Neutrophil-to-lymphocyte ratio, nucleated red blood cells and erythrocyte modifications in canine systemic inflammatory response syndrome

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Abstract

Systemic inflammatory response syndrome (SIRS) is the manifestation of the systemic response to an infectious or non-infectious disease. We evaluated the association between erythrocyte and leukocyte ratio modifications (NLR, BLR and BNLR) in canine SIRS and the severity of illness and outcome. Dogs were divided in 3 groups: SIRS (n=90), healthy (n=50) and chronic group (n=50). A SIRS grading was obtained based on how many criteria were fulfilled. The Acute Patient Physiology and Laboratory Evaluation ($APPLE_{fast}$) was allocated in SIRS dogs. Survival rate was assessed 15 days after admission. Dogs with cytology or culture positive for active bacterial infections were recorded as septic. Dogs with hemolytic or hemorrhagic disorders were excluded. An $APPLE_{fast}$ score >25 (p=0.03) and a SIRS grading >2 (p=0.001) were associated with mortality. Thirty-two dogs (35%) were recorded as septic. SIRS group showed lower RBC, HCT and HGB values than the other two groups (p<0.001). Twenty-two of SIRS dogs (24%) showed circulating NRBCs. The NRBC count was higher only in the SIRS group than healthy dogs (p=0.0007). The occurrence of circulating NRBCs was associated with poor outcome in SIRS groups (p=0.005). NLR was higher in SIRS group than control groups (p<0.0001) and lower in septic dogs (p=0.016). $APPLE_{fast}$, SIRS grading and circulating NRBCs should be considered as negative prognostic factors in canine SIRS. NLR, BLR and BNLR could be useful tools in dogs with SIRS, especially NLR which was significantly lower in septic group. Further prospective, large-scale studies investigating BLR and BNLR in canine SIRS are warranted.

Keywords complete blood count; neutrophil-to-lymphocyte ratio; nucleated red blood cells; dog; systemic inflammatory response syndrome.

Introduction

Systemic inflammatory response syndrome (SIRS) is the manifestation of the systemic response to an infectious or non-infectious disease associated with a massive release of inflammatory mediators (Hauptman et al., 1997). Criteria for SIRS diagnosis in dogs have been previously reported and include modifications in body temperature, heart and respiratory rate, and leukocyte count (Hauptman et al., 1997). In human medicine, many reports have identified the hematopoietic system as the target of SIRS (Aird 2003; Goyette et al. 2004; Napolitano, 2017). Hematological disorders in critically ill patients are also associated with poor outcome (Aird, 2003).

Nucleated red blood cells (NRBCs) are immature erythrocytes produced in the bone marrow and are not generally present in the peripheral blood of healthy human beings or dogs (Constantino and Cogionis, 2000; Barger, 2010). Low numbers of circulating NRBCs can be found in Dachshunds and in Miniature Schnauzers as breed specific finding (Meyer and Harvey, 1998). In humans, as in dogs, after the neonatal period, the presence of NRBCs in the peripheral blood is usually associated with malignant neoplasms, bone marrow diseases, and other serious disorders (Constantino and Cogionis, 2000; Earl et al., 1973). In veterinary medicine, only two studies have investigated the pathological presence of circulating NRBCs. Aroch and colleagues studied the prevalence of circulating NRBCs as a prognostic marker in dogs with heatstroke, concluding that both the presence and number of circulating NRBCs were associated with poor prognosis (Aroch et al., 2009). Müller et al. reported that circulating NRBCs were associated with poor outcome in critically ill dogs (Müller et al., 2014). However, the presence of circulating NRBCs should not always be considered as a negative prognostic factor. Normally, circulating NRBCs, together with reticulocytes and Howell-Jolly bodies, can also be part of a regenerative response to anemia (Barger, 2010).

The neutrophil-to-lymphocyte ratio (NLR) has been used in human medicine as a readily accessible parameter that can be calculated using a complete blood count (Zahorec, 2001). NLR has been proposed as an independent predictor of poor survival in patients with tumors and cardiovascular diseases (Zahorec, 2011; Kang et al., 2014; Ayça, 2015). To the best of our knowledge, there are no specific studies on NLR in critically ill dogs. NLR has been analyzed only in oncological veterinary patients (Mutz et al., 2015; Macfarlane et al., 2016a, b; Skor et al., 2017). The aim of this study was to evaluate the association between erythrocyte and leukocyte modifications during canine SIRS compared to the severity of illness and outcome.

