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Prognostic Usefulness of Blood Leukocyte Changes in Canine Parvoviral Enteritis

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Background: Despite treatment, many dogs still die of complications related to canine parvoviral (CPV) enteritis. Effective prognostication would be beneficial in managing this disease.

Hypothesis: We hypothesize that the occurrence of leukocytopenias at admission and at 24 and 48 hours after admission, and changes in absolute leukocyte counts over time, could be used to predict outcome.

Animals: Sixty-two puppies with confirmed CPV.

Methods: A prospective study was performed. CBC was performed daily until discharge or death (in which case a postmortem examination was performed).

Results: Of the nonsurvivors (10/62; 16%), 9 died because of complications of the disease and 1 was euthanized because of a poor prognosis. There was a statistical significant difference in the occurrence of leukocytopenias between groups at 24 and 48 hours postadmission. The survivors showed a significant increase over time in certain leukocyte types (specifically lymphocytes) compared with values at admission. The positive predictive value for survivors was high. Nonsurvivors had marked thymic and lymphoid atrophy and marked bone marrow hypocellularity.

Conclusion: An accurate prognosis could be obtained at 24 hours after admission by evaluating the change in total leukocyte, band neutrophil, lymphocyte, monocyte, and eosinophil counts.

Key words: CBC; Eosinopenia; Prognosis; Leukopenia; Lymphopenia.

Canine parvoviral (CPV) enteritis is a common infectious disease primarily affecting puppies between 6 weeks and 6 months of age. Canine parvovirus (Parvoviridae type 2a and 2b) has a predilection to infect rapidly dividing cells of the gastrointestinal tract, lymphoid tissue, and bone marrow, leading to hemorrhagic diarrhea, vomiting, marked leukopenia, and immunosuppression.[1–5] The rate of lymphoid and intestinal cell turnover appears to be the main factor determining the severity of the disease. Stress factors, in particular parasitic and nonspecific factors (eg, weaning), may predispose dogs to clinical disease by increasing mucosal cell activity.[2,5–8] Susceptibility of puppies to viral infection increases as the maternally acquired antibody titer declines to nonprotective levels. Inadequate immunization to parvovirus during the 1st year of life is an additional risk factor for disease. In susceptible canine populations, parvovirus infection most often presents as a severe systemic and even life-threatening illness.[8] It is associated with a survival rate as low as 9% in the absence of treatment, and 64% with treatment.[4]

Because peripheral blood leukocyte counts and morphology are relatively stable in health, leukocyte responses can be useful clinically because they may change dramatically in disease. Although leukocyte responses seldom are 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.

Several reports of parvoviral-associated leukopenia and its underlying causes have been published over the years, but they have been inconclusive and contradictory. To our knowledge, there has been no published work on the use of the total leukocyte count (WBC) and differential leukocyte counts as indicators of prognosis. In the study reported here, we examined changes in total and differential leukocyte counts over time in 62 puppies with CPV enteritis. Our hypotheses were that (1) occurrence of leukocytopenias at admission and 24 and 48 hours after admission could be used to predict outcome, (2) increases over time in total WBC and absolute differential leukocyte counts would be associated with better outcome, and (3) the positive predictive value (PPV) for lack of cytopenias as measured on different days would be very high in survivors.

Materials and Methods

Sixty-two puppies with clinical signs of CPV enteritis that presented sequentially at the Onderstepoort Veterinary Academic Hospital (OVAH) over a 10-month period were prospectively included in the study. Puppies were included in the trial only after written consent was received from the owners. The research protocol was approved by the University of Pretoria's Animal Use and Care Committee. The puppies were between the ages of 6 and 24 weeks, of any breed and either sex, and exhibited clinical signs typical of CPV infection (eg, lethargy, anorexia, vomiting, hemorrhagic diarrhea, dehydration, collapse). All puppies were admitted to the

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Isolation Ward (OVAH). The diagnosis of CPV was confirmed within 24 hours by fecal transmission electron microscopy (EM). EM was also used to exclude other enteric viruses (eg, coronavirus, canine distemper virus). Peripheral blood smear examination was used to exclude blood parasite infections (eg, canine babesiosis, canine ehrlichiosis). All puppies were treated according to a standard treatment protocol adapted from Macintire Smith-Carr.[9] Briefly, IV fluids with additional potassium and dextrose were administered as indicated, as were broad-spectrum antibiotics IV, antiemetic therapy, deworming PO, and enteral feeding.

