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STANDARD ARTICLE

Evaluation of plasma angiopoietin-2 and vascular endothelial growth factor in healthy dogs and dogs with systemic inflammatory response syndrome or sepsis

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Background: Angiopoietin-2 (Ang-2) and vascular endothelial growth factor (VEGF) are regulators of endothelial permeability.

Objective: Plasma concentrations of Ang-2 and VEGF are increased in dogs with systemic inflammatory response syndrome (SIRS) and sepsis and are correlated with disease severity and outcome.

Animals: Healthy dogs (n = 18) and client-owned dogs with SIRS (n = 34) or sepsis (n = 25).

Methods: Prospective observational study. Ang-2 and VEGF concentrations in admission plasma samples were compared between healthy dogs and dogs with SIRS or sepsis, and between survivors and non-survivors. Correlations with the acute patient physiologic and laboratory evaluation ($APPLE_{fast}$) disease severity score were examined.

Results: Median Ang-2 was significantly higher in dogs with SIRS (19.3; interquartile range [IQR]: 8.6-25.7 ng/mL) and sepsis (21.2; IQR: 10.3-30.1 ng/mL) compared to healthy dogs (7.6; IQR: 6.7-9.8 ng/mL). Ang-2 was significantly higher in non-survivors (24.1; IQR: 11.9-50.0 ng/mL) than survivors (10.2; IQR: 7.2-21.5 ng/mL) but did not correlate with the $APPLE_{fast}$ score. Admission Ang-2 predicted negative outcome in dogs with SIRS and sepsis with reasonable accuracy (area under the curve [AUC]: 0.75, confidence interval [CI]: 0.59-0.85; sensitivity: 0.5, CI: 0.29-0.71; specificity: 0.87, CI: 0.75-0.95); differentiation between sepsis and SIRS was poor (AUC: 0.58). Plasma VEGF was significantly higher in dogs with sepsis (45; IQR: 14-107.5 pg/mL) than in dogs with SIRS (3.3; IQR: 0-35.6 pg/mL) or healthy dogs (0; IQR: 0 pg/mL; $P = 0.008$). VEGF was significantly ($P = .0004$) higher in non-survivors (34.5; IQR: 0-105.7 pg/mL) than in survivors (0; IQR: 0-55.2 pg/mL). The ability of VEGF to predict a negative outcome was poor.

Conclusions and Clinical Importance: Ang-2 may represent a useful additional prognostic marker in dogs with SIRS.

KEYWORDS

$APPLE_{fast}$ score, biomarker, canine, inflammation, outcome, prognostic

1 | INTRODUCTION

Systemic inflammatory response syndrome (SIRS) and sepsis are common in both humans and animals.[1,2] Systemic inflammation, endothelial activation and dysfunction causing increased vascular permeability, loss of vascular tone, and impaired coagulation are hallmarks of the pathophysiology of SIRS and sepsis.[3,4] If uncontrolled, SIRS and sepsis

ABBREVIATIONS: AHDS, acute hemorrhagic diarrhea syndrome; ALT, alanine aminotransferase; Ang-2, angiopoietin-2; APPLE, acute patient physiologic and laboratory evaluation; AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; CV%, coefficient of variation; GDV, gastric dilatation volvulus; IQR, interquartile range; LLD, lower limit of detection; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; ROC, receiver-operating characteristics; SIRS, systemic inflammatory response syndrome; VEGF, vascular endothelial growth factor; WBC, white blood cell.

may lead to distributive shock, multi-organ dysfunction, and death. Early recognition and aggressive intervention are therefore crucial.[5]

Angiopoietin (Ang)-2 and vascular endothelial growth factor (VEGF) are major players in the regulation of vascular endothelial activation and dysfunction.[6–8] Ang-2 is predominantly synthesized by endothelial cells, stored in cytoplasmic Weibel-Palade bodies and released into the bloodstream upon endothelial activation.[7,9] The balance between the agonistic ligand Ang-1 and the antagonistic Ang-2 regulates baseline endothelial barrier function and its response to injury. Although Ang-1 promotes vessel stability, inhibits inflammation, and limits permeability,[10–14] Ang-2 promotes apoptosis, inflammation, adhesion, and vascular dysfunction.[11,15–17]