Materials and methods

Over a 4-year period, from January 2012 to November 2016, client-owned dogs with SIRS, which presented to the intensive care unit of our veterinary teaching hospital in Pisa (Italy), were retrospectively enrolled. Data were collected with the owners' informed consent. Our study included 3 groups of dogs: 1 with SIRS (SIRS group), 1 with healthy dogs (healthy group), and 1 with chronic diseases (chronic group).

Dogs were required to fulfill at least two of the following criteria to be included in the SIRS group: heart rate >120 bpm; respiratory rate >20 bpm; rectal core temperature <38°C or >39.2°C; and white blood cell count (WBC) $<6\times10^9$/L or $>16\times10^9$/L, or band neutrophils >3% (Hauptman et al., 1997). The SIRS grading was obtained based on how many criteria were fulfilled, thus the SIRS score ranged from 2 to 4. Dogs with ≥2 criteria of SIRS along with a documented underlying infectious cause were recorded as septic (Hardy et al., 2018). The $\text{APPLE}_{\text{fast}}$ (acute patient physiology and laboratory evaluation) score was applied in the SIRS group. The $\text{APPLE}_{\text{fast}}$ is a clinical score validated for hospitalized canine patients, which evaluates serum glucose, albumin, lactate, platelet count, and the mentation score (Table 1-2) (Hayes et al., 2010). Dogs were divided in two groups according to APPLEfast (<25 and ≥25) and SIRS (=2 and >2) to compare their outcome. Dogs were included in the chronic group if they did not satisfy SIRS criteria, had a diagnosis of chronic disease (i.e. chronic kidney disease, inflammatory bowel disease, chronic pancreatitis) and showed no increase in serum C-reactive protein (CRP), leukocytosis and no band neutrophils. Healthy dogs were chosen from those actively enrolled in the blood bank of the veterinary teaching hospital (during the study period), whose physical examination was normal, complete blood count (CBC) and serum biochemistry were within reference intervals, and serology tests for *Leishmania* spp., *Ehrlichia canis* and *Anaplasma phagocytophila* were negative. Dogs with disorders where the appearance of NRBCs in the peripheral blood is common, such as regenerative anemia (immune mediated hemolytic anemia and hemorrhagic process), were excluded from the study.

For each dog the NLR, band neutrophil-to-lymphocyte ratio (BLR) and band neutrophil-to-neutrophil-to-lymphocyte ratio (BNLR) were calculated. Each dog had a CBC at admission using a laser cell counter (Procyte DX, IDEXX Laboratories, Westbrook, USA) and a blood smear microscopic examination stained with May-Grunwald Giemsa (Aerospray Wescor, Delcon, Milan, Italy) and the NRBC count was assessed manually. Dogs with RBC and/or hematocrit (HCT) and/or hemoglobin (HGB) below the reference range ($5.65\ \text{x}10^{12}$/L, 0.37 L/L, and 131 g/L, respectively) were considered anemic. Anemia was characterized using mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Serum total protein, albumin, and CRP were evaluated using a biochemistry analyzer (Liasys, Assel SRL, Rome, Italy). $\text{APPLE}_{\text{fast}}$ parameters, such as glucose and lactate, were evaluated from the venous blood gas analysis (ABL 700, A. DeMori, Milan, Italy). Platelet count was evaluated from the CBC at admission and confirmed by smear evaluation. Mentation score was evaluated at admission before sedation/analgesic administration by the same clinician (GC) (Table 2). Rate of survival was assessed during hospitalization, at 15 days after their admission.

For all continuous parameters, the normality of data distribution was evaluated by the D'Agostino-Pearson test. Normally and non-normally distributed continuous parameters were reported as mean±standard deviation (SD) and as median and 2.5-97.5 percentile range, respectively, and were compared between the survivors and non-survivors using the Student t-test or Mann-Whitney test, respectively. Fisher's exact test was used to compare categorical variables between group outcomes. The presence of anemia and NRBCs, SIRS and $APPLE_{fast}$ grading groups were compared to outcome. NRBCs count, NLR, BLR, BNLR were compared to the outcome. Age and sex were compared between study population groups using the Kruskal-Wallis test. RBC, HGB, HCT, WBC, NRBCs, NLR were evaluated among study populations using a one-way ANOVA or Kruskal-Wallis test and Dunn's multiple comparison test. P-value <0.05 was considered as statistically significant (GraphPad Prism 6, GraphPad Software, California, USA).