Experimental Procedure

At admission and on every subsequent day of hospitalization, each puppy underwent a full clinical examination and special attention was paid to mentation, appetite, vomiting, fecal consistency, mucous membrane color, and capillary refill time. At admission and on every subsequent day until discharge or death, blood was collected in EDTA[a] from the jugular vein. A CBC was performed by means of an automated cell counter (CELL-DYN 3700 System[b]) on each sample. Leukocyte differential counts were performed manually by an experienced veterinary hematology technologist who counted 50–100 total cells depending on the severity of the leukopenia. Leukopenia in this study was defined as total WBC count $<4.5 \times 10^3/\mu L$; segmented neutrophil count $<3.0 \times 10^3/\mu L$; band neutrophil count = 0; lymphocyte count $<1.0 \times 10^3/\mu L$; monocyte count $<0.15 \times 10^3/\mu L$; and eosinophil count $<0.10 \times 10^3/\mu L$. The reference intervals used as cutoff values were taken from the Clinical Pathology Laboratory, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa. To avoid bias, the primary investigator did not have access to the hematologic data until after discharge or death of the patient.

Full postmortem examinations were performed on all puppies that died or were euthanized. Lesions were described and the following samples were collected in 10% formalin for routine hematoxylin and eosin processing: small intestine (duodenum, proximal, and distal ileum), spleen, lymph nodes (mesenteric and cervical), thymus, bone marrow from the proximal femur, liver, and central nervous system.

Statistical Analyses

Data were statistically analyzed using Stata[c] statistical software. The variables analyzed were total WBC count, segmented neutrophils, band neutrophils, lymphocytes, monocytes, and eosinophils. Nonparametric statistical tests were used after evaluating the Shapiro-Wilk test for normality. For cytopenias in WBC and differential leukocyte counts, the distribution by group (survivors versus nonsurvivors) for the first 3 days was represented in box plots and median was used as a measure of central location. Fisher's exact test was used to compare the frequency of cytopenias for groups (survivors versus nonsurvivors) on days 0 (day of admission) through 3, with respect to WBC and differential leukocyte counts, after dichotomizing the counts using the lower reference limits. Increases over time in groups for WBC and differential leukocyte counts were compared with respect to change from baseline (value at admission) in a nonparametric analysis of covariance (ANCOVA for ranks), with baseline ranks as covariate. For all comparisons, differences were considered significant when $P < .05$.

The PPV of a lack of cytopenia for survival was calculated on each day for WBC and differential leukocyte counts using the formula PPV = TP/(TP+FP) × 100, where TP is the true positives (survivors with absolute leukocyte counts ≥ the cutoff values provided) and FP is false positives (puppies that did not survive with absolute leukocyte counts ≥ the cutoff values provided).

Results

Of the 62 puppies admitted to the trial and treated for CPV enteritis, 52 (84%) survived, 9 died within the first 3 days of hospitalization because of complications of the disease, and 1 was euthanized on day 3 because of a poor prognosis. Thus, data were statistically analyzed up to the 3rd day. Two of the puppies that were included in the study (1 nonsurvivor and 1 survivor) did not have blood collected for a CBC at admission. Admission data were thus available for 60 of the 62 cases (Table 1).

The median WBC of the survivors never decreased below the lower reference limit ($4.05 \times 10^3/\mu L$) and started increasing 24 hours after admission. The median WBC of the nonsurvivors at 24 and 48 hours postadmission was markedly below the lower reference limit of $4.05 \times 10^3/\mu L$. There were significant differences between survivors and nonsurvivors with respect to the proportion of cases below the lower reference limit for WBC on specific days. The most significant differences were seen at 24 hours ($P = .022$; 58 versus 100%; Fisher's exact test) and 48 hours postadmission ($P = .005$; 42 versus 100%) (Fig 1). Significant differences were also observed between groups with regard to change over time from the day of admission. At 24 hours postadmission, the WBC for survivors was significantly higher than the count at admission as compared with nonsurvivors ($P = .0061$; ANCOVA for ranks). The same was true at 48 hours ($P = .0062$). The PPV of a lack of cytopenia for survival, using a cutoff value of WBC $\geq 4.5 \times 10^3/\mu L$, at 24 and 48 hours postadmission was 100 and 97%, respectively (Table 2).