Vascular endothelial growth factor is a key regulator of vasculogenesis and angiogenesis, promoting endothelial cell survival, growth, and migration.[18] It is produced by synovial cells, macrophages, leukocytes, platelets, and endothelial cells.[19] During inflammation, VEGF promotes vasodilatation and permeability thus playing a crucial role in disruption of the endothelial barrier function. Vascular endothelial growth factor and Ang-2 are interrelated in that Ang-2 is required for the induction of vascular leakage in response to pro-inflammatory cytokines and VEGF.[20]

A strong association between increased serum concentrations of Ang-2 with increasing clinical severity and mortality has been demonstrated in numerous studies in human medicine including patients with sepsis,[10,16,21–30] multiple trauma,[31,32] neoplasia,[33,34] chronic kidney disease,[12] and ventilator-associated pneumonia.[28]

Although most authors agree that plasma VEGF concentration is increased in systemic inflammation in humans, studies evaluating whether VEGF correlates with disease severity and outcome have yielded conflicting results.[35–37]

Besides the potential roles of Ang-2 and VEGF as diagnostic and prognostic markers, they are interesting tools to better understand the pathophysiology of vascular leakage syndromes. Furthermore, the availability of pharmacologic agents to block VEGF, Ang-2, or both may provide novel therapeutic strategies for conditions leading to endothelial dysfunction, such as SIRS and sepsis.[8,24]

The purpose of our study was to compare Ang-2 and VEGF concentrations in dogs with SIRS and sepsis and to examine correlations between Ang-2 and VEGF concentrations with disease severity and outcome. We hypothesized that plasma concentrations of Ang-2 and VEGF would be significantly higher in dogs with SIRS or sepsis compared to healthy dogs and that non-survivors would have significantly higher plasma concentration of Ang-2 and VEGF compared to survivors. Furthermore, we hypothesized that there would be a significant correlation between Ang-2 and VEGF and other known markers that have been studied in the context of systemic inflammation including C-reactive protein (CRP), lactate, albumin, bilirubin, and Acute Patient Physiologic and Laboratory Evaluation ($APPLE_{fast}$) score, a 5-variable disease severity model based on glucose, albumin, mentation score, platelet count, and lactate.

2 | MATERIALS AND METHODS

2.1 | Study design

Healthy dogs and dogs meeting SIRS criteria (see below) admitted to the intensive care unit of a university referral hospital between March 2015 and April 2016 were included in this prospective observational study. The study protocol was approved by the local ethics committee. Owner consent for the collection of blood samples from healthy dogs and the use of clinical data and surplus samples from dogs with SIRS or sepsis was obtained.

2.2 | Animals

Dogs were deemed healthy based on an uneventful medical history, normal physical examination, and unremarkable CBC and plasma biochemistry, including normal CRP. A diagnosis of SIRS was based on published clinical criteria for SIRS in dogs.[38] Briefly, dogs were considered to have SIRS if they fulfilled ≥ 2 of the following criteria at admission: body temperature < 38.1°C or > 39.2°C; heart rate > 120/min; respiratory rate > 20/min; white blood cell (WBC) count < 6.0×10^9/L (6 000/μL) or > 16.0×10^9/L (16 000/μL), and percentage of bands > 3% of the total WBC count. Sepsis was diagnosed in dogs meeting SIRS criteria, if a viral, bacterial, or protozoal infection could be identified by serology. Polymerase chain reaction (PCR), cytology, histopathology, bacterial culture, intraoperative evidence of a septic focus or if an infectious cause was strongly suspected and clear improvement with antibiotic treatment occurred.

The severity of illness was assessed at presentation using the $APPLE_{fast}$ score. This model is based on 5 variables—glucose, albumin, mentation score, platelet count, and lactate—and has been shown to predict the probability of death in critically ill dogs independent of their diagnosis.[39] The mentation score was assessed at admission before any analgesics or sedatives had been given. The $APPLE_{fast}$ score ranges from 0 to 50, and the algorithm for calculation of the score is shown in Supporting Information Supplemental Table 1. In the original validation cohort, the $APPLE_{fast}$ score showed a specificity of 85% to predict a negative outcome when a score > 25 was used as a cutoff value.[39]

2.3 | Sample collection and handling

Blood was collected at admission from the cephalic, saphenous, or jugular vein into tubes containing ethylenediaminetetraacetic acid or lithium-heparin (Microvette 200 μL, Lithium-Heparin, orange; Sarstedt AG, Nümbrecht, Germany). Heparinized blood was centrifuged at 4 000 rpm for 10 minutes at 4°C to separate plasma from cellular components. Plasma then was used for biochemistry, and the remaining sample aliquoted and frozen at −80°C.

