Neutrophilia is associated with a poorer clinical outcome in dogs with chronic hepatitis

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Abstract
Background Liver disease is a common cause of morbidity and mortality in dogs. Currently, it is challenging to prognosticate in these cases. The aim of this study was to evaluate the utility of the haematological variables in dogs with chronic hepatitis.

Methods Dogs with chronic hepatitis confirmed on histopathology had presenting haematological values retrospectively obtained and evaluated against survival time. Eighty-two dogs met the inclusion criteria and their data analysed.

Results Neutrophilic patients, with a count greater than 12×10⁹/l, controlled for sex and age, had a shorter survival time (P≤0.01). In dogs, neutrophilia at presentation predicted a poor outcome, whereas the other haematological parameters were not prognostically informative. When the dogs were split into even quarters on the basis of their neutrophil count, those within the higher quartiles had poorer survival times. Neutrophilia was associated with a poorer survival time in comparison to those patients with a lower count.

Conclusion The relationship between neutrophils, inflammation and clinical outcome is deserving of future study in dogs with chronic hepatitis.

Introduction Chronic hepatitis is a common and clinically important disease in dogs, encompassing inflammatory disorders of the hepatic parenchyma.¹ The underlying aetiology and pathogenesis frequently remains elusive, with a definitive diagnosis often based on histopathological evaluation.² The treatment is largely supportive, and based on nutraceuticals and dietary management. Targeted therapy is often performed based on histopathological findings, with chelation therapy being used for copper storage disorders or immunosuppressive or antimicrobial therapy being instigated dependent on the nature of the predominant inflammatory cell population and culture results.²⁻⁴ The possible sequelae of hepatic dysfunction, such as hepatic encephalopathy and ascites, often necessitate treatment in their own right, and can have a significant impact on quality of life in dogs with chronic hepatitis.⁵⁻⁸

Prognostication of chronic hepatitis is difficult with limited indicators available. Historically, the presence of ascites,⁶ increased total bilirubin⁹ and cirrhosis identified on histopathology¹⁰ have all been associated with a poorer prognosis. A recent study¹¹ using a crude measure of systemic inflammation, namely the systemic inflammatory response score (SIRS), found that dogs with liver disease and a high SIRS had a poorer clinical outcome than dogs with a low SIRS. However, the relationship between clinical outcome and inflammation using other objective markers of inflammation has received little attention in dogs with chronic hepatopathies.

Inflammatory markers such as C-reactive protein¹² and haptoglobin¹³ have previously been investigated in chronic hepatopathies. However, there is a paucity of data evaluating the utilisation of routinely available haematological variables in prognosticating dogs with chronic hepatitis. These parameters have the benefit of being widely available, relatively inexpensive to obtain without a requirement for specialised sample handling.
or equipment. In human medicine, these markers of inflammation have been evaluated in their ability to prognosticate in a number of conditions including myocardial infarction, strokes, surgical outcomes, snake envenomation and in a variety of neoplasms.

The aim of this study was to investigate whether the haematological evidence of inflammation was a prognostic factor in canine chronic liver disease. The hypothesis of the study was that dogs with haematological markers of inflammation at initial evaluation would have a shorter survival time.

Materials and methods

The hospital’s medical records were evaluated and searched from 2004 to 2018 to identify patients which met the diagnostic criteria of chronic hepatitis. Cases were included provided a histopathological diagnosis of chronic hepatitis was obtained, either via surgical biopsy or at post mortem examination. All of the cases were managed at the discretion of the attending clinician. Cases were excluded if it was reported that they had any comorbidities that would cause inflammation or were recorded to have been pretreated with corticosteroids in the clinical history. Cases were also excluded if they were receiving medications which could interfere with either haematology or serum biochemistry, such as phenobarbital. In order for cases to become part of the data set, they were also required to have a routine haematology and serum biochemistry obtained at presentation to the hospital in order to avoid variation introduced by differing machine analysers used at referring veterinary practices. To provide a control group, haematology was carried out in a cohort of healthy patients who were undergoing blood sampling for a haematology and biochemical health profile assessment.

The following data were collected from each case: age, breed, sex, neuter status as well as haematology (white blood cell, lymphocyte, neutrophil, monocyte, eosinophil, basophil and red blood cell counts, haemoglobin concentration, packed cell volume, mean cell volume, mean cell haemoglobin concentration and automated platelet count). The survival time (recorded in days from presentation to the referral institute until death), whether the cause of death was related to the chronic hepatitis or not (based on the clinician’s evaluation of the patient at the time of euthanasia and history) and the histopathological diagnosis was collected from all patients. Survival times were recorded from the clinical records, and if this information was not available, then the referring veterinary surgeons were contacted.