Severe neutropenia was observed in both survivors and nonsurvivors, but there was no significant difference, with respect to the proportion of cases below the lower reference limit, in segmented neutrophils between the groups by day. In both groups (survivors and nonsurvivors), the median segmented neutrophils remained below the lower reference limit ($3.0 \times 10^3/\mu L$) at 24 and 48 hours. There was also no significant difference in segmented neutrophils between groups with respect to change over time from the day of admission. The median band neutrophils of the nonsurvivors were significantly lower than the median band neutrophils of the survivors at 24 and 48 hours postadmission. There were significant differences between survivors and nonsurvivors with respect to the proportion of cases with counts equal to

Table 1. Number of survivors versus nonsurvivors by day for the first 4 days postadmission.

Time (hours)	Survivors	Nonsurvivors	Total (n)
Admission	51 (52)	9 (10)	60 (62)
24	52	8	60
48	52	7	59
72	43	1	44
96	33	0	33

The numbers in parentheses represent the total number of cases admitted to the study, of which 1 from each group did not have a CBC performed at admission. The fall in the number of puppies in the surviving group from 72 (hours) was attributable to discharge from hospital.

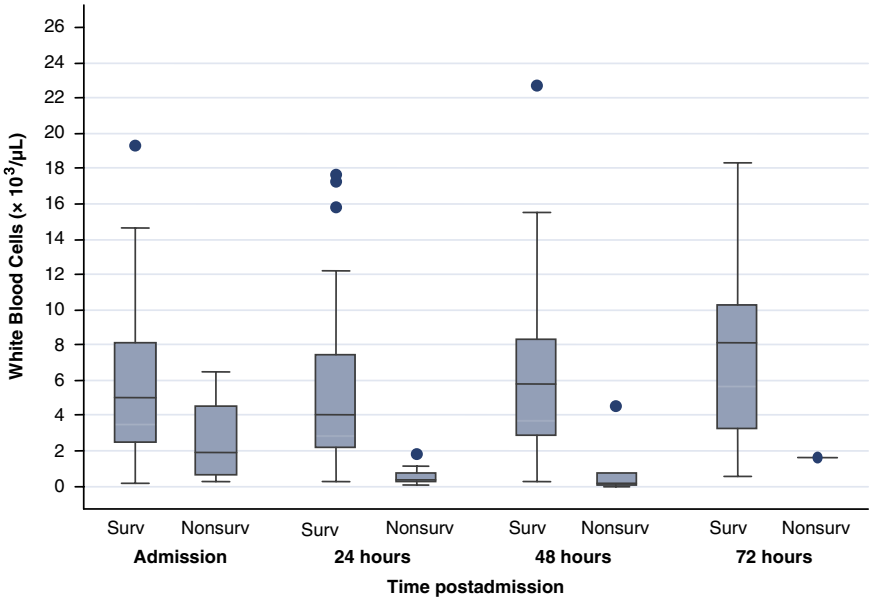

Fig 1. Box plot (representing the interquartile range) of the total WBC count over days in puppies (survivors and nonsurvivors) with canine parvoviral enteritis for the first 3 days postadmission. The box incorporates the middle 50% of the observations with the line inside the box as the median. The whiskers extend to the smallest and largest observations, indicating the range of the data. Outliers, values that are 1.5 times removed from the interquartile range, are plotted separately as dots. *Note*: The number of dogs evaluated decreased by day as indicated in Table 1.

zero, the lower reference limit for band neutrophils. The most significant differences were seen at 24 hours (P = .035; 23 versus 63%; Fisher's exact test) and 48 hours postadmission (P = .048; 19 versus 57%) (Fig 2). There was no significant difference in band neutrophils between groups with respect to change over time from the day of admission (ANCOVA for ranks). The PPV of a lack of cytopenia for survival, using a cutoff value of band neutrophils > 0, at 24 and 48 hours postadmission was 93% for both days (Table 2).

