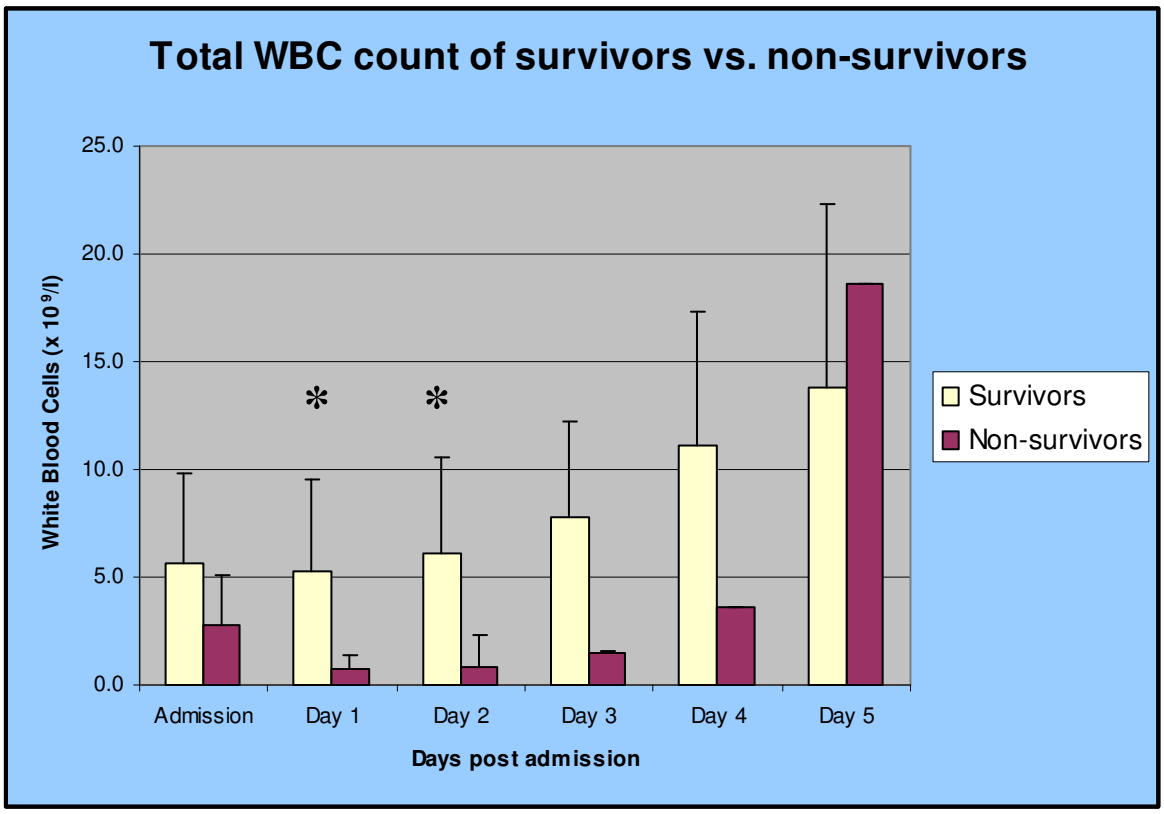

Figure 4.3 Comparison of groups (survivors vs. non-survivors) with respect to WBC over the first 5 days post admission. The * indicate significance ($p<0.05$). The "whiskers" represent standard deviation. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

Comparison of groups, by means of the Fisher's exact test, with respect to total WBC count is summarised in table 4.2. The data presented in the table are the number and percentage of dogs in each group with a WBC less than 4.5×10^9/l.

Table 4. 2 Comparison between groups (survivors vs. non-survivors) with respect to WBC that is less than 4.5×10^9/l (Fisher's exact test). Shaded values indicate significance ($p<0.05$)

Day	Survivors	Non-survivors	Fisher Exact test (p-value)
Day 0	48% (24/50)	70% (7/10)	0.302
Day 1	56.9% (29/51)	100% (9/9)	0.02
Day 2	41.2% (21/51)	100% (8/8)	0.002
Day 3	26.2% (11/42)	100% (2/2)	0.082
Day 4	25% (8/32)	100% (1/1)	0.273
Day 5	8.3% (2/24)	0% (0/1)	1.000

On day 1, groups (survivors vs. non-survivors) differed significantly ($p=0.02$; 56.9% vs. 100%) with respect to the percentage of animals with WBC $< 4.5 \times 10^9$/l.

On day 2, groups (survivors vs. non-survivors) differed significantly ($p=0.002$; 41.2% vs. 100%) with respect to the percentage of animals with WBC $< 4.5 \times 10^9$/l.

For WBC, groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.3 below). Mean was reported as adjusted to a given baseline value (i.e. 5.153). Here baseline value was used as covariate.

Table 4. 3 ANCOVA results when comparing groups with respect to WBC parameters. Adjusted mean baseline value of WBC on day 0=5.153. Shaded values indicate significance (p<0.05)

Parameter		Survivors Mean	Non-survivors Mean	p-Value
WBC Day 1 compared to Day 0	Unadjusted	- 0.320	- 2.350	0.0176
	Adjusted	- 0.087	- 3.391	
WBC Day 2 compared to Day 0	Unadjusted	0.536	- 2.586	0.0055
	Adjusted	0.955	- 4.148	
WBC Day 3 compared to Day 0	Unadjusted	3.129	- 4.150	0.0637
	Adjusted	2.556	- 3.557	
WBC Day 4 compared to Day 0	Unadjusted	6.742	- 1.20	0.2157
	Adjusted	5.602	- 1.820	
WBC Day 5 compared to Day 0	Unadjusted	8.943	13.80	0.6015
	Adjusted	8.765	13.117	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) differed significantly (p=0.0176) with respect to change from baseline in WBC on day 1 i.e. –0.087 vs. –3.391 (meaning that survivors showed a significant increase in WBC on day 1 compared to day 0 when compared to non-survivors) and the latter means were adjusted to a baseline value of 5.153.

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) differed significantly (p=0.0055) with respect to change from baseline in WBC on day 2 i.e. 0.955 vs. –4.148 (meaning that survivors showed a significant increase in WBC on day 2 compared to day 0 when compared to non-survivors) and the latter means were adjusted to a baseline value of 5.153.

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4.2 EVALUATION OF SPECIFIC LEUKOCYTE TYPES IN SURVIVORS AND NON-SURVIVORS WITH CANINE PARVOVIRAL ENTERITIS

4.2.1 SEGMENTED NEUTROPHILS

The box plot of the segmented neutrophils (Neut), displayed in fig. 4.4 below, indicates the distribution of the various values on a specific day. No significant differences were found in the segmented neutrophil kinetics between the survivors and non-survivors (see fig 4.5 below).

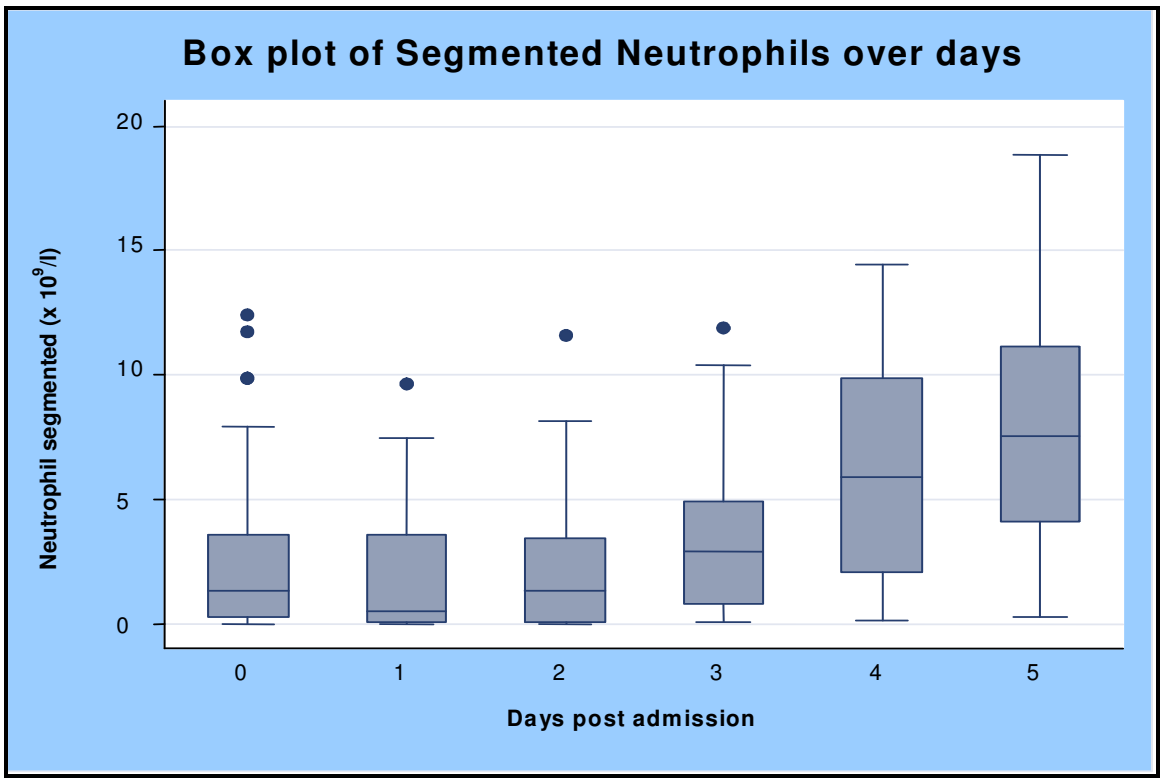

Figure 4.4 Box plot (representing the interquartile range) of the Neut over days in puppies (survivors and non-survivors) with canine parvoviral enteritis for the first 5 days post admission. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

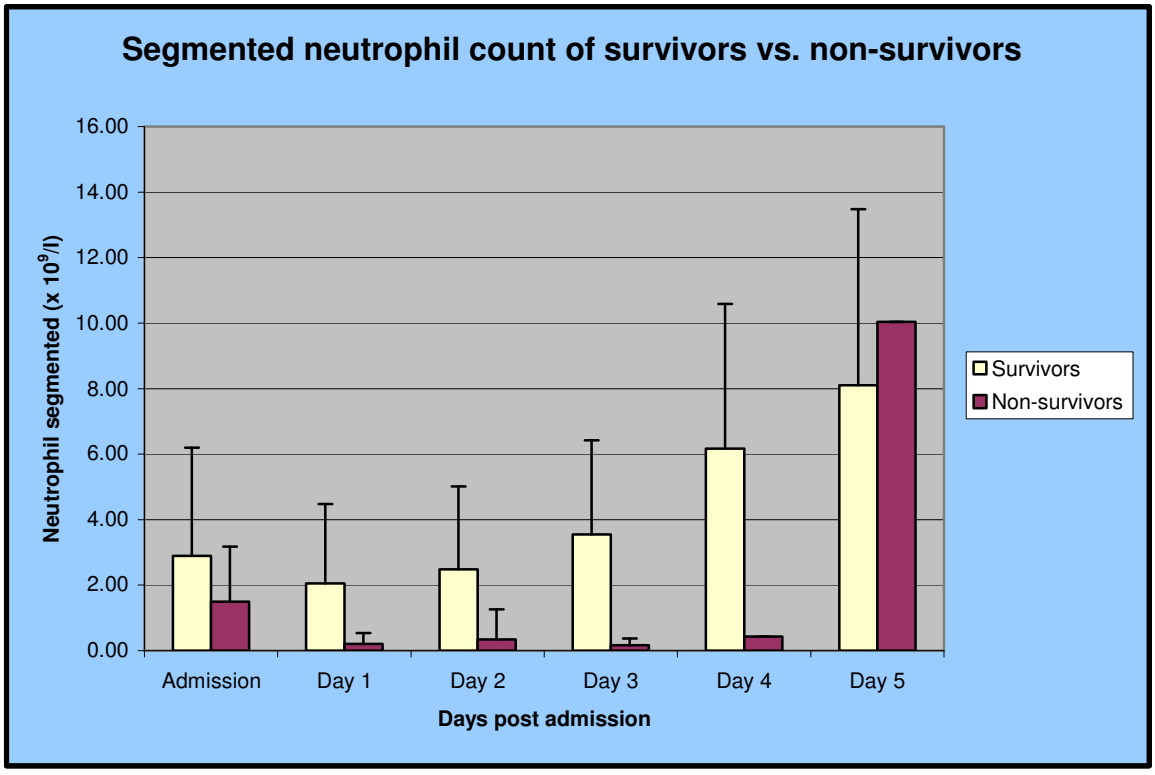

Figure 4.5 Comparison of groups (survivors vs. non-survivors) with respect to Neut over the first 5 days post admission. The "whiskers" represent standard deviation. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

Comparison of groups, by means of the Fisher's exact test, with respect to Neut is summarised in table 4.4. The data presented in the table are the number and percentage of dogs in each group with a Neut less than 3.0×10^9/l.

Table 4. 4 Comparison between groups (survivors vs. non-survivors) with respect to Neut that is less than 3.0×10^9/l (Fisher's exact test).

Day	Survivors	Non-survivors	Fisher Exact test (p-value)
Day 0	62% (31/50)	90% (9/10)	0.142
Day 1	68.6% (35/51)	100% (9/9)	0.096
Day 2	66.7% (34/51)	100% (8/8)	0.090
Day 3	50% (21/42)	100% (2/2)	0.489
Day 4	34.4% (11/32)	100% (1/1)	0.364
Day 5	20.8% (5/24)	0% (0/1)	1.000

The Fisher's exact test showed no significant differences in the Neut between survivors and non-survivors for the first 5 days post admission.

For Neut, groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.5 below). Mean was reported as adjusted to a given baseline value (i.e. 2.662). Here baseline value was used as covariate.

Table 4. 5 ANCOVA results when comparing groups with respect to Neut parameters. Adjusted mean baseline value of Neut on day 0=2.662. Shaded values indicate significance (p<0.05).

Parameter		Survivors Mean	Non-survivors Mean	p-Value
Neut Day 1 compared to Day 0	Unadjusted	- 0.807	- 1.476	0.0553
	Adjusted	- 0.684	- 1.991	
Neut Day 2 compared to Day 0	Unadjusted	- 0.376	- 1.514	0.0460
	Adjusted	- 0.174	- 2.169	
Neut Day 3 compared to Day 0	Unadjusted	1.360	- 3.90	0.1669
	Adjusted	0.780	- 2.097	
Neut Day 4 compared to Day 0	Unadjusted	3.846	- 2.310	0.1682
	Adjusted	3.392	- 2.173	
Neut Day 5 compared to Day 0	Unadjusted	5.346	7.30	0.7252
	Adjusted	5.895	7.46	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) differed significantly (p=0.046) with respect to change from baseline in Neut on day 2 i.e. –0.174 vs. –2.169 (meaning that survivors showed a significant increase in Neut on day 2 compared to day 0 when compared to non-survivors) and the latter means were adjusted to a baseline value of 2.662.

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4.2.2 BAND NEUTROPHILS

The box plot of the band neutrophils (Bands), displayed in fig. 4.6 below, indicates the distribution of the various values on a specific day. No significant differences were found in the band neutrophil kinetics, between the survivors and non-survivors (see fig 4.7 below).

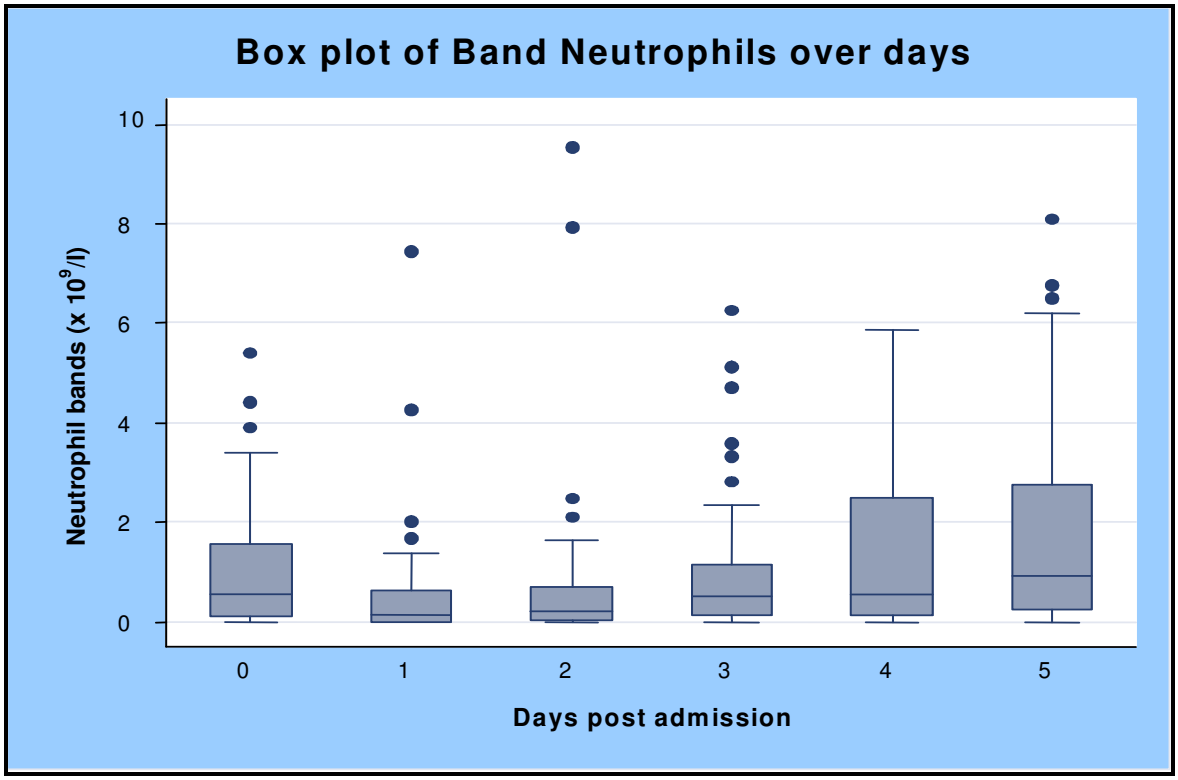

Figure 4.6 Box plot (representing the interquartile range) of the Bands over days in puppies (survivors and non-survivors) with canine parvoviral enteritis for the first 5 days post admission. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

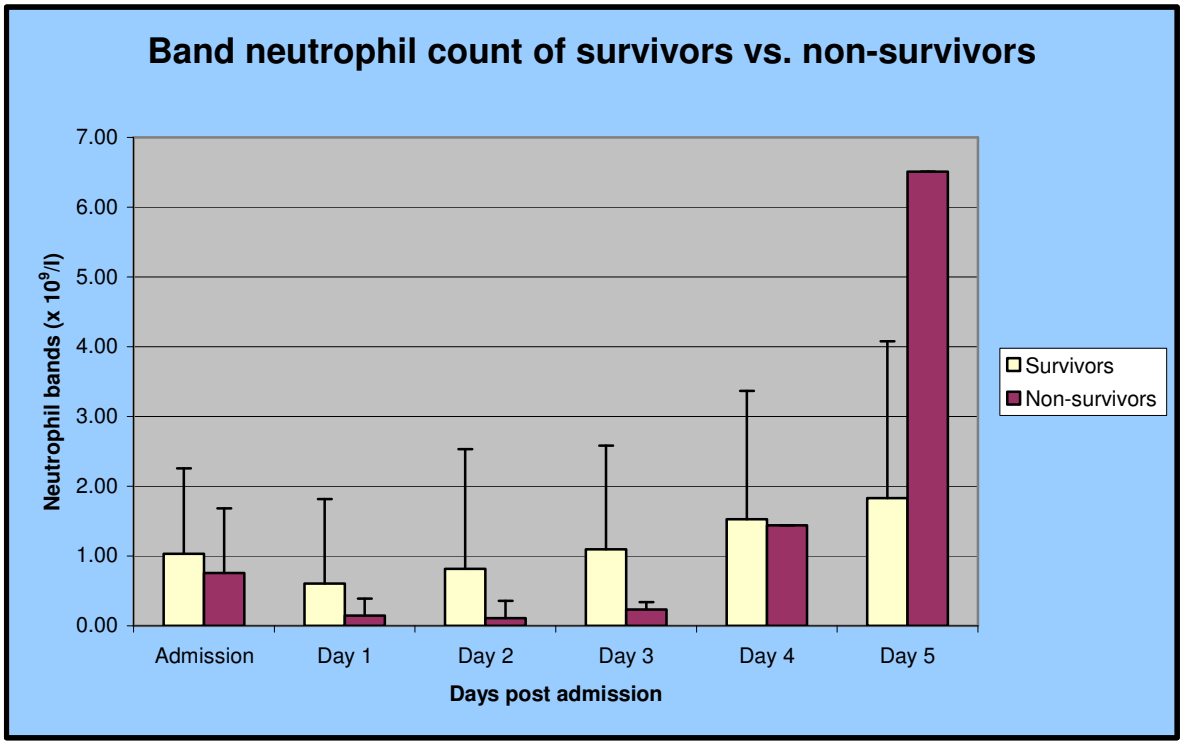

Figure 4.7 Comparison of groups (survivors vs. non-survivors) with respect to Bands over the first 5 days post admission. The "whiskers" represent standard deviation. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

Comparison of groups, by means of the Fisher's exact test, with respect to Bands is summarised in table 4.6. The data presented in the table are the number and percentage of dogs in each group with a Band that equals 0.

Table 4. 6 Comparison between groups (survivors vs. non-survivors) with respect to a Band that equals 0, i.e. no regenerative inflammatory response (Fisher's exact test).

Day	Survivors	Non-survivors	Fisher Exact test (p-value)
Day 0	6% (3/50)	10% (1/10)	0.528
Day 1	23.5% (12/51)	55.6% (5/9)	0.101
Day 2	19.6% (10/51)	50% (4/8)	0.081
Day 3	4.8% (2/42)	0% (0/2)	1.000
Day 4	9.4% (3/32)	0% (0/1)	1.000
Day 5	4.2% (1/24)	0% (0/1)	1.000

The Fisher's exact test showed no significant difference in the Bands between survivors and non-survivors for the first 5 days post admission.

For Bands, groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.7 below). Mean was reported as adjusted to a given baseline value (i.e. 0.988). Here baseline value was used as covariate.

Table 4. 7 ANCOVA results when comparing groups with respect to Band parameters. Adjusted mean baseline value of Bands on day 0=0.988.

Parameter		Survivors Mean	Non-survivors Mean	p-Value
Bands Day 1 compared to Day 0	Unadjusted	- 0.439	- 0.758	0.3189
	Adjusted	- 0.394	- 0.837	
Bands Day 2 compared to Day 0	Unadjusted	- 0.213	- 0.913	0.2898
	Adjusted	- 0.156	- 0.857	
Bands Day 3 compared to Day 0	Unadjusted	0.154	- 0.850	0.4541
	Adjusted	0.071	- 0.743	
Bands Day 4 compared to Day 0	Unadjusted	0.781	- 0.140	0.9540
	Adjusted	0.579	0.467	
Bands Day 5 compared to Day 0	Unadjusted	1.10	4.930	0.0833
	Adjusted	0.951	5.337	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) did not differ significantly with respect to change from baseline in Bands and the latter means were adjusted to a baseline value of 0.988.

4.2.3 LYMPHOCYTES

The box plot of the lymphocytes (Lymph), displayed in fig 4.8 below, indicates the distribution of the various values on a specific day. Significant differences were found in the lymphocyte kinetics between the survivors and non-survivors (see fig. 4.9 below).

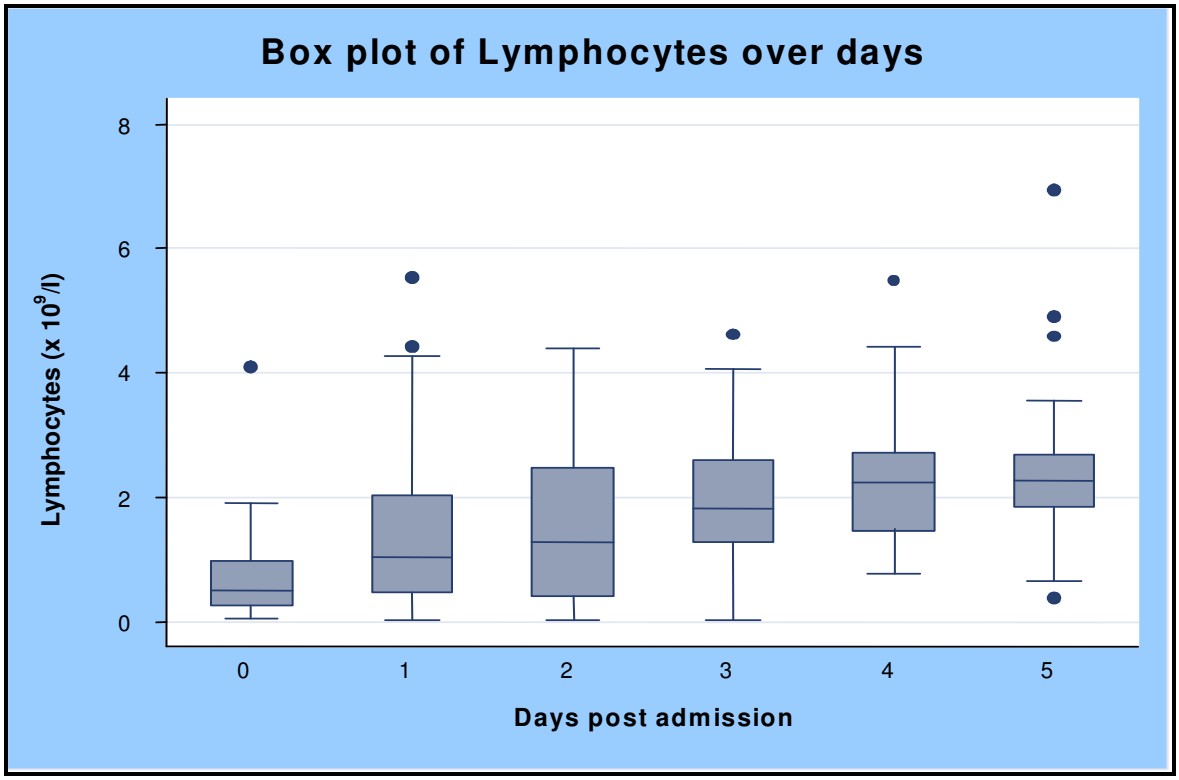

Figure 4.8 Box plot (representing the interquartile range) of the Lymph over days in puppies (survivors and non-survivors) with canine parvoviral enteritis for the first 5 days post admission. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

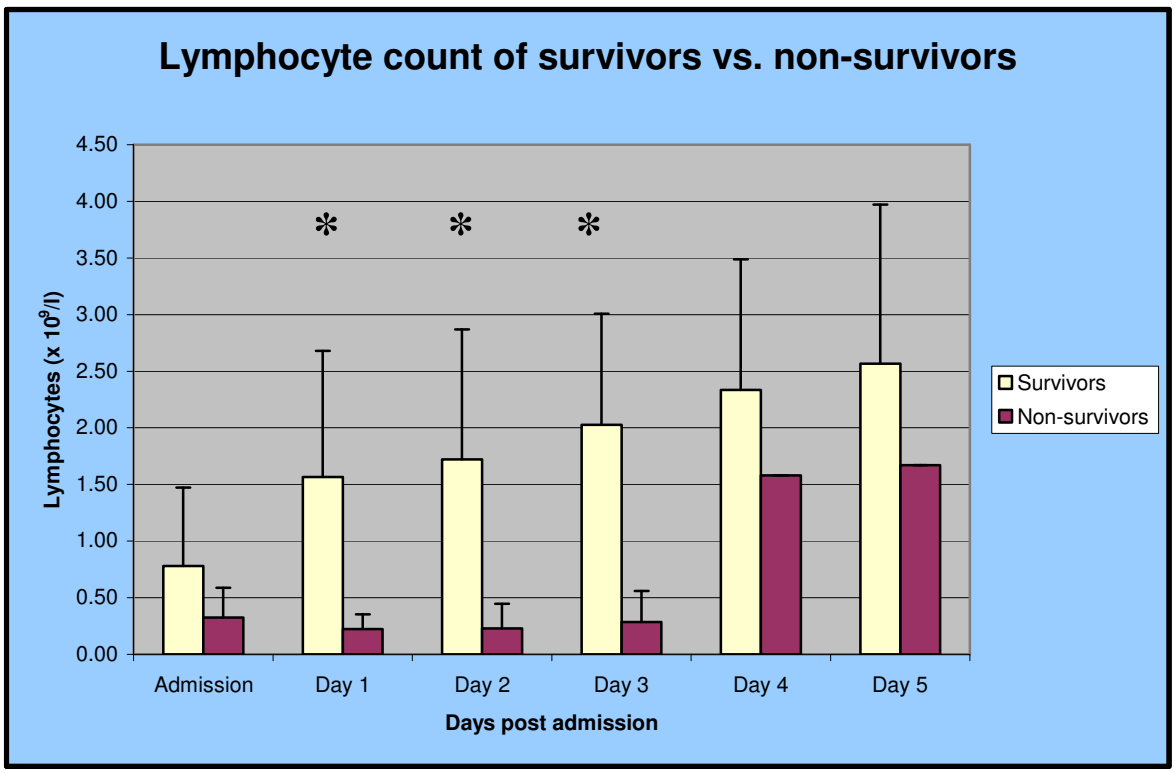

Figure 4.9 Comparison of groups (survivors vs. non-survivors) with respect to Lymph over the first 5 days post admission. The * indicates significance ($p<0.05$). The "whiskers" represent standard deviation. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

Comparison of groups, by means of the Fisher's exact test, with respect to Lymph is summarised in table 4.8. The data presented in the table are the number and percentage of dogs in each group with Lymph less than 1.0×10^9/l.

