Quantitative and qualitative leukocyte abnormalities in dogs with experimental and naturally occurring acute canine monocytic ehrlichiosis

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Key Words
Ehrlichia canis, inflammation, leukogram, lobularity index, MPXI

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Background: Canine monocytic ehrlichiosis (CME) is one of the most important tick-borne diseases worldwide. Cytopenias have been observed in both acute (nonmyelosuppressive) and chronic (myelosuppressive) CME; however, leukocyte abnormalities and indices have been incompletely described in dogs with acute CME.

Objectives: The aims of this study were to analyze temporal changes in differential leukocyte counts, leukocyte morphology, myeloperoxidase index (MPXI), and lobularity index (LI) in dogs with experimental and naturally occurring acute CME.

Methods: Differential leukocyte counts and morphology were evaluated in archived blood smears from 13 Beagle dogs experimentally infected with Ehrlichia canis and evaluated weekly for 42 days postinfection (DPI); 20 dogs with naturally occurring acute CME also were evaluated. MPXI and LI were obtained from ADVIA reports. Wilcoxon tests were used to assess changes over time; leukogram results in natural cases were assessed in comparison with reference intervals.

Results: In experimental dogs, significant decreases in neutrophil, monocyte, lymphocyte, and eosinophil counts, and a mild left shift occurred within 14 DPI. The MPXI decreased significantly between 14 and 21 DPI and remained low, while LI increased from 14 to 35 DPI. Lymphocyte counts rebounded at 21 DPI, normalizing total WBC counts. Neutrophil toxicity was seen rarely, but reactive lymphocytes were observed frequently. Dogs with natural infection had variable patterns of leukocyte changes.

Conclusions: Acute CME is associated with several discrete quantitative and qualitative leukogram changes indicative of concurrent inflammation, antigenic stimulation, and stress. Changes in MPXI and LI warrant further investigation in dogs with CME and other diseases.

Introduction

Canine monocytic ehrlichiosis (CME) is one of the most important canine tick-borne diseases worldwide. It is endemic in Europe, Asia, Africa, and the Americas (especially Latin America). The causative agent of CME is the intracellular, gram-negative bacterium Ehrlichia canis, which is transmitted by the tick Rhipicephalus sanguineus and infects mononuclear cells.¹ Following an incubation period of 8–20 days, the clinical course of CME can be sequentially divided into acute, subclinical, and chronic phases. Most untreated dogs recover spontaneously from the acute phase after 2–4 weeks, entering a subclinical phase that may last several months to years.¹ Immunocompetent dogs may eliminate the infection during this period, but some will eventually develop the chronic phase of the disease, typically characterized by aplastic pancytopenia and high mortality due to septicemia or severe bleeding.²
Definitive diagnosis of CME is heavily based on serology and/or polymerase chain reaction (PCR) assay, however, a CBC and blood smear examination are important components of the diagnostic workup. In the chronic (myelosuppressive) phase of CME, profound thrombocytopenia, anemia, and leukopenia are typically found and can provide immediate diagnostic and prognostic information to veterinarians. In acute (nonmyelosuppressive) CME, mild to moderate thrombocytopenia and anemia are typically found, but reports of leukogram abnormalities are incomplete and contradictory, especially with regard to changes indicative of inflammation. An acute phase protein response has been reported in dogs with acute CME, suggesting an inflammatory process.

Although leukopenia and neutropenia are consistent findings in dogs with experimental or natural acute CME, leukocytosis, neutrophilia, monocytosis, lymphocytosis, and rarely, a left shift have been reported in up to 35% of naturally infected dogs, supportive of an inflammatory process. Many previous studies, however, did not assess band neutrophil counts or neutrophil toxicity, reporting only data from automated analyzers (most of which do not quantify bands or toxicity), and without examination of blood smears. Furthermore, 2 novel indices reported by the ADVIA hematologist analyzer, the myeloperoxidase index (MPXI) and lobularity index (LI), have promise for assessing an inflammatory response, but have not been examined previously in dogs with CME.