There were significant differences between survivors and nonsurvivors with respect to the proportion of cases below the lower reference limit for lymphocytes ($1.0 \times 10^3/\mu L$) on specific days. The most significant differences were seen at 24 hours (P = .001; 38 versus 100%; Fisher's exact test) and 48 hours postadmission (P = .002; 37 versus 100%) (Fig 3). The change over time in lymphocytes from the value at admission was also significantly different between survivors and nonsurvivors. At 24 hours postadmission, the lymphocyte count for survivors was significantly higher than the count at admission as compared with nonsurvivors (P = .0065; ANCOVA for ranks), as well as at 48 hours postadmission (P = .0049). The median lymphocyte count remained below the lower limit of the reference interval ($1.0 \times 10^3/\mu L$) at 24 and 48 hours postadmission in the nonsurvivors. The PPV of a lack of cytopenia for survival, using a cutoff value of lymphocytes $\geq 1.0 \times 10^3/\mu L$, at 24 and 48 hours postadmission was 100% for both days (Table 2).

Table 2. Positive predictive values (PPV) of a lack of cytopenia for survival by day in puppies suffering from CPV enteritis for the first 2 days postadmission.

Leukocyte Type	Admission (%)	24 Hours (%)	48 Hours (%)
WBC $\geq 4.5 \times 10^3/\mu L$	90	100	97
Neutrophil $\geq 3.0 \times 10^3/\mu L$	95	100	100
Bands > 0	86	93	93
Lymphocyte $\geq 1.0 \times 10^3/\mu L$	100	100	100
Monocyte $\geq 0.15 \times 10^3/\mu L$	91	92	98
Eosinophil $\geq 0.1 \times 10^3/\mu L$	93	97	100

CPV, canine parvoviral.

There were significant differences between survivors and nonsurvivors with respect to the proportion of cases below the lower reference limit for monocytes ($0.15 \times 10^3/\mu L$) on specific days. The most significant differences were seen on admission (P = .018; 16 versus 56%; Fisher's exact test) and 24 hours (P = .021; 12 versus 50%) and 48 hours postadmission (P = .001; 17 versus 86%) (Fig 4). There was no significant difference in monocytes between groups with respect to change over time from the day of admission (ANCOVA for ranks). The PPV of a lack of cytopenia for survival, using a cutoff value of monocytes $\geq 0.15 \times 10^3/\mu L$, at 24 and 48 hours postadmission was 92 and 98%, respectively (Table 2).

The median eosinophil counts of the nonsurvivors at 24 and 48 hours postadmission were significantly below the lower reference limit for eosinophils ($0.10 \times 10^3/\mu L$). There were significant differences between survivors and nonsurvivors with respect to the proportion of cases below the lower reference limit for eosinophils on specific days. The most significant differences were seen at 24 hours (P = .05; 46 versus 88%; Fisher's exact test) and 48 hours postadmission (P = .003; 38 versus 100%) (Fig 5). At 48 hours postadmission, the eosinophil counts for survivors were significantly higher than those at admission as compared with nonsurvivors (P = .028;

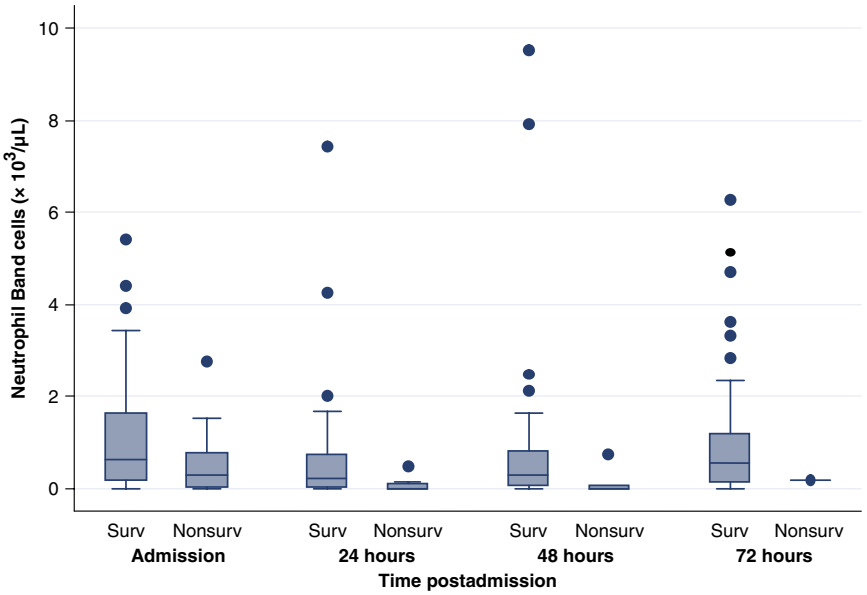

Fig 2. Box plot (representing the interquartile range) of the band neutrophil count over days in puppies (survivors and nonsurvivors) with canine parvoviral enteritis for the first 3 days postadmission. See Figure 1 legend for an explanation. *Note*: The number of dogs evaluated decreased by day as indicated in Table 1.