Table 4. 8 Comparison between groups (survivors vs. non-survivors) with respect to Lymph that is less than 1.0×10^9/l (Fisher's exact test). Shaded values indicate significance ($p<0.05$).

Day	Survivors	Non-survivors	Fisher Exact test (p-value)
Day 0	70% (35/50)	100% (10/10)	0.054
Day 1	37.3% (19/51)	100% (9/9)	<0.001
Day 2	35.3% (18/51)	100% (8/8)	0.001
Day 3	9.5% (4/42)	100% (2/2)	0.016
Day 4	9.4% (3/32)	0% (0/1)	1.000
Day 5	8.3% (2/24)	0% (0/1)	1.00

On day 1, groups (survivors vs. non-survivors) differed significantly ($p<0.001$; 37.3% vs. 100%) with respect to the percentage of animals with Lymph < 1.0×10^9/l.
On day 2, groups (survivors vs. non-survivors) differed significantly ($p=0.001$; 35.3% vs. 100%) with respect to the percentage of animals with Lymph < 1.0×10^9/l.
On day 3, groups (survivors vs. non-survivors) differed significantly ($p=0.016$; 9.5% vs. 100%) with respect to the percentage of animals with Lymph < 1.0×10^9/l.

For Lymph, groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.9 below). Mean was reported as adjusted to a given baseline value (i.e. 0.703). Here baseline value was used as covariate.

Table 4. 9 ANCOVA results when comparing groups with respect to Lymph parameters. Adjusted mean baseline value of Lymph on day 0=0.703. Shaded values indicate significance (p<0.05)

Parameter		Survivors Mean	Non-survivors Mean	p-Value
Lymph Day 1 compared to Day 0	Unadjusted	0.775	- 0.031	0.0179
	Adjusted	0.774	- 0.024	
Lymph Day 2 compared to Day 0	Unadjusted	0.942	- 0.054	0.007
	Adjusted	0.972	- 0.237	
Lymph Day 3 compared to Day 0	Unadjusted	1.311	- 7.45	0.0266
	Adjusted	1.330	- 0.304	
Lymph Day 4 compared to Day 0	Unadjusted	1.613	1.340	0.5814
	Adjusted	1.625	0.948	
Lymph Day 5 compared to Day 0	Unadjusted	1.761	1.430	0.5893
	Adjusted	1.851	1.021	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) differed significantly (p=0.0179) with respect to change from baseline in Lymph on day 1 i.e. 0.774 vs. –0.024 (meaning that survivors showed a significant increase in Lymph on day 1 compared to day 0 when compared to non-survivors) and the latter means were adjusted to a baseline value of 0.703.

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) differed significantly (p=0.007) with respect to change from baseline in Lymph on day 2 i.e. 0.972 vs. –0.237 (meaning that survivors showed a significant increase in Lymph on day 2 compared to day 0 when compared to non-survivors) and the latter means were adjusted to a baseline value of 0.703.

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) differed significantly (p=0.0266) with respect to change from baseline in Lymph on day 3 i.e.

1.330 vs. –0.304 (meaning that survivors showed a significant increase in Lymph on day 3 compared to day 0 when compared to non-survivors) and the latter means were adjusted to a baseline value of 0.703.

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4.2.4 MONOCYTES

The box plot of the monocytes (Mono), displayed in fig. 4.10 below, indicates the distribution of the various values on a specific day. Significant differences were found in the monocyte kinetics, between the survivors and non-survivors (see fig.4.11 below).

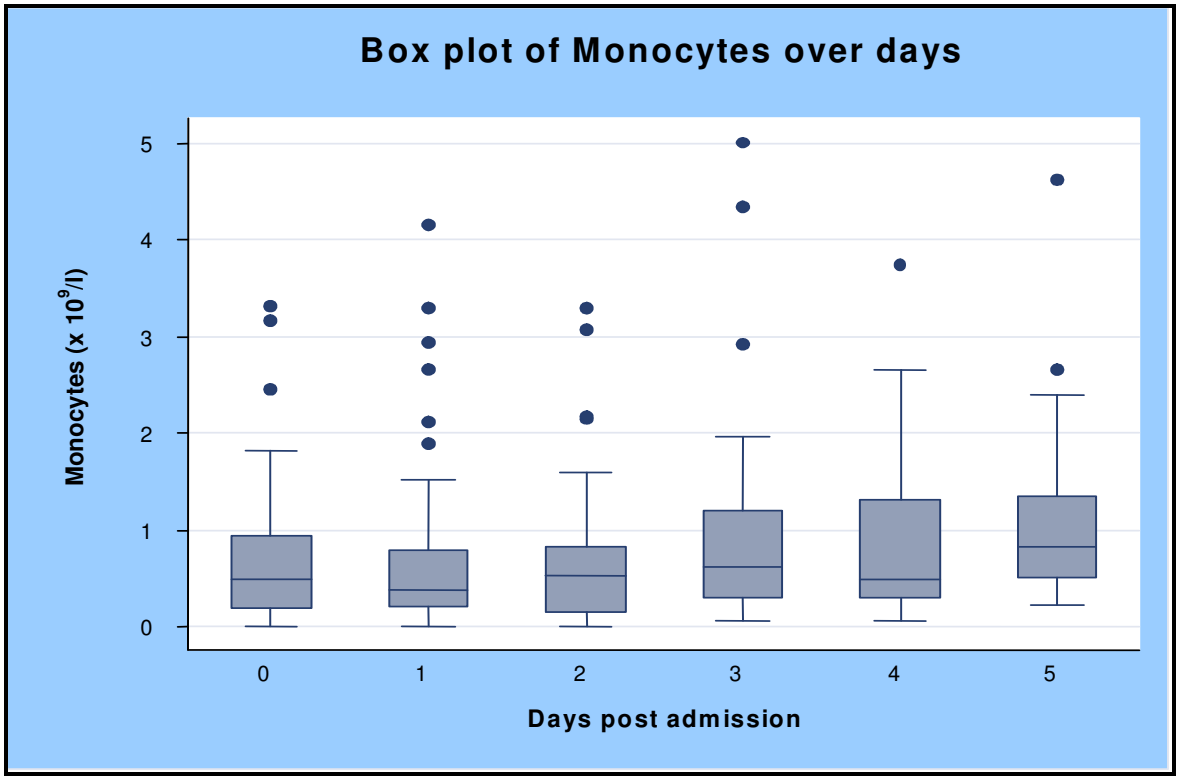

Figure 4.10 Box plot (representing the interquartile range) of the Mono over days in puppies (survivors and non-survivors) with canine parvoviral enteritis for the first 5 days post admission. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

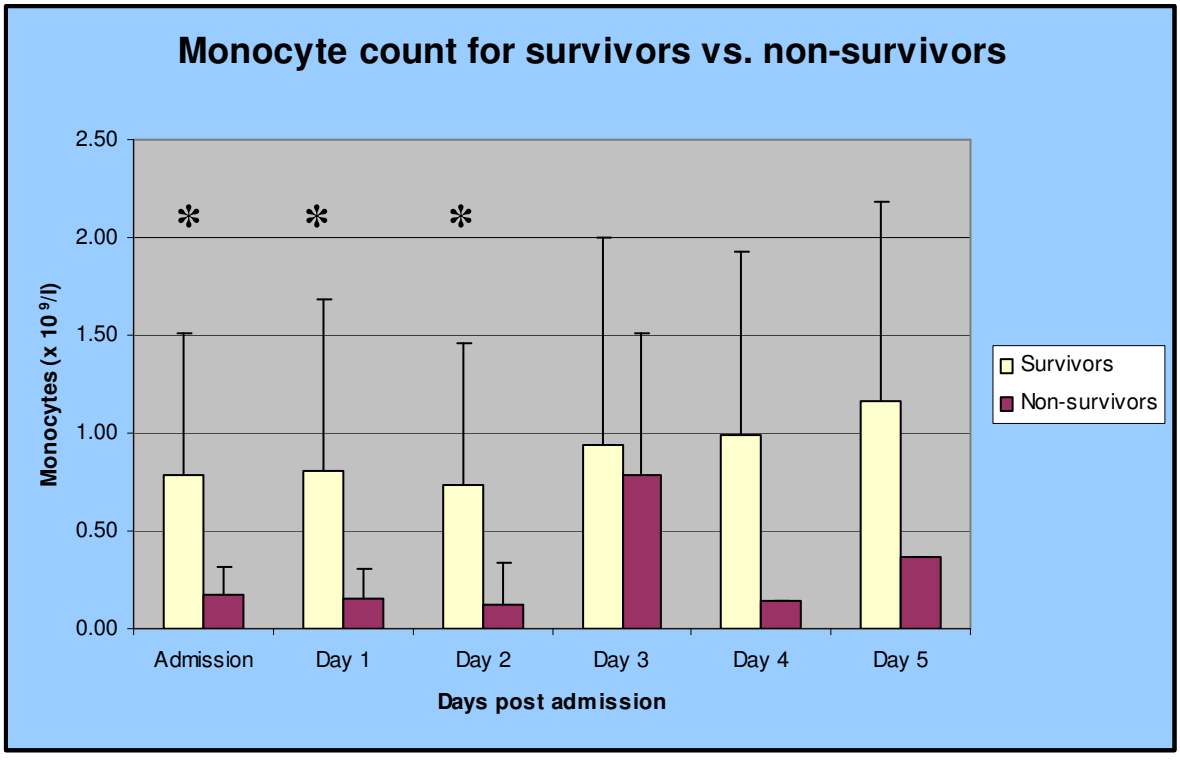

Figure 4.11 Comparison of groups (survivors vs. non-survivors) with respect to Mono over the first 5 days post admission. The * indicates significance ($p<0.05$). The "whiskers" represent standard deviation. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

Comparison of groups, by means of the Fisher's exact test, with respect to Mono is summarised in table 4.10. The data presented in the table are the number and percentage of dogs in each group with Mono less than 0.15×10^9/l.

Table 4. 10 Comparison between groups (survivors vs. non-survivors) with respect to Mono that is less than 0.15×10^9/l (Fisher's exact test). Shaded values indicate significance ($p<0.05$).

Day	Survivors	Non-survivors	Fisher Exact test (p-value)
Day 0	16% (8/50)	50% (5/10)	0.031
Day 1	11.8% (6/51)	44.4% (4/9)	0.034
Day 2	15.7% (8/51)	87.5% (7/8)	<0.001
Day 3	16.7% (7/42)	0% (0/2)	1.000
Day 4	6.3% (2/32)	100% (1/1)	0.091
Day 5	no values	no values	no p-value

On day 0, groups (survivors vs. non-survivors) differed significantly ($p=0.031$; 16% vs. 50%) with respect to the percentage of animals with Mono $< 0.15 \times 10^9$/l.

On day 1, groups (survivors vs. non-survivors) differed significantly ($p=0.034$; 11.8% vs. 44.4%) with respect to the percentage of animals with Mono $< 0.15 \times 10^9$/l.

On day 2, groups (survivors vs. non-survivors) differed significantly ($p<0.001$; 15.7% vs. 87.5%) with respect to the percentage of animals with Mono $< 0.15 \times 10^9$/l.

For Mono, groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.11 below). Mean was reported as adjusted to a given baseline value (i.e. 0.681). Here baseline value was used as covariate.

Table 4. 11 ANCOVA results when comparing groups with respect to Mono parameters. Adjusted mean baseline value of Mono on day 0=0.681.

Parameter		Survivors Mean	Non-survivors Mean	p-Value
Mono Day 1 compared to Day 0	Unadjusted	0.094	- 0.035	0.8482
	Adjusted	0.008	- 0.027	
Mono Day 2 compared to Day 0	Unadjusted	- 0.052	- 0.034	0.4350
	Adjusted	- 0.022	- 0.189	
Mono Day 3 compared to Day 0	Unadjusted	0.199	0.635	0.5355
	Adjusted	0.201	0.600	
Mono Day 4 compared to Day 0	Unadjusted	0.445	- 0.10	0.5680
	Adjusted	0.462	- 0.050	
Mono Day 5 compared to Day 0	Unadjusted	0.655	0.130	0.6411
	Adjusted	0.710	0.265	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) did not differ significantly with respect to change from baseline in Mono and the latter means were adjusted to a baseline value of 0.681.

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4.2.5 EOSINOPHILS

The box plot of the eosinophils (Eos), displayed in fig. 4.12 below, indicates the distribution of the various values on a specific day. Significant differences were found in the eosinophil kinetics, between the survivors and non-survivors (see fig. 4.13 below).

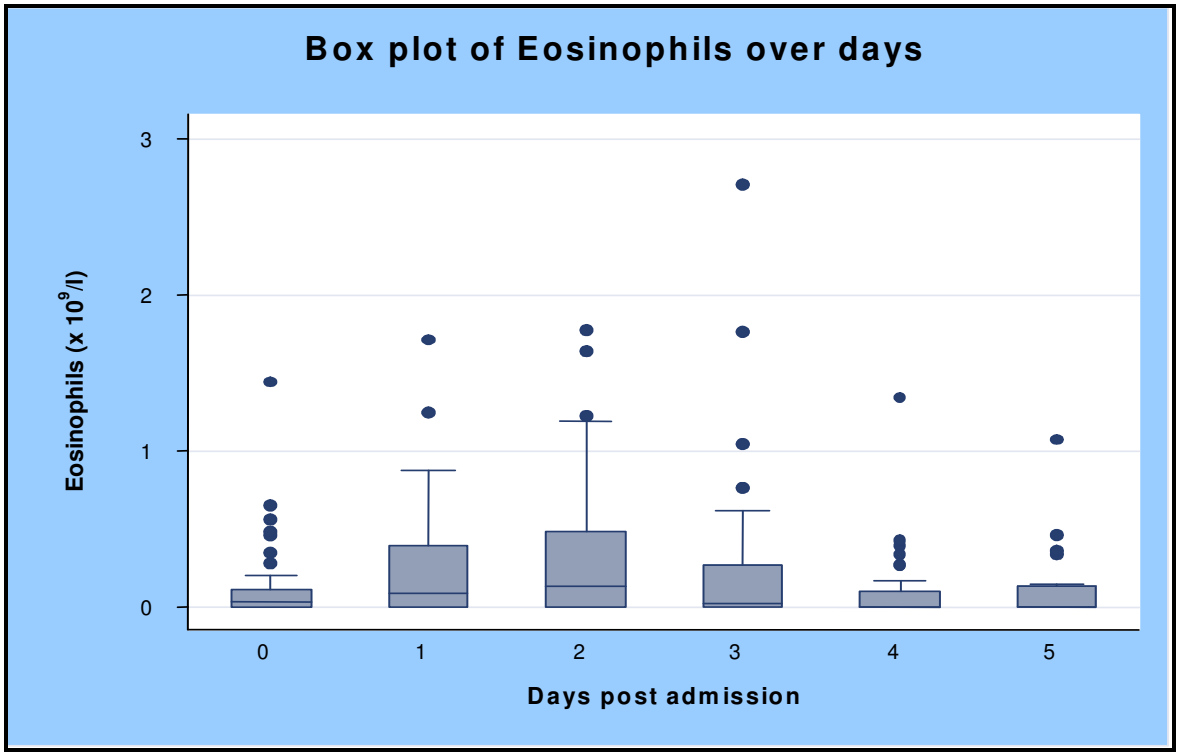

Figure 4.12 Box plot (representing the interquartile range) of the Eos over days in puppies (survivors and non-survivors) with canine parvoviral enteritis for the first 5 days post admission. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

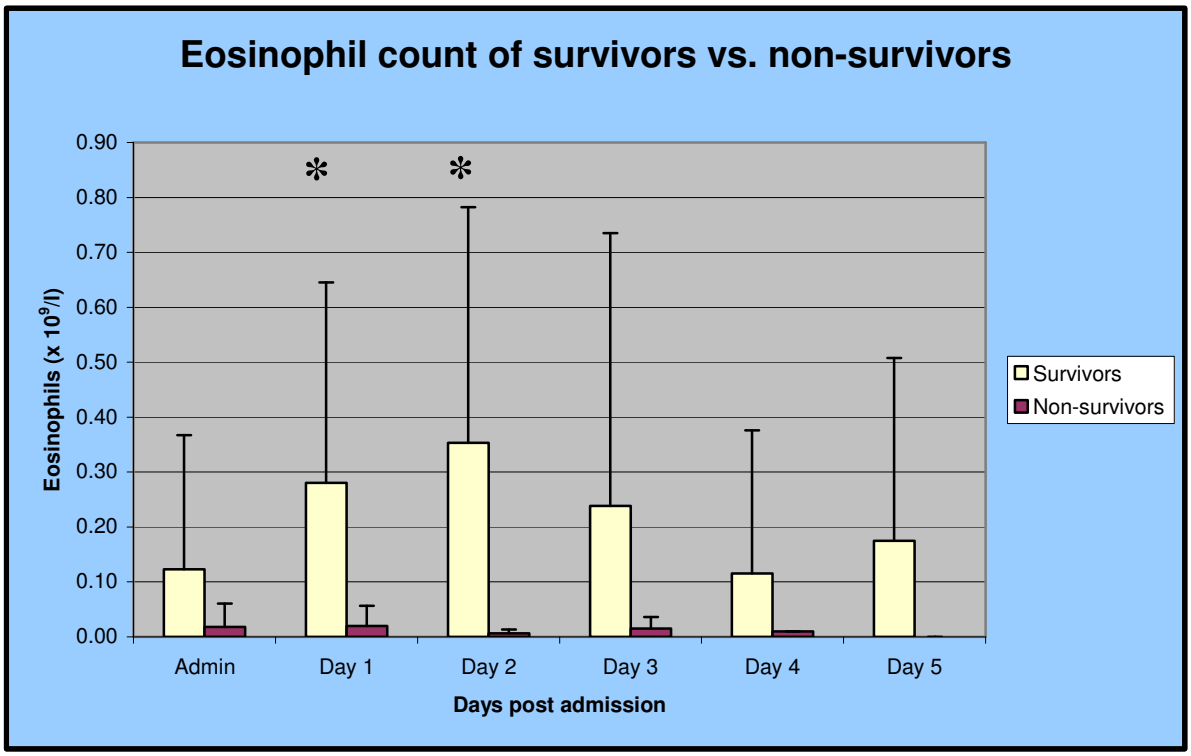

Figure 4.13 Comparison of groups (survivors vs. non-survivors) with respect to Eos over the first 5 days post admission. The * indicate significance ($p<0.05$). The "whiskers" represent standard deviation. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

Comparison of groups, by means of the Fisher's exact test, with respect to eosinophils (Eos) is summarised in table 4.12. The data presented in the table are the number and percentage of dogs in each group with Eos less than 0.1×10^9/l.

Table 4. 12 Comparison between groups (survivors vs. non-survivors) with respect to Eos that is less than 0.1×10^9/l (Fisher's exact test). Shaded values indicate significance ($p<0.05$).

Day	Survivors	Non-survivors	Fisher Exact test (p-value)
Day 0	72% (36/50)	90% (9/10)	0.426
Day 1	45.1% (23/51)	88.9% (8/9)	0.027
Day 2	37.3% (19/51)	100% (8/8)	0.001
Day 3	57.1% (24/42)	100% (2/2)	0.505
Day 4	71.9 (23/32)	100% (1/1)	1.000
Day 5	70.8% (17/24)	100% (1/1)	1.000

On day 1, groups (survivors vs. non-survivors) differed significantly ($p=0.027$; 45.1% vs. 88.9%) with respect to the percentage of animals with Eos $< 0.1 \times 10^9$/l.

On day 2, groups (survivors vs. non-survivors) differed significantly ($p=0.001$; 37.3% vs. 100%) with respect to the percentage of animals with Eos $< 0.1 \times 10^9$/l.

For eosinophils (Eos), groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.13 below). Mean was reported as adjusted to a given baseline value (i.e. 0.106). Here baseline value was used as covariate.

Table 4. 13 ANCOVA results when comparing groups with respect to Eos parameters. Adjusted mean baseline value of Eos on day 0=0.106.

Parameter		Survivors Mean	Non-survivors Mean	p-Value
Eos Day 1 compared to Day 0	Unadjusted	0.147	0.001	0.0869
	Adjusted	0.160	- 0.066	
Eos Day 2 compared to Day 0	Unadjusted	0.229	- 0.011	0.0703
	Adjusted	0.239	- 0.064	
Eos Day 3 compared to Day 0	Unadjusted	0.117	- 0.050	0.5897
	Adjusted	0.128	- 0.020	
Eos Day 4 compared to Day 0	Unadjusted	0.046	0.010	0.7603
	Adjusted	0.025	- 0.057	
Eos Day 5 compared to Day 0	Unadjusted	0.062	0	0.6260
	Adjusted	0.004	- 0.124	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) did not differ significantly with respect to change in Eos from baseline and the latter means were adjusted to a baseline value of 0.106.

4.2.6 BASOPHILS

Too few cases presented with basophils in circulation to do statistical analysis.

4.3 EVALUATION OF RED BLOOD CELL PARAMETERS IN SURVIVORS AND NON-SURVIVORS WITH CANINE PARVOVIRAL ENTERITIS

Very few significant differences were found in the red blood cell (RBC) parameters between survivors and non-survivors. Only the red cell count (RCC) on admission and the mean corpuscular haemoglobin concentration (MCHC) on admission were significantly higher in the survivors than the non-survivors.

Comparison of groups, by means of the Fisher's exact test, with respect to all RBC parameters is summarised in table 4.14. The data presented in the table are the number and percentage of dogs in each group with a value higher or lower then the reference interval for that specific RBC parameter.

Table 4.14 Comparison between groups (survivors vs. non-survivors) that had abnormal values (either higher or lower than the normal reference values) with respect to all RBC parameters (Fisher's exact test). Shaded values indicate significance ($p<0.05$).

Parameter	Day	Survivors (percentage and number of dogs with RBC parameter outside the indicated range)	Non-survivors (percentage and number of dogs with RBC parameter outside the indicated range)	Fisher Exact test (p-value)
Haemoglobin (Hb)	Day 0	46% (23/50)	80% (8/10)	0.082
(g/l)	Day 1	62.8% (32/51)	66.7% (6/9)	1.000
(Hb < 120 / > 180)	Day 2	70.6% (36/51)	87.5% (7/8)	0.427
	Day 3	71.4% (30/42)	100% (2/2)	1.000
	Day 4	71.9% (23/32)	100% (1/1)	1.000
	Day 5	83.3% (20/24)	100% (1/1)	1.000
Red Cell Count (RCC)	Day 0	32% (16/50)	70% (7/10)	0.035
($\times 10^{12}$/l)	Day 1	64.7% (33/51)	66.7% (6/9)	1.000
(RCC < 5.5 / > 8.5)	Day 2	68.6% (35/51)	87.5% (7/8)	0.417
	Day 3	73.8% (31/42)	100% (2/2)	1.000
	Day 4	75% (24/32)	100% (1/1)	1.000
	Day 5	75% (18/24)	100% (1/1)	1.000
Haematocrit (Ht)	Day 0	54% (27/50)	80% (8/10)	0.171
(l/l)	Day 1	66.7% (34/51)	55.6% (5/9)	0.706
(Ht < 0.37 / > 0.55)	Day 2	76.5% (39/51)	87.5% (7/8)	0.671
	Day 3	81% (34/42)	100% (2/2)	1.000
	Day 4	78.1% (25/32)	100% (1/1)	1.000
	Day 5	87.5% (21/24)	100% (1/1)	1.000

Parameter	Day	Survivors (percentage and number of dogs with RBC parameter outside the indicated range)	Non-survivors (percentage and number of dogs with RBC parameter outside the indicated range)	Fisher Exact test (p-value)
Mean Corpuscular Volume (MCV) (fl) (MCV < 60 / > 77)	Day 0	26% (13/50)	20% (2/10)	1.000
	Day 1	27.5% (14/51)	33.3% (3/9)	0.704
	Day 2	29.4% (15/51)	37.5% (3/8)	0.690
	Day 3	28.6% (12/42)	0% (0/2)	1.000
	Day 4	34.4% (11/32)	0% (0/1)	1.000
	Day 5	37.5% (9/24)	0% (0/1)	1.000
Mean Corpuscular Haemoglobin Concentration (MCHC) (g/dl rbc) (MCHC < 32 / > 36)	Day 0	0% (0/50)	20% (2/10)	0.025
	Day 1	7.8% (4/51)	11.1% (1/9)	0.570
	Day 2	5.9% (3/51)	12.5% (1/8)	0.451
	Day 3	2.4% (1/42)	0% (0/2)	1.000
	Day 4	no values	no values	no p-value
	Day 5	no values	no values	no p-value
Red Cell Distribution Width (RDW) (%) (RDW < 15.5 / > 19.5)	Day 0	30% (15/50)	20% (2/10)	0.709
	Day 1	39.2% (20/51)	22.2% (2/9)	0.464
	Day 2	41.2% (21/51)	25% (2/8)	0.464
	Day 3	38.1% (16/42)	50% (1/2)	1.000
	Day 4	46.9% (15/32)	100% (1/1)	0.485
	Day 5	41.7% (10/24)	0% (0/1)	1.000

On day 0, groups (survivors vs. non-survivors) differed significantly (p=0.035; 32% vs. 70%) with respect to the percentage of animals with RCC < 5.5×10^{12}/l. On this day groups also differed significantly (p=0.025; 0% vs. 20%) with respect to the percentage of animals with MCHC < 32 g/dl rbc.

For haemoglobin (Hb), groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.15 below). Mean was reported as adjusted to a given baseline value (i.e. 128.667). Here baseline value was used as covariate.

Table 4.15 ANCOVA results when comparing groups with respect to Hb. Adjusted mean baseline value of Hb on day 0=128.667.

Parameter		Survivors Mean	Non-survivors Mean	p-Value
Hb Day 1 compared to Day 0	Unadjusted	- 16.180	- 7.375	0.2379
	Adjusted	- 15.450	- 9.187	
Hb Day 2 compared to Day 0	Unadjusted	- 20.800	- 13.286	0.9087
	Adjusted	- 19.873	- 20.495	
Hb Day 3 compared to Day 0	Unadjusted	- 25.293	- 15.000	0.7681
	Adjusted	- 23.151	- 19.456	
Hb Day 4 compared to Day 0	Unadjusted	- 27.065	- 13.000	0.6964
	Adjusted	- 25.420	- 17.723	
Hb Day 5 compared to Day 0	Unadjusted	- 26.304	- 31.000	0.6934
	Adjusted	- 27.373	- 35.373	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) did not differ significantly with respect to change from baseline in Hb and the latter means were adjusted to a baseline value of 128.667.

For red cell count (RCC), groups were compared with respect to change from baseline in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.16 below). Mean was reported as adjusted to a given baseline value (i.e. 6.054). Here baseline value was used as covariate.

Table 4.16 ANCOVA results when comparing groups with respect to RCC. Adjusted mean baseline value of RCC on day 0=6.054.

Parameter		Survivors Mean	Non-survivors Mean	p-Value
RCC Day 1 compared to Day 0	Unadjusted	- 0.809	- 2.266	0.0878
	Adjusted	- 0.776	- 0.354	
RCC Day 2 compared to Day 0	Unadjusted	- 0.995	- 0.577	0.8993
	Adjusted	- 0.949	- 0.918	
RCC Day 3 compared to Day 0	Unadjusted	- 1.229	- 0.485	0.7177
	Adjusted	- 1.091	- 0.890	
RCC Day 4 compared to Day 0	Unadjusted	- 1.290	- 0.190	0.6819
	Adjusted	- 1.171	- 0.815	
RCC Day 5 compared to Day 0	Unadjusted	- 1.279	- 1.060	0.6910
	Adjusted	- 1.239	- 1.603	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) did not differ significantly with respect to change from baseline in RCC and the latter means were adjusted to a baseline value of 6.054.

For haematocrit (Ht), groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.17 below). Mean was reported as adjusted to a given baseline value (i.e. 0.376). Here baseline value was used as covariate.

Table 4. 17 ANCOVA results when comparing groups with respect to Ht. Adjusted mean baseline value of Ht on day 0=0.376.