The MPXI is an estimate of myeloperoxidase (MPO) content per leukocyte, as derived from the peroxidase channel of the ADVIA; MPO content is affected by degranulation, toxicity, and maturity of neutrophils. The LI is derived from the basophil channel and correlates with segmentation and density of neutrophil nuclei. Although these indices have not been well characterized in dogs, changes in neutrophil maturity and toxicity in dogs with acute CME could result in changes in MPXI and LI that coincide with other changes in the leukogram.

We hypothesized that significant leukocyte changes indicative of inflammation would be found in the peripheral blood of dogs with acute CME. To evaluate this, we assessed serial differential leukocyte counts, leukocyte morphology, MPXI, and LI in dogs with acute CME, using archived blood smears and data from a previous study of experimentally infected dogs. We also examined archived blood smears from 2 groups of dogs with naturally occurring nonmyelosuppressive (likely acute) CME in California and in Greece. A better understanding of expected quantitative and qualitative leukogram abnormalities in dogs with acute CME has the potential to improve the diagnostic and prognostic usefulness of a CBC in this disease.

Materials and Methods

Experimental study population

Thirteen Beagle dogs experimentally infected with E canis were included in the study. All dogs had participated in a previous study that evaluated the efficacy of rifampin in experimental acute CME (approved by the Research and Ethical Committee, School of Veterinary Medicine, Aristotle University of Thessaloniki [AUTH], 458/23-6-2009). The dogs comprised 7 females and 6 males, with an age range from 5 to 49 months (median 11 months). All dogs were current in vaccinations (latest vaccination was given one month [4 dogs] to several months [9 dogs] prior to experimental inoculation) and subject to strict endoparasite and ectoparasite control throughout the study. Prior to infection, the dogs were assessed as clinically healthy based on physical examination and lack of clinicopathologic abnormalities. All dogs were seronegative for E canis, Babesia canis, and Leishmania infantum antibodies (via indirect fluorescence antibody [IFA] tests) and Dirofilaria immitis antigens (SNAP 3DX, IDEXX, Westbrook, ME, USA), and were negative by PCR assay for E canis DNA in blood, spleen, and bone marrow aspirates.

Each dog had serial clinical examinations prior to and on the day of inoculation (day 0); thereafter, a clinical examination was performed every 2 days until 42 days post infection (DPI). As previously reported, all infected dogs became clinically ill by 21 DPI (with pallor [n = 2], depression [n = 4], anorexia [n = 4], lymphadenomegaly [n = 7], palpable splenomegaly [n = 10], fever [n = 13]), and/or thrombocytopenia (46,000–101,000 platelets/μL, median 73,000/μL, reference interval 200,000–500,000/μL); seroconverted to E canis antigens (reciprocal IFA titers 200–800, median 200, cutoff value ≥ 100); and were PCR-positive in at least one tissue (blood, bone marrow, or spleen) throughout the study period (through 42 DPI). Five dogs were treated with rifampicin from 21 DPI (after blood sampling) to 42 DPI; therefore, results from these dogs on 28, 35, and 42 DPI were excluded from the study.

Serial CBCs (ADVIA 120 Hematology System, Bayer, Tarrytown, NY, USA) were done within 4 h post sampling, prior to infection (day 0; n = 13 dogs) and on 7 (n = 9), 14 (n = 12), 21 (n = 13), 28 (n = 8),
35 (n = 8), and 42 (n = 6) DPI. Archived blood smears were not available for all dogs at all sampling periods. Blood smears prepared at the time of blood collection were air-dried and stained with Giemsa. For this study, total WBC count, MPXI, and LI were recorded and blood smears were examined retrospectively to determine differential WBC counts and evaluate WBC morphology.