Results

Ninety dogs fulfilled the inclusion criteria for the SIRS group. The healthy and chronic groups were composed of 50 dogs each. There were no differences between groups in terms of age and sex (p>0.05).

The healthy group was composed of 25 females (50%), five spayed, and 25 males (50%), one neutered. The most represented breeds in the healthy group were Boxer (19), German Shepherd and Golden Retriever (4 dogs each), Dobermann Pinscher (3), English Setter (2) and 7 dogs of other breeds (Dachshund and Miniature Schnauzers were not occurring). Eleven dogs were mixed breeds. The median age of the healthy group was 6 years (range 1-12 years).

The chronic group was composed of 50 dogs: 29 females (58%) including 13 spayed, and 22 males (42%), none of which had been castrated. The most represented breeds in chronic group were: Boxer (6), Poodle (4), Springer Spaniel (2), Cocker Spaniel (2), Bolognese (2), Bernese Mountain Dog (2), Chihuahua (2), Miniature Pinscher (2) and 14 dogs of other breeds (only one Dachshund was included in the list). Fifteen were mixed breeds. The median age of the chronic group was 8.5 (range 1-14 years).

SIRS group was composed of 42 females (47%) including 11 spayed, and 48 males (53%) including four that had been castrated. The most represented breeds were: Cocker Spaniel (5), German Shepherd (5), Dachshund (4), Yorkshire Terrier (4), Labrador Retriever (3), and Golden Retriever (2). Twenty-seven dogs were mixed breeds.

The 40 other breeds were each represented by one dog. Median age of SIRS group was 8 (range 0.2-15 years). Thirty-two dogs (35%) were included in the septic group, and the remaining 58 in the non-septic group. In the SIRS group, thirty-nine dogs (43%) died during the study period. Among the non-survivor dogs, thirty dogs (77%) died in the intensive care unit, 8 dogs (21%) died during the first 7 days after discharge, and one dog died during the first 15 days after discharge. Dogs in the SIRS group were sub-grouped using the SIRS score: 48% in 2/4 and 52% in >2/4. $APPLE_{fast}$ scores ranged from 14 to 37 points (median=25 points). Dogs with $APPLE_{fast}$ scores >25 (p=0.03) and SIRS grading >2 (p=0.001) were associated with an increased mortality rate.

In the SIRS group, anemia was present in 56 dogs (62%) and was not associated with the outcome (p=0.8279). Based on the evaluation of HCT, anemia was mild (0.3≤HCT≤0.37) in 24 dogs (43%), moderate (0.2≤HCT≤0.29) in 23 dogs (41%), and severe (HCT<0.19) in only 7 dogs (13%). Microcytic anemia (MCV<61.6 fL) was present in 55% (n=31) of anemic dogs, normocytic anemia (61.6≤MCV≤73.3 fL) was present in 41% of dogs (n=23), and only 2 dogs had macrocytic anemia. Four dogs (7%) showed hypochromic (MCHC<320 g/L) anemia and all the other anemic dogs (n=52; 93%) had normochromic anemia (320≤MCHC≤379 g/L) (Tveden, 2004). Dogs in the SIRS group showed lower RBC, HCT and HGB values compared to the other two groups (p<0.0001) (Table 3), and the presence of anemia was not associated with poor outcome. No statistical differences were found between septic and non-septic dogs, SIRS grading groups, and $APPLE_{fast}$ scores groups. No statistical differences were found between $APPLE_{fast}$ score groups (> and <25) for RBC, HGB and HCT, NLR, WBC and between septic and non-septic dogs.