Parameter		Survivors Mean	Non-survivors Mean	p-Value
Ht Day 1 compared to Day 0	Unadjusted	- 0.048	- 0.010	0.0736
	Adjusted	- 0.045	- 0.017	
Ht Day 2 compared to Day 0	Unadjusted	- 0.060	- 0.031	0.8806
	Adjusted	- 0.057	- 0.055	
Ht Day 3 compared to Day 0	Unadjusted	- 0.075	- 0.035	0.7138
	Adjusted	- 0.067	- 0.054	
Ht Day 4 compared to Day 0	Unadjusted	- 0.080	- 0.020	0.6276
	Adjusted	- 0.074	- 0.045	
Ht Day 5 compared to Day 0	Unadjusted	- 0.081	- 0.080	0.7397
	Adjusted	- 0.083	- 0.103	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) did not differ significantly with respect to change from baseline in Ht and the latter means were adjusted to a baseline value of 0.376.

For mean corpuscular volume (MCV), groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.18 below). Mean was reported as adjusted to a given baseline value (i.e. 62.195). Here baseline value was used as covariate.

Table 4.18 ANCOVA results when comparing groups with respect to MCV. Adjusted mean baseline value of MCV on day 0=62.195. Shaded values indicate significance ($p<0.05$).

Parameter		Survivors Mean	Non-survivors Mean	p-Value
MCV Day 1 compared to Day 0	Unadjusted	0.212	0.887	0.0202
	Adjusted	0.213	0.879	
MCV Day 2 compared to Day 0	Unadjusted	0.164	0.686	0.2631
	Adjusted	0.164	0.682	
MCV Day 3 compared to Day 0	Unadjusted	0.051	- 1.350	0.2228
	Adjusted	0.047	- 1.315	
MCV Day 4 compared to Day 0	Unadjusted	- 0.458	- 3.000	0.1513
	Adjusted	- 0.466	- 2.816	
MCV Day 5 compared to Day 0	Unadjusted	- 0.926	- 2.300	0.7885
	Adjusted	- 1.094	- 1.619	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) differed significantly ($p=0.0202$) with respect to change from baseline in MCV on day 1 i.e. 0.213 vs. 0.879 (meaning that survivors showed a significant increase in MCV on day 1 compared to day 0 when compared to non-survivors) and the latter means were adjusted to a baseline value of 62.195.

For mean corpuscular haemoglobin concentration (MCHC), groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.19 below). Mean was reported as adjusted to a given baseline value (i.e. 34.263). Here baseline value was used as covariate.

Table 4.19 ANCOVA results when comparing groups with respect to MCHC. Adjusted mean baseline value of MCHC on day 0=34.263. Shaded values indicate significance (p<0.05).

Parameter		Survivors Mean	Non-survivors Mean	p-Value
MCHC Day 1 compared to Day 0	Unadjusted	0.050	- 1.113	0.0021
	Adjusted	0.036	- 0.916	
MCHC Day 2 compared to Day 0	Unadjusted	- 0.060	- 0.914	0.0192
	Adjusted	- 0.070	- 0.849	
MCHC Day 3 compared to Day 0	Unadjusted	0.085	- 0.750	0.4892
	Adjusted	0.052	- 0.379	
MCHC Day 4 compared to Day 0	Unadjusted	0.155	- 1.200	0.5349
	Adjusted	0.081	- 0.427	
MCHC Day 5 compared to Day 0	Unadjusted	0.296	- 0.700	0.9098
	Adjusted	0.142	0.249	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) differed significantly (p=0.0021) with respect to change from baseline in MCHC on day 1 i.e. 0.036 vs. – 0.916 (meaning that survivors showed a significant increase in MCHC on day 1 compared to day 0 when compared to non-survivors) and the latter means were adjusted to a baseline value of 34.263.

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) differed significantly (p=0.0192) with respect to change from baseline in MCHC on day 2 i.e. – 0.070 vs. – 0.849 (meaning that survivors showed a significant increase in MCHC on day 2 compared to day 0 when compared to non-survivors) and the latter means were adjusted to a baseline value of 34.263.

For red cell distribution width (RDW), groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.20 below). Mean was reported as adjusted to a given baseline value (i.e. 17.107). Here baseline value was used as covariate.

Table 4. 20 ANCOVA results when comparing groups with respect to RDW. Adjusted mean baseline value of RDW on day 0=17.107.

Parameter		Survivors Mean	Non-survivors Mean	p-Value
RDW Day 1 compared to Day 0	Unadjusted	- 0.342	- 0.200	0.952
	Adjusted	- 0.321	- 0.294	
RDW Day 2 compared to Day 0	Unadjusted	- 0.428	- 0.229	0.9119
	Adjusted	- 0.402	- 0.349	
RDW Day 3 compared to Day 0	Unadjusted	- 0.615	- 0.150	0.8064
	Adjusted	- 0.534	- 0.365	
RDW Day 4 compared to Day 0	Unadjusted	- 0.629	0.400	0.5125
	Adjusted	- 0.594	0.130	
RDW Day 5 compared to Day 0	Unadjusted	- 0.435	1.500	0.3834
	Adjusted	- 0.233	0.931	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) did not differ significantly with respect to change from baseline in RDW and the latter means were adjusted to a baseline value of 17.107.

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4.4 EVALUATION OF THE PLATELET COUNT IN SURVIVORS AND NON-SURVIVORS WITH CANINE PARVOVIRAL ENTERITIS

The box plot of the platelets (PLT) displayed in fig. 4.14 below, indicates the distribution of the various values on a specific day. Significant differences were found in the platelet count between the survivors and non-survivors (see fig. 4.15 below).

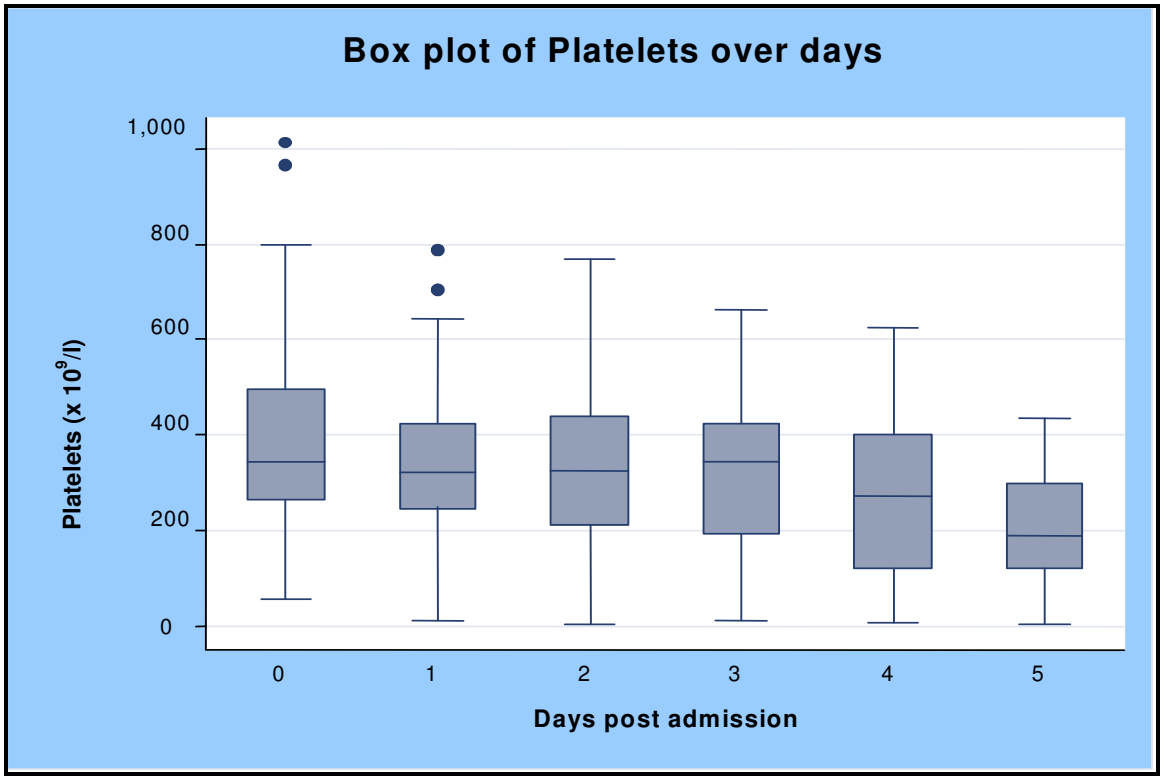

Figure 4.14 Box plot (representing the interquartile range) of the PLT over days in puppies (survivors and non-survivors) with canine parvoviral enteritis for the first 5 days post admission. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

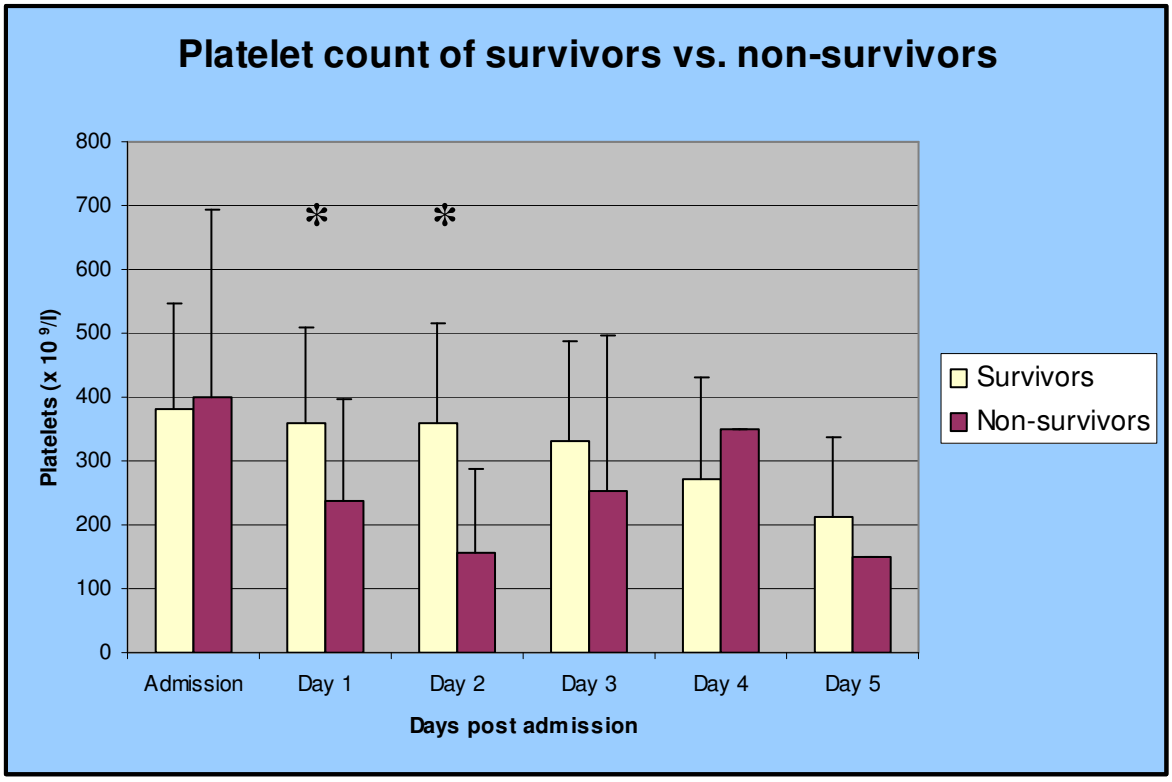

Figure 4.15 Comparison of groups (survivors vs. non-survivors) with respect to PLT over the first 5 days post admission. The * indicate significance ($p<0.05$). The "whiskers" represent standard deviation. **Note:** the number of dogs evaluated decreased by day as indicated in table 4.1.

Comparison of groups, by means of the Fisher's exact test, with respect to PLT is summarised in table 4.21. The data presented in the table are the number and percentage of dogs in each group with PLT less than 200×10^9/l.

Table 4. 21 Comparison between groups (survivors vs. non-survivors) with respect to PLT that is less than 200×10^9/l (Fisher's exact test). Shaded values indicate significance ($p<0.05$).

Day	Survivors	Non-survivors	Fisher Exact test (p-value)
Day 0	6% (3/50)	30% (3/10)	0.052
Day 1	7.8% (4/51)	44.4% (4/9)	0.013
Day 2	15.7% (8/51)	62.5% (5/8)	0.010
Day 3	23.8% (10/42)	50% (1/2)	0.442
Day 4	40.6% (13/32)	0% (0/1)	1.000
Day 5	54.2% (13/24)	100% (1/1)	1.000

On day 1, groups (survivors vs. non-survivors) differed significantly (p=0.013; 7.8% vs. 44.4%) with respect to the percentage of animals with PLT $< 200 \times 10^9$/l.

On day 2, groups (survivors vs. non-survivors) differed significantly (p=0.010; 15.7% vs. 62.5%) with respect to the percentage of animals with PLT $< 200 \times 10^9$/l.

For platelet count (PLT), groups were compared with respect to change from baseline (admission) in an analysis of covariance (ANCOVA), with baseline as covariant (see table 4.22 below). Mean was reported as adjusted to a given baseline value (i.e. 384.562). Here baseline value was used as covariate.

Table 4. 22 ANCOVA results when comparing groups with respect to PLT. Adjusted mean baseline value of PLT on day 0=384.562. Shaded values indicate significance (p<0.05).

Parameter		Survivors Mean	Non-survivors Mean	p-Value
PLT Day 1 compared to Day 0	Unadjusted	- 17.432	- 91.763	0.0037
	Adjusted	- 18.225	- 112.778	
PLT Day 2 compared to Day 0	Unadjusted	- 18.710	- 158.757	0.0005
	Adjusted	- 19.956	- 194.576	
PLT Day 3 compared to Day 0	Unadjusted	- 35.515	- 23.70	0.7175
	Adjusted	- 42.803	- 79.790	
PLT Day 4 compared to Day 0	Unadjusted	- 71.781	58.0	0.553
	Adjusted	- 93.828	0.180	
PLT Day 5 compared to Day 0	Unadjusted	- 97.926	- 143.0	0.6235
	Adjusted	- 160.309	- 225.051	

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) differed significantly (p=0.0037) with respect to change from baseline in PLT on day 1 i.e. –18.225 vs. –112.778 (meaning that non-survivors showed a significant decrease in PLT on day 1 compared to day 0 when compared to survivors) and the latter means were adjusted to a baseline value of 384.562.

In an analysis of covariance (ANCOVA) groups (survivors; non-survivors) differed significantly (p=0.0005) with respect to change from baseline in PLT on day 2 i.e. –19.956 vs. –194.576 (meaning that non-survivors showed a significant decrease in PLT on day 2 compared to day 0 when compared to survivors) and the latter means were adjusted to a baseline value of 384.562.

4.5 COMPARISON OF SIMILARITY IN NUMBERS OF DIFFERENT LEUKOCYTE TYPES BETWEEN CENTRAL AND PERIPHERAL BLOOD

From this study it was shown that, except for segmented and band neutrophils, the overall comparison of the specific leukocyte types were relatively good on the central- and peripheral blood (see table 4.23 below).

Table 4. 23 Comparison between the specific blood leukocyte types on central and peripheral blood.

Parameter	Day	Central blood		Peripheral blood		Comparison	
		Value (number of central samples/total number of samples)	Percentage	Value (number of peripheral samples/total number of samples)	Percentage	Value (peripheral samples similar to central/total number of samples)	Percentage
Neutrophil mature (Neut)	Day 0	40/60	66.7%	37/56	66.1%	35/56	62.5%
(Neut < 3.0 × 10^9/l)	Day 1	44/60	73.3%	47/60	78.3%	43/60	71.7%
	Day 2	42/59	71.2%	42/58	72.4%	40/58	68.9%
	Day 3	23/44	52.3%	24/44	54.6%	19/44	43.2%
	Day 4	12/33	36.4%	12/33	36.4%	12/33	36.4%
	Day 5	5/25	20%	7/25	28%	4/25	16.0%
Neutrophil band (Bands)	Day 0	4/60	6.7%	9/56	16.1%	2/56	3.6%
(Bands = 0)	Day 1	17/60	28.3%	19/60	31.7%	10/60	16.7%
	Day 2	14/59	23.7%	22/58	37.9%	10/58	17.2%
	Day 3	2/44	4.6%	6/44	13.6%	1/44	2.3%
	Day 4	3/33	9.1%	6/33	18.2%	1/33	3.0%
	Day 5	1/25	4.0%	4/25	16.0%	1/25	4.0%
Lymphocytes (Lymph)	Day 0	45/60	75%	44/56	78.6%	46/56	82.1%
(Lymph < 1.0 × 10^9/l)	Day 1	28/60	46.7%	35/60	58.3%	50/60	83.3%
	Day 2	26/59	44.1%	29/58	50.0%	46/58	79.3%
	Day 3	6/44	13.6%	16/46	34.8%	33/44	75.0%
	Day 4	3/33	9.1%	12/35	34.3%	16/33	48.5%
	Day 5	2/25	8.0%	6/27	22.2%	20/25	80.0%
Monocytes (Mono)	Day 0	13/60	21.7%	8/56	14.3%	35/56	62.5%
(Mono < 0.15 × 10^9/l)	Day 1	10/60	16.7%	7/60	11.7%	37/60	61.7%
	Day 2	15/59	25.4%	6/58	10.3%	31/58	53.5%
	Day 3	7/44	15.9%	1/44	2.3%	26/44	59.1%
	Day 4	3/33	9.1%	0/25	0%	19/32	59.4%
	Day 5	0/25	0%	0/25	0%	11/25	44.0%

Parameter	Day	Central blood		Peripheral blood		Comparison	
		Value (number of central samples/total number of samples)	Percentage	Value (number of peripheral samples/total number of samples)	Percentage	Value (peripheral samples similar to central/total number of samples)	Percentage
Eosinophils (Eos)	Day 0	45/60	75.0%	44/56	78.6%	46/56	82.1%
(Eos < 0.1 × 10^9/l)	Day 1	31/60	51.7%	35/60	58.3%	51/60	85.0%
	Day 2	27/59	45.8%	28/58	48.3%	46/58	79.3%
	Day 3	26/44	59.1%	27/44	61.2%	36/44	81.8%
	Day 4	24/33	72.7%	25/32	78.1%	25/32	78.1%
	Day 5	18/25	72.0%	21/25	84.0%	22/25	88.0%

On day 0, 62.5% (35/56) of cases (central and peripheral) compared similarly with respect to Neut < 3.0×10^9/l; Bands did not compare similarly (3.6%; 2/56) with respect to Bands = 0; 82.1% (46/56) of cases (central and peripheral) compared similarly with respect to Lymph < 1.0×10^9/l; 62.5% (35/56) of cases (central and peripheral) compared similarly with respect to Mono < 0.15×10^9/l; and 82.1% (46/56) of cases (central and peripheral) compared similarly with respect to Eos < 0.1×10^9/l.

On day 1, 71.7% (43/60) of cases (central and peripheral) compared similarly with respect to Neut < 3.0×10^9/l; Bands did not compare similarly (16.7%; 10/60) with respect to Bands = 0; 83.3% (50/60) of cases (central and peripheral) compared similarly with respect to Lymph < 1.0×10^9/l; 61.7% (37/60) of cases (central and peripheral) compared similarly with respect to Mono < 0.15×10^9/l; and 85.0% (51/60) of cases (central and peripheral) compared similarly with respect to Eos < 0.1×10^9/l.

On day 2, 68.9% (40/58) of cases (central and peripheral) compared similarly with respect to Neut < 3.0×10^9/l; Bands did not compare similarly (17.2%; 10/58) with respect to Bands = 0; 79.3% (46/58) of cases (central and peripheral) compared similarly with respect to Lymph < 1.0×10^9/l; 53.5% (31/58) of cases (central and peripheral) compared similarly with respect to Mono < 0.15×10^9/l; and 79.3% (46/58) of cases (central and peripheral) compared similarly with respect to Eos < 0.1×10^9/l.

On day 3, Neut did not compare similarly (43.2%; 19/44) with respect to Neut < 3.0×10^9/l; Bands did not compare similarly (2.3%; 1/44) with respect to Bands = 0; 75.0% (33/44) of cases (central and peripheral) compared similarly with respect to Lymph <

1.0 × 10^9/l; 59.1% (26/44) of cases (central and peripheral) compared similarly with respect to Mono < 0.15 × 10^9/l; and 81.8% (36/44) of cases (central and peripheral) compared similarly with respect to Eos < 0.1 × 10^9/l.

On day 4, Neut did not compare similarly (36.4%; 12/33) with respect to Neut < 3.0 × 10^9/l; Bands did not compare similarly (3.0%; 1/33) with respect to Bands = 0; Lymph did not compare similarly (48.5%; 16/33) with respect to Lymph < 1.0 × 10^9/l; 59.4% (19/32) of cases (central and peripheral) compared similarly with respect to Mono < 0.15 × 10^9/l; and 78.1% (25/32) of cases (central and peripheral) compared similarly with respect to Eos < 0.1 × 10^9/l.

On day 5, Neut did not compare similarly (16.0%; 4/25) with respect to Neut < 3.0 × 10^9/l; Bands did not compare similarly (4.0%; 1/25) with respect to Bands = 0; 80.0% (20/25) of cases (central and peripheral) compared similarly with respect to Lymph < 1.0 × 10^9/l; Mono did not compare similarly (44.0%; 11/25) with respect to Mono < 0.15 × 10^9/l; and 88.0% (22/25) of cases (central and peripheral) compared similarly with respect to Eos < 0.1 × 10^9/l.

4.6 HISTOPATHOLOGY

A complete data set is provided in Appendix M.

All the puppies that died (9), or were euthanized (2), were subjected to a full post mortem (PM) and histopathological examination. Changes in the following organs are described in this chapter: lymph nodes (peripheral and mesenteric); thymus; spleen and bone marrow. These organs are discussed in this chapter because they are relevant in the objectives of this study.

For more detail of the above mentioned organs or a description of other organs see **Appendix M**.

4.6.1 LYMPH NODES

Macroscopically, only one case (1/11) had marked lymph node congestion.
Histopathological examination of the peripheral lymph nodes showed that 73% (8/11) had depleted or no lymphoid follicles in the lymph nodes and 73% (8/11) had moderate to severe depletion of cortical lymphocytes (cortical atrophy).

Of the mesenteric lymph nodes, 82% (9/11) had depleted or no lymphoid follicles in the lymph nodes and 91% (10/11) had moderate to severe depletion of cortical lymphocytes (cortical atrophy). See fig.4.16 for histopathology sections of normal lymph node tissue and lymph node tissue affected by CPV.

4.6.2 THYMUS

Moderate to severe thymic atrophy was observed macroscopically in 73% (8/11) of the cases, often presenting just as a few small nodules scattered within a gelatinous mediastinum.

Histopathology of the thymus showed that 91% (10/11) of the cases had moderate to massive loss of cortical lymphocytes and severe collapse of the remaining stroma i.e. loss of the normal architecture of the thymus, and most of the lobules were made up of mostly supporting tissue. See fig.4.17 for histopathology slides of normal thymic tissue and thymic tissue affected by CPV.

4.6.3 SPLEEN

No macroscopic changes were found in the spleens of any cases.

Histopathology of the spleen showed that 82% (9/11) of cases had moderate to severe white pulp depletion or atrophy, which indicated near total loss of small lymphocytes within the white pulp. See fig.4.18 for histopathology slides of normal splenic tissue and splenic tissue affected by CPV.

4.6.4 BONE MARROW

Macroscopically, marked congestion of the bone marrow was observed in 45% (5/11) of the cases and 27% (3/11) of the cases showed gelatinous changes in the bone marrow.

Histopathological examination of the bone marrow showed moderate to severe hypocellularity to complete atrophy in 82% (9/11) of the cases. In most of the cases both the myeloid and erythroid series were equally affected and in some of the cases the megakaryocytes were low in number or even absent. See fig.4.19 for histopathology slides of normal bone marrow tissue and bone marrow tissue affected by CPV.

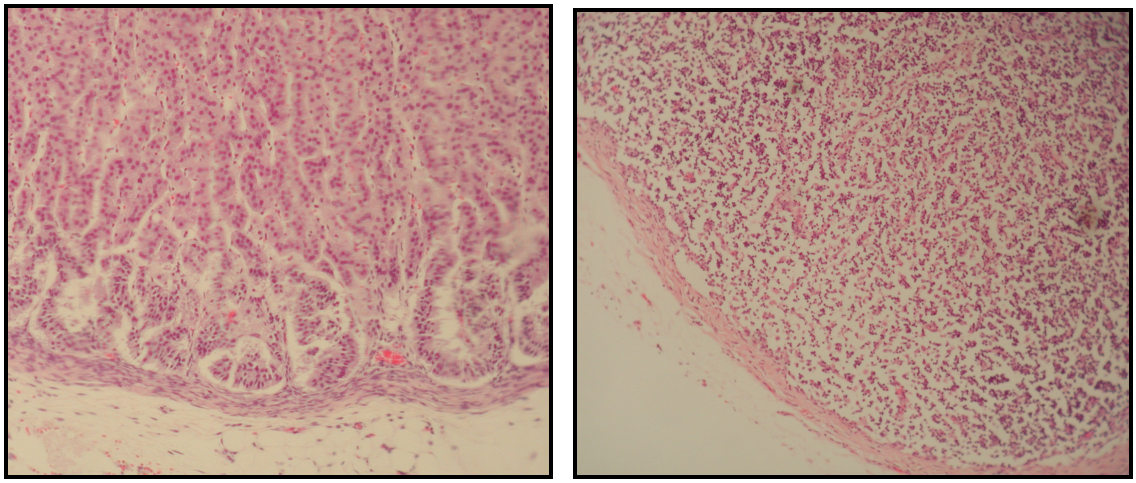

Figure 4.16 Histopathology sections of the cortex of a normal lymph node (left) and that of a lymph node affected by CPV (right). Note the cortical depletion of lymphocytes in the lymph node affected by CPV. (H&E processing; 10× objective was used).

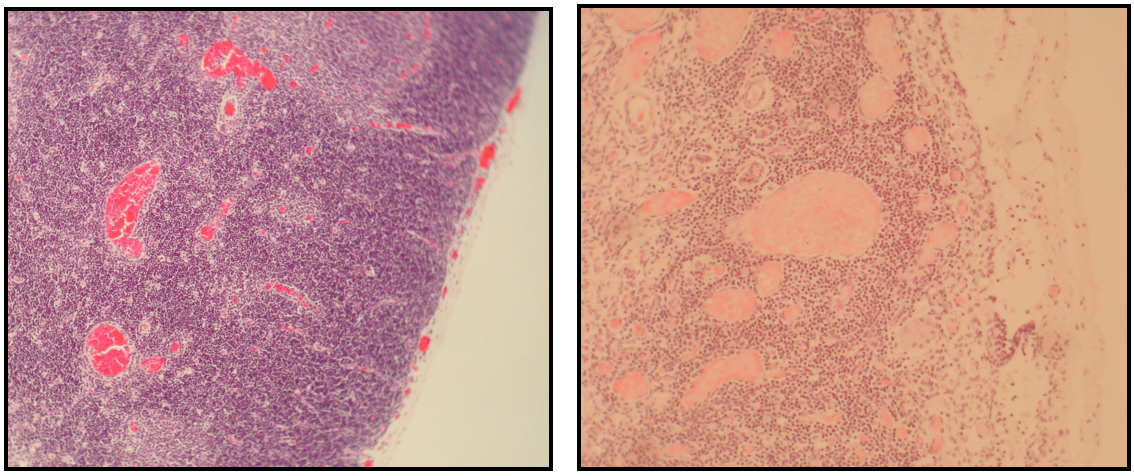

Figure 4. 17 Histopathology sections of the cortex of a normal thymus (left) and that of a thymus affected by CPV (right). Note the massive loss of cortical lymphocytes in the affected thymus, as well as the loss of the normal thymic architecture. (H&E processing; 10× objective was used).

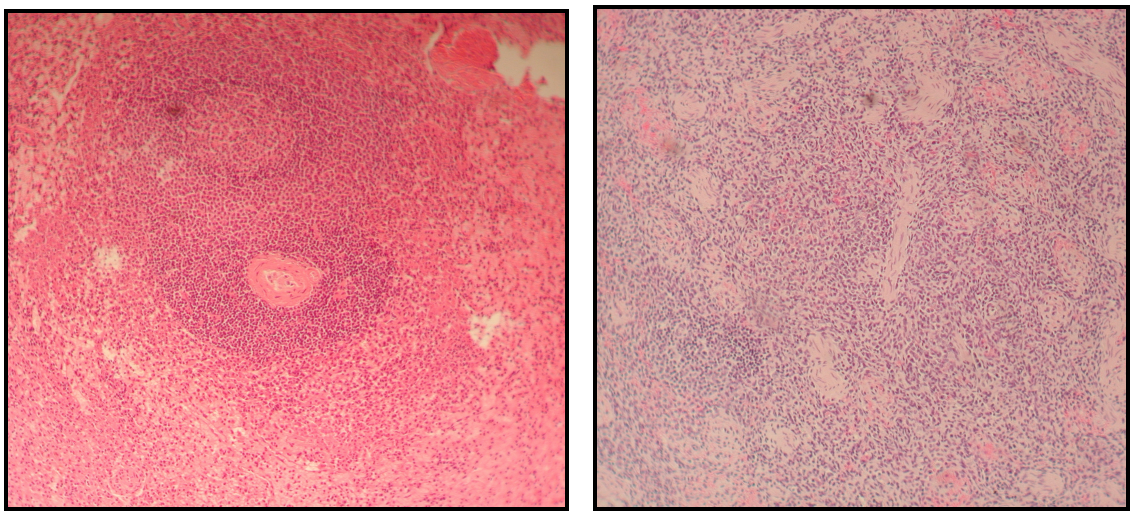

Figure 4. 18 Histopathology sections of normal spleen (left) and that of spleen affected by CPV (right). Note the marked white pulp depletion of the spleen affected by CPV. (H&E processing, 10× objective was used).