**Natural infection study populations**

Eleven dogs with nonmyelosuppressive (likely acute) CME were identified in a search of the electronic medical record system of the Veterinary Medical Teaching Hospital at the University of California (UC) Davis from January 1997 (the earliest year for which archived blood smears were available) to May 2014. Dogs included 6 females (4 spayed) and 5 males (3 neutered) ranging from 3 months to 14 years of age (median 3.5 years). Breeds included 5 mixed breeds, 2 Border Collies, and one each of Chow, Cocker Spaniel, Bichon Frise, and St. Bernard. Cases were enrolled if they met the following criteria: (1) clinical signs (fever, lymphadenomegaly, splenomegaly, platelet-related bleeding tendency) and laboratory abnormalities (thrombocytopenia) compatible with acute CME; (2) seropositivity to *E canis* antigens (SNAP 4Dx, IDEXX) or PCR-based amplification of *E canis* DNA using specific primers, with or without observation of *Ehrlichia* morulae in blood or cytology smears; and (3) reasonable exclusion of concurrent vector-borne or systemic noninfectious diseases (including *Anaplasma*, *Borrelia*, and *Dirofilaria*) that may potentially affect CBC results. Cases were excluded if the diagnosis of *E canis* infection was historical and not the reason for CBC evaluation. Three of the dogs had bone marrow evaluations confirming normal to mildly hyperplastic hematopoietic activity. All dogs but one were discharged with doxycycline treatment; 5 dogs were lost to follow-up and 6 had follow-up examinations with clinical and laboratory evidence of recovery. Total WBC count (for calculating leukocyte differential counts), MPXI, and LI, obtained using an ADVIA 120 analyzer, were recorded for comparison with intralaboratory reference intervals established using 60 clinically healthy dogs. Archived blood smears stained with Wright–Giemsa were retrospectively examined for WBC differential counts and morphology (see below).

Blood smears from 9 dogs with nonmyelosuppressive (likely acute) CME were retrieved from the archives of the Clinic of Companion Animals (CCA)-AUTH. Dogs included 7 males and 2 females ranging from 5 months to 4 years of age (median 14 months). Breeds included 3 mixed breeds, 2 German Shepherd dogs, and one each of Rottweiler, St. Bernard, English Setter, and Doberman breeds. Cases were enrolled if they met the following criteria: (1) the diagnosis of CME was confirmed (fever, lymphadenomegaly, splenomegaly, and thrombocytopenia combined with seropositivity to *E canis* (IFA, cut-off titer 1:100 and/or ELISA [ImmunoComb, Biogal-Galed, Israel]), and positive PCR assay for *E canis* 16S rDNA in bone marrow aspirates or observation of *Ehrlichia* morulae in buffy coat smears); (2) bone marrow aspirate samples indicated normo- or hypercellularity; (3) no cytologic, serologic, or PCR evidence of concurrent vector-borne infections (*Leishmania* sp., *Babesia* sp., *Anaplasma* sp., or *Dirofilaria immitis*) or laboratory evidence of noninfectious systemic diseases; and (4) the dogs rapidly recovered based on clinical and laboratory evidence following treatment with doxycycline. Availability of a good quality blood smear was also required for inclusion in the study. Total WBC count, obtained using a QBC analyzer (QBC VetAutoread Hematology System; IDEXX) was recorded. Blood smears for differential WBC counts at CCA-AUTH were stained with a rapid Romanowsky stain (Hemacolor; Merck) or Giemsa.

Because laboratory-specific reference intervals were not available for the QBC analyzer, total and differential WBC counts (from the QBC and blood smears, respectively) were obtained from 27 clinically healthy dogs presented to the CCA-AUTH for routine vaccination or elective surgery and used as preliminary reference intervals (reported as minimum-maximum values) for comparison. The clinically healthy dogs included 21 purebreds and 6 mixed-breed dogs, 17 males and 10 females, with an age range of 5 months to 11 years (median 1.5 years).

**Leukocyte differential counts and morphology**

A 200-cell manual leukocyte differential count was done on all blood smears by a single observer (AG) blinded to the origin of the smear. The observer was trained by certified medical technologists and supervised by a board-certified clinical pathologist (MMC) who assisted when questions arose regarding leukocyte classification or morphology. Band neutrophils were defined strictly as having nuclei with parallel sides and no evidence of segmentation. Absolute counts were calculated by multiplying the differential percentages by the total WBC count. If nucleated RBC (NRBC) were observed, the total WBC count was corrected.