Dogs with elevated copper levels, or rhodanine staining of copper within zone 3 consistent with a copper storage disorder were excluded from analysis. Dogs with bacterial growth on liver biopsy or bile culture were excluded, as were those with neutrophilic or granulomatous hepatitis on histopathology.

The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the absolute number of neutrophils \((\times10^9/l)\) by the absolute number of lymphocytes \((\times10^9/l)\). For the hospital haematology analyser, the reference interval for neutrophils was \(3.6–12.0\times10^9/l\) and for lymphocytes was \(0.7–4.8\times10^9/l\). If a lymphocyte count of \(0\times10^9/l\) was recorded, this was converted to \(0.01\times10^9/l\) to allow the NLR to be calculated.

The raw data were analysed using a Cox proportional hazards model, to assess the influence of the absolute neutrophil, monocyte, lymphocyte, eosinophil and basophil counts and the NLR on survival time, in days, as sole variables alongside age and sex. Each of these variables were then split equally into the upper and lower halves. These two groups were then evaluated for each variable’s effect on survival time using a Kaplan-Meier plot. A Mann-Whitney U test was used to compare the signalment and NLR of the healthy dogs with those diagnosed with liver disease. A significance of \(P<0.05\) was set. Statistical analysis was performed in R statistical software package (R Development Core Team (2012)).

Results

One hundred and fifteen cases were initially identified as having the potential to be included within the study. Thirty-one cases were excluded as had a hepatopathy other than chronic hepatitis, 4 had concurrent comorbidities and they were receiving medications at the time of sampling which could have altered the haematological variables and 2 had positive bile cultures. The remaining 78 dogs had confirmed hepatitis with complete records and recorded survival times, and were further analysed.

The median age of the dogs was 6 years, with a range of 6 months to 15 years. The breed distribution was as follows: Labrador retriever (17), crossbreed (11), cocker spaniel (9), springer spaniel (7), border terrier (3), German shepherd dog (3), Border collie (2), Doberman pinscher (2), miniature schnauzer (2), West Highland white terrier (2) and one each of Akita, Bedlington terrier, boxer, bracco Italiano, dachshund, Dalmatian, flat-coated retriever, Havanese, Jack Russell terrier, Lhasa apso, Newfoundland, Old English sheepdog, pug, rottweiler, rough collie, Samoyed, Shetland sheepdog, Skye terrier, Staffordshire bull terrier and Tibetan terrier.

The healthy control group had a median age of 8 years, with a range of 2–11 years. The breeds comprising this group were: crossbreeds (5), greyhounds (2), lurchers (2) and one each of Border collie, cocker spaniel, dachshund, Labrador and Hungarian vizsla.

There was no significant difference in age (\(P=0.37\)) or sex/neuter status (\(P=0.61\)) between the study population and controls.
The median neutrophil count was $10.19 \times 10^9/l$ in the dogs with chronic hepatitis. The upper limit of the laboratory reference range for the neutrophil count of $12.0 \times 10^9/l$ was used in the chronic hepatitis group, with patients split into two groups, those above and those below this value. There was a significant difference in survival time using this cut-off point ($P=0.02$). When evaluated as continuous numbers rather than dichotomised, this was also significant ($P=0.03$). These remained significant when modelled to account for age and sex. The Kaplan-Meier plot for this is shown in figure 1. The survival times are shown with the proportion of dogs surviving to each point in table 1.

The median lymphocyte count was $1.16 \times 10^9/l$ in those with chronic hepatitis. There was no significant difference in survival time when separated based on those within or above the reference range ($<1.5 \times 10^9/l$) ($P=0.49$) (figure 2). The median eosinophil count was $0 \times 10^9/l$ in those with chronic hepatitis. There was no significant difference in survival time when separated based on eosinophil count ($P=0.11$). The median monocyte count was $1.55 \times 10^9/l$ in the dogs with chronic hepatitis. There was no significant difference in survival when based on monocyte count ($P=0.28$).

The median NLR of those with liver disease was 8.78, ranging from 1.23 to 2211.6. The control group had a median NLR of 2.08, ranging from 1.67 to 4.31. The NLR was significantly higher in dogs with liver disease compared with healthy control dogs ($P<0.01$). When the dogs were split above and below the median of the NLR and compared, there was no statistical difference ($P=0.36$) between survival times (figure 3).