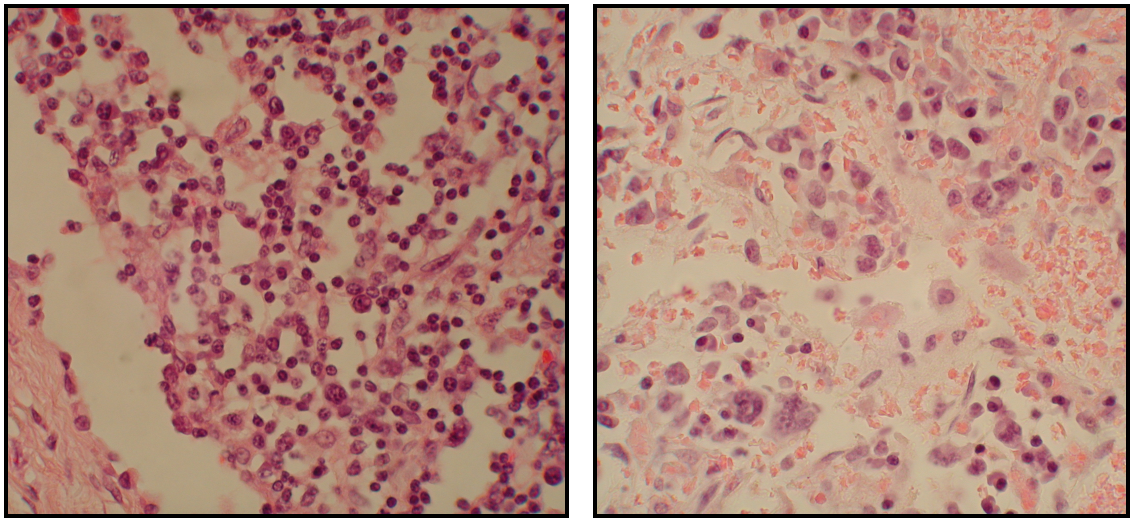

Figure 4. 19 Histopathology sections of normal bone marrow (left) and that of bone marrow affected by CPV (right). Note the marked hypocellularity of the bone marrow affected by CPV. (H&E processing; 50× oil objective was used).

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CHAPTER 5 DISCUSSION

Parvovirus remains an important cause of morbidity and mortality in young dogs. Despite the availability of an effective vaccine, the only treatment available is supportive care for affected dogs. Without treatment, the survival rate of dogs experimentally infected with CPV is 9.1%, but with supportive care the survival rate of clinically affected dogs is 64 – 79%.[35] Intensive care improves survival; however, it is usually very costly. This study represents an attempt to document the possibility of determining a prognosis early in the course of the disease in puppies infected with canine parvovirus (CPV) enteritis, in order to avoid high costs, by using the total white blood cell (WBC) count and the differential leukocyte counts.

Parvoviruses (Parvoviridae), specifically canine parvovirus 2 (CPV-2) is a significant worldwide pathogen in dogs and the most common cause of viral enteritis in this canine species[36]. They are small, non-enveloped, single-stranded DNA viruses that replicate in rapidly dividing cells (i.e. enterocytes, bone marrow precursor cells, lymphoid tissue and myocardiocytes)[1-6,36].

Most adult dogs are immune to the disease, either via natural infection or immunization. Immunity is long-lived, even lifelong, which leaves only a pool of susceptible puppies, whose ages approximate between 6 weeks and 6 months.[1,36] Factors predisposing puppies to parvoviral infection include lack of protective immunity, unsanitary or overcrowded environments and endoparasitism.[1,3,9,36] Certain breeds are also at increased risk for severe CPV enteritis, including the Rottweiler, Doberman Pinscher, American Staffordshire terrier, Labrador retriever and German Shepherd dog.[36]

Total WBC counts during CPV enteritis are generally characterised as being low to severely leukopaenic, a phenomenon that is widely accepted to be due to destruction of haematopoietic progenitor cells of the various leukocyte types primarily in the bone marrow, and also in other lymphoproliferative organs i.e. the thymus, lymph nodes and spleen, resulting in inadequate compensation for the massive demand for leukocytes (specifically neutrophils) in the inflamed gastrointestinal tract. Lymphopaenia, and in

severe cases panleukopaenia, occur secondary to lymphoid necrosis and destruction of myeloproliferative cells in the bone marrow.[36] The high mortality in dogs with continuing severe leukopaenia can largely be attributed to their high susceptibility to secondary bacterial infections that can lead to septicaemia.

As was stated in the introductory chapter, leukocyte responses are seldom pathognomonic for a specific disease, but can provide clinical information to establish a list of differential diagnoses, to assess a patient's response to treatment or to suggest, as was shown in this study, a fairly accurate prognosis. Another very important point that was also gained from the results of this study is that it is necessary to perform multiple leukograms over a period of several days in order to establish a specific response pattern over time.[16]

Several reports have been published over the years regarding the leukopaenia seen in canine parvoviral enteritis as well as the underlying causes, but no specific publications have appeared with regards to the use of the WBC and the changes in different leukocyte types as indicators of prognosis. Woods (1980)[7], Potgieter (1981)[28] and O'Sullivan (1984)[12] all agreed that leukopaenia was associated with a poor prognosis or with patients that needed aggressive treatment. Potgieter found that the leukopaenia was mostly due to a severe neutropaenia and that the lymphocytes only dropped to 50% of normal values, therefore he came to the conclusion that the neutrophils are the most important leukocyte type to monitor. However, some authors have disagreed with the conclusions of the above mentioned authors. Mason (1987)[29] found that the leukopaenia should not be used as the sole criterion to determine prognosis, and McCaw (1996)[34] found that neutropaenia (even when very severe) was not a significant prognostic indicator. None of these latter authors did serial haematology determinations on a daily basis on their patients.

In this study there were several statistically significant differences found between those puppies that did survive and those that did not survive, in the WBC as well as most of the specific leukocyte types.

5.1 THE EVALUATION OF TOTAL WHITE BLOOD CELL COUNT IN SURVIVORS AND NON-SURVIVORS OF CANINE PARVOVIRUS ENTERITIS

Because neutrophils are the most numerous leukocytes in dog blood, it is said that a change in the neutrophil count will usually result in a change in the total WBC count.[16] But it has been shown in numerous studies over the years that in CPV the severe leukopaenia can be attributed to a marked decrease in all the leukocyte types, specifically neutrophils, lymphocytes, monocytes and eosinophils.

The use of granulocyte colony-stimulating factor (G-CSF) has been advocated for the treatment of dogs with leukopaenia. G-CSF is a cytokine produced by the bone marrow and endothelial cells (amongst others), that functions to release granulocytes from the storage pool in the bone marrow, shorten neutrophil maturation time and enhance granulopoiesis. A study by Mischke et. al. [37] found no benefit in using this product in CPV. Reasons postulated for the lack of a treatment effect included depletion of the storage pool and of more mature progenitor cells in the bone marrow, lack of G-CSF receptors secondary to granulopoietic progenitor cell depletion, and a lag time of 2-3 days before the effect of G-CSF on accelerated maturation of progenitor cells is measurable in the blood.[36,37]

Statistical analysis of the data from this study has shown that, not only were a large percentage of CPV cases leukopaenic, but also that there were statistically significant differences between the puppies that survived and those that did not survive in the various leukocyte types, specifically the lymphocyte, monocyte and eosinophil counts.

In the cases that survived, the mean WBC did not drop below 4.5×10^9/l, a cut-off value reported by Woods et. al.[7] In fact, in these cases the mean WBC never dropped below 5.0×10^9/l. In the cases that survived, the WBC also started rising within 24 – 48 hours after admission and often resulted in a rebound leukocytosis, as high as 84.0×10^9/l in some cases. In the cases that died due to the disease (excluding the 2 cases that were euthanized), the mean WBC never went higher than 2.0×10^9/l. The mean values in these cases at admission, 24 hours and 48 hours post admission were 2.8-, 0.7- and 0.8×10^9/l respectively.

The most significant differences, between the survivors and non-survivors, were seen on day 1 (24 hours post admission) and day 2 (48 hours post admission) with p=0.02 and p=0.002 respectively. This means that as early as 24 – 48 hours post admission, based on the WBC, a fairly accurate prediction can be made with regards to the outcome of these cases. If the WBC remains less than 2.0×10^9/l 24 – 48 hours post admission, the chances that the patient will survive or that it will leave the hospital within 7 days is poor.

Besides the differences by day there were also significant differences, between the survivors and non-survivors, with regards to the change over time from the day of admission. The most significant findings were in the first 24 – 48 hours post admission where the WBC of the puppies that survived was significantly higher than their WBC at admission. For the puppies that did not survive there was no significant change (increase) in the WBC from the WBC at admission until death.

5.2 EVALUATION OF SPECIFIC LEUKOCYTE TYPES IN SURVIVORS AND NON-SURVIVORS OF CANINE PARVOVIRUS ENTERITIS

5.2.1 NEUTROPHILS: Segmented and band cells

Neutrophils are one of the first lines of the host defence against invading pathogens, particularly bacteria. They are produced in the bone marrow and can be divided into two compartments. The proliferation (mitotic) compartment and the maturation and storage compartment where neutrophil metamyelocytes, bands, and segmenters are stored for a variable period of time while the cells undergo maturation. From here mature neutrophils are released into the blood in an age-ordered fashion.[4,16-18] Neutrophils are distributed in one of two dynamic sub pools in the blood: (1) the circulating pool consisting of cells in the mainstream of circulation, usually sampled by venipuncture, and (2) the marginal pool consisting of cells that move slowly along the endothelial surface of small capillaries and venules because of reduced blood flow and adhesion molecules on neutrophils and endothelial cells. The distribution of neutrophils between the circulating and marginal pools is approximately 1:1 in dogs.[4,16-18]

The most common pathophysiological mechanisms for neutropaenia are one or a combination of the following: (1) deficient neutrophil production in the bone marrow; (2) a shift in neutrophils from the circulating to the marginal neutrophil pool; and (3) emigration of neutrophils from the blood into the tissues at a rate that exceeds neutrophil replacement into the blood from the bone marrow.[16 4,17,18,21] In a retrospective study conducted by Brown et. al[38] causes for neutropaenia were grouped into six etiological categories, which included non-bacterial infectious disease; increased demand due to marked inflammation, bacterial sepsis, or endotoxaemia; drug-associated neutropaenia; primary bone-marrow disease; immune-mediated neutropaenia; and diseases of unclear etiology. This study showed that the largest single category associated with the development of neutropaenia was non-bacterial infectious disease, of which CPV accounted for almost 50% of all the cases.

Not only can the severe neutropaenia seen in CPV be attributed to the destruction of mitotically active myeloblasts in the bone marrow by a direct effect of the virus, but it can also be related to endotoxaemia and possible sepsis, as well as massive loss of neutrophils through the intestinal wall.[2,5,15,16,26,38,39] It has been reported that even in normal dogs there is considerable loss of neutrophils into the intestinal lumen and that this loss may be greatly increased in enteric disease.[5,15,26] The most important complication of severe neutropaenia is increased susceptibility to infection, which often results from organisms found as part of the normal flora of the gastrointestinal tract, nasopharynx and skin.[38]

In both the cases that survived and those that did not survive, the neutropaenia was quite profound, but there was no significant difference between the groups. In neither one of the groups did the segmented neutrophils increase to above 3.0×10^9/l for the first 24 – 48 hours. Although the group that survived did show an increase in band neutrophils (left-shift) by day 2 after admission, there was no statistically significant difference in band neutrophils between the cases that survived and those that did not survive. These findings confirm those of McCaw et. al.[34] who found that neutropaenia (even when very severe) was not a significant prognostic indicator in CPV enteritis. It has to be added that the various combinations of a regenerative or degenerative left shift together with neutrophilia as prognostic indicators were not examined in this study.

5.2.2 LYMPHOCYTES

Because lymphocytes are essential components in humoral and cell-mediated immune responses they are the second most numerous blood leukocyte found.[16] In immature animals certain T-lymphocyte precursors migrate from the bone marrow, where they develop from pluripotential stem cells, to the thymus, the central lymphoid organ, where they are educated and selected for self-tolerance.[22] T- and B-lymphocytes are involved in cell-mediated and humoral immunity respectively, by modulating the activity of other cells and by producing antibodies.[4,16,18,19]

Various mechanisms exists that can cause severe lymphopaenia: (1) Corticosteroid-associated lymphopaenia can develop from endogenous release of the hormone cortisol, as seen with severe stress, as well as exogenous administration of glucocorticoids; (2) lymphopaenia of acute infection may be the result of endogenous release of cortisol leading to redistribution of lymphocytes, the trapping of lymphocytes in draining lymph nodes to promote antigen contact, or the direct effect of viruses such as canine distemper and CPV leading to atrophy or destruction of lymphoid tissue; (3) loss, sequestration, or blockage of flow of lymphocyte-rich lymph may produce a lymphopaenia due to sequestration or loss of the recirculating lymphocyte population, as seen in protein-losing enteropathy.[4,16,19]

Previous studies have shown that, while local antibody production is detectable early in the course of CPV infection, it is the systemic humoral immune response that confers protection, as the virus enters the intestinal tract by way of the bloodstream as opposed to via the intestinal lumen. Circulating antibodies to CPV are usually detectable at the commencement of clinical signs, and peak during the course of clinical illness. Disease severity and duration is largely determined by the rapidity of this systemic immune response.[1,6,36]

In this study significant differences were found in the lymphocyte count over time between the cases that survived and those that did not survive. The most significant results were seen 24 – 72 hours post admission. On day 1 (12 – 24 hours post admission) all of the cases that did not survive had lymphocyte counts less than 1.0×10^9/l, compared with only 37% of those that survived ($p<0.001$). On day 2 (48 hours post admission) all of the cases that did not survive again had lymphocyte counts less

than 1.0×10^9/l, compared with only 35% of those that survived (p=0.001). On day 3 (72 hours post admission) all of the cases that did not survive still had lymphocyte counts less than 1.0×10^9/l, compared with only 9.5% of those that survived (p=0.016). These findings were even more significant than those of the WBC and probably contributed significantly to the leukopaenia, making the lymphocyte count a very important leukocyte parameter to observe during the course of the disease to determine prognosis in CPV enteritis.

The change over time of the lymphocyte count from the value at admission was also significantly different between survivors and non-survivors. The most significant findings were also for the first 24 – 72 hours when the lymphocyte count of the cases that survived was significantly higher than their lymphocyte count at admission. From day 1 (12 – 24 hours post admission) the mean lymphocyte count for the cases that survived was > 1.0×10^9/l. For the cases that did not survive there was no significant change (increase) in the lymphocyte count from admission until death. The mean lymphocyte count remained less than 1.0×10^9/l for the first 24 – 72 hours post admission.

5.2.3 MONOCYTES

Monocytes / tissue macrophages comprise the mononuclear phagocyte system (MPS) and function in the phagocytosis and digestion of cellular debris, micro-organisms and particulate matter; secretion of inflammatory mediators, and antigen presentation to lymphocytes. They share a common progenitor cell with neutrophils in the bone marrow but there are no maturation and storage pools for monocytes in the bone marrow.[4,18,23] Monocytosis is a common finding in acute and chronic inflammatory conditions, whereas monocytopaenia is rarely seen and of little importance.[4,16,18,23] Although monocytes and neutrophils share a common progenitor cell, the time it takes to produce a monocyte in the bone marrow (3 days) is a lot shorter than the time it takes to produce a neutrophil (6 days). Therefore, the recovery of monocyte numbers will precede that of neutrophils in the blood. This is especially true in the panleukopaenia seen secondary to CPV infection where monocytopaenia followed by a recovering monocyte count precedes the return of neutrophil production. Thus, monitoring the monocyte count in

the blood may be beneficial in evaluating the recovery from a leukopaenic state in patients suffering from CPV enteritis.[4,19,23]

Not only are monocyte numbers affected in CPV enteritis, but in a study done by Decaro et. al.[40] it appeared that the phagocytic ability of these cells was also affected. The study looked at 2 pups over 2 weeks that were naturally infected with canine parvovirus type 1 (CPV 1). The CPV 1 infection led to a marked reduction of monocyte phagocytosis in both pups. Also monocyte killing was impaired and in one pup this function was completely absent.

In this study more than 55% of the puppies (survivors and non-survivors) also did not show any microscopic morphologic evidence of monocyte activity during the 5 day period that was statistically analysed. This could be a factor contributing to why puppies infected with parvo virus are more susceptible to secondary infections.

There were significant differences in monocyte count over time between the cases that survived and those that did not survive the disease. The most significant differences were seen at admission, as well as on day 1 (12 – 24 hours post admission) and day 2 (48 hours post admission), similar to the WBC and the lymphocyte count. At admission 50% of the cases that did not survive had a monocyte count less than 0.15×10^9/l, compared with only 16% of the cases that survived ($p=0.031$). On day 1, 44% of the cases that did not survive had a monocyte count less than 0.15×10^9/l, compared with only 12% of the cases that survived ($p=0.034$). Day 2 was the most significant: 87.5% of the cases that did not survive had a monocyte count less than 0.15×10^9/l compared with only 16% of the cases that survived ($p<0.001$). This confirms the statement made earlier in this section that, if the bone marrow is not too severely affected, the return of monocytes in circulation should precede that of the neutrophils. In the cases that did not survive the monocyte count remained low.

Even though there were significant differences in monocyte count over time between survivors and non-survivors, the change in the monocyte count from the value at admission was not significantly different between the cases that survived and those that did not survive.

5.2.4 EOSINOPHILS

Newly formed eosinophils are stored in the bone marrow in a similar fashion to neutrophils i.e. a maturation and storage pool exists for eosinophils in the bone marrow. An important fact to take into account is that eosinophil production in the bone marrow is controlled by T-lymphocytes.[16,18] Eosinopaenia of acute infection is frequently seen, and although it has never been verified, this finding has been attributed to endogenous release of cortisol.[16,18,24] Therefore, the eosinopaenia seen in CPV infection could be due to a combination of myelosuppression, a lack of T-lymphocytes to stimulate eosinophil production by the bone marrow, as well as massive release of endogenous cortisol.

Schoeman et. al. (Personal communication, 2006), on basal cortisol as a prognostic indicator in CPV enteritis, has shown that there was no difference in the mean basal cortisol (MBC) level between the cases that survived and those that did not survive at admission. But, the cases that survived showed a marked reduction in MBC on days 1 and 2, whereas the cases that did not survive showed no reduction in MBC concentrations between admission and day 2. This suggests that patients that are critically ill, such as the CPV cases that did not survive, have very high MBC concentrations that may account for the very low eosinophil counts.

In this study, the difference in eosinophil count over time between the cases that survived and those that did not survive was also significant and can probably be attributed to two possibilities, myelosuppression and severe endogenous release of cortisol. The most significant findings were on day 1 (12 – 24 hours post admission) and day 2 (48 hours post admission). On day 1, 89% of the cases that did not survive had an eosinophil count less than 0.1×10^9/l, compared with only 45% of those that survived ($p=0.027$). On day 2, 100% of the cases that did not survive had an eosinophil count less than 0.1×10^9/l, compared with only 37% of those that survived ($p=0.001$).

As for the monocyte count, there was no significant difference between the survivors and non-survivors in the eosinophil count over time compared with the value at admission.

5.2.5 BASOPHILS

Basophils are not a representative leukocyte type in CPV enteritis.

5.3 HISTOPATHOLOGY

The findings on PM and histopathology were consistent with those seen in the blood, corroborating the hypothesis that haemopoetic cellular depletion in the different tissues (i.e. lymph nodes, thymus, spleen and bone marrow), lead to the irreversible leukopaenia in the non-survivors.

These findings also confirm the findings of Potgieter et.al.[28], as well as Boosinger et. al.[31]. Potgieter showed that CPV resulted in marked depletion of granulocytes in the bone marrow, but Boosinger was the first to report alterations in the myeloid, erythroid and megakaryocytic cell lines.

5.4 EVALUATION OF RED BLOOD CELL PARAMETERS IN SURVIVORS AND NON-SURVIVORS WITH CANINE PARVOVIRAL ENTERITIS

During the course of the disease the red blood cell (RBC) parameters can be slightly affected, but as reported by others, severe anaemia is seldom a significant finding in CPV. The reason why patients suffering from CPV seldom present with or develop severe anaemia is because the mature red blood cells have a long life span in circulation relative to the short period the virus suppresses production in the bone marrow.[28] A decreasing haematocrit through the course of the disease is probably due to a combination of severe intestinal haemorrhage and the dilutional effect of the rehydration therapy.[3,5]

Similar findings were seen in this study in that the RBC parameters were minimally affected. Only two of the RBC parameters showed significant differences between the survivors and non-survivors. Only the red cell count (RCC) on admission ($p=0.035$) and

the mean corpuscular haemoglobin concentration (MCHC) on admission (p=0.025) were significantly higher in the survivors than the non-survivors. This could be an indication that the cases that did not survive already suffered from hypochromic anaemia before contracting the disease. Internal parasites are a common problem in young animals in the geographical area where the samples were collected and previous studies have already shown that co-pathogenic factors like stress (e.g. weaning) and internal parasites may predispose the puppies to clinical disease by increasing mucosal cell activity.[1,5,6,10,12] It has also been hypothesized that these factors may play an important role in the clinical expression of the disease. [5]

Several puppies in this study had pale mucous membranes on clinical examination, which did not always relate to anaemia. Reticulocyte counts were not evaluated, but polychromasia was not observed on blood smears, suggesting the anaemia was non-regenerative during the course of the study.[13]

5.5 EVALUATION OF THE PLATELET COUNT IN SURVIVORS AND NON-SURVIVORS WITH CANINE PARVOVIRAL ENTERITIS

The most important role of the platelet (PLT) is the maintenance of normal vascular endothelial integrity. Thrombocytopaenia secondary to infection is a clinicopathologic finding of many viral infections. Virus-induced thrombocytopaenia can occur due to decreased platelet production (e.g. infection of megakaryocytes) or as a result of direct action of viruses and/or immunologic components on platelets or endothelium (e.g. peripheral depletion and/or consumption), respectively. Besides haemorrhagic manifestations, subclinical thrombocytopaenia may affect vascular permeability. Increased permeability of the vasculature as seen with thrombocytopaenia associated with viral disease may potentiate extravascular dissemination of the virus.[41]

In a study on the thrombocytopaenia seen in canine distemper virus (CDV) by Axthelm et.al.[41], thrombocytopaenia was found to be mediated by virus-antibody immune complexes on platelet membranes. Decreased platelet production due to a direct viral effect on megakaryocytes was a likely contributing factor. The fact that thrombocytopaenia, for most viral infections, frequently occurs coincident with, or

subsequent to, development of antiviral antibodies favours immunologic mechanisms of depletion.

In this study none of the puppies, survivors or non-survivors, showed any sign of petechiae on their clinically visible mucosal surfaces. The only evidence of petechiae was seen during necropsy on the small intestinal surfaces, which could also have been due to the viral infection.

This study showed significant differences in the platelet count between survivors and non-survivors. On day 1 (12 – 24 hours post admission) 44% of the cases that did not survive had a platelet count of less than 200×10^9/l, compared with only 8% of the cases that survived (p=0.013). On day 2 (48 hours post admission) 62.5% of the cases that did not survive had a platelet count of less than 200×10^9/l, compared with only 16% of the cases that survived (p=0.010). These findings correlate with those seen in the leukocytes and can therefore serve as a contributing parameter in determining prognosis.

A caveat to the interpretation of this finding is the fact that some of the samples showed signs of platelet aggregation. Fortunately, none of the thrombocytopaenic samples of the cases that died showed microscopic evidence of platelet aggregation, making the results more valid.

5.6 COMPARISON OF THE SIMILARITY IN NUMBERS OF DIFFERENT LEUKOCYTE TYPES BETWEEN CENTRAL AND PERIPHERAL BLOOD

Because clinicians in practice often only have a peripheral blood smear at hand it is also important to determine whether peripheral blood smear findings will compare well with that seen on central blood. The WBC can be estimated from a stained peripheral blood smear. The estimate can be made by examining the area of the smear used for the leukocyte differential count, using the 50× oil immersion objective. The average number of leukocytes in ten fields of view is determined. A semi-quantitative estimate of the WBC per microliter (/µl) is calculated by multiplying the average number of

leukocytes per field of view by 2000.[4] It is very important to remember that an estimated WBC is not a substitute for a properly performed quantitative count.

The findings of this study showed that the comparison between central and peripheral blood, with regards to the different leukocyte types, were relatively good. What was also an important finding was that the comparison between central and peripheral blood was good on the days when the most accurate prediction with regards to the outcome could be made.

On day 1 (72%) and day 2 (69%) the Neut count showed a relatively good comparison with that of the central blood. The Band count did not show a good comparison on any of the days. The reason for this finding could be due to the fact that these cells sequester more in the peripheral capillaries and would therefore not be seen in high numbers in central blood. Or possibly the numbers are too low, so coefficients of variation are very high.

The Lymph count showed very good comparison between central and peripheral blood at admission (82%), day 1 (83%), day 2 (79%) and day 3 (75%). This is a very important finding, seeing as the lymphocyte count was shown to be a very accurate leukocyte type to use as a prognostic indicator.

The Mono count showed a relatively poor comparison between central and peripheral blood at admission (63%), day 1 (62%) and day 2 (54%).

The Eos count showed very good comparison between central and peripheral blood at admission (82%), day 1 (85%), day 2 (79%) and day 3 (82%). Together with the lymphocyte count these leukocyte types could be very useful to monitor on the peripheral smear to determine prognosis in patients suffering from CPV enteritis.

University of Pretoria etd – Goddard, A (2006)

CHAPTER 6 CONCLUSION

Canine parvoviral (CPV) enteritis is an economically important disease in South Africa as well as globally. More effective prediction of the outcome of the disease will thus have an economic impact if a prognosis can be determined early in the course of the disease.

Leukopaenia has always been a typical finding in CPV and several studies over the years have speculated on its use as a prognostic indicator. As was stated before, leukocyte responses are seldom pathognomonic for a specific disease, but can aid in establishing a fairly accurate prognosis.

This study has extended the findings of others and looked at the individual leukocyte types and found that several of the leukocyte parameters can be used successfully, within the first 24 – 48 hours after commencement of treatment, as prognostic indicators for CPV enteritis. These parameters include: total white blood cell (WBC) count, lymphocyte count (Lymph), monocyte count (Mono) and eosinophil count (Eos).

Despite the finding of Woods et. al.[7] that cases with a WBC $< 4.5 \times 10^9$/l had a poorer prognosis, this study has shown that a WBC $< 2.0 \times 10^9$/l, a Lymph $< 1.0 \times 10^9$/l, a Mono $< 0.15 \times 10^9$/l and a Eos $< 0.10 \times 10^9$/l during the first 24 – 48 hours after admission and commencement of treatment, are accurate predictors of a poor outcome, which include death or hospital stay of more than 7 days.

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University of Pretoria etd – Goddard, A (2006)

APPENDICES

APPENDIX A

Consent Form for Parvoviral Enteritis Trial

I, ……..
(Full Names)
Herewith give permission for the dog under my care

…….
(Name of dog)

a ……..
(breed, sex, colour, age)

to participate in the study on whole blood parameters in parvoviral enteritis in the Department of Companion Animal Clinical Studies, Section Clinical Pathology, Faculty of Veterinary Science, University of Pretoria.

The trial has been explained to me and I understand that the blood samples drawn are routine and safe. I understand, furthermore, that the costs of the additional tests will be borne by the trial fund, and that I will only be liable for costs pertaining to the treatment that would in any event be required by my dog, including any complications that may arise as a result of the parvoviral enteritis.

I hereby also give permission that a full post mortem examination can be performed on my dog in the event of death / euthanasia.

Signed at: …….. (place) on the …… day

of …… 20…….

……..
Signature of Owner or Authorized person

Toestemmingsvorm vir Parvovirus Enteritis Projek

Hiermee gee ek,…….
(Volle naam en van)
toestemming vir die hond in my sorg,

……..
(Naam van hond)

'n……
(ras, geslag, kleur, ouderdom)

om deel te neem in die studie oor heelbloed parameters in parvovirus entritis, in die Departement van Geselskapsdiere Kliniese Studies, Seksie Kliniese Patologie, Fakulteit Veeartsenykunde, Universiteit van Pretoria.

Die doel van die projek is aan my verduidelik, en ek verstaan dat die bloedmonster versamelings van my hond veilige, en roetiene prosedures is. Ek verstaan verder ook dat al die kostes van die addisionel toetse deur die projek-fonds gedra sal word. Ek verstaan dat ek aanspreeklik is vir die kostes van standaard behandeling en diagnostiese toetse wat in elk geval deur my hond benodig sou word, insluitende enige komplikasies wat as gevolg van die parvovirus enteritis sou mag ontstaan.