During differential counting, leukocytes were examined for morphologic abnormalities. Toxic
changes in band and segmented neutrophils were scored as 1+ (few Döhle bodies or slight cytoplasmic basophilia in a few cells), 2+ (moderate Döhle bodies and diffuse cytoplasmic basophilia or vacuolation in a few cells), 3+ (many Döhle bodies, cytoplasmic basophilia, and foamy vacuolation in many cells) to 4+ (many Döhle bodies, severe cytoplasmic basophilia, and foamy vacuolation in many to most cells). Reactive lymphocytes and monocytes were subjectively estimated as none, rare, few, or many. Reactive lymphocytes were defined as those having many clear cytoplasmic vacuoles, with or without immature chromatin.

**Statistical analysis**

Data were analyzed statistically using JMP (v. 11.2; SAS Institute Inc., Raleigh, NC, USA). Quantitative data were analyzed using Wilcoxon tests to compare time points (preinfection and 7–42 DPI in experimentally infected dogs). Linear regression was used to assess correlation between MPXI, LI, and differential leukocyte counts. Qualitative data (toxicity, reactivity) were compared by using chi-square analysis. $P < .05$ was considered as statistically significant.

**Results**

A total of 103 blood smears from dogs in the experimental infection study were reviewed. Four smears were excluded because of leukocyte deterioration (pyknosis), poor smear quality, or insufficient numbers of leukocytes available for evaluation. Thirty-four smears were duplicates from the same blood sample, so the results were averaged. The remaining 65 smears were from day 0 (preinfection, $n=13$), and 7 (9), 14 (12), 21 (11), 28 (8), 35 (6), and 42 (6) DPI.

Significant changes were found in all leukocyte counts during the course of experimental infection as compared with preinfection counts (Figure 1). Nucleated RBCs were observed in low numbers (median 1 [range 0–4]/100 WBCs) at all time points. The MPXI decreased significantly at 21 DPI and remained low through 42 DPI, while LI increased significantly at 14 DPI and remained high through 28 DPI (Figure 2). The MPXI correlated positively with the concentration of segmented neutrophils ($r^2 = .14$, $P = .0021$) and correlated inversely with the concentration of lymphocytes ($r^2 = .22$, $P < .0001$). The LI correlated inversely with the concentration of segmented neutrophils ($r^2 = .24$, $P = .0004$, chi-square) (Figure 3). Rare to few activated monocytes were seen in 22/65 (33.8%) smears at 0, 7, 14, 21, and 35 DPI. One dog had many reactive monocytes at 35 DPI. Ehrlichial morulae were not observed in any of the smears.

**Discussion**

We identified several discrete quantitative and qualitative leukocyte abnormalities in dogs with experimental and naturally occurring acute CME that were consistent with a concurrent inflammatory response, antigenic stimulation, and stress. As expected, neutropenia was the predominant leukocyte abnormality in experimentally infected animals, but a significant (albeit small) increase in band neutrophils and...
reactive lymphocytes and decreases in monocyte, lymphocyte, and eosinophil counts also were observed during the first 2–3 weeks of infection. The increase in LI indicated a decrease in nuclear density or segmentation, which corroborated the left shift. A marked decrease in MPXI occurred at 3 weeks post infection and persisted for the duration of the study. In contrast with experimentally infected dogs, hematologic findings in natural infections were more variable, with mild neutrophilia observed as frequently as neutropenia, and a few dogs having an increased MPXI. Overall, these findings support the presence of a weak inflammatory response and the potential value of MPXI and LI in assessing and monitoring inflammation in dogs with nonmyelosuppressive acute CME. Our findings also raise important questions about hematologic differences between experimental and naturally occurring CME.

The experimental *E canis* study was advantageous for evaluating leukocytes in acute CME, because other infections had been ruled out and the time course of leukocyte changes could be followed closely. Even with a relatively small sample size, clear patterns of quantitative and qualitative changes were demonstrated in all leukocyte types, and in leukocyte indices. As in previous studies, neutropenia and leukopenia comprised the most common leukogram pattern in dogs with acute CME. The cause of neutropenia in