2.4 | Clinical evaluation

All dogs underwent standard diagnostic evaluation including clinical examination, CBC, and plasma biochemistry including CRP (Canine CRP assay; Randox Reagents, London, United Kingdom). Concentrations of electrolytes, ionized calcium, and lactate were measured on whole blood immediately after blood collection (RapidPoint 500 System; Siemens Healthineers, Erlangen, Germany). Urinalysis, microbiologic analysis, serologic testing, diagnostic imaging, surgery, or some combinations of these was performed as required based on the individual patient and at the discretion of the treating veterinarian.

2.5 | Measurements of Ang-2

Plasma Ang-2 concentration was measured according to the manufacturer's instructions using a commercial ELISA test kit (Human Angiopoietin-2 Immunoassay; R&D Systems, Minneapolis, Minnesota. Product datasheet available at: https://www.rnsystems.com/products/human-angiopoietin-2-quantikine-elisa-kit_dang20, accessed March 5, 2017) for use in humans that was validated for the use in dogs.[40] Tests were run in duplicate and by the same person. If the coefficient of variation (CV%) between duplicates was > 10%, measurements were repeated. Plasma samples initially were diluted 1:5 with Calibrator Diluent (Human Angiopoietin-2 Immunoassay; R&D Systems. Product datasheet available at: https://www.rnsystems.com/products/human-angiopoietin-2-quantikine-elisa-kit_dang20, accessed March 5, 2017). If the measured Ang-2 concentration was outside the range of the standard curve (21-3 000 pg/mL), samples were further diluted (up to 50 times) and measurements were repeated. Assay lower limit of detection (LLD) was 21.3 pg/mL. Intra-assay and inter-assay variability were assessed in a previous study and were 4% and 6.7%, respectively.[41]

2.6 | Measurement of VEGF

Plasma VEGF concentration was measured using a validated commercial VEGF ELISA kit (https://www.rndsystems.com/products/canine-vegf-quantikine-elisa-kit_cave00) for dogs. Plasma samples were diluted in 1:2 ratio with 1% bovine serum albumin in phosphate-buffered saline. Because no result was outside the upper range of the standard curve, further dilution was not necessary. If the CV% between duplicates was > 10%, measurements were repeated. Because plasma was first used to perform biochemistry and additional testing followed by Ang-2, VEGF could only be measured in 54 of 77 dogs because of insufficient sample volume in the remaining 23 dogs.

As part of our own pre-analytical validation, canine peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood of a healthy dog by Biocoll density gradient centrifugation (Biocoll density 1.077; Biochrom, www.biochrom.de). The PBMCs were seeded at 2×10^6/mL in Roswell Park Memorial Institute Glutamax 1640 medium supplemented with 10% inactivated fetal calf serum (Gibco; www.thermofisher.com), 100 IU/mL penicillin, and 100 μg/mL streptomycin (SO613, Biochrom) and cultured for 6 days with 5 μg/mL Concanavalin A (Sigma-Aldrich; www.sigmaaldrich.com). The supernatant was harvested and stored at −80°C in aliquots until analysis. This supernatant then was used as a positive control and to estimate inter-assay variability, which was 4.6%. Intra-assay variability was assessed on 15 aliquots measured in parallel on the same plate and was 4.9%. Serum VEGF was stable during 3 freeze-thaw cycles (Supporting Information Supplemental Table 2). The LLD of the assay was 15 pg/mL. Values below the LLD were set at 15 pg/mL for analysis.

2.7 | Statistical analysis

Data were evaluated using a commercial statistical software package (NCSS[11] Statistical Software, 2016; NCSS; LLC, Kaysville, Utah, available at: ncss.com/software/ncss). Patient characteristics including sex, neuter status, age, and body weight were compared between healthy dogs and dogs with SIRS or sepsis. Shapiro Wilk testing indicated non-normal distribution for several continuous variables, which therefore were reported as median and interquartile range (IQR).