Hiermee gee ek ook toestemming dat 'n volledige nadoodse ondersoek op my hond uitgevoer mag word in die geval van dood / genadedood.

Geteken te ……. (plek) op die …… dag

van …… 20 ……..

……..
Handtekening van eienaar of gemagtigde persoon

APPENDIX B

PARVO-VIRAL ENTERITIS TRIAL

Client Information Sheet

From the clinical examination and laboratory tests so far performed on your dog, it seems most likely that it is suffering from a viral infection, called canine parvovirus or the so called "cat flu". This virus causes damage to the intestines such that the normal intestinal lining is lost, bleeding occurs and food cannot be absorbed. It also causes other problems such as a decrease in the white blood cell count due to bone marrow suppression.

It has been advised that your dog should be admitted to the Onderstepoort Isolation unit for intensive treatment. Your dog will be treated with intravenous fluids (drips containing glucose and various salts), antibiotics, drugs that suppress nausea and vomiting, deworming drugs, and if needed, blood- or plasma transfusions.

In this study we wish to measure the various cellular components (Full blood count) on a daily basis from being admitted until discharge or death. We will be comparing these data with the severity of illness the puppy is showing and with the eventual outcome. With this information we hope to learn more about the disease in order to enable us to predict the outcome of the disease more accurately and thus make treatment more cost effective.

This trial will cost you no more money that it would usually cost you to treat your dog. We are paying for all the additional blood and faecal tests performed on your puppy.
This study has been approved by the Ethics Committee of the Faculty of Veterinary Science, University of Pretoria.

Thank you for allowing your puppy to be included in the study. If you have any further questions please feel free to ask the clinician on duty, or myself [Tel: (012) 529 8293(w)].

Dr Amelia Goddard BVSc (Hons.)

PARVOVIRUS ENTERITIS PROJEK

Klient Informasievorm

Vanuit die kliniese ondersoek en laboratoriumtoetse sover uitgevoer op u hond, lyk dit asof u hond aan ‘n virusinfeksie lei, genaamd hond parvovirus of die sogenaamde “katgriep” virus. Hierdie virus veroorsaak skade aan die dermkanaal, so erg dat die normale dermvoering verlore gaan, bloeding voorkom en kos nie meer geabsorbeer kan word nie. Dit veroorsaak ook ander probleme soos ‘n verlaging in die witseltelling as gevolg van beenmurgonderdrukking.

Dit word aanbeveel dat u hond opgeneem moet word na die Onderstepoort Isolasie-eenheid vir intensiewe behandeling. U hond sal behandel word met binne-aarse vloeistowwe (drips wat glukose en verskeie soute bevat), antibiotika, middels wat naarheid en vomisie onderdruk, ontwurmingsmiddels, en indien nodig, bloed- of plasma oortapping.

In hierdie studie wil ons die verskeie sellulêre komponente (Volbloedtelling) op ‘n daaglikse basis meet, vanaf opname tot ontslag of dood. Ons sal hierdie data vergelyk met die ergheidsgraad van die siekte wat die hondjie wys sowel as die uiteindelike uitkoms van die siekte. Ons hoop om met hierdie informasie meer te leer oor die siekte om ons instaat te stel om ‘n meer akkurate voorspelling van die uitkoms te maak en dus behandeling ook meer koste effektief te maak.

Die studie sal u niks meer kos as wat dit u in elk geval sou kos om u hond te behandel nie. Ons sal vir al die **addisionele** bloed- en fekale toetse, op u hond gedoen, betaal.
Hierdie studie is goedgekeur deur die Etiese komittee van die Fakulteit Veeartsenykunde, Universiteit van Pretoria.

Dankie dat u toelaat dat u hond ingesluit kan word in die studie. Indien u enige verdere navrae het kan u gerus die klinikus aan diens vra of myself kontak [Tel: (012) 529 8293 (w)]

Dr Amelia Goddard BVSc (Hons.)

APPENDIX C

General treatment guidelines for CPV enteritis

(Adapted from current OVAH parvoviral treatment protocol, and Macintire et. al.[42])

General treatment for all dogs will consist of the following:

- All dogs will have an intravenous catheter placed at time of admission.

- Initial intravenous fluid therapy will be aimed at correcting dehydration within 6 hours. Degree of dehydration (%) will be determined according to accepted guidelines **(Appendix D**). The fluid used for this purpose will be 1L Ringer's Lactate®, with 20-50ml of 50% dextrose solution, and 1 vial (20mEq) potassium chloride (Sabax Potassium Chloride)[d] added. The volume of fluid needed to effect rehydration will be determined using the following formula: (Body mass x 10 x % dehydration = volume in ml). Following this initial period of fluid replacement, serum potassium and glucose concentration will be determined, and potassium chloride and glucose added to the intravenous fluid according to deficits **(Appendix E).**

- Once the rehydration phase has been completed (approximately 6 hours post-admission), the patients will be started on enteral feeding either via syringe feeding, or via a naso-oesophageal tube. Enteral feeding will consist of any of the following: Hill's a/d; Hill's can i/d; Eukanuba intestinal for puppies; skinless chicken.

  Daily food (calorie) requirement will be based on the following calculation:

  IER (Illness energy requirements) = BER (basal energy requirement) x I-factor (Illness factor)

  BER = (body mass in kg x 30) + 70 = kcal/24 hours

  I-factor: 1.25 for inactivity
  1.5 for moderate cage activity, sepsis, trauma, surgery

- Once the rehydration phase had been completed, the maintenance phase of intravenous fluid therapy will be initialized: The fluid used for this purpose will be Ringer's Lactate®, spiked with potassium chloride and 50% dextrose. Daily serum potassium and glucose concentration will be determined for this purpose, and potassium chloride and dextrose added to the intravenous fluid according to deficits **(Appendix E)**. The rate of administration of this fluid will be tailored to the individual patient's needs. The total amount of daily fluid requirement (enteral and parenteral) will be estimated as follows: maintenance fluid requirements **(Appendix F)** + ongoing losses. The ongoing loss due to the diarrhoea is estimated to be 10-20 ml/kg/24 hrs initially, and will be adjusted during the course of treatment.

[d] Sabax Potassium Chloride, Adcock Ingram Critical Care, PO Box 6888, Johannesburg, 2000

- Antibiotic administration:
  - Amoxicillin
    (a) Initially Amoxil®[e] intravenous (i/v), 15mg/kg, tid. Once rehydration had been effected and peripheral perfusion judged adequate (absence of hypothermia, capillary refill time > 2 seconds, normalized skin tugor), the formulation will be changed to amoxycillin per os (capsules or suspension)
    (b) Clamoxyl RTU®[f] injectable suspension subcutaneous (s/c), 20mg/kg, bid, and once no vomition had occurred for 24 hours, the formulation will be changed to
    (c) Clamoxyl tablets®[g] per os, 20g/kg, bid, or Amoxycillin suspension per os, 20mg/kg, bid.

    The amoxycillin treatment will be combined with gentamicin treatment, to effect greater gram-negative spectrum to the antibiotic regime, as follows:
  - Gentamicin (Genta® 20 PHENIX)[h]
    (a) Due to the risk of acute renal failure development, gentamicin will only be administered after the rehydration phase of therapy had been completed (see above).
    (b) Dose: 2.2 mg/kg, i/v, tid.

- Anti-emetic therapy:
  - Metoclopramide (Clopamon®)[i] at 0.2-0.4 mg/kg every 6-8 hours OR 2mg/kg/24hr via continuous rate i/v infusion as standard treatment,
  - or prochlorperazine (Stemetil®)[j] at 0.1 mg/kg, i/v. every 4-6 hours if metoclopramide is ineffective in controlling vomiting.

- Sucralfate (Ulsanic® suspension)[k] at 1ml per 3kg, p/o, q6h, to persistently vomiting dogs to prevent reflux oesophagitis.

- Fenbendazole (Panacur® BS)[l] 50mg/kg, oid, p/o for 5d, irrespective of whether helminth eggs are identified on faecal flotation.

- Heated cages if hypothermia is present.

- Daily intravenous potassium supplementation if hypokalaemia is present **(Appendix E).**

[e] Amoxil® injectable, SmithKline Beecham, PO Box 347, Bergvlei, 2012
[f] Clamoxyl® RTU injectable suspension, Pfizer Animal Health, PO Box 783720, Sandton, 2146
[g] Clamoxyl® palatable tablets, Pfizer Animal Health, PO Box 783720, Sandton, 2146
[h] Genta® 20 PHENIX Aqueous injectable solution, Logos Agvet, Private Bag X115, Halfway House, 1685
[i] Clopamon®, Intramed, PO Box 2251, Randburg, 2125
[j] Stemetil® Injection, Rhône-Poulenc Rorer SA (Pty) Ltd, PO Box 1130, Port Elizabeth, 6000
[k] Ulsanic® Suspension, Continental Ethicals (Pty) Ltd, PO Box 55307, Northlands, 2116
[l] Panacur® BS, Hoechst Roussel Vet Specialities (Pty) Ltd, PO Box 6065, Halfway House, 1685

- Plasma transfusion (20 ml/kg) if albumin < 15 g/l or total serum protein < 35 g/l, or if clinically indicated according to the primary investigator or the duty clinician at the outpatient clinic. In some patients with albumin below this cut-off level a transfusion may not be clinically indicated.

- Whole blood transfusion if haematocrit < 15%, or if clinically indicated according to the primary investigator or the duty clinician at the outpatient clinic **(Appendix G)**. In some patients with Ht below this cut-off level a transfusion may not be clinically indicated.

APPENDIX D

Guidelines for estimating degree of dehydration (%)

Percentage dehydration	Clinical abnormalities
< 5%	No detectable abnormalities
5%	Slight loss of skin turgor
10%	Skin tents momentarily when lifted, mucous membranes slightly dry, capillary refill time (CRT) normal to slightly prolonged, moderate depression but still alert
>10%	Skin remains tented when lifted, mucous membranes dry and tacky, eyes sunken into orbits, CRT prolonged, tachycardia, weak pulse, markedly depressed and dull

(Classification of dehydration adapted from Strombeck's Small animal gastroenterology)

APPENDIX E

Guideline for potassium supplementation in IV fluids

Serum Potassium (mEq/L)	Potassium Chloride added per litre fluid	Number of KCl vials added/L	Maximum infusion rate (ml/kg/hr)*
3.6 – 5.0	20 mEq	1	25
3.1 – 3.5	30 mEq	1.5	17
2.6 – 3.0	40 mEq	2	12
2.1 – 2.5	60 mEq	3	8
< 2.0	80 mEq	4	6

* In order to prevent total hourly potassium administration from exceeding 0.5 mEq/kg body weight.

APPENDIX F

Daily caloric and maintenance water requirements for normal dogs

BODY WEIGHT (kg)	TOTAL kcal/day or TOTAL WATER (ml/day)	Kcal/kg/day or WATER/kg/day (ml/kg/day)
1	132	132
2	214	107
3	285	95
4	348	87
5	407	81
6	463	77
7	515	74
8	566	71
9	615	68
10	662	66
11	707	64
12	752	63
13	795	61
14	837	60
15	879	59
16	919	57
17	959	56
18	998	55
19	1037	55
20	1075	54

APPENDIX G

Whole blood transfusion therapy – volume to be transfused

Whole blood transfusion therapy will be performed if the patient's Ht<15% and the amount to be transfused calculated by the following formula {Kristensen AT, Feldman BF: Blood banking and transfusion medicine, in Ettinger SJ, Feldman BF (eds): *Textbook of Veterinary Internal Medicine*. Philadelphia, W.B. Saunders; 1995:347-360.}[A value of 25% will be taken for the Ht (desired).]

$$\text{Volume (ml) blood needed} = \frac{\text{Ht (desired)} - \text{Ht (patient)}}{\text{Ht (donor)}} \times 90 \times \text{Body weight (kg)}$$

APPENDIX H

Clinical Examination (Admission)

Client Name: ..... F Number: .....

Patient Name: .....

Breed: .....

Sex: ..... Age: ..... Weight: .....

Vaccination Dates: .....

Date of Admission: .....

Time of Admission: .....

Number of days depressed	1	2	3	>3
Number of days anorectic	1	2	3	>3
Number of days vomiting	1	2	3	>3
Vomiting episodes per day	1	2	3	>3
Description of vomitus				
Number of days diarrhoea	1	2	3	>3
Diarrhoea episodes per day	1	2	3	>3
Description of diarrhoea				
Habitus	1+	2+	3+	4+
% Dehydrated	0-5%	5%	10%	>10%
Mucosae	moist		dry	
	pale	pink	congested	
Oral ulcerations	yes		no	
Temperature				
Pulse rate				
Pulse quality	weak	strong	waterhammer	
Pulse rhythm	regular		irregular	
Respiratory rate				
Depth of respiration	normal	laboured	shallow	
Abnormal lung sounds	yes		no	
If yes, describe				
Abdominal palpation	tense		easily palpable	
	painful		not painful	
	thickened gut loops		fluid filled gut loops	
	gas in intestines		intussusception	
Blood smear	parasites		leukopaenia	
	thrombocytopaenia		reticulocytes present	
Faecal flotation				
Faecal wet preparation				
Faecal smear	leukocytes	erythrocytes	protozoa	
	spirochaetes	fungi	normal flora	

APPENDIX I

Clinical scoring assessment

Patient F-number:

Date:

Day number: 0 (admission), 1,2,3,4,5,6,7 or 8. (Encircle choice)

Temp: Pulse: Resp: Weight:

Encircle the applicable choice under 1 – 6 below:

1) Habitus	1	Collapsed / moribund
	2	Severe depression
	3	Mild-to-moderate depression
	4	Normal
2) Appetite:	1	No interest in food
	2	Voluntarily eats small amounts of food offered
	3	Voluntarily eats moderate amounts of food offered (but not normal)
	4	Normal
3) Vomiting:	1	Severe (≥ 6 times per 12h)
	2	Moderate (3-5 times per 12h)
	3	Mild (1-2 times per 12h)
	4	Absent
4) Faecal consistency:	1	Watery diarrhoea, bloody
	2	Watery diarrhoea, not bloody
	3	Soft
	4	Well-formed
5) Mucous membranes	1	Congested
	2	Pale
	3	Normal
6) Capillary Refill Time	1	> 2 seconds
	2	< 1 second
	3	1-2 seconds

APPENDIX J

Patient Outcome

Patient: ……..

E.M. positive for parvovirus? ……

E.M. positive for Coronavirus? ……

Died / Recovered

Date died / recovered: …….

Time died / recovered: …….

Days to recovery / death? ……..

Complications developed? …….

If so, describe …….

……

……

……

…….

APPENDIX K

Full Blood Count (FBC)

A Full blood count is the measurement of the concentration of cells per volume of blood. It can be determined manually via haemocytometer, or electronically via an automated cell counter.

The automated cell counter that was used in this study is the CELL-DYN® 3700 System to measure, count, and calculate the haematologic parameters. The CELL-DYN® 3700 System is a multi-parameter, automated haematology analyzer designed for in vitro diagnostic use in clinical laboratories.

Parameters that will be included:

- Haemoglobin (Hb)
- Red Cell Count (RCC)
- Haematocrit (Ht)
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Haemoglobin Concentration (MCHC)
- Total White Blood Cell count (WBC)
- Neutrophil (Segmented) absolute count (Neut)
- Neutrophil (Bands) absolute count (Bands)
- Lymphocyte absolute count (Lymph)
- Monocyte absolute count (Mono)
- Eosinophil absolute count (Eos)
- Basophil absolute count (Baso)
- Platelet count (PLT)

Principles of operation

Four independent measurements are used in the CELL-DYN® 3700 System to obtain the haematologic parameters.

- The WBC (White Blood Cell) Optical Count (WOC) and the WBC differential data are measured in the optical flow channel.
- The WBC Impedance Count (WIC) is measured in one electrical impedance channel.
- The RBC (Red Blood Cell) and PLT (Platelet) data are measured in a second electrical impedance channel.
- The HGB (Haemoglobin) is measured in the spectrophotometric channel.

During each instrument cycle, the sample is aspirated, diluted, and mixed, and the measurements for each parameter are performed.

APPENDIX L

LEUKOCYTE PARAMETERS: Total White Cell Count (WBC)

Patient no.	WCCA	WCC1	WCC2	WCC3	WCC4	WCC5	WCC6	WCC7	WCC8	WCC9	WCC10	WCC11	WCC12	WCC13	Stay	Outcome
1	3.1	3.3	1.2	2.5	10.0	15.8	24.4	28.8							7	Alive
2	9.5	7.2	7.6	7.3											3	Alive
3	4.8	1.9	0.6	1.4	3.6	18.6									5	Dead
4	9.1	6.5	8.6												3	Alive
5	5.9	4.1	8.0	10.8											3	Alive
6	5.6	3.0	6.6	13.5	14.0										4	Alive
7	1.2	1.9	5.9	10.7	12.7	12.1	22.9								7	Alive
8	2.2	2.5	0.4	2.6	8.2	10.3	12.8	13.8							7	Alive
9	7.5	4.3	3.0	8.3	13.2	17.8	19.2	30.2							7	Alive
10	7.3	7.6	7.0	3.3	2.8	4.6									5	Alive
11	0.5	1.1	3.9	6.5	9.1										4	Alive
12	8.8	6.4	6.7												2	Alive
13	3.5	4.3	7.1	1.8	1.0	1.3	6.2	3.0	4.1	26.1					9	Alive
14	2.5	6.5	5.8	11.4	19.1										4	Alive
15	1.9	1.8	4.5												2	Dead
16	13.2	15.8	10.3	8.8	8.9	7.4									5	Alive
17	10.6	8.6	6.2	6.1	4.3	5.7									5	Alive
18	0.2	1.3	22.7	14.5	17.4	12.5									5	Alive
19	5.1	17.7	10.0	6.5											3	Alive
20	8.1	3.0	0.3	0.5	4.3	6.6	11.0								6	Alive
21	4.3	0.9	1.1	10.0	15.6	14.7									5	Alive
22	1.1	2.6	6.2	4.8											3	Alive
23	3.3	4.3	15.5	18.4	19.1	29.5	27.6								6	Alive
24	0.5	0.3	0.0												3	Dead
25	5.0	2.9	0.3	5.6	11.4	16.3	21.3								6	Alive
26	5.2	6.3	4.7	3.3	2.7	10.2	18.6	22.1	23.9						8	Alive
27	0.6	0.4	3.3	8.1	17.2	21.5	34.5	77.2	62.4	72.3	84.0	75.0	79.9	75.4	13	Alive
28	5.0	10.4	10.0	8.8											3	Alive
29		1.1	0.7												2	Dead
30	2.3	1.9	4.7	10.3	17.0										4	Alive
31	6.9	12.2	11.8	16.1											3	Alive
32	3.1	0.9	1.3	5.4	19.6	38.5	72.1	117.0	84.7	52.1					9	Alive
33	3.8	17.3	15.3	9.3											3	Alive

Patient no.	WCCA	WCC1	WCC2	WCC3	WCC4	WCC5	WCC6	WCC7	WCC8	WCC9	WCC10	WCC11	WCC12	WCC13	Stay	Outcome
34	2.4														1	Dead
35	6.8	4.0	5.9	8.4	14.4										4	Alive
36	6.5	0.5	0.2	1.6											3	Dead
37	1.2	1.1	2.8	5.3	8.3										4	Alive
38	11.6	9.7	5.9	8.5											3	Alive
39	1.1	1.7	5.0	8.1											3	Alive
40	0.3	0.2	0.2												2	Dead
41	1.2														1	Dead
42	11.6	7.9	8.6												2	Alive
43	4.5	0.1	0.1												2	Dead
44	19.3	8.9	10.9												2	Alive
45	11.8	5.1	4.0												2	Alive
46	2.8	3.2	2.8	7.8	14.0	20.4	20.6								6	Alive
47	3.4	3.5	5.7	12.4	15.3	13.3									5	Alive
48	2.9	4.6	7.2												2	Alive
49	0.6	0.4													1	Dead
50	11.8	6.6	4.3												2	Alive
51	1.8	2.6	10.2	15.4	20.2										4	Alive
52	5.5	0.9	0.5	3.1	4.7	10.0									5	Alive
53	14.6	8.3	4.3	2.8	2.6	3.0									5	Alive
54	2.5	1.8	2.0	8.4	11.5										4	Alive
55	4.1	2.7	3.9	16.2	22.6	19.6	27.4	41.2							7	Alive
56	0.6	0.3	0.5	2.7	3.3	10.5									5	Alive
57	2.0	3.5	3.5	4.1											3	Alive
58	5.0	10.8	11.1												2	Alive
59		5.8	4.2	8.4	7.7	8.9									5	Alive
60	9.3	10.0	11.8												2	Alive
61	5.3	0.4	0.1												2	Dead
62	7.0	2.8	1.6	1.5	4.1	20.5	26.0	29.4	48.9						8	Alive

LEUKOCYTE PARAMETERS: Segmented Neutrophil Count (Nmat)

Patient no.	NmatA	Nmat1	Nmat2	Nmat3	Nmat4	Nmat5	Nmat6	Nmat7	Nmat8	Nmat9	Nmat10	Nmat11	Nmat12	Nmat13	Stay	Outcome
1	1.55	1.39	0.05	0.10	2.10	7.27	6.59	18.14							7	Alive
2	4.85	1.66	2.36	2.56											3	Alive
3	2.74	0.72	0.10	0.31	0.43	10.04									5	Dead
4	5.46	2.67	4.04												3	Alive
5	3.19	1.07	3.04	5.18											3	Alive
6	1.46	0.06	0.92	4.59	6.02										4	Alive
7	0.31	0.49	0.83	5.03	8.38	7.14	18.78								7	Alive
8	0.31	0.05	0.01	0.42	2.62	6.70	9.09	8.28							7	Alive
9	2.70	1.12	0.78	2.82	9.90	12.64	12.48	19.63							7	Alive
10	5.48	3.72	4.20	1.72	0.81	1.75									5	Alive
11	0.10	0.02	2.07	4.55	6.01										4	Alive
12	6.42	3.58	2.68												2	Alive
13	2.17	3.83	6.50	0.47	0.14	0.42	4.38	2.16	3.08	21.30					9	Alive
14	1.28	1.76	2.26	2.96	11.27										4	Alive
15	0.72	0.83	2.61												2	Dead
16	7.92	9.64	6.08	5.46	5.87	3.33									5	Alive
17	9.88	7.45	4.59	3.60	2.37	2.91									5	Alive
18	0.01	0.03	11.58	11.89	14.44	9.75									5	Alive
19	0.20	5.13	3.40	3.19											3	Alive
20	6.32	1.14	0.03	0.18	1.81	4.62	7.26								6	Alive
21	1.38	0.02	0.07	4.80	10.76	11.32									5	Alive
22	0.11	0.10	1.61	0.86											3	Alive
23	0.26	0.26	3.26	8.28	11.08	18.29	17.11								6	Alive
24	0.07	0.00	0.00												3	Dead
25	3.00	0.12	0.02	1.68	4.56	8.64	11.72								6	Alive
26	3.59	3.72	2.40	0.53	0.54	2.04	9.49	15.43	17.69						8	Alive
27	0.12	0.03	0.92	2.59	11.70	16.13	28.67	64.08	48.05	62.68	70.14	61.50	63.12	62.58	13	Alive
28	0.75	3.85	4.40	4.40											3	Alive
29		0.18	0.00												2	Dead
30	0.18	0.27	1.03	5.36	8.16										4	Alive
31	0.97	3.54	4.48	8.16											3	Alive
32	0.93	0.05	0.05	0.97	5.88	18.87	49.03	70.20	66.07	34.91					9	Alive
33	0.46	3.98	8.11	5.02											3	Alive
34	0.77														1	Dead

Patient no.	NmatA	Nmat1	Nmat2	Nmat3	Nmat4	Nmat5	Nmat6	Nmat7	Nmat8	Nmat9	Nmat10	Nmat11	Nmat12	Nmat13	Stay	Outcome
35	2.58	1.52	1.89	3.86	8.50										4	Alive
36	5.40	0.07	0.00	0.03											3	Dead
37	0.37	0.17	0.73	3.18	4.15										4	Alive
38	4.87	5.63	2.36	2.81											3	Alive
39	0.21	0.07	0.80	4.70											3	Alive
40	0.06	0.00	0.01												2	Dead
41	0.68														1	Dead
42	6.89	5.85	5.25												2	Alive
43	2.43	0.01	0.00												2	Dead
44	11.77	5.52	6.76												2	Alive
45	7.91	1.43	1.32												2	Alive
46	0.65	0.26	0.34	4.60	8.54	15.30	12.36								6	Alive
47	0.41	0.98	2.28	5.21	11.17	7.85									5	Alive
48	0.29	0.46	1.15												2	Alive
49	0.13	0.00													1	Dead
50	9.86	4.09	1.85												2	Alive
51	0.02	0.16	4.69	9.24	12.32										4	Alive
52	3.52	0.23	0.00	0.81	1.32	6.60									5	Alive
53	12.41	7.06	2.32	1.15	0.42	0.30									5	Alive
54	1.05	0.14	0.10	1.76	5.87										4	Alive
55	0.98	0.54	0.47	10.37	14.10	11.17	17.26	28.43							7	Alive
56	0.22	0.01	0.00	0.59	2.11	7.56									5	Alive
57	0.44	0.11	0.14	0.62											3	Alive
58	2.00	6.40	7.22												2	Alive
59		0.29	0.50	2.86	3.77	4.09									5	Alive
60	3.63	2.60	4.48												2	Alive
61	1.91	0.02	0.01												2	Dead
62	3.36	0.45	0.06	0.06	0.82	9.84	15.86	23.23	42.05						8	Alive

LEUKOCYTE PARAMETERS: Band Neutrophil Count (Nimm)

Patient no.	NimmA	Nimm1	Nimm2	Nimm3	Nimm4	Nimm5	Nimm6	Nimm7	Nimm8	Nimm9	Nimm10	Nimm11	Nimm12	Nimm13	Stay	Outcome
1	1.24	0.53	0.02	0.70	4.50	3.32	12.69	8.06							7	Alive
2	3.42	2.02	0.00	0.15											3	Alive
3	1.58	0.65	0.06	0.31	1.44	6.51									5	Dead
4	0.09	0.07	0.26												3	Alive
5	0.18	0.33	0.40	0.86											3	Alive
6	1.90	0.84	0.79	0.54	0.56										4	Alive
7	0.29	0.11	2.48	3.32	2.67	2.06	0.46								7	Alive
8	0.13	0.00	0.00	1.14	3.77	0.82	0.13	0.55							7	Alive
9	3.90	1.38	0.72	2.82	0.53	0.18	1.54	4.83							7	Alive
10	0.22	1.67	0.07	0.00	0.00	0.18									5	Alive
11	0.04	0.02	0.66	0.46	0.00										4	Alive
12	1.50	0.26	0.40												2	Alive
13	0.42	0.00	0.14	0.04	0.02	0.26	1.36	0.24	0.08	0.26					9	Alive
14	0.03	1.17	1.57	5.13	1.53										4	Alive
15	0.76	0.47	0.72												2	Dead
16	0.79	0.32	0.00	0.00	0.00	0.00									5	Alive
17	0.21	0.17	0.31	0.12	0.09	0.06									5	Alive
18	0.00	0.52	9.53	0.73	0.35	0.50									5	Alive
19	1.22	4.25	0.80	0.39											3	Alive
20	0.00	0.84	0.02	0.15	0.95	0.92	0.77								6	Alive
21	1.63	0.02	0.31	3.60	2.50	1.32									5	Alive
22	0.11	0.31	0.93	0.43											3	Alive
23	0.53	0.60	7.91	6.26	5.16	6.20	5.52								6	Alive
24	0.01	0.00	0.00												3	Dead
25	0.20	0.00	0.00	1.46	2.74	1.96	1.70								6	Alive
26	0.31	0.82	0.00	0.03	0.19	2.75	1.49	1.11	0.24						8	Alive
27	0.02	0.00	1.29	4.70	3.61	2.15	1.38	4.63	3.12	3.62	3.36	3.75	5.59	0.75	13	Alive
28	0.85	0.62	0.70	0.09											3	Alive
29		0.13	0.03												2	Dead
30	1.06	0.15	0.56	1.34	2.04										4	Alive
31	1.79	1.10	0.94	0.32											3	Alive
32	0.74	0.02	0.10	1.62	5.88	8.09	10.09	25.74	5.93	3.65					9	Alive
33	0.38	7.44	1.38	0.65											3	Alive
34	0.29														1	Dead