ANCOVA for ranks). The PPV of a lack of cytopenia for survival, using a cutoff value of eosinophils $\geq 0.1 \times 10^3/\mu L$, at 24 and 48 hours postadmission was 97 and 100%, respectively (Table 2). Basophils were rare in both groups and were not analyzed further.

Findings on Postmortem Examination

Macroscopically, 1 puppy (1/10) had marked lymph node congestion. Moderate to severe thymic atrophy was observed in 7 of 10 puppies, often indicated by a few small nodules scattered within a gelatinous mass in the mediastinum. No macroscopic changes were found in the spleen. Marked congestion (5/10) and gelatinous changes (3/10) were observed in the bone marrow.

On histopathology, 7 of 10 puppies had peripheral lymph nodes that had depleted or no lymphoid follicles, with moderate to severe depletion of cortical lymphocytes (cortical atrophy). Eight of 10 puppies had mesenteric lymph nodes that had depleted or no lym-

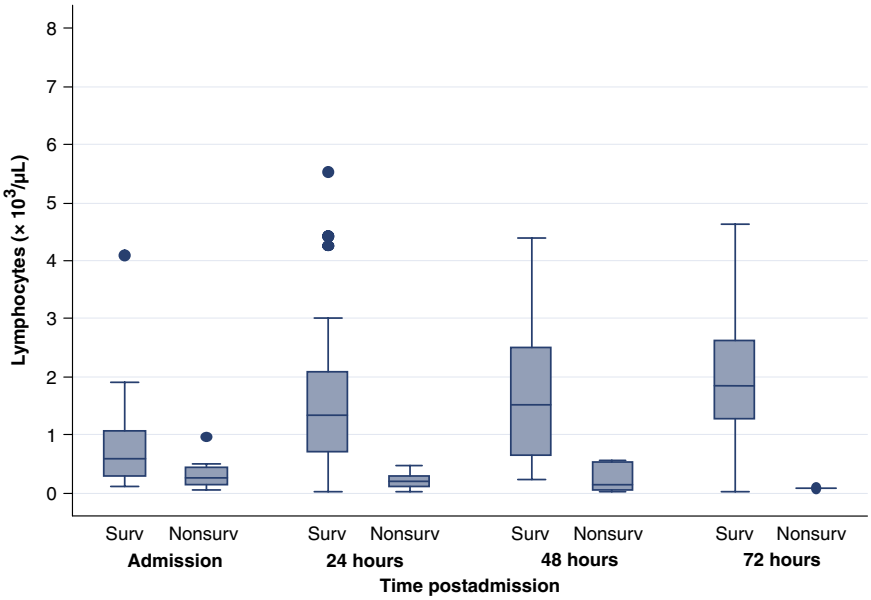

Fig 3. Box plot (representing the interquartile range) of the lymphocyte count over days in puppies (survivors and nonsurvivors) with canine parvoviral enteritis for the first 3 days postadmission. See Figure 1 legend for an explanation. *Note*: The number of dogs evaluated decreased by day as indicated in Table 1.