Differences in plasma Ang-2 and VEGF concentrations between dogs with SIRS or sepsis and healthy dogs and survivors and non-survivors were assessed using the Kruskal-Wallis multiple-comparison Z-value test. The Kruskal-Wallis multiple-comparison Z-value test also was used to assess the differences in multiple variables including $APPLE_{fast}$ score, neutrophil count, band neutrophils, urea, creatinine, lactate, albumin, bilirubin, and CRP between survivors and non-survivors.

The VEGF concentrations of healthy dogs and dogs with SIRS and sepsis and survivors and non-survivors were compared using Fisher's exact test. To correct for multiple comparisons, Bonferroni correction was used. Results below the LLD were set at 15 pg/mL for analysis.

In general, data points were classified as outliers if they were more than 1.5 times the IQR above the third quartile or below the first quartile. Dogs euthanized for financial reasons rather than intractable disease were excluded from the outcome analysis.

Because the non-normal distribution of the data, the correlation of Ang-2 and VEGF, and the associations of Ang-2 and VEGF with clinical and laboratory variables and the $APPLE_{fast}$ score were assessed using the Spearman rank correlation test.

Statistical significance was set at $P < 0.05$.

The performance of Ang-2 and VEGF to predict a negative outcome was assessed using receiver-operating characteristics (ROC) analysis. For comparison, ROC analysis also was performed for CRP, lactate, albumin, and $APPLE_{fast}$ score.

Optimal cut-off points for sensitivity and specificity were determined using the most favorable cost-benefit ratios to obtain the largest possible proportion of correctly classified dogs while ensuring that sensitivity and specificity were > 0.3.

3 | RESULTS

3.1 | Study population

Seventy-seven dogs were enrolled in the study: 34 dogs were diagnosed with SIRS, 25 with sepsis, and 18 dogs were healthy. No significant differences were found between groups regarding age, neuter status, and body weight (Table 1). Ten dogs were crossbreds and 67 were purebreds belonging to 37 different breeds, with the most common breeds being Bernese Mountain dog (n = 7), Siberian Husky (n = 7), Labrador Retriever (n = 5), Malinois (n = 4), Border Collie (n = 3), German Shepherd (n = 3), and Cocker Spaniel (n = 3).

The most common sources of sepsis were septic peritonitis (n = 8), followed by pyometra (n = 4), pyelonephritis (n = 4), pneumonia (n = 2), and 1 of each of the following: pyothorax, prostatic abscess, babesiosis, ehrlichiosis, parvovirus infection, cholangitis, and bacterial cystitis with urosepsis. Babesiosis was diagnosed based on a positive *Babesia* PCR result. The dog with ehrlichiosis had an increased antibody titer using 2 different antibody tests (SNAP 4Dx Plus Test; Idexx, Westbrook, Maine, and IFA). Bacterial cholangitis was diagnosed based on the presence of neutrophilic inflammation and bacteria on histopathologic examination of the gall bladder wall after cholecystectomy.

TABLE 1 Patient characteristics of healthy dogs and dogs with SIRS or sepsis

	Healthy		SIRS		Sepsis	
Variable	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**n**	**Median (IQR)**
Age (y)	18	6.9 (4.9-9.0)	34	8.7 (6.3-10.4)	25	7.5 (5-9.5)
Body weight (kg)	18	25.8 (22.8-29.9)	34	27.4 (16.3-36.7)	25	23.5 (12.7-26.9)
Sex	18		34		25	
Male (n, intact/neutered)		9 (5/4)		18 (11/7)		21 (10/11)
Female (n, intact/neutered)		9 (6/3)		16 (9/7)		14 (2/12)
$APPLE_{fast}$ score		NA	34	25.3 (24-27.4)	25	26.3 (24.9-27.5)
Neutrophil count (10^9/L)	18	4.9 (4.1-5.4)	34	16.2 (11.1-22.9)	25	11.5 (7–14)
Band neutrophils (%)	18	0.1 (0-0.3)	34	5 (2.7-9.3)	25	15.4 (7-28.6)#
Albumin (g/L)	18	34.5 (32.5-35.7)	34	24.5 (21.2-29.5)	25	24.5 (21-29.8)
Bilirubin (μmol/L)	18	1.4 (1-1.7)	34	3.2 (2.1-5.4)**	25	5 (2.3-25.9)**
CRP (mg/L)	18	2.7 (0.7-5.9)*	34	106.2 (68.1-143.3)**	25	123 (71.4-1624)**
Lactate (mmol/L)		NA	34	2.5 (2-3.8)	25	2.6 (2.1-3.2)
Duration of hospital stay (d)			30	3 (1-6.5)	24	3 (2.6.8)
Outcome						
Alive to discharge, n (%)		NA	23	23 (68)	12	12 (48)
Euthanized/died, n (%)		NA	11	7/4 (32)	13	9/4 (52)