Patient no.	NimmA	Nimm1	Nimm2	Nimm3	Nimm4	Nimm5	Nimm6	Nimm7	Nimm8	Nimm9	Nimm10	Nimm11	Nimm12	Nimm13	Stay	Outcome
35	2.31	0.16	0.47	0.50	0.14										4	Alive
36	0.59	0.07	0.06	0.16											3	Dead
37	0.63	0.29	0.84	0.11	0.42										4	Alive
38	4.41	0.19	0.00	0.09											3	Alive
39	0.04	0.00	0.80	0.16											3	Alive
40	0.00	0.00	0.00												2	Dead
41	0.04														1	Dead
42	2.90	0.00	0.00												2	Alive
43	1.53	0.00	0.00												2	Dead
44	5.40	0.00	0.00												2	Alive
45	2.01	0.20	0.12												2	Alive
46	0.28	0.00	0.50	0.62	2.10	1.84	0.41								6	Alive
47	1.77	0.77	2.11	0.87	0.31	0.27									5	Alive
48	1.10	0.87	0.65												2	Alive
49	0.02	0.00													1	Dead
50	0.47	0.00	0.09												2	Alive
51	0.09	0.16	1.63	0.92	1.21										4	Alive
52	0.44	0.04	0.00	0.19	0.19	0.20									5	Alive
53	0.73	0.25	0.22	0.06	0.10	0.12									5	Alive
54	0.75	0.00	0.10	1.18	0.35										4	Alive
55	1.64	0.05	0.62	0.97	5.88	2.74	1.92	3.71							7	Alive
56	0.00	0.00	0.08	0.65	0.07	0.32									5	Alive
57	0.52	0.14	0.21	0.25											3	Alive
58	0.05	0.00	0.22												2	Alive
59		1.04	0.59	2.35	0.15	0.89									5	Alive
60	0.74	0.70	0.12												2	Alive
61	2.76	0.00	0.00												2	Dead
62	2.24	0.39	0.00	0.15	0.90	6.77	5.98	2.65	2.45						8	Alive

LEUKOCYTE PARAMETERS: Lymphocyte Count (Lymph)

Patient no.	LymphA	Lymph1	Lymph2	Lymph3	Lymph4	Lymph5	Lymph6	Lymph7	Lymph8	Lymph9	Lymph10	Lymph11	Lymph12	Lymph13	Stay	Outcome
1	0.12	0.86	0.86	1.65	3.00	4.90	3.66	1.73							7	Alive
2	0.19	2.02	2.51	2.34											3	Alive
3	0.24	0.30	0.28	0.48	1.58	1.67									5	Dead
4	1.82	2.54	2.75												3	Alive
5	1.24	1.93	3.28	3.78											3	Alive
6	0.78	1.08	2.31	4.05	4.34										4	Alive
7	0.29	0.95	0.59	1.71	0.89	1.94	1.37								7	Alive
8	1.28	1.83	0.39	0.99	1.48	1.85	2.56	3.73							7	Alive
9	0.30	1.03	0.90	1.66	2.24	2.67	2.50	1.81							7	Alive
10	1.02	1.44	1.96	1.02	1.29	1.89									5	Alive
11	0.24	1.03	0.74	1.37	2.37										4	Alive
12	0.62	2.24	3.02												2	Alive
13	0.70	0.33	0.38	1.21	0.77	0.39	0.17	0.42	0.66	2.74					9	Alive
14	0.30	0.85	1.04	2.74	2.48										4	Alive
15	0.04	0.18	0.54												2	Dead
16	4.09	5.53	4.02	2.82	2.58	3.55									5	Alive
17	0.23	0.63	0.68	1.28	1.46	1.43									5	Alive
18	0.11	0.03	0.68	1.31	1.74	1.63									5	Alive
19	0.20	4.43	4.40	1.37											3	Alive
20	1.22	0.90	0.24	0.03	1.12	0.66	1.65								6	Alive
21	1.03	0.68	0.55	1.00	1.25	1.47									5	Alive
22	0.35	0.94	2.29	2.83											3	Alive
23	1.06	2.15	1.40	2.76	2.67	2.36	3.86								6	Alive
24	0.44	0.30	0.03												3	Dead
25	1.60	2.49	0.28	1.68	3.99	3.42	5.54								6	Alive
26	0.78	1.13	1.88	2.18	1.51	4.59	4.84	2.54	4.06						8	Alive
27	0.22	0.10	0.53	0.73	1.72	1.94	2.38	2.62	4.99	2.82	3.11	6.75	4.79	5.28	13	Alive
28	1.90	4.26	3.80	3.52											3	Alive
29		0.46	0.57												2	Dead
30	0.14	0.65	1.50	1.85	2.72										4	Alive
31	0.69	1.59	2.48	2.00											3	Alive
32	0.50	0.63	0.96	1.94	5.49	6.93	5.77	10.53	6.78	10.42					9	Alive
33	1.14	2.94	2.91	1.77											3	Alive
34	0.96														1	Dead

Patient no.	LymphA	Lymph1	Lymph2	Lymph3	Lymph4	Lymph5	Lymph6	Lymph7	Lymph8	Lymph9	Lymph10	Lymph11	Lymph12	Lymph13	Stay	Outcome
35	0.75	1.84	2.48	2.60	3.74										4	Alive
36	0.33	0.03	0.16	0.09											3	Dead
37	0.37	0.42	0.22	1.27	2.66										4	Alive
38	0.70	3.01	2.66	3.40											3	Alive
39	0.74	1.50	2.90	2.19											3	Alive
40	0.22	0.17	0.15												2	Dead
41	0.49														1	Dead
42	0.26	1.11	1.29												2	Alive
43	0.27	0.07	0.05												2	Dead
44	1.35	2.05	2.18												2	Alive
45	0.59	1.73	1.56												2	Alive
46	0.50	2.11	0.34	2.26	2.24	2.45	5.56								6	Alive
47	0.14	0.35	0.34	1.98	1.53	2.26									5	Alive
48	0.64	2.07	4.10												2	Alive
49	0.14	0.23													1	Dead
50	0.28	1.85	2.02												2	Alive
51	0.90	1.88	3.16	4.62	4.44										4	Alive
52	1.43	0.47	0.31	1.86	2.91	2.60									5	Alive
53	0.58	0.75	1.51	1.26	1.30	2.01									5	Alive
54	0.25	1.22	1.12	3.70	3.57										4	Alive
55	0.49	0.59	1.79	2.11	2.01	3.53	5.75	4.94							7	Alive
56	0.35	0.28	0.42	1.40	0.86	2.10									5	Alive
57	0.48	2.31	2.56	2.62											3	Alive
58	1.85	1.43	2.22												2	Alive
59		2.15	1.76	1.43	2.46	2.58									5	Alive
60	1.67	2.60	2.36												2	Alive
61	0.11	0.26	0.06												2	Dead
62	0.49	0.95	1.22	0.81	1.89	2.46	3.38	3.23	0.98						8	Alive

LEUKOCYTE PARAMETERS: Monocyte Count (Mono)

Patient no.	MonoA	Mono1	Mono2	Mono3	Mono4	Mono5	Mono6	Mono7	Mono8	Mono9	Mono10	Mono11	Mono12	Mono13	Stay	Outcome
1	0.12	0.33	0.12	0.05	0.30	0.32	1.46	0.86							7	Alive
2	0.86	1.37	0.15	1.97											3	Alive
3	0.24	0.23	0.14	0.28	0.14	0.37									5	Dead
4	1.27	0.78	1.20												3	Alive
5	0.65	0.49	1.04	0.22											3	Alive
6	0.78	1.02	1.39	1.62	2.66										4	Alive
7	0.26	0.27	1.53	0.64	0.76	0.61	2.06								7	Alive
8	0.13	0.20	0.00	0.05	0.33	0.93	1.02	1.24							7	Alive
9	0.53	0.60	0.18	0.91	0.40	1.25	1.92	2.72							7	Alive
10	0.58	0.38	0.70	0.40	0.53	0.69									5	Alive
11	0.12	0.02	0.39	0.13	0.64										4	Alive
12	0.26	0.32	0.47												2	Alive
13	0.21	0.15	0.07	0.07	0.06	0.23	0.24	0.18	0.16	1.31					9	Alive
14	0.75	1.89	1.51	0.46	2.48										4	Alive
15	0.38	0.32	0.63												2	Dead
16	0.40	0.32	0.10	0.44	0.18	0.37									5	Alive
17	0.25	0.34	0.50	0.73	0.34	0.86									5	Alive
18	0.08	0.73	0.68	0.58	0.87	0.50									5	Alive
19	2.45	2.66	0.60	0.52											3	Alive
20	0.49	0.12	0.02	0.15	0.43	0.40	0.22								6	Alive
21	0.26	0.09	0.15	0.60	1.09	0.59									5	Alive
22	0.48	0.73	0.68	0.67											3	Alive
23	1.45	0.77	2.17	0.74	0.19	2.66	0.83								6	Alive
24	0.00	0.00	0.00												3	Dead
25	0.20	0.29	0.00	0.78	0.11	2.28	2.34								6	Alive
26	0.47	0.44	0.14	0.30	0.46	0.82	2.60	2.98	1.91						8	Alive
27	0.17	0.27	0.59	0.08	0.17	1.29	2.07	5.17	6.24	2.82	5.46	3.00	8.79	6.79	13	Alive
28	1.30	1.14	0.60	0.44											3	Alive
29		0.29	0.10												2	Dead
30	0.92	0.76	1.60	1.75	3.74										4	Alive
31	3.31	4.15	3.30	5.01											3	Alive
32	0.93	0.18	0.16	0.86	2.35	4.62	7.21	10.53	5.08	3.13					9	Alive
33	1.82	2.94	2.14	1.58											3	Alive
34	0.34														1	Dead

Patient no.	MonoA	Mono1	Mono2	Mono3	Mono4	Mono5	Mono6	Mono7	Mono8	Mono9	Mono10	Mono11	Mono12	Mono13	Stay	Outcome
35	1.02	0.24	0.83	1.09	2.02										4	Alive
36	0.07	0.36	0.00	1.30											3	Dead
37	0.17	0.17	0.56	0.74	1.00										4	Alive
38	1.51	0.68	0.71	1.70											3	Alive
39	0.06	0.14	0.50	1.05											3	Alive
40	0.03	0.02	0.03												2	Dead
41	0.01														1	Dead
42	0.12	0.55	1.29												2	Alive
43	0.27	0.04	0.03												2	Dead
44	0.77	0.80	0.33												2	Alive
45	1.65	1.12	0.52												2	Alive
46	1.33	0.51	0.62	0.31	1.12	0.82	2.27								6	Alive
47	1.09	1.23	1.14	4.34	2.30	2.39									5	Alive
48	0.81	1.09	0.65												2	Alive
49	0.30	0.15													1	Dead
50	1.00	0.40	0.26												2	Alive
51	0.76	0.42	0.71	0.62	2.22										4	Alive
52	0.06	0.14	0.19	0.25	0.28	0.60									5	Alive
53	0.88	0.25	0.26	0.14	0.39	0.24									5	Alive
54	0.40	0.29	0.54	1.60	1.73										4	Alive
55	0.98	1.46	0.94	2.92	0.36	2.16	2.19	4.12							7	Alive
56	0.04	0.01	0.00	0.05	0.26	0.53									5	Alive
57	0.44	0.21	0.39	0.49											3	Alive
58	0.75	2.11	0.67												2	Alive
59		1.51	0.92	1.76	1.31	1.34									5	Alive
60	3.16	3.30	3.07												2	Alive
61	0.11	0.00	0.03												2	Dead
62	0.63	0.73	0.16	0.45	0.49	1.44	0.78	1.47	3.42						8	Alive

LEUKOCYTE PARAMETERS: Eosinophil Count (Eos)

Patient no.	EosA	Eos1	Eos2	Eos3	Eos4	Eos5	Eos6	Eos7	Eos8	Eos9	Eos10	Eos11	Eos12	Eos13	Stay	Outcome
1	0.06	0.20	0.14	0.00	0.10	0.00	0.00	0.00							7	Alive
2	0.19	0.14	1.22	0.29											3	Alive
3	0.00	0.00	0.02	0.03	0.01	0.00									5	Dead
4	0.46	0.46	0.34												3	Alive
5	0.65	0.29	0.24	0.76											3	Alive
6	0.56	0.00	1.19	2.70	0.42										4	Alive
7	0.02	0.08	0.47	0.00	0.00	0.36	0.23								7	Alive
8	0.48	0.43	0.00	0.00	0.00	0.00	0.00	0.00							7	Alive
9	0.08	0.17	0.42	0.08	0.13	1.07	0.77	1.21							7	Alive
10	0.05	0.38	0.07	0.17	0.17	0.09									5	Alive
11	0.00	0.00	0.04	0.00	0.09										4	Alive
12	0.00	0.00	0.13												2	Alive
13	0.00	0.00	0.01	0.02	0.01	0.00	0.04	0.00	0.12	0.50					9	Alive
14	0.15	0.85	0.93	0.11	1.34										4	Alive
15	0.00	0.00	0.00												2	Dead
16	0.00	0.00	0.10	0.09	0.27	0.15									5	Alive
17	0.02	0.01	0.12	0.31	0.04	0.46									5	Alive
18	0.00	0.00	0.23	0.00	0.00	0.13									5	Alive
19	0.00	1.24	0.80	1.04											3	Alive
20	0.08	0.00	0.00	0.00	0.00	0.00	0.00								6	Alive
21	0.00	0.09	0.02	0.00	0.00	0.00									5	Alive
22	0.04	0.73	0.68	0.00											3	Alive
23	0.00	0.52	0.78	0.37	0.00	0.00	0.28								6	Alive
24	0.00	0.00	0.00												3	Dead
25	0.00	0.00	0.00	0.00	0.00	0.00	0.00								6	Alive
26	0.05	0.19	0.28	0.26	0.01	0.00	0.19	0.02	0.00						8	Alive
27	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.77	0.00	0.43	1.93	0.00	0.00	0.00	13	Alive
28	0.20	0.52	0.50	0.35											3	Alive
29		0.04	0.00												2	Dead
30	0.00	0.08	0.00	0.00	0.34										4	Alive
31	0.14	1.71	0.59	0.61											3	Alive
32	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.85	0.00					9	Alive
33	0.00	0.00	0.77	0.28											3	Alive
34	0.05														1	Dead

Patient no.	EosA	Eos1	Eos2	Eos3	Eos4	Eos5	Eos6	Eos7	Eos8	Eos9	Eos10	Eos11	Eos12	Eos13	Stay	Outcome
35	0.14	0.24	0.00	0.34	0.00										4	Alive
36	0.13	0.02	0.00	0.00											3	Dead
37	0.00	0.00	0.28	0.00	0.08										4	Alive
38	0.12	0.19	0.18	0.17											3	Alive
39	0.00	0.00	0.01	0.00											3	Alive
40	0.00	0.00	0.01												2	Dead
41	0.00														1	Dead
42	1.44	0.40	0.77												2	Alive
43	0.00	0.00	0.01												2	Dead
44	0.00	0.53	1.64												2	Alive
45	0.00	0.51	0.48												2	Alive
46	0.04	0.32	0.28	0.00	0.00	0.00	0.00								6	Alive
47	0.00	0.00	0.00	0.00	0.00	0.13									5	Alive
48	0.06	0.10	0.65												2	Alive
49	0.00	0.01													1	Dead
50	0.06	0.26	0.09												2	Alive
51	0.04	0.00	0.00	0.00	0.00										4	Alive
52	0.06	0.02	0.00	0.00	0.00	0.00									5	Alive
53	0.00	0.00	0.00	0.20	0.39	0.33									5	Alive
54	0.05	0.14	0.14	0.17	0.00										4	Alive
55	0.00	0.05	0.08	0.00	0.27	0.00	0.27	0.00							7	Alive
56	0.00	0.00	0.00	0.00	0.00	0.00									5	Alive
57	0.12	0.74	0.21	0.12											3	Alive
58	0.35	0.87	0.78												2	Alive
59		0.81	0.42	1.76	0.00	0.00									5	Alive
60	0.09	0.80	1.77												2	Alive
61	0.00	0.11	0.01												2	Dead
62	0.28	0.22	0.13	0.03	0.00	0.00	0.00	0.00	0.00						8	Alive

LEUKOCYTE PARAMETERS: Peripheral Segmented Neutrophil Count (PNmat)

Patient no.	PNmat0	PNmat1	PNmat2	PNmat3	PNmat4	PNmat5	PNmat6	PNmat7	PNmat8	PNmat9	PNmat10	PNmat11	PNmat12	PNmat13	Stay	Outcome
1	1.36	0.99	0.10	0.10	2.10	7.58	13.91	22.75							7	Alive
2	3.99	2.16	2.81	1.83											3	Alive
3	3.17	0.46	0.07	0.39	0.50	7.81									5	Dead
4	4.91	1.89	3.35												3	Alive
5		1.64	3.28	3.56											3	Alive
6	0.78	0.06	0.13	2.70	6.16										4	Alive
7	0.31	0.15	1.65	3.85	5.84	5.08	17.40								7	Alive
8	0.18	0.00	0.00	0.78	2.46	1.96	7.04	6.35							7	Alive
9	1.95	0.95	1.62	3.15	9.37	13.88	12.29	18.72							7	Alive
10	4.89	2.20	2.80	0.63	0.42	1.06									5	Alive
11	0.03	0.02	0.94	0.91	4.82										4	Alive
12	7.74	4.03	3.89												2	Alive
13	3.22	3.27	5.47	0.58	0.30	0.36	4.34	2.55	1.52	21.14					9	Alive
14	0.05	0.91	0.99	3.08	8.02										4	Alive
15	0.65	0.79	2.61												2	Dead
16	8.45	11.06	7.00	6.42	5.16	3.48									5	Alive
17	9.33	6.11	2.60	1.28	2.58	1.60									5	Alive
18		0.05	12.94	9.72	14.27	7.63									5	Alive
19	0.05	5.49	3.60	3.77											3	Alive
20	6.32	1.14	0.17	0.05	0.43	2.71	6.05								6	Alive
21	2.41	0.04	0.09	5.20	8.89	7.79									5	Alive
22	0.11	0.05	0.68	1.34											3	Alive
23	0.26	0.09	5.27	10.12	13.37	23.90	19.87								6	Alive
24	0.05	0.05	0.01												3	Dead
25	2.90	0.20	0.05	2.18	4.90	7.17	16.40								6	Alive
26	3.12	2.39	1.97	0.50	0.30	3.77	10.97	15.69	15.54						8	Alive
27	0.10	0.04	0.86	2.59	10.32	15.27	23.12	66.39	43.06	62.18	80.64	63.00	65.52	62.58	13	Alive
28	0.30	2.70	5.00	3.34											3	Alive
29		0.07	0.00												2	Dead
30	0.46	0.15	0.47	2.99	6.63										4	Alive
31		1.34	4.60	6.92											3	Alive
32	0.12	0.00	0.03	0.86	7.25	18.87	36.77	91.26	66.91	42.20					9	Alive
33	0.53	6.40	11.32	4.19											3	Alive
34	1.25														1	Dead

Patient no.	PNmat0	PNmat1	PNmat2	PNmat3	PNmat4	PNmat5	PNmat6	PNmat7	PNmat8	PNmat9	PNmat10	PNmat11	PNmat12	PNmat13	Stay	Outcome
35	2.24	1.24	1.53	3.53	8.21										4	Alive
36	5.20	0.07	0.00	0.03											3	Dead
37	0.34	0.06	0.28	3.07	4.73										4	Alive
38	5.10	5.43	2.12	3.32											3	Alive
39	0.21	0.09	1.50	3.24											3	Alive
40	0.05	0.02	0.02												2	Dead
41	0.54														1	Dead
42	6.84	4.58	5.68												2	Alive
43		0.01	0.00												2	Dead
44	12.93	4.63	6.65												2	Alive
45	6.14	1.28	1.52												2	Alive
46	0.14	0.13	0.28	1.56	5.46	12.04	15.04								6	Alive
47	0.34	0.56	1.48	3.22	6.12	8.11									5	Alive
48	0.93	0.41	1.80												2	Alive
49	0.05	0.05													1	Dead
50	9.44	3.89	2.19												2	Alive
51	0.11	0.10	3.88	9.39	10.91										4	Alive
52	4.07	0.20	0.02	0.25	1.41	4.50									5	Alive
53	11.97	5.40	1.55	0.95	0.83	0.48									5	Alive
54	0.70	0.04	0.04	2.02	6.79										4	Alive
55	1.23	0.03	0.47	4.86	10.85	10.78	17.81	29.66							7	Alive
56	0.17	0.01	0.03	0.76	1.75	8.19									5	Alive
57	0.66	0.07	0.18	1.97											3	Alive
58	1.80	6.70	7.33												2	Alive
59		0.17	0.29	3.61	4.00	2.76									5	Alive
60	2.70	3.30	6.37												2	Alive
61	1.75	0.06													2	Dead
62	3.57	0.45	0.13	0.21	0.57	10.05	17.94	21.76	44.99						8	Alive

LEUKOCYTE PARAMETERS: Peripheral Band Neutrophil Count (PNimm)

Patient no.	PNimm0	PNimm1	PNimm2	PNimm3	PNimm4	PNimm5	PNimm6	PNimm7	PNimm8	PNimm9	PNimm10	PNimm11	PNimm12	PNimm13	Stay	Outcome
1	0.62	0.92	0.10	0.30	6.00	5.85	6.34	3.46							7	Alive
2	3.33	1.15	0.00	0.51											3	Alive
3	0.96	0.53	0.07	0.34	2.16	7.81									5	Dead
4	0.00	0.00	0.09												3	Alive
5		0.25	0.00	0.54											3	Alive
6	1.23	0.24	0.53	0.27	0.00										4	Alive
7	0.12	0.65	2.12	2.35	2.29	2.06	0.23								7	Alive
8	0.04	0.00	0.01	0.68	2.54	0.21	0.00	0.41							7	Alive
9	3.75	0.52	0.36	2.99	0.40	0.36	0.58	0.91							7	Alive
10	0.00	0.61	0.00	0.00	0.03	0.09									5	Alive
11	0.01	0.02	0.55	0.52	0.09										4	Alive
12	0.26	0.13	0.00												2	Alive
13	0.04	0.04	0.00	0.00	0.00	0.10	0.25	0.09	0.04	0.00					9	Alive
14	1.50	1.30	1.45	1.14	1.72										4	Alive
15	0.34	0.47	0.45												2	Dead
16	0.13	0.00	0.00	0.09	0.00	0.00									5	Alive
17	0.11	0.00	0.12	0.00	0.00	0.06									5	Alive
18		0.52	6.81	0.73	0.00	0.00									5	Alive
19	0.36	4.96	0.30	0.07											3	Alive
20	0.16	0.72	0.01	0.12	1.38	0.40	0.77								6	Alive
21	0.95	0.14	0.26	3.00	1.72	0.29									5	Alive
22	0.04	0.05	0.56	0.00											3	Alive
23	0.66	0.26	5.89	3.31	4.20	1.48	1.38								6	Alive
24	0.00	0.00	0.00												3	Dead
25	0.00	0.00	0.00	2.24	2.28	1.30	0.64								6	Alive
26	0.21	1.01	0.00	0.10	0.11	3.16	1.49	0.66	0.72						8	Alive
27	0.00	0.01	1.58	3.73	2.58	3.23	2.42	4.63	1.25	0.72	0.84	3.00	4.00	0.75	13	Alive
28	0.55	0.42	0.00	0.44											3	Alive
29		0.26	0.00												2	Dead
30	1.15	0.21	0.19	1.13	0.85										4	Alive
31		1.10	0.35	0.16											3	Alive
32	0.93	0.00	0.00	2.59	3.92	11.17	7.93	2.34	0.85	0.52					9	Alive
33	0.53	4.84	0.77	0.65											3	Alive
34	0.48														1	Dead

Patient no.	PNimm0	PNimm1	PNimm2	PNimm3	PNimm4	PNimm5	PNimm6	PNimm7	PNimm8	PNimm9	PNimm10	PNimm11	PNimm12	PNimm13	Stay	Outcome
35	1.16	0.16	0.30	0.34	0.14										4	Alive
36	0.52	0.00	0.00	0.54											3	Dead
37	0.56	0.03	0.92	0.69	0.08										4	Alive
38	3.48	0.00	0.00	0.17											3	Alive
39	0.02	0.02	0.50	0.00											3	Alive
40	0.00	0.00	0.00												2	Dead
41	0.15														1	Dead
42	0.70	0.00	0.00												2	Alive
43		0.00	0.00												2	Dead
44	1.35	0.09	0.00												2	Alive
45	0.71	0.00	0.08												2	Alive
46	0.87	0.06	0.31	1.09	1.82	0.61	0.21								6	Alive
47	1.09	0.35	1.14	1.24	0.46	0.40									5	Alive
48	0.93	0.46	0.36												2	Alive
49	0.02	0.02													1	Dead
50	0.35	0.00	0.00												2	Alive
51	0.14	0.26	2.45	0.31	0.40										4	Alive
52	0.22	0.00	0.01	0.12	0.47	0.20									5	Alive
53	0.00	0.08	0.09	0.00	0.00	0.00									5	Alive
54	0.70	0.07	0.00	1.01	0.35										4	Alive
55	0.98	0.00	0.70	1.78	0.68	1.96	1.10	2.06							7	Alive
56	0.00	0.00	0.02	0.70	0.23	0.00									5	Alive
57	0.60	0.07	0.04	0.25											3	Alive
58	0.00	0.00	0.00												2	Alive
59		0.70	0.50	1.09	0.39	0.45									5	Alive
60	1.30	0.50	0.00												2	Alive
61	1.17	0.00													2	Dead
62	1.89	0.22	0.10	0.15	0.49	2.26	1.04	0.59	0.00						8	Alive

LEUKOCYTE PARAMETERS: Peripheral Lymphocyte Count (PLymph)

Patient no	PLymph0	PLymph1	PLymph2	PLymph3	PLymph4	PLymph5	PLymph6	PLymph7	PLymph8	PLymph9	PLymph10	PLymph11	PLymph12	PLymph13	Stay	Outcome
1	0.43	0.56	0.67	2.00	1.50	1.58	2.93	0.58							7	Alive
2	0.29	2.59	2.28	2.70											3	Alive
3	0.24	0.30	0.24	0.20	0.65	1.49									5	Dead
4	2.00	2.15	2.32												3	Alive
5		1.23	2.48	3.46											3	Alive
6	1.18	0.78	2.18	4.46	5.04										4	Alive
7	0.41	0.23	0.71	3.64	1.14	2.06	1.83								7	Alive
8	1.41	2.08	0.33	0.78	2.71	4.94	3.58	4.14							7	Alive
9	0.45	2.06	0.54	0.50	1.19	1.07	1.73	1.81							7	Alive
10	1.75	1.98	1.40	1.29	1.18	2.02									5	Alive
11	0.36	0.77	1.09	3.45	2.91										4	Alive
12	0.53	1.92	1.94												2	Alive
13	0.21	0.47	0.57	0.79	0.44	0.34	0.99	0.03	1.56	3.13					9	Alive
14	0.35	0.91	0.81	6.16	6.30										4	Alive
15	0.23	0.32	0.72												2	Dead
16	4.49	4.11	1.96	1.58	2.67	3.26									5	Alive
17	0.53	0.77	0.81	2.01	0.65	1.43									5	Alive
18		0.13	1.14	1.16	0.70	2.25									5	Alive
19	0.61	2.30	2.90	0.52											3	Alive
20	0.65	0.78	0.08	0.04	0.69	0.40	1.65								6	Alive
21	0.56	0.29	0.31	0.40	0.47	1.47									5	Alive
22	0.22	0.57	1.86	1.54											3	Alive
23	0.79	1.98	1.24	1.66	0.76	1.48	2.21								6	Alive
24	0.47	0.23	0.03												3	Dead
25	1.50	2.20	0.23	0.39	1.37	3.26	1.07								6	Alive
26	1.20	2.02	1.60	1.52	1.13	1.33	3.16	2.65	1.20						8	Alive
27	0.29	0.09	0.26	0.97	1.72	0.86	3.11	3.09	5.62	4.34	0.84	3.00	4.00	2.26	13	Alive
28	1.60	2.60	1.40	2.02											3	Alive
29		0.15	0.27												2	Dead
30	0.14	0.51	1.22	1.55	3.23										4	Alive
31		0.12	0.83	1.77											3	Alive
32	0.50	0.70	1.04	0.65	2.55	5.78	8.65	9.36	10.16	5.21					9	Alive
33	0.68	2.42	1.38	1.86											3	Alive
34	0.48														1	Dead

