Assessment of Novel Haematological Inflammatory Markers (NLR, SII, and SIRI) as Predictors of SIRS in Dogs with Canine Monocytic Ehrlichiosis

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Abstract

Canine monocytic ehrlichiosis (CME) is a tick-borne and fatal disease that induces a systemic inflammatory response syndrome (SIRS), which underscores the importance of assessing the level of inflammation. In this study, we aimed to evaluate changes in the SII and SIRI indexes in dogs with CME based on the presence of SIRS criteria. A total of 53 dogs affected with CME and 13 healthy dogs were included in the study. Haematological data, including neutrophil to lymphocyte ratio (NLR), systemic inflammatory index (SII), and systemic inflammatory response index (SIRI), were collected and statistically compared between groups based on SIRS status. The NLR and SIRI indexes were significantly higher in dogs with CME that meet the criteria for SIRS compared to dogs without SIRS and healthy controls. In summary, monitoring the SIRI index can aid in evaluating SIRS criteria in dogs with CME, which can help improve their treatment and overall prognosis.

Key words: *canine monocytic ehrlichiosis; neutrophil to lymphocyte ratio; systemic inflammatory index; systemic inflammatory response index*

Introduction

Canine monocytic ehrlichiosis (CME) is a tick-borne disease caused by *Ehrlichia canis*, which primarily infects monocytes and macrophages (Ewing et al., 1995). CME is a widespread disease with significant clinical and economic impacts on the dog population worldwide (Harrus and Waner, 2011). The disease may present with acute, subclinical, or chronic manifestations, depending on the severity of the infection and the immune status of the host (Harrus et al., 2003).

Clinical signs of CME may vary depending on the phase of the infection, and can include hyperthermia, anorexia, weight loss, oedema, haemorrhage, lymph adenomegaly, splenomegaly, mucosal pallor, uveitis, and blindness, mild anaemia, thrombocytopenia, and leukopenia (Sangione et al., 2011). In the chronic

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(myelosuppressive) phase of CME, veterinarians typically find pronounced thrombocytopenia, anaemia, and leukopenia, which can provide immediate diagnostic and prognostic information (Mylonakis et al., 2011). In the acute (non-myelosuppressive) phase, mild to moderate thrombocytopenia and anaemia are typically found (Mylonakis et al., 2011), along with several discrete quantitative and qualitative leukogram changes that are indicative of concurrent inflammation, antigenic stimulation, and stress (Gianopoulos et al., 2016). While leukopenia and neutropenia are common findings in dogs with acute CME, leukocytosis, neutrophilia, monocytosis, lymphocytosis, and rarely, a left shift, have been reported in up to 35% of naturally infected dogs, supporting an inflammatory process (Mylonakis et al., 2011; Gianopoulos et al., 2016). Also, the virulent stage of CME is linked to several complications associated with inflammatory mediators such as cytokines, acute-phase proteins, nitric oxide, and oxidative stress. This leads to a systemic inflammatory response syndrome (SIRS) (Asawapattanakul et al., 2021).

Inflammatory biomarkers are important tools for monitoring various diseases, with the potential of predicting disease severity and treatment outcomes, particularly after SIRS (Rejec et al., 2017, Pierini et al., 2019). Several novel inflammatory markers have been described, including the systemic inflammatory response index (SIRI), aggregate index of systemic inflammation (AISI), and systemic inflammatory index (SII), which involve neutrophils, monocytes, lymphocytes, and platelets (Hamad et al., 2019). These novel indexes are based on the main components of well-known inflammatory markers, such as neutrophils, monocytes, lymphocytes, and platelets (Pierini et al., 2019).

After conducting a review of the literature, the authors concluded that no studies to date have investigated the determination of the SII and SIRI index in dogs with CME. As a result, the objective of this study was to assess changes in SII and SIRI indexes based on the presence of SIRS in dogs with the disease, and to evaluate these indexes with respect to SIRS criteria.

Materials and methods

Animal material

The study was designed retrospectively between January 2019 and January 2023 from dogs presented to Aydın Adnan Menderes University, Faculty of Veterinary Medicine Animal Hospital with complaints of weakness, hind limb weakness, epistaxis, and ocular lesions. Due to the retrospective nature of the study, ethical approval was not obtained but owners were informed about the study and necessary permissions were obtained.