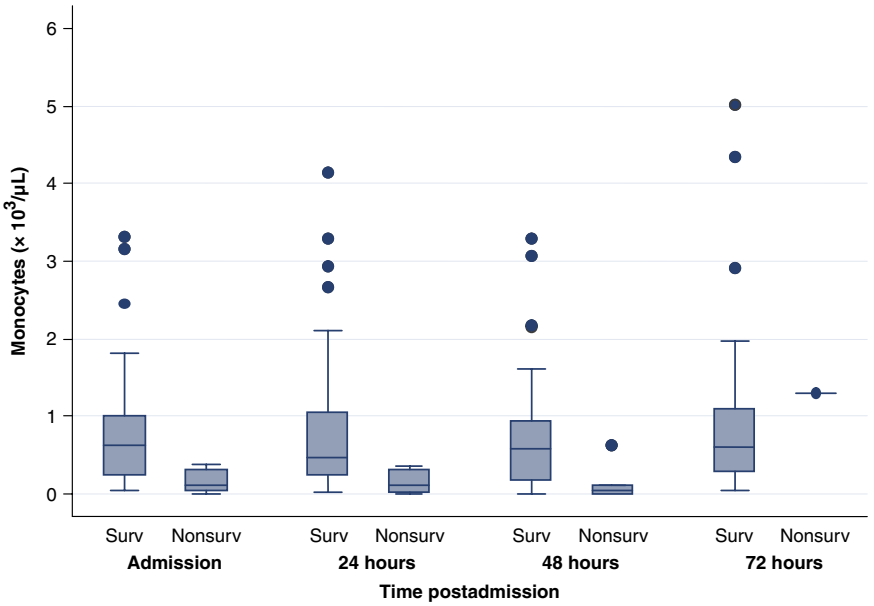

Fig 4. Box plot (representing the interquartile range) of the monocyte count over days in puppies (survivors and nonsurvivors) with canine parvoviral enteritis for the first 3 days postadmission. See Figure 1 legend for an explanation. *Note*: The number of dogs evaluated decreased by day as indicated in Table 1.

phoid follicles and 10 of 10 puppies had moderate to severe depletion of cortical lymphocytes (cortical atrophy). In 9 of 10 cases, the thymus had moderate to massive loss of cortical lymphocytes and severe collapse of the remaining stroma (ie, loss of the normal architecture of the thymus), and most of the lobules were made up only of supporting tissue. The spleen in 8 of 10 puppies had moderate to severe white pulp depletion or atrophy, with near total loss of small lymphocytes within the white pulp. Bone marrow was moderately to severely hypocellular in 9 of 10 puppies. In most puppies, the myeloid and erythroid series were equally affected, and in some puppies megakaryocyte numbers were low or even zero.

Discussion

The WBC during CPV enteritis is generally characterized as being low to severely low. This finding is widely accepted to be attributable to destruction of hematopoietic progenitor cells of the various leukocyte types primarily in the bone marrow, but also in other lymphoproliferative organs such as the thymus, lymph

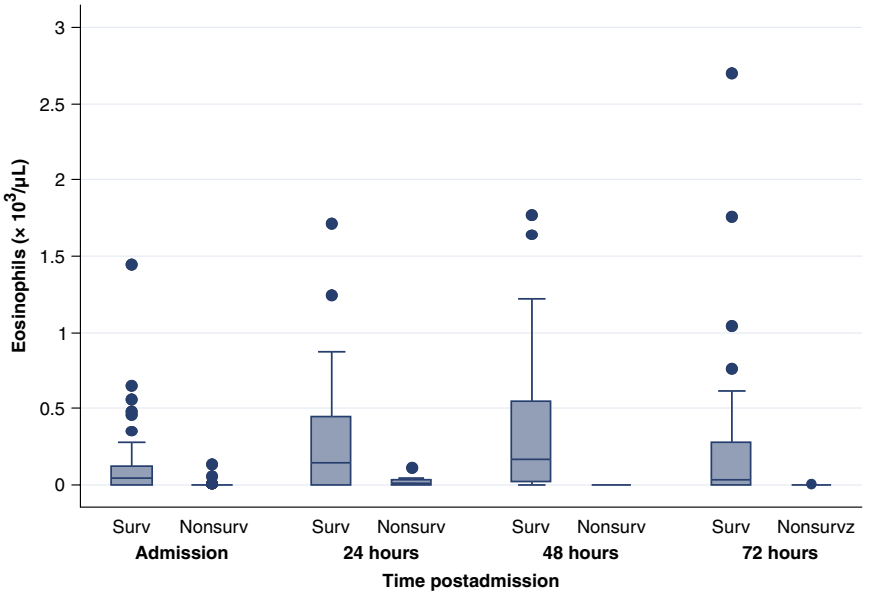

Fig 5. Box plot (representing the interquartile range) of the eosinophil count over days in puppies (survivors and nonsurvivors) with canine parvoviral enteritis for the first 3 days post admission. See Figure 1 legend for an explanation. *Note*: the number of dogs evaluated decreased by day as indicated in Table 1.