Twenty-two of the 90 dogs (24%) showed circulating NRBCs (range 0-30, median 0). The NRBC count was significantly higher in the SIRS group compared to the healthy group (p=0.0007) and no differences were found between the SIRS group and the chronic group and between the healthy and chronic groups (Table 3). No statistically significant differences were found between $APPLE_{fast}$ score groups and SIRS grading groups. In the SIRS group, the occurrence of circulating NRBCs was associated with poor outcome (p=0.005).

Sixty-five percent of dogs with SIRS (n=59) showed leukocytosis, 10% (n=9) showed leukopenia, and the remaining dogs did not show any WBC alterations. Band neutrophils >3% occurred in 52 dogs (58%). Dogs with SIRS showed a higher WBC count compared to the control groups (p<0.0001) (Table 3). WBC counts were not statistically different between survivors and non-survivors, septic and non-septic dogs, SIRS grading groups, and $APPLE_{fast}$ scores groups.

NLR was significantly higher in the SIRS group compared to the control groups (p<0.0001) (Table 3) and not associated with outcome. NLR was not statistically different between SIRS grading groups or $APPLE_{fast}$ scores groups. NLR was significantly lower in septic dogs (*p*=0.0272) (Fig. 1). BLR and BNLR were evaluated in association with outcome, sepsis and $APPLE_{fast}$ groups and no associations were found.

Discussion

In our study, dogs in the SIRS group were chosen from the ICU using at least two out the 4 criteria (Hauptman et al., 1997), as reported in a recent study on the topic (Müller et al., 2014). Unlike the study on NRBCs in critically ill patients (Müller et al., 2014), we decided to exclude patients with disorders that might have involved the presence NRBCs in the peripheral blood. In fact, in some diseases, such as immune mediated hemolytic anemia and acute hemorrhagic disorders, circulating NRBCs are common, thus creating a bias in the interpretation of results (Tveden, 2010).

In our study, anemia was present in almost 60% of SIRS dogs. The most frequent types of anemia were mild-moderate (87% of dogs), microcytic (55%), normocytic (41% of dogs), and normochromic (93%) anemia. Within our anemic population, only 7 dogs had severe anemia, making this type of anemia infrequent in this category of dogs. This result is similar to the prevalence in human patients (Aird, 2003; Goyette et al., 2004) and higher compared to a recent study on hospital-acquired anemia in critically ill dogs (Lynch et al., 2016). This difference could be due to different inclusion criteria. In fact, we included dogs with SIRS, whereas the other study (Lynch et al., 2016) included hospitalized patients that may have had less serious diseases. Anemia was not associated with the outcome in this study; however, because of its high prevalence, anemia has to be taken into account in critically ill patients because it could affect patient management. Dogs with SIRS showed a significant reduction in RBC, HCT and HGB as these parameters globally assess the erythron compared to healthy and chronic dogs. This suggests that the active inflammatory status of SIRS dogs could play a key role in the development of anemia. A mild, micro-normocytic normochromic anemia was the most frequent type of anemia. This type of anemia is typical of chronic diseases (anemia of chronic inflammation); however, in SIRS, it may be a common feature because of the concurrent or acute impairment of a chronic state.

NRBCs are a negative prognostic marker in human beings with critical illnesses (Constantino and Cogionis, 2000). However, there are only two studies on the prognostic importance of circulating NRBCs in dogs (Aroch et al., 2009; Müller et al., 2014). Investigating the prognostic relevance of peripheral NRBCs in dogs with heatstroke, the authors of one study concluded that the presence of peripheral NRBCs is common in these dogs and is associated with a worse outcome (Aroch et al., 2009). Authors of the other study reported that for critically ill dogs, NRBC positive patients had a higher mortality compared to NRBC negative ones (Müller et al., 2014).

According to the literature, our results highlight that NRBCs were significantly higher in the SIRS group compared to healthy dogs (Müller et al., 2014). Among dogs with SIRS, the occurrence of circulating NRBCs was associated with poor outcome, confirming that circulating NRBCs are a negative prognostic marker. The exclusion of dogs with disorders characterized by the appearance of NRBCs in the peripheral blood should increase the significance of this result.

The pathogenesis of circulating NRBCs in critically ill patients in human and veterinary medicine is not well understood. The most common substantiated theory for their presence in critically ill patients is inflammation and/or decreased tissue oxygenation, inducing a damage in the blood-bone marrow barrier, allowing for their release in the circulation (Stachon et al. 2003, 2005, 2006, 2007; Müller et al., 2014).