Canine monocytic ehrlichiosis is defined by the detection of positive results using the SNAP® 4Dx® test kit (IDEXX Laboratories, Inc., Westbrook, ME, USA). This test kit is used to detect the presence of antibodies against *Ehrlichia canis*, as well as *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, and *Dirofilaria immitis*. In order to eliminate co-infections, Snap Leishmania tests were also performed on the animals, and those with positive results for Leishmania, *Anaplasma* spp., *Dirofilaria* spp., and Lyme disease were excluded from the study.

As a result, of the 120 animals evaluated, a total of 53 animals were identified as infected only with Ehrlichiosis.

To confirm the diagnoses of animals identified as monoinfected with Ehrlichiosis, an indirect fluorescent antibody test (IFAT) was applied, and dogs with positive IFAT results (cut-off value of 1:50)

were included in the study. In addition, physical examinations were performed on dogs that tested positive for *E. canis* to assess: (i) the presence of ticks on their bodies, (ii) the effectiveness of ectoparasite control measures, and (iii) their daily living conditions, whether in an enclosed or open area. The animal material in the study consisted of two groups, a mono-infected group (*n*:53) and a healthy group (*n*:13).

The inclusion and exclusion criteria for animals included in the study are described below.

Dogs with at least two clinical symptoms associated with epistaxis, anaemia, weakness in the hind legs, petechial haemorrhage, fatigue, tick infestation, and who tested positive on the rapid test for CME,

i. Age between 1-10 years,
ii. No previous treatment before presenting,
iii. Non-pregnant and non-lactating dogs,
iv. Animals without severe complications,
v. Animals with negative PCR, and
vi. Only infected with ehrlichiosis.

Figure 1 provides a summary of the workflow diagram utilised for the study.

Laboratory analyses

Blood samples were collected from dogs diagnosed with CME via a rapid test kit and confirmed by IFAT, using appropriate technique by *Vena Jugularis* to EDTA, lithium heparin, and serum tubes in a total volume of 4 mL.

Haematological analyses were performed using Abacus Vet5 (Diatron, Hungary). Following sampling and laboratory analyses, infected dogs were classified

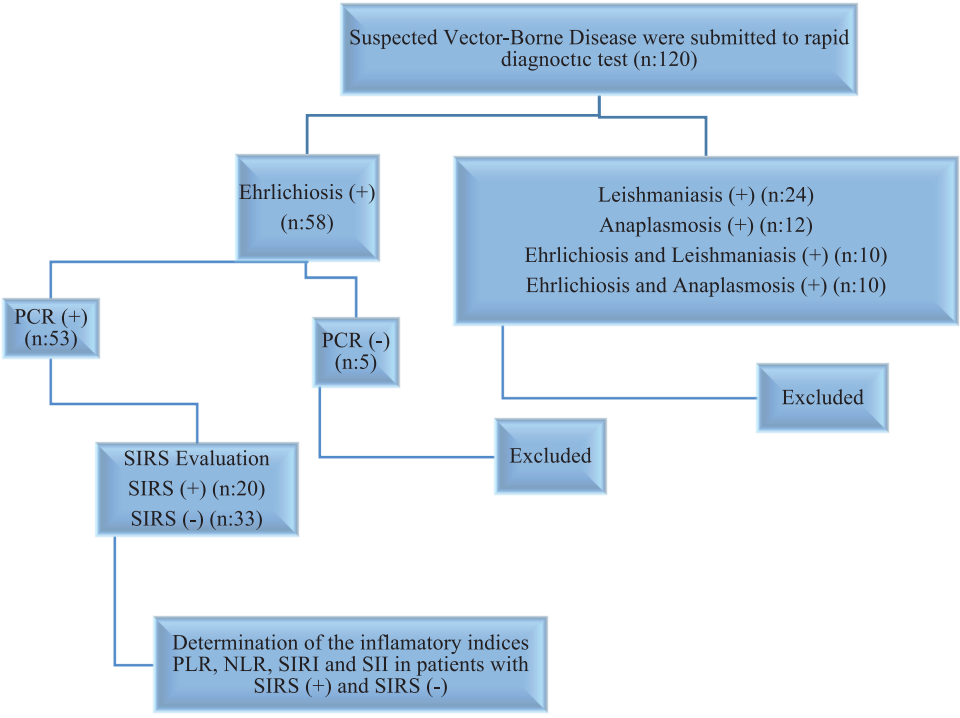

Figure 1. The method followed in the selection of animals for the study.

as positive or negative based on the SIRS criteria, requiring the fulfilment of at least two conditions as reported by Hauptman et al. (1997):

i. Body temperature < 37.8°C or > 39.4°C,
ii. Heart rate > 140 bpm,
iii. Respiratory rate > 30 breaths/min or pCO_2 < 32 mmHg, and
iv. WBC < 6000 or > 16,000 cells/μL, or > 3% band neutrophils.