nodes, and spleen, resulting in inadequate compensation for the massive demand for leukocytes (specifically neutrophils) in the inflamed gastrointestinal tract.[10] The high mortality in dogs with severe leukopenia can largely be attributed to their high susceptibility to secondary bacterial infections that can lead to septicemia.[10] The results of this study indicate that a WBC $\geq 4.5 \times 10^3/\mu L$, a lymphocyte count $\geq 1.0 \times 10^3/\mu L$, a monocyte count $\geq 0.15 \times 10^3/\mu L$, and an eosinophil count $\geq 0.10 \times 10^3/\mu L$ as well as the presence of a left shift, as early as 24 hours after admission and commencement of treatment, are accurate predictors of a better outcome in CPV enteritis. The WBC and lymphocyte count, in particular, had 100% PPV of a lack of cytopenias for survival 24 hours postadmission.

In previous studies, Woods et al,[11] Potgieter et al,[12] and O'Sullivan et al[7] all agreed that leukopenia was associated with a poor prognosis or with the need for aggressive treatment. Potgieter found that leukopenia was mostly because of severe neutropenia and that the lymphocyte counts decreased to only 50% of normal values; he concluded that neutrophils were the most important leukocyte to monitor. Mason et al,[13] however, reported that leukopenia should not be used as the sole criterion of prognosis, and McCaw et al[d] found that neutropenia (even when very severe) was not a significant prognostic indicator. Only a few of these studies included serial CBC. In this study, we found several significant differences in WBC and differential leukocyte counts between puppies that survived and those that did not survive. The fact that we may be dealing with 2 different strains of viruses (ie, CPV-2a or CPV-2b) is a possible explanation for the differences in leukocyte numbers. Because none of the above-mentioned studies determined the strain of virus that caused disease, and because the specific viral strain was not determined in our study, it would be impossible to comment on this possibility. This study has shown that marked leukopenia, lymphopenia, monocytopenia, and eosinopenia as well as the absence of a left shift could be indicators of a poor prognosis as early as 24 hours after treatment has started.

Neutrophils are the most numerous leukocyte type in dog blood and are responsible for the destruction of bacteria, fungi, yeast, algae, parasites, and viruses as well as induction of antibody-dependent cellular cytotoxicity. A change in the neutrophil count will usually result in a change in the WBC count.[2–4,14–19] In CPV enteritis, severe neutropenia can not only be attributed to destruction of mitotically active myeloblasts in bone marrow as a direct effect of the virus but may also be related to endotoxemia and possible sepsis leading to neutrophil margination, as well as a massive loss of neutrophils through the intestinal wall.[1,3,11,20–23] Although neutropenia in both groups was severe, there was no significant difference in segmented neutrophils between puppies that survived and those that did not. Survivors, however, developed a degenerative left shift compared with nonsurvivors. This finding could be an indication that the bone marrow of survivors was less affected by the virus, that tissue demand in nonsurvivors was more profound, or that the disease process was too acute for a bone marrow response to occur in those that did not survive.

Lymphocytes are the second most common blood leukocyte in healthy dogs and are essential components of humoral and cell-mediated immune responses.[14] Mechanisms that can cause severe lymphopenia in CPV enteritis include the following: (1) lymphopenia of acute infection that may be the result of endogenous release of cortisol leading to redistribution of lymphocytes and the trapping of lymphocytes in draining lymph nodes to promote antigen contact; (2) direct effect of the virus leading to atrophy or destruction of lymphoid tissue; and (3) loss, sequestration, or blockage of flow of lymphocyte-rich lymph as seen in protein-losing enteropathy.[11,22,24] In this study, lymphopenia in the puppies was most probably attributable to a direct effect of the virus leading to atrophy or destruction of lymphoid tissue or endogenous release of high concentrations of cortisol.[14,23] Lymphopenia in nonsurvivors was most severe ($<1.0 \times 10^3/\mu L$) at 24 and 48 hours postadmission, indicating that these puppies probably did not develop an immune response. The puppies that survived had a significant increase in lymphocytes between the time of admission and 24 hours postadmission, and the count remained significantly higher than that of nonsurvivors at all time points thereafter. This finding was even more significant statistically than the WBC count and probably contributed substantially to the leukopenia, making the lymphocyte count a very important leukocyte parameter to monitor during the course of the disease to determine prognosis in CPV enteritis.