During SIRS, there is an increase in WBC, neutrophils and monocytes and a simultaneous reduction in lymphocytes and eosinophils (Jilma et al., 2012). Thus, in the present study SIRS dogs showed a higher WBC count compared to the control groups and in septic dogs, the WBC count should be lower compared to non-septic dogs due to neutropenia and lymphopenia. In contrast to the literature, WBC were not statistically different between septic and non-septic dogs (Schultze, 2010). This difference could be due to an early or late assessment of the leukocyte pattern compared to the beginning of sepsis.

High NLR has been proposed as an independent predictor of poor survival in various human clinical conditions observed in oncological patients (Zahorec, 2001; Kang et al., 2014) and in patients with cardiovascular diseases (Kang et al., 2014). Another recent work, showed how both a high NLR at hospitalization, and a persistently low or high NLR during the first two days after admission were associated with increased risk of 28-day mortality (Hwang et al., 2017). In veterinary medicine, NLR has only been analyzed in oncologic veterinary patients (Mutz et al., 2015; Macfarlane et al., 2016a, b; Skor et al., 2017), and there have been no investigations in SIRS dogs. Mutz et al. (2015) evaluated the potential prognostic significance of NLR in a population of dogs with newly diagnosed multi-centric lymphoma, concluded that NLR does not act like a prognostic marker in this tumor (Mutz et al., 2015). More recently, another study investigated the ability of NLR to differentiate soft tissue sarcoma from benign soft tissue tumors (Macfarlane et al., 2016a). In this study, dogs with soft tissue sarcoma, NLR was significantly higher compared to those with benign tumors (Macfarlane et al., 2016a). Two other papers assessed the utility of NLR in predicting the histopathological grade and outcome of canine cutaneous mast cell tumor (Macfarlane et al., 2016b; Skor et al., 2017). Dogs with high-grade mast cell tumors and a shorter progression-free interval had higher NLR (Macfarlane et al., 2016b; Skor et al., 2017).

To the best of our knowledge, this is the first study evaluating NLR in critically ill dogs. NLR was significantly higher in SIRS dogs compared to the other two study groups, and was not associated with the outcome. Moreover, NLR was significantly lower in the septic group compared to the non-septic group. This data is in contrast to human studies, that report higher NLR in septic patients. Our findings could be explained by the different leukocyte patterns between dogs and human beings. In septic dogs, the most common findings were neutropenia and lymphopenia, which reduces NLR in these patients (Schultze, 2010). Another possible explanation could be the presence of a "stress leukogram", a pattern that has not been reported in human patients (Deleand and Welte, 2017).

Our study had some limitations. Firstly, using Hauptman's SIRS criteria, false-positive in SIRS group could occur. We believe that the interpretation of anemia could be underestimated due to dehydration, which was not taken into account in this study, together with the assessment of RBC regeneration by means of reticulocyte values in relationship to the degree of the anemia. Given the retrospective nature of the present study, we were not able to analyze the comorbidities and cause of SIRS. Especially pre-existing chronic and subclinical diseases could have altered the interpretation of the hematological alterations. Not all dogs had an evaluation of antibiotic treatments pre-hospitalization that could have affected sepsis diagnosis in our population. We did not collect information on the treatments of SIRS dogs, and this could have affected the dog's prognosis. We were unable to standardize the SIRS management, although all the dogs in the present study received a similar medical management.

Conclusions

Dogs with SIRS showed a significant reduction in RBC, HCT and HGB compared to healthy and chronic dogs. This suggests that the active inflammatory status of SIRS dogs could play a key role in the development of anemia. A mild, micro-normocytic normochromic anemia was the most frequent type of anemia. Our results also suggest that the occurrence of circulating NRBCs could has additional negative prognostic values.

The evaluation of NLR, BLR and BNLR could be interesting tools that could be evaluated in dogs with SIRS, where they were significantly higher compared to the healthy and chronic dogs. Moreover, NLR was significantly lower in the septic group compared to the non-septic group. Further prospective, large-scale studies investigating the role of NLR, BLR and BNLR in canine SIRS are warranted.

Declaration of interest

The Authors declare no conflict of interest. This paper was not supported by grants.

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