Abbreviations: $APPLE_{fast}$ score, Five-parameter acute physiologic and laboratory evaluation score (0-50); CRP: C-reactive protein; IQR, interquartile range; NA, not applicable; SIRS, systemic inflammatory response syndrome; VEGF, vascular endothelial growth factor.
**P < 0.001 vs healthy control; *P < 0.05 vs healthy control.; #P < .05 vs SIRS.

Dogs with SIRS were diagnosed with hemangiosarcoma (n = 8); renal disease including amyloidosis (n = 1), glomerulonephritis (n = 1), ethylene glycol intoxication (n = 1), and acute kidney injury of unknown origin (n = 1); pancreatitis (n = 3); steroid-responsive meningitis-arteritis (n = 2); aspiration pneumonia (n = 2); acute hemorrhagic diarrhea syndrome (AHDS) (n = 2); foreign body ileus (n = 2); gastric dilatation volvulus (GDV) (n = 2); and, 1 of each ulcerative gastritis, chronic active hepatitis, immune-mediated hemolytic anemia, Evan's syndrome, immune-mediated polyarthritis, myositis, vasculitis, pulmonary eosinophilic granuloma, and snake bite. In the 2 dogs with aspiration pneumonia, the diagnosis was based on consistent historical, clinical, and radiographic findings. These 2 dogs were classified in the SIRS group instead of the sepsis group because aspiration pneumonia is not primarily a consequence of bacterial infection, but an inflammatory response to the aspirated material.

Population characteristics, $APPLE_{fast}$ score, and outcome for dogs with SIRS and sepsis are shown in Table 1. As expected, SIRS and sepsis animals significantly differed from healthy dogs in plasma concentrations of albumin, bilirubin, and CRP. Dogs with sepsis had a significantly lower total neutrophil count, with a higher number of band neutrophils compared to dogs with SIRS. No significant differences were found in $APPLE_{fast}$ score and duration of hospitalization or outcome between the SIRS and sepsis groups.

Differences in baseline blood variables between survivors and non-survivors are shown in Table 2. Median $APPLE_{fast}$ score, band neutrophils, and concentrations of urea, creatinine, lactate, bilirubin, and CRP were significantly higher and albumin were significantly lower in non-survivors compared to survivors.

3.2 | Ang-2, VEGF, CRP, and lactate concentrations

Median Ang-2 concentration was significantly higher in dogs with SIRS and sepsis compared to healthy dogs (Figure 1A). Non-survivors had significantly higher plasma Ang-2 concentrations compared to dogs that survived to discharge (Figure 1B).

Plasma concentrations of VEGF were below the level of detection in all 7 healthy dogs tested, in 20 of 29 dogs with SIRS, and in 6 of 18 dogs with sepsis. Median plasma concentrations of VEGF were higher in dogs with sepsis compared to dogs with SIRS and healthy dogs (Figure 2A) and in non-survivors compared to survivors (Figure 2B). After Bonferroni correction, differences were nonsignificant.

Median CRP and lactate concentrations are shown in Table 1. Median CRP concentration was significantly higher in dogs with SIRS and sepsis compared to healthy dogs (Table 1) and significantly higher in non-survivors compared to survivors (Table 2). No significant difference in CRP was found between dogs with SIRS and those with sepsis.

Non-survivors had significantly higher lactate concentrations (3.5; IQR: 2.3-5.3 mmol/L) compared to survivors (1.0; IQR: 1.5-2.8 mmol/L).