![Figure 1. Total and differential leukocyte counts in dogs experimentally infected with *Ehrlichia canis* on day 0 and monitored for 42 days post infection. Box plots indicate the median and interquartile range, whiskers indicate minimum and maximum values, and outliers are shown as individual dots. *Significantly lower than other time points; **significantly higher than other time points (P = .0169 bands; P = .0140 eosinophils; P < .0001 other leukocytes; Wilcoxon).](image-url)
acute CME is not known; impaired granulopoiesis (decreased neutrophil production), accelerated neutrophil egress and utilization, or immune-mediated neutrophil destruction resulting from anti-neutrophil antibodies\textsuperscript{21,22} are possible mechanisms. The small but significant increase in band neutrophils and concurrent increase in LI indicated a mild transient inflammatory response concurrent with the development of neutropenia. Mild and transient left shifts (combined with neutropenia) were reported previously in one experimental study of dogs infected with \textit{E canis}.\textsuperscript{15} Neutrophil toxicity has not been previously described in experimental ehrlichiosis and was rare in our study.

In contrast with controlled experimental \textit{E canis} infections\textsuperscript{14,15,23,24}, but similar to other retrospective studies of naturally infected dogs in which 6–35\% of dogs had mild to moderate neutrophilia\textsuperscript{5,10,13}, several naturally infected dogs at UC Davis had mild to moderate neutrophilia (ranging from 14,000 to 21,000/\(\mu\)L) and subsequent leukocytosis. A mild left shift also was seen in 25\% of dogs from UC Davis, but more often in conjunction with a normal or increased neutrophil count. Mild and transient left shifts have been reported in 10–26\% of naturally infected dogs, most commonly associated with neutrophilia.\textsuperscript{8,9} Only one dog with natural infection in our study had neutrophil toxicity, but the rapid Romanowsky stain used at CCA-AUTH may be less sensitive for detecting mild toxic change.\textsuperscript{25}

Several dogs with CME were found in a retrospective survey of dogs with neutrophil toxicity, although it was not specified whether the dogs had acute or chronic CME.\textsuperscript{26} The cause of these discrepancies between experimentally and naturally infected dogs (and between dogs in Greece and California) cannot be explained easily. Concurrent infections with other organisms or inflammatory complications of CME (such as uveitis, meningitis, and pneumonia) may have led to neutrophilia.\textsuperscript{27–29} Further, in previous retrospective cases series\textsuperscript{7–10,13}, seropositivity for \textit{E canis} was the only inclusion criterion, without a systematic effort to exclude concurrent vector-borne infections. The development of neutrophilia also may depend on the virulence of the \textit{E canis} strain.\textsuperscript{12}

The left shifts in acute CME in our study were mild, comprising fewer than 800 bands/\(\mu\)L, and thus may not be of diagnostic value. However, our results suggest the leukocyte indices MPXI and LI could help in identifying or corroborating inflammation and for investigating the pathogenesis of neutropenia in CME. The MPXI, an estimate of MPO content per leukocyte, decreased dramatically in experimental dogs when neutropenia was at its most severe, and remained low (below the lower limit of the UC Davis reference interval) for the duration of the study. Currently, the validity of MPXI in dogs is uncertain because the underlying software calculations are made in comparison with human neutrophils.\textsuperscript{30} The MPXI was lower in 29 dogs with infections causing severe leukocyte consumption and acquired MPO deficiency\textsuperscript{18}, but has not previously been examined in CME. In one retrospective analysis, mean MPXI was
Table 1. Median (minimum–maximum) of leukocyte variables including myeloperoxidase index (MPXI) and lobularity index (LI) in dogs with naturally occurring nonmyelosuppressive (likely acute) canine monocytic ehrlichiosis (Ehrlichia canis) at the Clinic of Companion Animals, Aristotle University of Thessaloniki (CCA-AUTh) (n = 9) and at the University of California (UC) Davis (n = 11).