The hemograms of the dogs included in the study were evaluated and the SIRI and SII values were calculated using the following formulas:

SIRI = neutrophil count × monocyte/lymphocyte

SII = platelet/lymphocyte × neutrophil

Statistical analyses

Descriptive statistics were performed on the index data obtained from haematological data, and then tabulated with mean and standard error. Despite the logarithmic transformation of the data, it was determined that they did not show a normal distribution according to the Shapiro Wilk test. Non-parametric tests, specifically the Kruskal-Wallis ANOVA test, were used for group comparisons. Results with a p-value less than 0.05 were considered statistically significant. All analyses and graphs were performed using GraphPad Prism 9.0 software.

Results and discussion

The haematological data was used to calculate the NLR, SII, and SIRI indexes. Comparations of groups were shown in Figure 2. Significant statistical distinctions were noted in the NLR values among SIRS-positive patients, SIRS-negative patients, and the healthy control group.

The results of the statistical analysis on the data collected for the SII index indicated that there were no notable differences between the healthy control group, SIRS negative patients, and SIRS positive patients.

When analysing the data collected for the SIRI indexes, it was observed that the SIRI levels of dogs with SIRS were signifi-

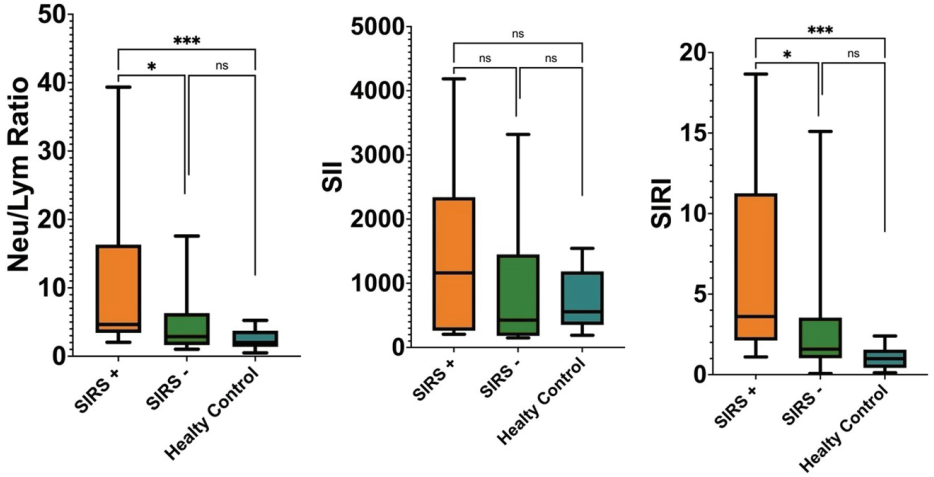

Figure 2. Comparison of NLR, SII and SIRI in the healthy and infected groups, categorised based on the presence or absence of SIRS

Ns: non-significant, *P<0.05, ***P<0.01.

Table 1. Mean ±standard error values of haematological indexes across groups with respect to SIRS status

	Neu/Lym Ratio ($\bar{X}$ ± SE)	SII ($\bar{X}$ ± SE)	SIRI ($\bar{X}$ ± SE)
SIRS +	9.9 ± 2.6[a]	1332 ± 372.0	6.4 ± 1.6[a]
SIRS -	4.8 ± 1.0[b]	946.2 ± 253.5	3.1 ± 1.0[b]
Healthy Control	2.4 ± 0.4[b]	722.5 ± 122.8	1.0 ± 0.2[b]

[a,b]: Values with different letters in the same column are statistically different

cantly higher compared to both SIRS negative dogs and healthy control dogs.

When using new measurement parameters for the determination of sepsis, it was observed that the data obtained from calculation tools including platelet counts may be insufficient (Table 1).