TABLE 2 Differences between survivors (n = 35) and non-survivors (n = 24)

Variable	Survivors	Non-survivors
$APPLE_{fast}$ score	23 (21-27)	29.5 (24.3-31)**
Neutrophil count (10^9/L)	10.6 (5.2-20.5)	11.2 (7.6-16.6)
Band neutrophils (%)	1.5 (0.2-7.8)	12.5 (5.5-26.1)**
Urea (mmol/L)	6.4 (4.8-10)	12.3 (8.0-35.2)**
Creatinine (μmol/L)	75.5 (54.5-93.8)	149.5 (69.8-275.3)*
Lactate (mmol/L)	2.0 (1.5-2.8)	3.5 (2.3-5.3)*
Albumin (g/L)	30.2 (24.3-34.3)	23.3 (19.1-25.5)**
Bilirubin (μmol/L)	2.2 (1.3-3.6)	5.0 (2.6-23.3)**
CRP (mg/L)	60 (4.6-116)	112.2 (78.8-171)*

Abbreviations: $APPLE_{fast}$ score, five-parameter acute physiologic and laboratory evaluation score (0–50); CRP, C-reactive protein.
Data are shown as medians and interquartile ranges unless stated otherwise.
**P < 0.001; *P < 0.05.

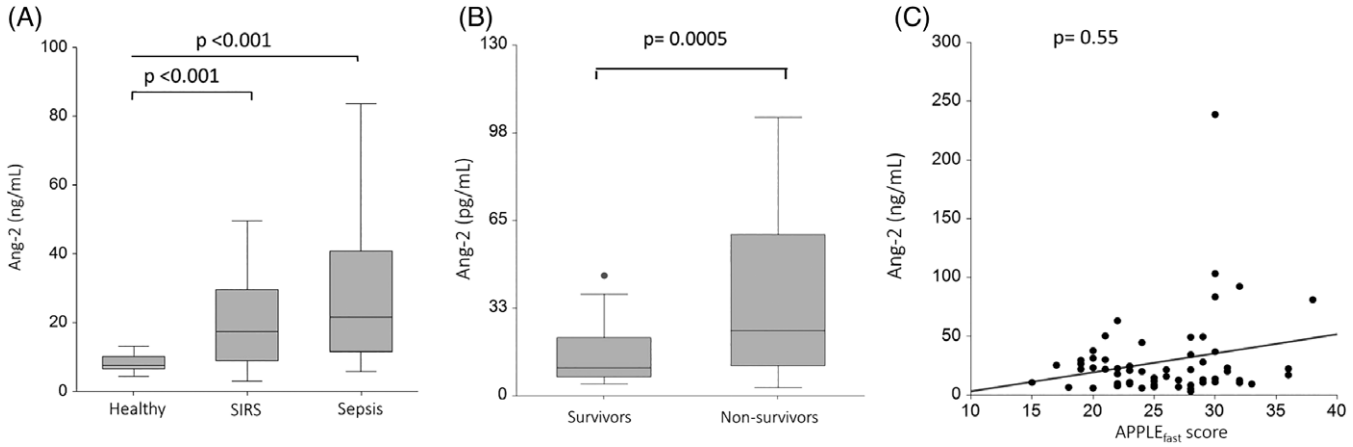

FIGURE 1 Plasma Ang-2 concentrations in dogs (A) Comparison of plasma Ang-2 concentrations among healthy control dogs (n = 18), dogs with SIRS (n = 32), and dogs with sepsis (n = 28). (B) Comparison of plasma Ang-2 concentration between survivors (n = 35) and non-survivors (n = 24). (C) Lack of a statistically significant correlation between plasma Ang-2 concentrations and APPLE$_{fast}$ score in dogs with SIRS or sepsis. The central lines in the boxes represent the median values, and the top and bottom of the boxes represent the 75th and 25th percentiles, respectively. Data points were classified as outliers if they were more than 1.5 times the interquartile range above the third quartile or below the first quartile. Abbreviations: Ang-2, angiopoietin-2; APPLE$_{fast}$ score: acute patient physiologic and laboratory evaluation score (five-variable disease severity model based on glucose, albumin, mentation score, platelet count, and lactate); SIRS: systemic inflammatory response syndrome

No significant difference between dogs with SIRS and dogs with sepsis could be detected.