Patient no	PLymph0	PLymph1	PLymph2	PLymph3	PLymph4	PLymph5	PLymph6	PLymph7	PLymph8	PLymph9	PLymph10	PLymph11	PLymph12	PLymph13	Stay	Outcome
35	0.88	1.36	2.12	2.18	3.02										4	Alive
36	0.13	0.02	0.03	0.16											3	Dead
37	0.22	0.28	0.84	0.58	2.08										4	Alive
38	0.93	2.43	1.06	3.66											3	Alive
39	0.44	1.22	1.60	2.59											3	Alive
40	0.13	0.07	0.07												2	Dead
41	0.49														1	Dead
42	1.16	1.11	1.29												2	Alive
43		0.07	0.00												2	Dead
44	3.09	2.23	1.64												2	Alive
45	0.94	1.28	0.80												2	Alive
46	0.50	0.56	2.11	1.54	0.34	0.62	2.26	2.03	2.24	1.96	2.45	1.43	5.56	3.50	6	Alive
47	0.14	0.27	0.35	0.14	0.34	0.34	1.98	0.50	1.53	3.37	2.26	1.33			5	Alive
48	0.64	0.35	2.07	2.02	4.10	2.09									2	Alive
49	0.06	0.09													1	Dead
50	0.28	0.35	1.85	1.25	2.02	0.90									2	Alive
51	0.47	0.78	1.73	1.39	0.40										4	Alive
52	0.22	0.59	0.17	1.61	1.13	3.60									5	Alive
53	0.29	1.58	0.77	0.45	0.57	1.14									5	Alive
54	0.45	1.30	0.88	1.85	1.84										4	Alive
55	1.23	0.30	0.39	2.27	7.23	3.92	3.56	3.30							7	Alive
56	0.14	0.11	0.25	0.27	1.06	1.16									5	Alive
57	0.36	2.70	2.73	1.48											3	Alive
58	1.40	2.38	2.89												2	Alive
59		0.46	0.80	1.76	1.46	1.69									5	Alive
60	0.56	1.60	2.01												2	Alive
61	0.90	0.13													2	Dead
62	0.14	0.39	0.61	0.21	0.74	3.28	2.60	3.53	0.98						8	Alive

LEUKOCYTE PARAMETERS: Peripheral Monocyte Count (Pmono)

Patient no.	PMono0	PMono1	PMono2	PMono3	PMono4	PMono5	PMono6	PMono7	PMono8	PMono9	PMono10	PMono11	PMono12	PMono13	Stay	Outcome
1	0.56	0.69	0.22	0.10	0.40	0.79	1.22	2.02							7	Alive
2	1.43	0.86	1.82	2.19											3	Alive
3	0.24	0.61	0.17	0.34	0.22	1.49									5	Dead
4	1.64	2.28	2.58												3	Alive
5		0.78	2.00	2.81											3	Alive
6	1.57	1.86	2.51	3.78	1.82										4	Alive
7	0.31	0.80	1.42	0.86	3.43	2.42	3.44								7	Alive
8	0.13	0.23	0.06	0.36	0.49	3.19	2.05	2.90							7	Alive
9	1.28	0.77	0.48	1.33	2.11	2.14	3.07	7.25							7	Alive
10	0.66	2.81	2.38	1.35	1.18	1.38									5	Alive
11	0.10	0.29	1.33	1.63	1.27										4	Alive
12	0.18	0.13	0.60												2	Alive
13	0.04	0.52	0.92	0.36	0.24	0.49	0.62	0.27	0.86	1.83					9	Alive
14	0.35	2.60	2.32	0.80	2.87										4	Alive
15	0.68	0.18	0.72												2	Dead
16	0.13	0.63	1.34	0.70	0.89	0.67									5	Alive
17	0.64	1.55	2.23	2.62	0.82	2.39									5	Alive
18		0.60	1.82	2.90	2.44	2.63									5	Alive
19	3.98	3.72	2.00	1.24											3	Alive
20	0.97	0.36	0.04	0.29	1.81	3.10	2.53								6	Alive
21	0.34	0.26	0.44	1.40	4.52	5.15									5	Alive
22	0.68	1.66	2.91	1.92											3	Alive
23	1.58	1.72	2.79	3.13	0.76	2.66	4.14								6	Alive
24	0.00	0.02	0.00												3	Dead
25	0.60	0.49	0.02	0.78	2.85	4.56	2.98								6	Alive
26	0.62	0.63	0.94	0.96	1.16	1.94	2.98	3.09	6.45						8	Alive
27	0.17	0.26	0.59	0.81	2.58	2.15	5.87	3.09	11.86	3.62	1.68	9.00	9.59	6.79	13	Alive
28	2.35	4.37	3.00	2.38											3	Alive
29		0.62	0.38												2	Dead
30	0.55	0.99	2.54	4.53	5.95										4	Alive
31		9.39	5.78	7.08											3	Alive
32	1.55	0.20	0.21	1.30	5.88	2.70	18.03	14.04	6.78	3.65					9	Alive
33	2.05	3.63	1.68	2.42											3	Alive
34	0.19														1	Dead

Patient no.	PMono0	PMono1	PMono2	PMono3	PMono4	PMono5	PMono6	PMono7	PMono8	PMono9	PMono10	PMono11	PMono12	PMono13	Stay	Outcome
35	2.52	0.96	1.77	2.18	3.02										4	Alive
36	0.33	0.35	0.19	0.85											3	Dead
37	0.10	0.61	0.70	0.90											4	Alive
38	2.09	1.55	2.66	1.19											3	Alive
39	0.38	0.34	1.40	2.27											3	Alive
40	0.03	0.11	0.11												2	Dead
41	0.05														1	Dead
42	2.90	0.55	1.29												2	Alive
43		0.04	0.10												2	Dead
44	1.74	1.60	1.09												2	Alive
45	4.01	2.50	1.12												2	Alive
46	1.12	1.34	1.48	3.12	4.76	6.32	1.85								6	Alive
47	1.70	2.45	2.74	7.44	5.36	3.46									5	Alive
48	0.70	1.61	1.94												2	Alive
49	0.47	0.25													1	Dead
50	1.65	1.25	0.90												2	Alive
51	1.08	1.46	2.14	4.31	8.28										4	Alive
52	0.17	0.11	0.30	1.12	1.69	1.70									5	Alive
53	2.34	1.25	1.89	1.29	1.12	1.20									5	Alive
54	0.65	0.07	1.08	3.53	2.53										4	Alive
55	1.48	2.38	2.69	7.29	3.84	2.94	4.93	6.18							7	Alive
56	0.28	0.17	0.20	0.97	0.26	1.16									5	Alive
57	0.32	0.21	0.49	0.41											3	Alive
58	1.50	0.86	0.89												2	Alive
59		3.48	2.39	1.76	1.85	3.92									5	Alive
60	4.74	4.20	1.77												2	Alive
61	1.22	0.14													2	Dead
62	1.26	1.57	0.58	0.93	2.30	4.92	4.42	3.53	2.93						8	Alive

LEUKOCYTE PARAMETERS: Peripheral Eosinophil Count (PEos)

Patient no.	PEos0	PEos1	PEos2	PEos3	PEos4	PEos5	PEos6	PEos7	PEos8	PEos9	PEos10	PEos11	PEos12	PEos13	Stay	Outcome
1	0.12	0.13	0.12	0.00	0.00	0.00	0.00	0.00							7	Alive
2	0.08	0.43	0.68	0.07											3	Alive
3	0.00	0.00	0.05	0.14	0.07	0.00									5	Dead
4	0.55	0.20	0.26												3	Alive
5		0.21	0.24	0.43											3	Alive
6	0.84	0.06	1.25	1.76	0.98										4	Alive
7	0.05	0.08	0.00	0.00	0.00	0.48	0.00								7	Alive
8	0.00	0.20	0.00	0.00	0.00	0.00	0.13	0.00							7	Alive
9	0.08	0.00	0.00	0.33	0.13	0.36	1.54	1.51							7	Alive
10	0.00	0.00	0.42	0.03	0.00	0.05									5	Alive
11	0.00	0.00	0.00	0.00	0.00										4	Alive
12	0.09	0.19	0.27												2	Alive
13	0.00	0.00	0.14	0.07	0.02	0.00	0.00	0.06	0.12	0.00					9	Alive
14	0.25	0.78	0.23	0.23	0.19										4	Alive
15	0.00	0.04	0.00												2	Dead
16	0.00	0.00	0.00	0.00	0.18	0.00									5	Alive
17	0.00	0.17	0.43	0.18	0.26	0.23									5	Alive
18		0.00	0.00	0.00	0.00	0.00									5	Alive
19	0.00	1.24	1.20	0.91											3	Alive
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00								6	Alive
21	0.04	0.04	0.00	0.00	0.00	0.00									5	Alive
22	0.04	0.26	0.19	0.00											3	Alive
23	0.00	0.26	0.31	0.18	0.00	0.00	0.00								6	Alive
24	0.00	0.00	0.00												3	Dead
25	0.00	0.00	0.00	0.00	0.00	0.00	0.21								6	Alive
26	0.05	0.25	0.19	0.23	0.00	0.00	0.00	0.00	0.00						8	Alive
27	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.62	1.45	0.00	0.00	0.80	1.51	13	Alive
28	0.20	0.31	0.60	0.62											3	Alive
29		0.00	0.04												2	Dead
30	0.00	0.04	0.28	0.10	0.34										4	Alive
31		0.24	0.24	0.16											3	Alive
32	0.00	0.00	0.03	0.00	0.00	0.00	0.72	0.00	0.00	0.52					9	Alive
33	0.00	0.00	0.15	0.19											3	Alive
34	0.00														1	Dead

Patient no.	PEos0	PEos1	PEos2	PEos3	PEos4	PEos5	PEos6	PEos7	PEos8	PEos9	PEos10	PEos11	PEos12	PEos13	Stay	Outcome
35	0.00	0.28	0.18	0.17	0.00										4	Alive
36	0.33	0.02	0.00	0.00											3	Dead
37	0.00	0.06	0.14	0.05											4	Alive
38	0.00	0.19	0.06	0.17											3	Alive
39	0.00	0.03	0.00	0.00											3	Alive
40	0.00	0.00	0.00												2	Dead
41	0.00														1	Dead
42	0.00	0.08	0.34												2	Alive
43		0.00	0.00												2	Dead
44	0.19	0.36	1.53												2	Alive
45	0.00	0.05	0.48												2	Alive
46	0.11	0.13	0.11	0.00	0.00	0.00	0.00								6	Alive
47	0.00	0.00	0.00	0.00	0.00	0.00									5	Alive
48	0.00	0.09	1.01												2	Alive
49	0.00	0.00													1	Dead
50	0.00	0.20	0.30												2	Alive
51	0.00	0.00	0.00	0.00	0.20										4	Alive
52	0.06	0.00	0.00	0.00	0.00	0.00									5	Alive
53	0.00	0.00	0.00	0.11	0.08	0.18									5	Alive
54	0.00	0.32	0.00	0.00	0.00										4	Alive
55	0.16	0.00	0.04	0.00	0.00	0.00	0.00	0.00							7	Alive
56	0.01	0.00	0.00	0.00	0.00	0.00									5	Alive
57	0.04	0.46	0.14	0.00											3	Alive
58	0.30	0.86	0.00												2	Alive
59		0.99	0.21	0.17	0.00	0.09									5	Alive
60	0.00	0.40	1.65												2	Alive
61	0.27	0.06													2	Dead
62	0.14	0.17	0.19	0.00	0.00	0.00	0.00	0.00	0.00						8	Alive

THROMBOCYTE PARAMETERS: Thrombocyte Count (Thr)

Patient no.	ThrA	Thr1	Thr2	Thr3	Thr4	Thr5	Thr6	Thr7	Thr8	Thr9	Thr10	Thr11	Thr12	Thr13	Stay	Outcome
1	228.00	284.00	300.00	371.00	186.00	366.00	207.00	233.00							7	Alive
2	293.00	334.00	352.00	421.00											3	Alive
3	292.00	313.00	401.00	425.00	350.00	149.00									5	Dead
4	663.00	645.00	534.00												3	Alive
5	963.00	704.00	758.00	662.00											3	Alive
6	440.00	289.00	439.00	584.00	624.00										4	Alive
7	513.00	552.00	622.00	644.00	553.00	435.00	546.00								7	Alive
8	146.00	205.00	241.00	214.00	178.00	124.00	57.80	43.90							7	Alive
9	497.00	563.00	429.00	416.00	273.00	269.00	287.00	358.00							7	Alive
10	77.30	136.00	234.00	268.00	266.00	269.00									5	Alive
11	494.00	503.00	402.00	243.00	287.00										4	Alive
12	280.00	223.00	227.00												2	Alive
13	400.00	300.00	289.00	330.00	307.00	191.00	88.10	50.60	8.20	147.00					9	Alive
14	382.00	422.00	471.00	424.00	402.00										4	Alive
15	188.00	11.50	2.10												2	Dead
16	352.00	319.00	314.00	389.00	415.00	375.00									5	Alive
17	293.00	259.00	223.00	325.00	231.00	266.00									5	Alive
18	410.00	332.00	206.00	163.00	113.00	189.00									5	Alive
19	307.00	315.00	331.00	325.00											3	Alive
20	222.00	259.00	303.00	226.00	77.10	4.60	43.60								6	Alive
21	577.00	549.00	504.00	406.00	281.00	213.00									5	Alive
22	147.00	211.00	174.00	173.00											3	Alive
23	230.00	212.00	175.00	131.00	122.00	121.00	184.00								6	Alive
24	607.00	380.00	143.00												3	Dead
25	279.00	293.00	199.00	137.00	110.00	133.00	178.00								6	Alive
26	244.00	304.00	320.00	363.00	396.00	375.00	318.00	371.00	472.00						8	Alive
27	238.00	56.70	44.80	11.40	9.30	6.40	11.10	10.50	10.00	97.70	127.00	114.00	145.00	140.00	13	Alive
28	378.00	424.00	334.00	396.00											3	Alive
29		400.00	260.00												2	Dead
30	313.00	284.00	326.00	352.00	331.00										4	Alive
31	339.00	335.00	354.00	337.00											3	Alive
32	263.00	187.00	138.00	88.10	114.00	154.00	250.00	233.00	285.00	231.00					9	Alive
33	316.00	237.00	133.00	134.00											3	Alive
34	1012.00														1	Dead

Patient no.	ThrA	Thr1	Thr2	Thr3	Thr4	Thr5	Thr6	Thr7	Thr8	Thr9	Thr10	Thr11	Thr12	Thr13	Stay	Outcome
35	557.00	386.00	494.00	443.00	545.00										4	Alive
36	264.00	339.00	212.00	83.60											3	Dead
37	506.00	513.00	507.00	558.00	496.00										4	Alive
38	368.00	350.00	372.00	376.00											3	Alive
39	799.00	786.00	580.00	522.00											3	Alive
40	626.00	401.00	139.00												2	Dead
41	511.00														1	Dead
42	486.00	563.00	606.00												2	Alive
43	78.40	108.00	73.70												2	Dead
44	483.00	555.00	768.00												2	Alive
45	337.00	428.00	477.00												2	Alive
46	249.00	314.00	247.00	244.00	222.00	105.00	129.00								6	Alive
47	319.00	206.00	144.00	95.70	106.00	117.00									5	Alive
48	285.00	295.00	375.00												2	Alive
49	353.00	94.10													1	Dead
50	427.00	388.00	408.00												2	Alive
51	554.00	355.00	322.00	237.00	180.00										4	Alive
52	281.00	373.00	461.00	454.00	401.00	394.00									5	Alive
53	353.00	275.00	333.00	381.00	300.00	334.00									5	Alive
54	355.00	314.00	395.00	467.00	427.00										4	Alive
55	223.00	355.00	387.00	466.00	413.00	297.00	263.00	240.00							7	Alive
56	599.00	500.00	293.00	138.00	55.70	76.00									5	Alive
57	462.00	519.00	480.00	521.00											3	Alive
58	331.00	321.00	338.00												2	Alive
59		137.00	146.00	141.00	98.50	112.00									5	Alive
60	593.00	451.00	511.00												2	Alive
61	58.00	85.70	31.30												2	Dead
62	233.00	229.00	274.00	307.00	181.00	160.00	104.00	122.00	194.00						8	Alive

ERYTHROCYTE PARAMETERS: Haemoglobin Concentration (Hb)

Patient no.	Hb0	Hb1	Hb2	Hb3	Hb4	Hb5	Hb6	Hb7	Hb8	Hb9	Hb10	Hb11	Hb12	Hb13	Stay	Outcome
1	158	186	166	169	152	165	155	160							7	Alive
2	173	120	114	143											3	Alive
3	119	122	103	102	106	88									5	Dead
4	117	109	104												3	Alive
5	119	103	96	97											3	Alive
6	137	133	139	143	124										4	Alive
7	100	99	90	96	88	86	82								7	Alive
8	145	120	119	110	110	108	117	119							7	Alive
9	113	108	96	104	125	119	119	117							7	Alive
10	132	139	127	121	130	128									5	Alive
11	118	108	106	95	106										4	Alive
12	138	121	117												2	Alive
13	83	75	75	79	73	71	68	70	68	68					9	Alive
14	163	147	146	144	164										4	Alive
15	96	72	71												2	Dead
16	110	105	116	114	111	114									5	Alive
17	120	108	122	123	108	111									5	Alive
18	98	89	74	71	68	63									5	Alive
19	160	135	118	129											3	Alive
20	121	94	104	100	86	78	82								6	Alive
21	116	93	85	79	81	87									5	Alive
22	141	133	113	111											3	Alive
23	161	131	116	90	93	96	94								6	Alive
24	104	92	100												3	Dead
25	123	112	110	98	91	97	92								6	Alive
26	109	111	113	116	115	108	99	102	107						8	Alive
27	115	115	104	96	89	83	73	77	71	71	69	69	73	72	13	Alive
28	130	112	111	109											3	Alive
29		166	144												2	Dead
30	175	127	119	117	127										4	Alive
31	176	147	134	122											3	Alive
32	186	126	128	104	111	124	122	99	91	88					9	Alive
33	98	82	75	66											3	Alive
34	100														1	Dead

Patient no.	Hb0	Hb1	Hb2	Hb3	Hb4	Hb5	Hb6	Hb7	Hb8	Hb9	Hb10	Hb11	Hb12	Hb13	Stay	Outcome
35	109	89	87	75	75										4	Alive
36	120	129	114	107											3	Dead
37	195	151	143	148	135										4	Alive
38	134	95	100	108											3	Alive
39	109	101	96	91											3	Alive
40	106	108	115												2	Dead
41	99														1	Dead
42	85	90	93												2	Alive
43	135	115	93												2	Dead
44	82	85	94												2	Alive
45	82	86	77												2	Alive
46	141	135	126	129	124	111	121								6	Alive
47	198	150	149	127	130	141									5	Alive
48	134	132	123												2	Alive
49	196	183													1	Dead
50	132	99	93												2	Alive
51	152	144	123	124	117										4	Alive
52	92	90	102	100	89	87									5	Alive
53	102	83	87	101	98	92									5	Alive
54	139	108	107	108	101										4	Alive
55	130	133	125	107	87	80	80	89							7	Alive
56	117	108	90	80	72	75									5	Alive
57	123	110	93	90											3	Alive
58	154	129	130												2	Alive
59		94	99	93	97	96									5	Alive
60	155	114	123												2	Alive
61	110	106	101												2	Dead
62	135	106	97	85	74	76	82	90	79						8	Alive

ERYTHROCYTE PARAMETERS: Red Cell Count (RCC)

Patient no.	RCC0	RCC1	RCC2	RCC3	RCC4	RCC5	RCC6	RCC7	RCC8	RCC9	RCC10	RCC11	RCC12	RCC13	Stay	Outcome
1	7.22	8.35	7.40	7.69	6.95	7.41	7.04	7.17							7	Alive
2	7.28	4.89	4.68	5.81											3	Alive
3	4.95	5.47	4.59	4.59	4.76	3.89									5	Dead
4	5.94	5.48	5.24												3	Alive
5	5.56	4.89	4.41	4.37											3	Alive
6	5.62	5.55	5.57	5.81	5.16										4	Alive
7	4.91	4.47	4.37	4.58	4.36	4.24	3.95								7	Alive
8	6.64	5.38	5.41	5.01	5.12	4.90	5.40	5.50							7	Alive
9	5.28	5.01	4.49	4.77	5.71	5.43	5.38	5.50							7	Alive
10	6.03	6.35	5.83	5.60	6.05	5.77									5	Alive
11	5.33	4.87	4.78	4.28	4.83										4	Alive
12	6.15	5.35	5.17												2	Alive
13	4.26	3.77	3.85	3.97	3.74	3.65	3.47	3.63	3.52	3.38					9	Alive
14	6.90	6.07	6.15	6.13	6.91										4	Alive
15	4.39	3.56	3.48												2	Dead
16	5.12	4.96	5.50	5.40	5.23	5.52									5	Alive
17	5.12	4.60	5.27	5.34	4.71	4.89									5	Alive
18	6.11	5.29	4.54	4.38	4.25	4.01									5	Alive
19	8.16	6.74	5.99	6.49											3	Alive
20	5.89	4.61	5.18	4.97	4.34	3.89	4.09								6	Alive
21	5.86	4.76	4.37	4.00	4.15	4.55									5	Alive
22	7.01	6.70	5.63	5.56											3	Alive
23	7.69	6.38	5.55	4.28	4.42	4.50	4.51								6	Alive
24	5.05	4.47	4.77												3	Dead
25	6.17	5.58	5.50	4.94	4.62	4.90	4.64								6	Alive
26	5.07	5.19	5.43	5.50	5.49	5.11	4.71	4.83	5.05						8	Alive
27	5.78	5.77	5.37	4.92	4.50	4.24	3.86	3.82	3.62	3.64	3.52	3.43	3.54	3.45	13	Alive
28	5.69	4.96	4.90	4.87											3	Alive
29		8.51	7.37												2	Dead
30	8.15	5.83	5.18	5.12	5.58										4	Alive
31	8.02	6.59	5.89	5.40											3	Alive
32	8.23	5.63	5.72	4.65	4.98	5.53	5.58	4.53	4.21	4.05					9	Alive
33	4.89	4.08	3.78	3.30											3	Alive
34	4.92														1	Dead

Patient no.	RCC0	RCC1	RCC2	RCC3	RCC4	RCC5	RCC6	RCC7	RCC8	RCC9	RCC10	RCC11	RCC12	RCC13	Stay	Outcome
35	5.85	4.71	4.69	3.89	3.93										4	Alive
36	5.72	6.06	5.37	5.11											3	Dead
37	8.81	6.89	6.54	6.83	6.18										4	Alive
38	6.42	4.46	4.75	5.10											3	Alive
39	4.87	4.60	4.49	4.09											3	Alive
40	4.93	5.01	5.36												2	Dead
41	4.47														1	Dead
42	3.89	4.19	4.14												2	Alive
43	6.60	5.63	4.51												2	Dead
44	3.70	3.80	4.18												2	Alive
45	3.94	4.20	3.74												2	Alive
46	7.56	7.27	6.79	6.88	6.57	5.80	6.52								6	Alive
47	11.50	8.58	8.55	7.37	7.50	8.15									5	Alive
48	5.92	5.78	5.45												2	Alive
49	8.94	8.52													1	Dead
50	5.77	4.31	4.01												2	Alive
51	6.48	5.23	5.30	5.30	5.07										4	Alive
52	4.26	4.13	4.82	4.73	4.16	4.13									5	Alive
53	4.55	3.67	3.85	4.53	4.43	4.24									5	Alive
54	5.98	4.57	4.66	4.67	4.42										4	Alive
55	5.94	6.12	5.77	4.95	3.94	3.70	3.71	4.25							7	Alive
56	5.62	5.20	4.36	3.89	3.46	3.55									5	Alive
57	6.12	5.51	4.60	4.55											3	Alive
58	6.66	5.57	5.64												2	Alive
59		4.57	4.80	4.46	4.73	4.63									5	Alive
60	7.22	5.25	5.75												2	Alive
61	5.84	5.57	5.36												2	Dead
62	6.27	4.83	4.43	3.91	3.43	3.56	3.81	4.17	3.76						8	Alive

ERYTHROCYTE PARAMETERS: Haematocrit (Ht)

Patient no.	Ht0	Ht1	Ht2	Ht3	Ht4	Ht5	Ht6	Ht7	Ht8	Ht9	Ht10	Ht11	Ht12	Ht13	Stay	Outcome
1	0.45	0.52	0.46	0.48	0.44	0.46	0.43	0.45							7	Alive
2	0.49	0.33	0.32	0.40											3	Alive
3	0.33	0.37	0.31	0.30	0.31	0.25									5	Dead
4	0.34	0.31	0.30												3	Alive
5	0.34	0.29	0.27	0.27											3	Alive
6	0.39	0.39	0.39	0.41	0.36										4	Alive
7	0.31	0.27	0.26	0.28	0.26	0.25	0.26								7	Alive
8	0.42	0.35	0.34	0.32	0.32	0.33	0.34	0.35							7	Alive
9	0.34	0.32	0.28	0.30	0.38	0.34	0.33	0.34							7	Alive
10	0.39	0.41	0.39	0.38	0.39	0.37									5	Alive
11	0.34	0.31	0.30	0.27	0.31										4	Alive
12	0.40	0.35	0.33												2	Alive
13	0.25	0.22	0.23	0.24	0.22	0.21	0.20	0.21	0.20	0.20					9	Alive
14	0.45	0.40	0.40	0.41	0.46										4	Alive
15	0.26	0.21	0.21												2	Dead
16	0.32	0.30	0.33	0.32	0.31	0.33									5	Alive
17	0.34	0.30	0.34	0.35	0.31	0.32									5	Alive
18	0.30	0.26	0.22	0.21	0.20	0.19									5	Alive
19	0.46	0.38	0.34	0.36											3	Alive
20	0.36	0.29	0.32	0.31	0.26	0.23	0.25								6	Alive
21	0.34	0.27	0.25	0.22	0.23	0.25									5	Alive
22	0.41	0.39	0.33	0.32											3	Alive
23	0.46	0.39	0.33	0.26	0.27	0.27	0.27								6	Alive
24	0.31	0.27	0.29												3	Dead
25	0.36	0.33	0.32	0.29	0.26	0.28	0.27								6	Alive
26	0.32	0.32	0.34	0.34	0.34	0.32	0.29	0.30	0.31						8	Alive
27	0.34	0.34	0.32	0.29	0.27	0.26	0.23	0.24	0.23	0.23	0.22	0.21	0.22	0.22	13	Alive
28	0.36	0.32	0.32	0.32											3	Alive
29		0.50	0.43												2	Dead
30	0.52	0.38	0.34	0.33	0.36										4	Alive
31	0.51	0.43	0.38	0.35											3	Alive
32	0.55	0.38	0.38	0.30	0.33	0.36	0.37	0.30	0.28	0.26					9	Alive
33	0.29	0.25	0.23	0.20											3	Alive
34	0.31														1	Dead

Patient no.	Ht0	Ht1	Ht2	Ht3	Ht4	Ht5	Ht6	Ht7	Ht8	Ht9	Ht10	Ht11	Ht12	Ht13	Stay	Outcome
35	0.33	0.27	0.27	0.22	0.22										4	Alive
36	0.35	0.38	0.33	0.31											3	Dead
37	0.57	0.44	0.42	0.44	0.39										4	Alive
38	0.41	0.29	0.30	0.33											3	Alive
39	0.32	0.30	0.29	0.27											3	Alive
40	0.30	0.32	0.34												2	Dead
41	0.29														1	Dead
42	0.25	0.28	0.27												2	Alive
43	0.41	0.35	0.28												2	Dead
44	0.24	0.25	0.28												2	Alive
45	0.24	0.26	0.23												2	Alive
46	0.41	0.40	0.38	0.38	0.37	0.32	0.36								6	Alive
47	0.57	0.44	0.43	0.37	0.37	0.41									5	Alive
48	0.39	0.38	0.36												2	Alive
49	0.54	0.53													1	Dead
50	0.39	0.29	0.27												2	Alive
51	0.43	0.42	0.35	0.35	0.36										4	Alive
52	0.27	0.26	0.30	0.29	0.26	0.25									5	Alive
53	0.30	0.24	0.26	0.30	0.29	0.28									5	Alive
54	0.41	0.32	0.34	0.34	0.30										4	Alive
55	0.41	0.42	0.40	0.32	0.26	0.23	0.23	0.27							7	Alive
56	0.34	0.32	0.27	0.24	0.21	0.21									5	Alive
57	0.37	0.32	0.27	0.27											3	Alive
58	0.44	0.37	0.38												2	Alive
59		0.27	0.29	0.27	0.28	0.28									5	Alive
60	0.45	0.33	0.37												2	Alive
61	0.34	0.33	0.32												2	Dead
62	0.41	0.32	0.29	0.25	0.22	0.22	0.24	0.26	0.23						8	Alive

ERYTHROCYTE PARAMETERS: Mean Corpuscular Volume (MCV)