Systemic inflammatory response syndrome is a clinical process that develops due to the release of high levels of inflammatory mediators in a disease state (Hauptman et al., 1997). For dogs, abnormalities in heart and respiratory rate, body temperature, and leukocyte count are used to determine SIRS status (Hauptman et al., 1997). Ehrlichiosis is a multisystem disease affecting various organs and tissues in dogs. The most common findings compatible with the disease are petechiae, generalized lymphadenopathy, splenomegaly, hepatomegaly, cardiomyopathy and oedema (Mylonakis et al., 2011; Sangione et al., 2011). It has been reported that the inflammatory response of the host shaped with ehrlichiosis may occur due to infectious and non-infectious causes such as sepsis, malariasis, babesiosis, multiple trauma and burns (Mylonakis et al., 2011; Sangione et al., 2011). Mastrorilli et al. (2007) detected the presence of SIRS in nine of 16 dogs with leptospirosis and the presence of non-regenerative anaemia in six of these dogs. Our study aimed to assess the severity of inflammation induced by Ehrlichiosis and investigate the potential of haemato-biochemical indexes as a diagnostic biomarker for SIRS in dogs with Ehrlichiosis. We found that the SIRI and NLR indexes were effective in detecting SIRS in dogs with Ehrlichiosis, indicating their practical value in clinical diagnosis.

Under routine field conditions in both human and veterinary medicine, the complete blood count is a commonly performed laboratory analysis. The complete blood count allows for easy identification and interpretation of the relationship between various cell types. In human medicine, determining indexes such as the neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and monocyte to lymphocyte ratio (PLR) provides insight into the prognosis of neoplastic conditions and inflammatory processes (Qin et al., 2016; Guven et al., 2022). However, there is limited data on the potential value of these indexes in veterinary medicine. Thus far, only one study by Yazlik et al. (2022) has demonstrated the diagnostic significance of SII in identifying pyometra in dogs. However, to our knowledge, there are no studies investigating the potential utility of SII and SIRI as novel markers of inflammation in dogs with CME.

NLR was selected as a useful clinical indicator of immune function because it reflects both innate and adaptive immu-

nity and is widely available in clinical settings (Guthrie et al., 2013). Neutrophils play a crucial role in the innate immune system, directly attacking pathogens, releasing inflammatory substances, and activating T lymphocytes to combat infections (Lewis et al., 2012). Lymphocytes, on the other hand, are primarily involved in the adaptive immune system and cell-mediated responses (Ayala et al., 1996). Given the dynamic nature of the immune response, using ratios of cell types rather than absolute cell counts can more accurately reflect fluctuations in the immune system (Dourmashkin et al., 2023). Therefore, NLR can serve as a potential prognostic factor for various disease processes. According to recent veterinary literature, NLR has been shown to be a useful prognostic indicator for different types of cancer (Rejec et al., 2017) and inflammatory conditions (Benvenuti et al., 2020; Neumanni, 2021).

The present study found a direct relationship between NLR and the presence of SIRS. This is consistent with studies in humans that have reported higher NLR in patients with sepsis (Hwang et al., 2017). However, other studies in dogs have reported that septic dogs may have decreased NLR and neutropenia (Schultze et al., 2010; Pierini et al., 2019). In the present study, SIRS positive dogs with ehrlichiosis had significantly higher NLR than the non-SIRS and healthy control group. This may be due to the phase of the disease and characteristics of the study population. SIRS status was characterised by leukocytosis and increased NLR, which is consistent with previous reports (Zahorec, 2001). SIRS also resulted in lymphocytopenia, which is a significant decrease in the blood lymphocyte count (Jilma et al., 1999). The increase in NLR was due to increased neutrophil counts and decreased lymphocyte counts. Differential leukocyte counts have also been evaluated in dogs with ehrlichiosis compared to healthy dogs, and it was found that these dogs had generally decreased lymphocyte and increased neutrophil levels (Castro et al., 2004; Bhadesiya and Raval, 2015).