The cohort was split into four groups based on the neutrophil count: less than $5.87 \times 10^9/l$ (group 1), $5.87–8.11 \times 10^9/l$ (group 2), $8.12–11.5 \times 10^9/l$ (group 3) and more than $11.6 \times 10^9/l$ (group 4). In comparison to the dogs in group 1 with the lowest neutrophils, as the neutrophil count increased in groups 2, 3 and 4, the HR increased to 1.57 (95 per cent CI of 0.72 to 3.39), 1.38 (95 per cent CI of 0.67 to 2.84) and 1.82 (95 per cent CI of 0.91 to 3.62), respectively (figure 4). Additionally, for each $1.0 \times 10^9/l$ increase in neutrophil count, there is an HR of 1.042 (95 per cent CI 1.004 to 1.08, $P=0.02$).

Discussion
Neutrophil count was demonstrated to be prognostic in dogs with chronic hepatitis. There was a suggestion of a biphasic increase in the HR in those with both low and higher neutrophil counts resulting in a greater risk of death as would be expected to result from inflammatory states.

Neutrophilia can occur for a myriad of reasons in those with underlying hepatopathies. The

![Figure 1](image1.png)

**Figure 1** The Kaplan-Meier plot demonstrates the survival time difference between dogs with a neutrophil count above and below the upper end of the reference interval of $12 \times 10^9/l$. Those with a count above $12 \times 10^9/l$ (grey line, $N=18$) are shown against those with a neutrophil count below $12 \times 10^9/l$ (black line, $N=61$), $P=0.02$.

![Table 1](image2.png)

| Neutrophil number ($\times 10^9/l$) | Survival time in days at the time point a set percentage of dogs that have died |
|------------------------------------|--------------------------------------------------------------------------------|
|                                    | 25% | 50% | 75% |
| 0–5.88                             | 25  | 67  | 207 |
| 5.89–8.11                          | 85  | 27  | 153 |
| 8.12–11.5                          | 6.5 | 70  | 217 |
| $>11.5$                            | 2   | 5.5 | 147 |

![Figure 2](image3.png)

**Figure 2** The Kaplan-Meier plot demonstrates the survival time difference between dogs with a lymphocyte count above and below the upper end of the reference limit of $1.5 \times 10^9/l$. Those with a lymphocyte count below $1.5 \times 10^9/l$ (black line, $N=52$) are compared against those with a lymphocyte count above $1.5 \times 10^9/l$ (grey line, $N=27$), $P=0.49$.

![Figure 3](image4.png)

**Figure 3** The Kaplan-Meier plot demonstrates the survival time difference between dogs with a neutrophil-to-lymphocyte ratio above and below the maximum value in the control group of 4.3. Those with neutrophil-to-lymphocyte ratio above 4.3 (grey line, $N=52$) were compared against those below 4.3 (black line, $N=27$). $P=0.36$. 
Neutrophil count has been found to have several clinical utilities previously. In human paediatric medicine, the absolute count and morphological changes have been used in predicting bacterial infections. A limitation of this study was that it was retrospective and as such reliant on the accuracy and completeness of historical clinical records, although referring veterinary surgeons were also contacted if necessary.
to ensure accurate survival times were obtained. This also meant that the approach to each patient was not standardised and this may have had an impact on survival times. As with all veterinary studies using survival time as the primary end point, there is owner variation and clinician-dependent decision-making which can introduce variability as to when patients were euthanised. Again due to the retrospective nature, information regarding the treatment patients were receiving and body condition score at the time of blood sampling or liver biopsy was not always available, and these may have had an impact on the results. It would also have been ideal to evaluate the role of other clinical factors such as the presence of ascites or hepatic encephalopathy on survival times and relationship but the study was underpowered for this, and future multicentre or prospective studies would be necessary to interrogate this further.

To further evaluate the utility of a circulating neutrophilia as a prognostic indicator in canine chronic hepatitis, a prospective study would be necessary. Additionally, a prospective study in which the biomarkers that have previously been shown to be of importance in liver disease, such as interleukin-6 and C-reactive protein, are analysed alongside the previously identified prognostic indicators, in a cohort of dogs with chronic hepatitis with standardised treatment would be ideal. These variables could be combined and their relative importance assigned. The aim would be to generate a predictive model in order to provide a more accurate prognosis in these cases.

Conclusion

This retrospective study suggests that alterations in the absolute neutrophil count can be of prognostic importance. These findings support the hypothesis that systemic inflammation in the setting of chronic hepatitis is associated with a poorer prognosis. Prospective studies are warranted to ascertain the utility of this parameter as an independent prognostic indicator, and potentially as part of a predictive model, in canine chronic hepatitis.

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Competing interests

None declared.

Ethics approval

This study was approved by the institute’s Veterinary Ethical Review Committee.

Data availability statement

Data are available on reasonable request. © British Veterinary Association 2020. No commercial re-use. See rights and permissions. Published by BMJ.

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