Patient no.	MCV0	MCV1	MCV2	MCV3	MCV4	MCV5	MCV6	MCV7	MCV8	MCV9	MCV10	MCV11	MCV12	MCV13	Stay	Outcome
1	62.1	62.4	62.7	62.0	62.8	62.3	61.7	62.3							7	Alive
2	67.5	68.3	68.4	68.7											3	Alive
3	67.3	67.2	67.2	65.8	64.3	65.0									5	Dead
4	57.0	56.8	57.0												3	Alive
5	60.4	59.6	61.2	62.7											3	Alive
6	70.1	71.1	70.5	69.9	69.8										4	Alive
7	62.2	61.3	59.7	60.4	59.9	58.9	66.7								7	Alive
8	63.4	64.4	63.4	62.9	63.1	66.5	63.0	63.3							7	Alive
9	64.1	63.4	62.0	61.9	66.2	61.8	61.9	61.9							7	Alive
10	63.7	64.0	66.8	67.4	64.2	64.2									5	Alive
11	63.1	63.0	63.4	64.8	64.0										4	Alive
12	65.0	64.7	64.3												2	Alive
13	59.7	59.4	58.8	59.6	57.9	57.5	56.8	56.5	57.0	57.7					9	Alive
14	65.8	65.8	65.5	67.5	65.8										4	Alive
15	58.8	59.4	59.5												2	Dead
16	61.6	60.4	59.8	59.7	59.8	59.1									5	Alive
17	66.0	65.8	64.9	64.6	64.7	64.5									5	Alive
18	48.3	48.6	49.4	48.6	47.9	47.4									5	Alive
19	56.2	56.7	56.0	55.8											3	Alive
20	61.8	62.5	60.9	61.6	60.9	60.1	61.1								6	Alive
21	57.4	57.3	56.9	55.7	55.4	55.4									5	Alive
22	58.1	58.8	58.3	58.3											3	Alive
23	59.8	60.4	60.2	61.0	60.4	59.9	60.6								6	Alive
24	60.7	61.2	61.1												3	Dead
25	58.3	58.3	58.7	58.2	57.0	56.9	57.2								6	Alive
26	62.1	61.3	62.2	61.9	61.6	62.2	61.2	61.9	61.5						8	Alive
27	58.1	58.7	59.2	59.6	60.3	60.6	60.7	62.4	62.8	62.4	62.9	62.0	62.8	62.9	13	Alive
28	64.0	64.9	64.6	65.1											3	Alive
29		59.0	58.6												2	Dead
30	63.6	64.5	64.7	64.6	64.1										4	Alive
31	63.2	65.0	65.0	65.2											3	Alive
32	66.6	66.8	65.8	65.4	65.5	65.0	65.4	65.8	65.9	65.1					9	Alive
33	60.1	60.2	59.9	60.0											3	Alive
34	62.5														1	Dead

Patient no.	MCV0	MCV1	MCV2	MCV3	MCV4	MCV5	MCV6	MCV7	MCV8	MCV9	MCV10	MCV11	MCV12	MCV13	Stay	Outcome
35	56.5	56.8	56.8	56.1	55.8										4	Alive
36	61.2	62.1	60.8	60.0											3	Dead
37	65.2	64.5	64.0	63.9	63.1										4	Alive
38	64.0	63.9	63.3	64.1											3	Alive
39	66.3	65.5	65.2	66.5											3	Alive
40	61.6	63.3	63.9												2	Dead
41	65.3														1	Dead
42	65.3	66.1	65.4												2	Alive
43	62.1	62.2	62.5												2	Dead
44	64.4	65.8	65.8												2	Alive
45	60.5	61.0	60.7												2	Alive
46	54.4	55.1	55.5	55.1	56.1	55.7	55.3								6	Alive
47	49.7	50.9	50.3	50.3	49.5	50.2									5	Alive
48	65.5	65.7	65.8												2	Alive
49	60.2	62.4													1	Dead
50	67.2	67.0	67.1												2	Alive
51	66.4	66.8	66.7	66.3	70.0										4	Alive
52	63.0	62.9	62.2	62.2	61.5	61.4									5	Alive
53	65.4	65.3	67.2	65.2	65.2	65.0									5	Alive
54	69.0	69.1	72.2	72.4	67.6										4	Alive
55	68.2	68.6	69.1	63.9	64.7	62.4	63.1	63.4							7	Alive
56	61.0	60.7	61.0	61.7	59.6	60.2									5	Alive
57	59.8	58.2	58.4	58.3											3	Alive
58	65.8	66.8	66.7												2	Alive
59		59.6	60.9	60.0	59.8	60.4									5	Alive
60	62.2	63.1	63.9												2	Alive
61	58.3	59.5	59.8												2	Dead
62	64.6	66.1	64.4	63.8	62.6	63.0	62.9	62.8	62.2						8	Alive

ERYTHROCYTE PARAMETERS: Mean Corpuscular Haemoglobin Concentration (MCHC)

Patient no	MCHC0	MCHC1	MCHC2	MCHC3	MCHC4	MCHC5	MCHC6	MCHC7	MCHC8	MCHC9	MCHC10	MCHC11	MCHC12	MCHC13	Stay	Outcome
1	35.3	35.8	35.8	35.3	34.9	35.7	35.7	35.9							7	Alive
2	35.3	36.1	35.7	35.7											3	Alive
3	35.7	33.2	33.5	33.8	34.5	35.0									5	Dead
4	34.5	35.0	34.8												3	Alive
5	35.4	35.2	35.4	35.4											3	Alive
6	34.9	33.8	35.4	35.2	34.6										4	Alive
7	32.6	36.3	34.5	34.5	33.6	34.4	31.0								7	Alive
8	34.4	34.5	34.6	34.8	34.0	33.1	34.3	34.1							7	Alive
9	33.4	33.9	34.5	35.4	33.1	35.5	35.6	34.5							7	Alive
10	34.2	34.1	32.6	32.2	33.6	34.6									5	Alive
11	35.0	35.1	35.0	34.6	34.2										4	Alive
12	34.4	34.9	35.1												2	Alive
13	32.8	33.4	33.1	33.2	33.7	33.8	34.7	34.3	34.1	35.0					9	Alive
14	35.9	36.8	36.3	34.9	36.0										4	Alive
15	37.5	34.0	34.4												2	Dead
16	34.8	34.9	35.2	35.5	35.4	34.9									5	Alive
17	35.5	35.8	35.8	35.7	35.5	35.3									5	Alive
18	33.1	34.6	32.8	33.4	33.5	33.4									5	Alive
19	34.9	35.4	35.2	35.6											3	Alive
20	33.2	32.5	32.9	32.8	32.5	33.1	32.7								6	Alive
21	34.4	34.2	34.2	35.6	35.4	34.6									5	Alive
22	34.8	33.8	34.3	34.4											3	Alive
23	34.9	34.0	34.7	34.3	34.7	35.6	34.5								6	Alive
24	33.9	33.9	34.3												3	Dead
25	34.2	34.4	34.0	33.9	34.6	34.9	34.4								6	Alive
26	34.7	34.9	33.4	34.2	34.2	34.0	34.5	34.1	34.5						8	Alive
27	34.1	34.0	32.7	32.9	32.7	32.2	31.4	32.3	31.1	31.2	31.3	32.4	32.8	33.0	13	Alive
28	35.6	34.9	35.0	34.4											3	Alive
29		33.2	33.4												2	Dead
30	33.8	33.9	35.4	35.4	35.4										4	Alive
31	34.7	34.3	34.9	34.6											3	Alive
32	34.0	33.6	33.9	34.4	34.1	34.4	33.5	33.1	32.8	33.5					9	Alive
33	33.4	33.3	33.1	33.6											3	Alive
34	32.5														1	Dead

Patient no	MCHC0	MCHC1	MCHC2	MCHC3	MCHC4	MCHC5	MCHC6	MCHC7	MCHC8	MCHC9	MCHC10	MCHC11	MCHC12	MCHC13	Stay	Outcome
35	32.8	33.2	32.7	34.4	34.4										4	Alive
36	34.4	34.4	34.8	34.8											3	Dead
37	33.9	34.1	34.1	34.0	34.7										4	Alive
38	32.7	33.2	33.3	33.1											3	Alive
39	33.6	33.4	33.0	33.3											3	Alive
40	34.8	34.1	33.5												2	Dead
41	33.9														1	Dead
42	33.3	32.3	34.4												2	Alive
43	32.9	33.0	33.0												2	Dead
44	34.4	33.8	34.0												2	Alive
45	34.6	33.5	33.7												2	Alive
46	34.1	33.7	33.3	34.0	33.7	34.3	33.6								6	Alive
47	34.7	34.4	34.6	34.3	35.2	34.6									5	Alive
48	34.6	34.7	34.4												2	Alive
49	36.5	34.5													1	Dead
50	34.0	34.4	34.4												2	Alive
51	35.2	34.5	34.7	35.3	33.0										4	Alive
52	34.3	34.6	34.0	34.0	34.8	34.4									5	Alive
53	34.4	34.6	33.6	34.1	34.0	33.4									5	Alive
54	33.7	34.2	31.9	31.9	34.0										4	Alive
55	32.0	31.6	31.3	33.9	34.0	34.5	34.2	33.0							7	Alive
56	34.1	34.1	33.9	33.4	34.9	34.9									5	Alive
57	33.6	34.2	34.7	33.9											3	Alive
58	35.2	34.6	34.5												2	Alive
59		34.4	33.7	34.6	34.1	34.3									5	Alive
60	34.6	34.3	33.6												2	Alive
61	32.2	31.9	31.5												2	Dead
62	33.5	33.2	34.1	33.9	34.3	33.9	34.4	34.2	33.6						8	Alive

ERYTHROCYTE PARAMETERS: Red Cell Distribution Width (RDW)

Patient no.	RDW0	RDW1	RDW2	RDW3	RDW4	RDW5	RDW6	RDW7	RDW8	RDW9	RDW10	RDW11	RDW12	RDW13	Stay	Outcome
1	15.3	15.5	16.3	15.6	15.9	14.7	15.3	15.1							7	Alive
2	15.5	15.2	14.1	17.0											3	Alive
3	14.5	15.0	15.6	14.9	14.9	16.0									5	Dead
4	20.5	20.2	20.2												3	Alive
5	19.6	20.4	20.4	19.3											3	Alive
6	15.6	15.9	16.5	15.4	15.4										4	Alive
7	16.0	16.4	16.8	16.0	16.2	17.5	17.2								7	Alive
8	15.3	15.8	15.3	15.4	16.3	15.1	15.6	15.6							7	Alive
9	15.9	16.0	17.0	16.7	15.2	17.6	16.3	16.9							7	Alive
10	16.4	15.5	15.1	14.8	16.3	16.3									5	Alive
11	14.8	14.4	14.9	14.8	14.3										4	Alive
12	17.1	16.5	16.7												2	Alive
13	17.2	17.7	17.7	18.8	19.0	18.7	19.1	19.8	19.4	20.2					9	Alive
14	16.1	15.1	15.6	16.3	15.3										4	Alive
15	18.3	16.8	15.7												2	Dead
16	16.2	18.3	16.8	17.5	15.2	15.6									5	Alive
17	15.1	13.1	14.7	13.8	13.3	14.3									5	Alive
18	23.2	24.0	23.5	21.8	23.4	24.8									5	Alive
19	19.4	19.4	19.0	19.2											3	Alive
20	18.0	16.5	16.3	18.3	17.3	18.1	17.6								6	Alive
21	18.1	15.3	16.9	17.0	18.5	17.8									5	Alive
22	17.0	17.2	15.0	15.5											3	Alive
23	17.3	17.7	15.4	16.5	16.1	16.2	17.0								6	Alive
24	15.3	14.6	15.3												3	Dead
25	17.3	19.0	16.9	16.8	16.7	18.4	17.4								6	Alive
26	16.8	15.9	16.2	15.2	15.2	15.1	15.6	14.6	15.4						8	Alive
27	21.2	19.0	18.9	19.3	17.9	18.4	18.7	19.8	18.3	18.1	17.7	21.7	21.5	24.7	13	Alive
28	16.7	14.9	16.0	16.7											3	Alive
29		17.5	18.1												2	Dead
30	18.0	16.4	15.8	16.7	15.6										4	Alive
31	16.6	15.6	13.8	14.2											3	Alive
32	15.0	14.5	14.4	13.8	13.6	14.3	13.4	14.0	14.0	13.3					9	Alive
33	16.4	17.3	17.9	16.8											3	Alive
34	16.1														1	Dead

Patient no.	RDW0	RDW1	RDW2	RDW3	RDW4	RDW5	RDW6	RDW7	RDW8	RDW9	RDW10	RDW11	RDW12	RDW13	Stay	Outcome
35	16.2	17.8	17.1	17.3	17.1										4	Alive
36	18.2	18.0	18.3	17.5											3	Dead
37	16.0	13.9	14.1	15.2	14.3										4	Alive
38	16.0	15.0	15.1	15.7											3	Alive
39	18.0	17.0	17.9	16.7											3	Alive
40	16.5	16.7	17.5												2	Dead
41	16.6														1	Dead
42	16.0	16.8	17.2												2	Alive
43	15.8	16.3	14.2												2	Dead
44	17.1	18.5	19.4												2	Alive
45	16.4	15.3	15.8												2	Alive
46	22.2	21.8	21.2	19.6	21.9	21.7	20.8								6	Alive
47	35.0	29.6	28.7	28.9	32.3	29.6									5	Alive
48	15.6	15.8	16.7												2	Alive
49	16.8	16.4													1	Dead
50	13.7	14.6	14.2												2	Alive
51	14.9	14.9	14.8	13.4	14.8										4	Alive
52	17.1	16.3	16.4	16.3	15.9	16.7									5	Alive
53	17.0	15.9	16.5	15.6	17.7	17.2									5	Alive
54	14.6	14.8	16.0	15.2	14.2										4	Alive
55	16.3	18.2	16.8	14.9	14.9	15.2	15.1	16.0							7	Alive
56	16.1	15.6	15.1	16.4	15.5	15.6									5	Alive
57	16.9	16.9	17.5	16.7											3	Alive
58	14.8	16.0	13.0												2	Alive
59		20.1	19.3	20.7	18.0	19.8									5	Alive
60	16.2	13.8	14.6												2	Alive
61	17.9	17.9	18.3												2	Dead
62	16.7	16.1	16.8	16.7	16.1	15.8	16.1	16.5	16.5						8	Alive

APPENDIX M

All the puppies that died (natural & euthanized) were subjected to a full Post Mortem and histopathological examination.

1. Puppy no: P03 [PM473.04 (Patient no: 171770)]

Euthanized

Changes were consistent with CPV.

Gross PM changes:

Dehydration

Blood smear – regenerative left shift, neutrophilia, 3+ active monocytes

Pale mucous membranes

Stomach was empty

Small intestine – the serosa was roughened

Small intestine mucosa – dull, bile stained, fissured, no mucous were present

Thymus – very small and difficult to identify within the anterior mediastinum

Histopathology:

Duodenum – total loss of villi, collapse of lamina propria. Attempts at covering the damaged lamina propria were present in the form of a single layer of enterocytes. The lamina propria closest to the lumen showed a heavy infiltrate of lymphocytes. Moderate crypt loss with dilatation of the remaining ones was also present.

Ileum – massive loss of villi and collapse of the lamina propria together with crypt loss was present. The thin layer of lamina propria was covered by a single layer of enterocytes

Ileum anterior to the caecum – most of villi lost, lamina propria collapsed, covered by a single layer of cells. The few remaining crypts were dilated and lined by basophilic enterocytes. GALT showed 50% depletion.

Colon posterior caecum – nothing remarkable

Peripheral lymph node – no follicles were present, cortical lymphocytes were mildly depleted.

Mesenteric lymph node – no follicles were present, cortical lymphocytes were mildly depleted.

Spleen – moderate depletion of the white pulp, near total loss of small lymphocytes within the white pulp.

Thymus – marked loss of cortical lymphocytes, collapse of the remaining stroma.

Bone marrow – very cellular, marked increase in M: E ratio. Numerous megakaryocytes were seen.

CNS – no abnormalities were observed, no changes consistent with Distemper virus infection.

2. Puppy no: P15 [PM825.04 (Patient no: 175104)]

Changes were consistent with Infectious Canine Hepatitis (ICH), despite testing positive for CPV on electron microscopy.

Gross PM changes:

Blood smear – leukopaenia (mainly active monocytes present), thrombocytopaenia

Paintbrush haemorrhages on gastric serosa

Stomach full of black serous haemorrhagic content

Diffuse catarrhal enteritis

Marked lymph node congestion

Acute inhalation pneumonia (right hand side)

Histopathology

Duodenum – nothing remarkable

Ileum – nothing remarkable – moderate post mortal (PM) sloughing

Ileum anterior to caecum – nothing remarkable – moderate PM sloughing

Colon posterior to caecum – nothing remarkable

Peripheral lymph node – severely congested

Mesenteric lymph node –severely congested

Liver – extensive necrosis with intranuclear inclusion bodies - ICH

Spleen – nothing remarkable

Thymus – marked interlobular oedema, severe loss of thymic lymphocytes such that there was no cortico-medullary differentiation

Bone marrow – moderately hypocellular with both erythroid and myeloid lines being affected. No megakaryocytes were observed

CNS – multifocal gliosis within the spinal cord, scattered endothelial cell necrosis and intranuclear inclusion bodies were present.

3. Puppy no: P24 [PM1012.04 (Patient no: 133069)]

Changes were consistent with CPV

Gross PM changes

Dehydrated

Blood smear - marked leukopaenia, thrombocytopaenia

Pale mucous membranes

Stomach was full of mucoid material

Small intestine serosa was roughened and petechiated

Small intestine mucosa was roughened, mucous absent, mucosa petechiated

Thymus pale pink, only a few nodules were present in the anterior mediastinum

Bone marrow was congested

Histopathology

Duodenum – total loss of villi, collapse of lamina propria, massive loss of crypts, remaining crypts dilated, lined by large basophilic enterocytes and many contained necrotic debris

Ileum – total loss of lining enterocytes, stunting of some villi in some areas with loss of villi in others. The loss of crypts was not as severe as in the duodenum. GALT showed 80-90% loss of lymphocytes. Remaining crypts were dilated, some contained necrotic debris and they were all lined by young basophilic enterocytes.

Ileum anterior to caecum – villous necrosis over GALT marked with total loss of crypts. GALT showed 90% depletion of lymphocytes. Villi still present adjacent to GALT zone, only showed scattered mild crypt dilation containing minimal necrotic debris

Colon posterior to caecum – Mild loss of goblet cells

Peripheral lymph node – follicles depleted

Mesenteric lymph node – follicles depleted, para-cortical areas depleted, marked medullary congestion

Spleen – moderate depletion of white pulp, marked lymphocyte necrosis within the white pulp.

Thymus – scattered lobules of thymic tissue with marked interlobular oedema and congestion. Normal architecture of thymus was missing with severe loss of cortical lymphocytes and only a few surrounding hassles corpuscles, most of the lobule was being made up of supporting tissue.

Bone marrow - marked bone marrow atrophy – small islands of haemopoetic cells were scattered between dilated sinusoids. Red cell precursors were more so than myeloid. Plasma cell clumps present within some of the islands.

CNS – no abnormalities were observed, no changes consistent with Distemper virus infection.

4. Puppy no: P29 [PM1064.04 (Patient no: 127409)]

Changes were consistent with CPV

Gross PM changes

Blood smear – severe leukopaenia and thrombocytopaenia, 2+ active monocytes

Stomach empty

Small intestinal serosa roughened

Small intestinal mucosa dull, bile stained, fissured, no mucous present

Severe thymic atrophy

Pancreas showed scattered areas of haemorrhage between the lobules

Histopathology

Duodenum – total loss of villi, collapse of the lamina propria and near total loss of crypts. Scattered dilated crypt remnants were all that remained.

Ileum – villi collapsed and covered with large cuboidal enterocytes, lamina propria had collapsed and in some areas was bare. There was moderate crypt loss and mild dilatation of remaining crypts.

Ileum anterior to caecum – 90% depletion of lymphocytes from the GALT and marked villi and crypt loss over the GALT area

Colon posterior to caecum – increased basophilia and mild loss of goblet cells

Pancreas – massive interstitial haemorrhage with focal necrosis and neutrophil infiltrate – acute interstitial pancreatitis.

Peripheral lymph node – nothing remarkable
Mesenteric lymph node – moderate loss of follicles and mild cortical depletion of lymphocytes
Spleen – moderate depletion of white pulp, marked loss of small lymphocytes
Thymus – massive thymic atrophy with total absence of normal cortex

Bone marrow – moderately hypocellular, both erythroid and myeloid lines were affected, but the myeloid more so. Scattered megakaryocytes were present.

CNS – nothing remarkable, no changes consistent with Distemper virus infection.

5. Puppy no: P34 [PM1098.04 (Patient no: 114419)]

Changes were consistent with CPV.

Gross PM changes

Blood smear - marked leukopaenia, moderate thrombocytopaenia
Pale mucous membranes
Stomach empty
Small intestine mucosa sloughed, necrotic pseudomembrane was present, diffuse transmural congestion, and GALT was sunken.

Ileocaecal intussusception

Thymus very small, consisting of small lobules scattered within gelatinous mediastinum

Histopathology

Duodenum – moderate loss of villi with collapse of the underlying lamina propria and loss of associated crypts. The remaining mucosa had slightly collapsed villi and dilated crypts containing necrotic debris.
Ileum – transmural congestion, mild focal loss of villi and crypts.

Ileum anterior to caecum – transmural congestion, villi flat and stunted, covered with a single layer of basophilic cuboidal immature enterocytes. Crypts dilated and some contained necrotic debris. GALT showed 90% depletion.
Colon posterior to caecum – increased basophilia of lining cells and a decrease in the number of goblet cells

Peripheral lymph node – marked cortical lymphocyte depletion, no follicles were present.
Mesenteric lymph node – marked cortical lymphocyte depletion, no follicles were present.
Spleen – absent white pulp
Thymus – normal structure was absent due to massive loss of cortical lymphocytes, with only supporting stroma remaining

Bone marrow – there was marked atrophy with small islands of cells scattered between dilated sinusoids. Both erythroid and myeloid series were markedly affected. Megakaryocytes were present but small.

CNS – no abnormalities were observed, no changes consistent with Distemper virus infection.

6. Puppy no: P36 [PM22.05 (Patient no: 133695)]

Euthanized
Changes were consistent with CPV
Gross PM changes
Blood smear - moderate leukopaenia, thrombocytopaenia
Pale mucous membranes
Stomach empty
Small intestine serosa finely speckled with red (petechiae?)
Small intestine mucosa – thick, reddened, bile stained mucous strands in the jejenum. GALT was sunken. Contents were serous.
Thymus – moderate interlobular oedema and atrophy
Bone marrow – fatty with red streaks

Histopathology

Duodenum – total loss of villi and collapse of the lamina propria. Near total loss of crypts, remaining crypts dilated and lined by hypertrophic enterocytes. Some crypts contained necrotic debris.

Ileum – total loss of villi and crypts with the formation of fissures within the remnants of the mucosa.

Ileum anterior to caecum – there was total loss of villi and near total loss of crypts. GALT showed 80% depletion. Crypt remnants were dilated and contained debris.

Colon posterior to caecum – there was increased basophilia of lining cells with a decrease in goblet cell numbers.

Peripheral lymph node – mild lymphoid atrophy of the cortex, follicles were depleted

Mesenteric lymph node – mild lymphoid atrophy, follicles were depleted

Spleen – mild depletion of white pulp

Thymus – massive loss of cortical lymphocytes, no normal structure, just supporting stroma remained.

Bone marrow – mildly atrophic, erythroid more so than myeloid. Mature stages were absent.

CNS – no abnormalities were observed, no changes consistent with Distemper virus infection.

7. Puppy no: P40 [PM41.05 (Patient no: 178304)]

Changes were consistent with CPV

Gross PM changes

Blood smear - moderate leukopaenia

Pale mucous membranes

Small intestinal serosa – finely speckled with red spots

Small intestinal mucosa – scattered petechiae, bile stained serous fluid that covered the mucosa

Bone marrow – congested

Histopathology

Duodenum – there was total loss of villi and a moderate loss of crypts. Crypts were dilated and some contained necrotic debris.

Ileum – there were multifocal areas of villous collapse and crypt loss. In between these areas the villi appeared normal but the crypts were dilated and some contained debris.

Ileum anterior to caecum – there was total loss of villi and most crypts in 90% of the section. The remaining 10% had villi but their crypts were dilated and contained debris.

Colon posterior to caecum – increased basophilia and loss of goblet cells

Peripheral lymph node – marked lymphocyte depletion, no follicles were present

Mesenteric lymph node – moderate lymphocyte depletion, no follicles were present

Spleen – marked depletion of white pulp

Thymus – marked loss of cortical lymphocytes with collapse of the remaining supporting stroma

Bone marrow – there was marked atrophy with cells scattered between the dilated sinusoids

CNS – no abnormalities were observed, no changes consistent with Distemper virus infection.

8. Puppy no: P41 [PM31.05 (Patient no: 118092)]

Changes were consistent with CPV

Gross PM changes

Blood smear - moderate leukopaenia, moderate anisocytosis and hypochromasia.

Mucous membranes pale

Stomach full of mucous, contained a plastic foreign body, multifocal ecchymoses.

Small intestine serosa – speckled red

Small intestine mucosa – scattered fibrin strands and linear haemorrhages.

Bone marrow congested and slightly gelatinous

Histopathology

Duodenum – villi detail couldn't be determined due to autolysis but there was marked crypt loss with dilation of remaining crypts.

Ileum – villi collapsed and stunted. Marked loss of crypts.

Ileum anterior to caecum – there was total loss of villi and crypts. GALT showed 90% depletion

Colon posterior to caecum – there was increased basophilia and loss of goblet cells.

Peripheral lymph node – there was marked loss of lymphocytes, no follicles were present.

Mesenteric lymph node – there was marked depletion of lymphocytes, no follicles were present.

Thymus – massive loss of cortical lymphocytes, mainly supporting stroma was left.

Bone marrow – there was marked atrophy with cells scattered thinly amongst the dilated sinusoids. All lines were affected.

CNS – no abnormalities were observed, no changes consistent with Distemper virus infection.

9. Puppy no: P43 [PM40.04 (Patient no: 178467)]

Changes were consistent with CPV.

Gross PM changes

Blood smear - pancytopaenia

Stomach empty

Small intestine serosa – congested

Small intestine mucosa – bile stained material lined the mucosa. Underneath multifocal haemorrhages were present, especially within the duodenum and jejunum.

Thymus consisted of islands within a gelatinous mediastinum.

Histopathology

Duodenum – total loss of villi and near total loss of crypts with collapse of the lamina propria and marked bacterial colonization of damaged mucosa. Crypts were lined by markedly hypertrophic enterocytes.

Ileum – total loss of villi and near total loss of crypts with marked hypertrophy of crypt lining cells.

Ileum anterior to caecum – there was total loss of villi and near total loss of crypts. Crypt lining cells were markedly hypertrophic.

Colon posterior to caecum – there was increased basophilia and loss of goblet cells.

Peripheral lymph node – marked lymphocyte depletion, few remaining follicles

Mesenteric lymph node – moderate lymphocyte depletion, few remaining follicles

Spleen – marked depletion of white pulp

Thymus – there was marked depletion of cortical lymphocytes with only supporting stroma that remained.

Bone marrow – moderately cellular with absence of maturing and storage pools

CNS – no abnormalities were observed, no changes consistent with Distemper virus infection.

10. Puppy no: P49 [PM92.05 (178060)]

Changes were consistent with CPV.

Gross PM changes

Carcass was congested

Mild dehydration

Blood smear - panleukopaenia

Small intestine serosa - congested

Small intestine mucosa – congested, fissured, no mucous

Thymus – small lobules within an oedematous mediastinum

Histopathology

Duodenum – there was total loss of villi with collapse of the lamina propria and a heavy bacterial colonization of the lumen surface. Crypts were mostly absent with the remnants lined by hypertrophic enterocytes. Marked congestion with scattered haemorrhages within the muscle layers were also present.

Ileum – near total loss of villi and crypts. The lamina propria had collapsed and there was a heavy bacterial colonization at the lumen surface. Crypts were lined by

hypertrophic enterocytes. There was marked congestion with multifocal haemorrhages within the muscle layers.

Ileum anterior to caecum – villi associated with GALT lost or collapsed, crypts were mostly absent. GALT showed 90% loss of lymphocytes. Remaining foci of villi showed mucosal haemorrhage and crypt dilation.

Colon posterior to caecum – increased basophilia and loss of goblet cells.

Peripheral lymph node – mild lymphocyte depletion, follicles present

Mesenteric lymph node – mild lymphocyte depletion, follicles present

Spleen – mild white pulp atrophy

Thymus – marked loss of cortical lymphocytes with collapse of the remaining stroma

Bone marrow – moderate atrophy that involved all lines, no maturing or storage pools were present.

CNS – no abnormalities were observed, no changes consistent with Distemper virus infection.

11. Puppy no: P61 [PM345.05 (180954)]

Changes were consistent with CPV.

Gross PM changes

Blood smear - pancytopaenia

Pale mucous membranes

Stomach full of bile stained mucous

Small intestine serosa – finely speckled with red spots

Small intestine mucosa – fissured, tan in colour, no mucous

Thymus – small lobules in mediastinum

Bone marrow – congested and gelatinous

Histopathology

Duodenum – there was total loss of villi with collapse of the lamina propria and fissures within the mucosa as deep as the muscularis mucosa. Crypts were depleted with remaining ones lined by hypertrophic enterocytes.

Ileum – necrotic remnants of villi were still visible but there was near total depletion of crypts.

Ileum anterior to caecum – bare villous structures were present, collapsed and blunted. Near total loss of all crypts.

Colon posterior to caecum – increased basophilia and loss of goblet cells

Peripheral lymph node – moderate lymphocyte depletion, no follicles

Mesenteric lymph node – moderate lymphocyte depletion, no follicles

Spleen – moderate white pulp atrophy

Thymus – marked loss of cortical lymphocytes, collapse of the normal architecture

Bone marrow – marked atrophy with cells scattered between dilated sinusoids

CNS – no abnormalities were observed, no changes consistent with Distemper virus infection.