SII, a combined measure that includes platelet, neutrophil, and lymphocyte counts, has emerged as a valuable prognostic marker in a range of health conditions such as cancer, coronary artery disease, acute ischemic stroke, and premature rupture of membranes. It has been shown to be a useful indicator of both local immune response and systemic inflammation in human, specifically with higher levels in patients with inflammatory conditions (Incir et al., 2020), and its potential as a prognostic predictor has been supported by studies (Fang et al., 2020; Hou et al., 2021). Furthermore, a high SII value (≥ 834.89) can effectively differentiate postmenopausal osteoporosis patients at risk of experiencing osteoporotic fractures (Fang et al., 2020). The objective of this study was to assess the potential variation in SII measurements among animals infected with Ehrlichiosis, based on their SIRS status. Our findings suggest that although SII was greater in SIRS-positive dogs with CME, it did not exhibit significant variation relative to SIRS status in Ehrlichiosis-infected animals. Therefore, SII is not a suitable diagnostic parameter for determining SIRS status. Our finding was compatible with Yazlik et al. (2022), who observed the higher SII in dogs with pyometra, regardless of the SIRS status. Nevertheless, additional research is warranted to investigate the potential prognostic value of SII levels in these dogs.

The systemic inflammation index is an objective marker used to assess inflammation throughout the body and is calculated using a simple formula platelet×neutrophil/lymphocyte. In a study of dogs with CME, a Cox proportional hazards

regression model identified severe anaemia, severe leukopenia, pancytopenia, a tendency to bleed (particularly from the nose) and the German shepherd breed as important indicators of poor survival (Harrus et al., 1997). Another experimental study on CME found that infected animals exhibited significant decreases in red blood cell count, haemoglobin levels, and packed cell volume mean values during weeks 2 and 3, while mean corpuscular haemoglobin concentration was significantly higher during weeks 3 and 4 (de Castro et al., 2004). The fact that the timing of CME infection was unknown for the patients in our study, and that the immune response to the disease varied, may have contributed to differences in haematological indices.

In cases of acute non-myelosuppressive CME, mild to moderate thrombocytopenia and anaemia are commonly observed, but reports on leukogram abnormalities have been incomplete and inconsistent, particularly with respect to indicators of inflammation (Kuehn and Gaunt, 1985; Harrus et al., 1997; Mylonakis et al., 2011; Theodorou et al., 2013). Evidence of an acute phase protein response has been documented in dogs with acute CME, which suggests the presence of an inflammatory process (Mylonakis et al., 2011; Rudoler et al., 2015). While leukopenia and neutropenia are consistent findings in dogs with experimental or natural acute CME, up to 35% of naturally infected dogs have exhibited leukocytosis, neutrophilia, monocytosis, lymphocytosis, and, rarely, a left shift, which supports the presence of an inflammatory process (Harrus et al., 1997; De Castro et al., 2004; Mylonakis et al., 2011). The hematopoietic system has been found to be impacted by SIRS in several human studies (Aird, 2003). Similarly, in our study, significant changes were observed in the parameters calculated using the SIRI index in dogs with SIRS, which reflects the changes that occur in the hematopoietic system in response to SIRS.

Conclusion

Our study showed that the SIRI index is a useful parameter for evaluating SIRS criteria in dogs with CME, but further studies on a larger sample size and supported by other inflammatory markers are needed to determine its inclusion in SIRS criteria.

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Procjena novih hematoloških upalnih markera (NLR, SII i SIRI) kao prediktora Sindroma sustavnog upalnog odgovora (SSUO) u pasa s psećom monocitnom erlihiozom

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Pseća monocitna erlihioza (CME) je smrtonosna bolest koju prenose krpelji koja prouzroči sindrom sistemskog upalnog odgovora (SSUO), što naglašava važnost procjene razine upale. U ovoj studiji, cilj nam je bio procijeniti promjene u SII i SIRI indeksima u pasa s CME na temelju prisutnosti SSUO kriterija. Uključeno je ukupno 53 pasa s CME i 13 zdravih pasa. Prikupljeni su hematološki podatci, uključujući omjer neutrofila i limfocita (NLR), sistemski upalni indeks (SII), indeks sistemskog upalnog odgovora (SIRI) te su oni statistički uspoređeni između skupina na temelju SSUO statusa. NLR i SIRI indeksi bili su značajno veći u pasa s CME koji zadovoljavaju kriterije za SSUO u usporedbi s psima iz skupine bez SSUO i iz kontrolne skupine. Zaključno, naša studija ukazuje na to da praćenje SIRI indeksa može pomoći u procjeni SSUO kriterija u pasa s CME, što može pomoći poboljšati njihovo liječenje i sveukupnu prognozu.

Ključne riječi: *pseća monocitna erlihioza, omjer neutrofila i limfocita, sistemski upalni indeks, indeks sistemskog upalnog odgovora*