3.3 | Correlation analysis

Plasma Ang-2 concentration was weakly but significantly positively correlated with VEGF, CRP, the number of band neutrophils, plasma bilirubin concentration, and alanine aminotransferase (ALT) activity, and weakly negatively correlated with plasma albumin concentrations (Table 3, Figure 3). No significant positive correlation was found between Ang-2 and APPLE$_{fast}$ score (Figure 1C).

Plasma VEGF was moderately positively correlated with the number of band neutrophils, weakly positively correlated with Ang-2 and ALT activity, and weakly negatively correlated with plasma albumin concentration (Table 3). No correlation was found among VEGF, CRP, lactate, and APPLE$_{fast}$ score (Figure 2C).

3.4 | ROC analysis

To discriminate the healthy from diseased dogs, survivors from non-survivors, and dogs with SIRS from dogs with sepsis, optimum cutoff values, sensitivities, specificities, and area under the curve for Ang-2,

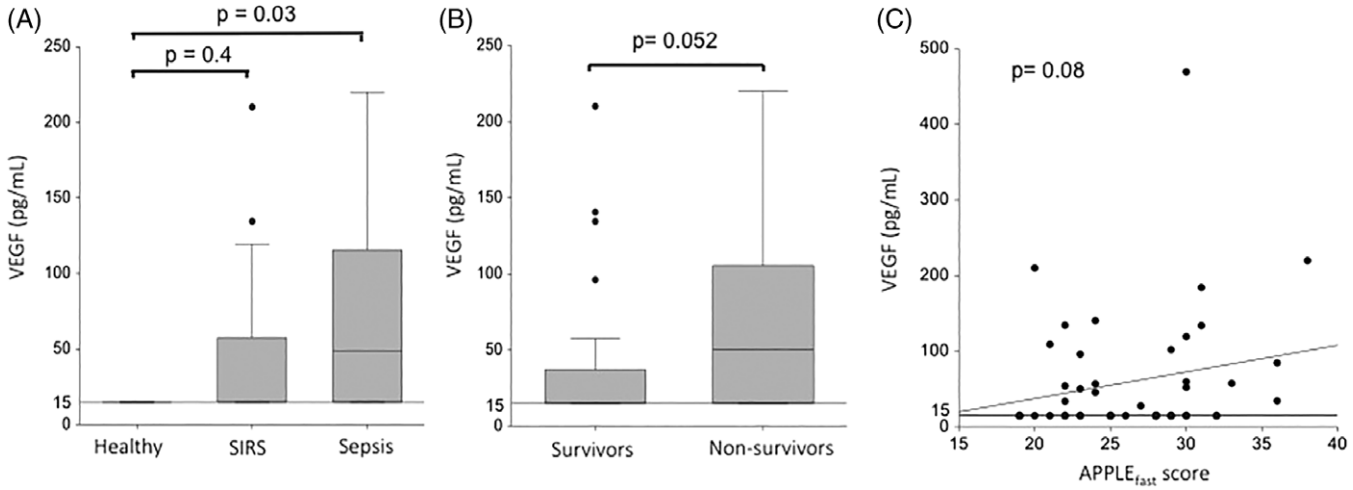

FIGURE 2 Plasma VEGF concentrations in dogs (A) Comparison of plasma VEGF concentrations among healthy control dogs (n = 7), dogs with SIRS (n = 32), and dogs with sepsis (n = 28). (B) Comparison of plasma VEGF concentration between survivors (n = 26) and non-survivors (n = 21). (C) Lack of a statistically significant correlation between plasma VEGF concentrations and APPLE$_{fast}$ score in dogs with SIRS or sepsis. The central lines in the boxes represent the median values, and the top and bottom of the boxes represent the 75th and 25th percentiles, respectively. Data points were classified as outliers if they were more than 1.5 times the interquartile range above the third quartile or below the first quartile. Cut-off for lower limit of detection (LLD) was 15 pg/mL; 27 of 53 samples were below the LLD. Abbreviations: Ang-2, angiopoietin-2; APPLE$_{fast}$ score, acute patient physiologic and laboratory evaluation score (five-variable disease severity model based on glucose, albumin, mentation score, platelet count, and lactate); SIRS: systemic inflammatory response syndrome; VEGF, vascular endothelial growth factor