Monocytes are part of the mononuclear phagocyte system and as macrophages function in the phagocytosis and digestion of cellular debris, microorganisms, and particulate matter; secretion of inflammatory mediators; and presentation of antigens to lymphocytes. Monocytosis is a common finding in acute and chronic inflammatory conditions, whereas monocytopenia is rarely seen and of little clinical importance.[2,9,11,21] Although monocytes and neutrophils share a common progenitor cell, the time taken to produce a monocyte in the bone marrow (3 days) is much shorter than the time taken to produce a neutrophil (6 days).[3,21,25] Therefore, the recovery of monocyte numbers will precede that of neutrophils in the blood. This is especially true in the panleukopenia seen secondary to CPV infection where monocytopenia followed by a recovering monocyte count precedes the return of neutrophil production. Thus, monitoring the monocyte count in the blood is beneficial in evaluating the recovery from a leukopenic state in patients suffering from CPV enteritis.[3,21,23] Monocytopenia in nonsurvivors was most severe ($<0.15 \times 10^3/\mu L$) at admission and at 24 and 48 hours postadmission. Not only are monocyte numbers affected in CPV enteritis, but in a study by Decaro et al[24] 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. Monocyte-mediated killing also was impaired, and in 1 pup this function was completely absent. In this study, more than half of the puppies (survivors and nonsurvivors) had no morphological evidence of monocyte activity (such as

cytoplasmic basophilia and vacuolization) during the 3-day evaluation period. Thus, impaired monocyte function could be a factor contributing further to susceptibility to secondary infections.

The production of eosinophils in the bone marrow is controlled by T lymphocytes.[25,26] Eosinopenia is frequently seen in acute infection, and although it has never been verified, this finding has been attributed to endogenous release of cortisol.[15,25,26] The marked eosinopenia seen in CPV infection could be caused by a combination of myelosuppression, a lack of T lymphocytes to stimulate eosinophil production by the bone marrow, and the endogenous release of high concentrations of cortisol. In a study on basal cortisol as a prognostic indicator in CPV enteritis, Schoeman et al[27] found no difference in the mean basal cortisol (MBC) concentrations between survivors and nonsurvivors at admission. But, the survivors showed a marked reduction in MBC at 24 and 48 hours postadmission, whereas the nonsurvivors showed no reduction in MBC concentrations between admission and 48 hours postadmission. This suggests that patients that are critically ill, such as the puppies with CPV that did not survive, have high MBC concentrations that may account for the very low eosinophil counts. In nonsurvivors, the mean eosinophil count remained $< 0.1 \times 10^3/\mu L$ (the lower limit of the reference interval) on all days, serving as a good prognostic indicator in CPV enteritis, specifically at 48 hours postadmission.

The histopathologic findings were consistent with what has been reported previously and with what was reflected in the cell counts in peripheral blood.[12,29] This supports the hypothesis that hemopoetic cellular depletion in multiple tissues (eg, lymph nodes, thymus, spleen, bone marrow) was partially responsible for the irreversible leukopenia in nonsurvivors.

This study has examined the individual leukocyte types and found that several leukocyte parameters can be used successfully within the first 24–48 hours after beginning treatment as prognostic indicators for CPV enteritis. These parameters include WBC count, band neutrophil count, lymphocyte count, monocyte count, and eosinophil count. The postmortem histopathological findings supported, at least partially, bone marrow and lymphoid tissue depletion as the underlying cause of the leukocyte changes.

A limitation to this study was that because of its clinical nature the investigators had no control over when in the course of the disease process a puppy was presented for treatment. This, as well as the degree of illness and other factors (such as vaccination status and internal parasitism), could partly explain the lack of uniformity in the total WBC count and differential leukocyte counts seen. However, based on clinical examinations carried out at admission in all cases, the 2 groups did appear to be equally ill at admission.

Footnotes

[a] EDTA vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ

[b] Cell Dyne 3700 System, Abbott Laboratories, Santa Clara, CA

[c] StataCorp. *Stata Statistical Software: Release 8*, StataCorp LP, College Station, TX, 2003

[d] McCaw DL, Harrington DP, Jones BD. A retrospective study of canine parvovirus gastroenteritis: 89 cases. J Vet Int Med 1996;10: 157 (abstract)

Acknowledgments

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