<table>
<thead>
<tr>
<th>Site</th>
<th>Analyte</th>
<th>Infected</th>
<th>Reference Values*</th>
<th>No. Below Reference Limit</th>
<th>No. Within Reference Limits</th>
<th>No. Above Reference Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCA-AUTh (QBC analyzer with manual differential)</td>
<td>WBCs (x10^9/L)</td>
<td>8811 (2517–12,513)</td>
<td>5800–16,500</td>
<td>1</td>
<td>8</td>
<td>0</td>
</tr>
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<td></td>
<td>Bands (x10^9/L)</td>
<td>0 (0–137)</td>
<td>Rare (&lt; 200)</td>
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<td>9</td>
<td>0</td>
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<tr>
<td></td>
<td>Neutrophils (x10^9/L)</td>
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<td>3000–10,800</td>
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<td></td>
<td>Lymphocytes (x10^9/L)</td>
<td>3204 (619–6757)</td>
<td>1600–8700</td>
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<td>6</td>
<td>0</td>
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<tr>
<td></td>
<td>Monocytes (x10^9/L)</td>
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<td>0–600</td>
<td>–</td>
<td>9</td>
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</tr>
<tr>
<td></td>
<td>Eosinophils (x10^9/L)</td>
<td>68 (0–125)</td>
<td>0–2000</td>
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<td>0</td>
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<tr>
<td></td>
<td>Nucleated RBCs</td>
<td>2 (0–28)</td>
<td>–</td>
<td>–</td>
<td>5</td>
<td>5</td>
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<td>UC Davis (ADVIA 120 with manual differential)</td>
<td>WBCs (x10^9/L)</td>
<td>10,090 (3293–24,690)</td>
<td>6000–13,000</td>
<td>2</td>
<td>4</td>
<td>5</td>
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<tr>
<td></td>
<td>Bands (x10^9/L)</td>
<td>66 (0–740)</td>
<td>Rare (&lt; 150)</td>
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<td>4</td>
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<td>1000–4000</td>
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<td>1</td>
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<tr>
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<td>Monocytes (x10^9/L)</td>
<td>154 (33–1975)</td>
<td>150–1200</td>
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<td>10</td>
<td>1</td>
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<tr>
<td></td>
<td>Eosinophils (x10^9/L)</td>
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<td>0–1500</td>
<td>–</td>
<td>11</td>
<td>0</td>
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<tr>
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<td>Nucleated RBCs</td>
<td>1 (0–4; 81)</td>
<td>0</td>
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<td>4</td>
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<tr>
<td></td>
<td>MPXI [n = 8]</td>
<td>22.7 (3.2–34.3)</td>
<td>7–22</td>
<td>2</td>
<td>2</td>
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<td>LI [n = 5]</td>
<td>3.3 (2.9–3.7)</td>
<td>2.6–3.7</td>
<td>0</td>
<td>5</td>
<td>0</td>
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</tbody>
</table>

*CCA-AUTh reference limits are the minimum–maximum values (rounded) from 27 clinically healthy dogs. UC Davis reference values are based on intra-laboratory reference intervals established using 60 clinically healthy adult dogs.

marginally higher in 399 dogs with a toxic left shift (compared with 6235 dogs without) but remained within the reference interval and hence was interpreted as having limited diagnostic value.30

In experimentally infected dogs in this study, the decrease in MPXI may have been associated with the persistent neutropenia or with neutrophils having lower MPO content due to maturational abnormalities or degranulation.18,19 Monocytopenia also could have contributed to the decreased MPXI, although neutrophils are the primary source of MPO in dogs. Purulent inflammation may lead to increased MPXI in dogs18, and may have been a secondary complication in the few naturally infected dogs with increased MPXI. Also in our study, the decrease in MPXI followed an increase in the LI, an indicator of immature cells (with less dense chromatin and lack of segmentation of nuclei) that coincided with the slight increase in band neutrophils (but did not exceed the upper limit of the UC Davis reference interval).19 The density of mononuclear cell nuclei also contributes to the LI, such that the increased LI in the dogs experimentally infected with E canis could have been the result of reactive monocytes and lymphocytes as well the left shift.19 The MPXI and LI results in our study raise questions regarding the mechanism of leukocyte abnormalities in acute CME, and warrant further investigation to assess their clinical usefulness.

Persistent monocytopenia was an unexpected finding in experimentally infected dogs and may have contributed to the development of leukopenia, although its clinical relevance is uncertain. Scant information is available regarding monocyte concentrations in dogs experimentally infected with E canis. In one experimental study evaluating differential leukocyte counts up to 5 weeks post infection, mild monocytosis was observed at isolated time points, and infected dogs had higher overall monocyte concentrations compared with uninfected controls.15 Both monocytopenia and monocytosis have been reported in retrospective case series of acute CME, and were also seen in the present study.3–10 Although reported previously3, our study is the first to semi-quantify activated monocytes in acute CME, which were seen in ~30% of observations in experimental dogs and in ~40% of natural infections. More than 60% of experimentally infected dogs had activated monocytes at 35 DPI, suggesting it may be a late onset change in acute CME as compared with the earlier peak of lymphoid reactivity. Of potential importance, median neutrophil and monocyte counts at 42 DPI remained significantly lower than preinfection, suggesting a persistent inhibitory effect on myelopoiesis, or persistent immune-mediated destruction.21,22,31

In the experimental dogs of this study, lymphocyte concentrations were significantly reduced at 7 and 14 DPI, consistent with inflammation and
possibly glucocorticoid-mediated stress. This was followed by mild rebound lymphocytosis for the duration of the study. In a previous experimental study of *E canis* infection, evaluating lymphocyte concentrations up to 5 weeks post infection, a significant decrease in lymphocyte count was observed at week 3 post infection, and infected dogs had overall lower lymphocyte concentrations compared with uninfected controls. Lymphopenia has been reported to occur more frequently (7–48% of cases) than lymphocytosis (3–11% of cases) in retrospective studies of acute CME; and was seen in ~30% of dogs with naturally occurring acute CME in this study. The finding of lymphopenia in natural cases could indicate an early (1–2 week) time point after infection. Reactive lymphocytes were observed in all dogs with naturally occurring acute CME and were especially prominent in experimentally infected dogs when lymphocyte counts were low, consistent with the antigenic effect of acute *E canis* infection. Lymphocyte reactivity with atypia has been described previously in a single case report of Panola Mountain Ehrlichia sp. infection. We did not observe the marked granular lymphocytosis (≥ 20,000/μL) or clonal T-cell expansion reported in naturally occurring CME. It is unknown whether this hematologic abnormality is a feature of acute CME or of more chronic infections.

Eosinopenia (and to an extent lymphopenia) likely resulted from adrenocortical stress response in experimentally infected dogs in our study, as frequently reported in previous clinical studies of CME. The clinical relevance of eosinopenia is unclear since reference intervals for eosinophils often have a lower limit of 0 cells/μL.

Although metarubricytosis is a rare finding in CME, careful manual differential counts should be done in dogs with CME to avoid spuriously increased leukocyte concentrations.

The absence of *E canis* morulae in blood smears of the experimental and clinical groups of dogs in this study was not surprising, as the diagnostic sensitivity of blood smear examination is low for this finding.

Confounding factors in the experimental study were the relatively young median age of the dogs and their recent vaccinations, which may have contributed to occasional preinfection lymphocytosis when compared with reference intervals for adult dogs. Recent vaccination also could have triggered an increase in the number of reactive lymphocytes, and enhanced the robustness of the rebound lymphocytosis. Furthermore, the inherent subjectivity in assessing activated monocytes and lymphocytes microscopically can contribute to variability in how cells are classified.

The small sample size of clinically healthy dogs used to define the reference limits at CCA-AUTH may have affected the sensitivity of identifying hematologic abnormalities in dogs with naturally occurring acute CME in Greece. Additionally, variability in the time to presentation and the potential for undetected concurrent disease may have contributed to the variability of hematologic findings in dogs defined as naturally infected. The use of 2 different types of hematology analyzers for the measurement of total WBC counts precluded direct comparison of dogs naturally infected with CME in California and in Greece. Additional limitations in this study were the low numbers of animals and the difficulty in defining acute CME in naturally infected dogs.

In conclusion, several discrete quantitative and qualitative leukocyte abnormalities were found in dogs with experimental and naturally occurring acute CME. These changes were consistent with a concurrent inflammatory response, antigenic stimulation, and stress. Changes in the novel indicators MPXI and LI suggested decreased MPO in leukocytes and the presence of immature leukocytes, and warrant further investigation in CME and other inflammatory diseases. These findings add to our understanding of leukocyte pathophysiology in dogs with nonmyelosuppressive acute CME, emphasize the value of differential counts and morphology in evaluating and monitoring this disease, and provide new findings in leukocyte indices that may have future diagnostic applicability.

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