TABLE 3 Correlation of plasma Ang-2 and VEGF with C-reactive protein, lactate (n = 77), and $APPLE_{fast}$ score (n = 59)

	Ang-2		VEGF	
Variable	**Spearman ρ**	***P*-value**	**Spearman ρ**	***P*-value**
Angiopoietin-2 (ng/mL)	…	…	0.35	**0.008**
VEGF (pg/mL)	0.35	**0.008**	…	…
$APPLE_{fast}$	0.1	0.55	0.56	0.08
CRP (mg/L)	0.5	**<0.001**	0.01	0.96
Lactate (mmol/L)	0.16	0.2	0.09	0.56
Bilirubin (μmol/L)	0.35	**0.002**	0.15	0.28
Albumin (g/L)	−0.43	**<0.001**	−0.47	**0.0003**
ALT (IU)	0.33	**0.003**	0.31	**0.02**
Band neutrophils (%)	0.44	**<0.001**	0.62	**<0.001**

Abbreviations: ALT, alanine aminotransferase; $APPLE_{fast}$ score, five-parameter acute physiologic and laboratory evaluation score (0–50); CRP, C-reactive protein; VEGF: Vascular endothelial growth factor.
Significance was set at $P < 0.05$. Significant *P*-values are printed in bold.

VEGF, CRP, and $APPLE_{fast}$ score were calculated and are shown in Table 4. The diagnostic accuracy of plasma Ang-2 concentration to differentiate between healthy and diseased dogs and between survivors and non-survivors can be considered good. However, Ang-2 did not have sufficient specificity to differentiate dogs with SIRS from those with sepsis.

Plasma VEGF concentration had fair accuracy in the differentiation of healthy and diseased dogs. The discriminatory power to differentiate survivors from non-survivors and septic dogs from dogs with SIRS was poor.

Creactive protein concentration had high diagnostic accuracy to differentiate between healthy and diseased animals but had very low sensitivity to differentiate between survivors and non-survivors, and between dogs with SIRS and those with sepsis.

The optimal cutoff value for the $APPLE_{fast}$ score to discriminate survivors from non-survivors in this cohort was 29 with a specificity of 89% and sensitivity of 63%. Specificity and sensitivity for the cutoff value of 25 for the $APPLE_{fast}$ score were 57% and 75%, respectively.

4 | DISCUSSION

Endothelial activation and dysfunction play a central role in the pathogenesis of SIRS and sepsis and can lead to the development of distributive shock, multi-organ dysfunction, and death.[36,41] In the context of systemic inflammation, both VEGF and Ang-2 have been shown to promote endothelial activation and permeability in human patients.[32,42,43] Sensitive markers to assess endothelial dysfunction could facilitate an early diagnosis of SIRS or sepsis and allow a better understanding of the role of the endothelium in critical illness with the ultimate goal of guiding targeted therapeutic interventions.

The purpose of our study was to assess the diagnostic utility of Ang-2 and VEGF in dogs with SIRS and sepsis and to evaluate associations of these 2 markers with disease severity and outcome.

In agreement with results in human patients with SIRS and sepsis,[15,21,24,29] admission plasma Ang-2 concentrations were significantly higher in dogs with SIRS or sepsis compared to healthy control dogs. Admission plasma Ang-2 concentration was significantly higher in non-survivors than in survivors and had good diagnostic accuracy to predict negative outcome with an excellent specificity of 87% and a moderate sensitivity of 50%. Admission Ang-2 was not useful to differentiate dogs with sepsis from those with SIRS, which is in agreement with findings of studies in human medicine.[44] In human patients, association of high Ang-2 concentrations with increased mortality in patients with a range of critical illnesses including SIRS, sepsis, multiple trauma, cancer, and acute and chronic kidney disease has been shown.[10,12,24,30,32,43] Although plasma Ang-2 did not correlate with the $APPLE_{fast}$ disease severity score in this cohort, most studies in human medicine report a strong positive relationship between serum Ang-2 and disease severity scores including Acute Physiology, Age, and Chronic Health Evaluation II, Sepsis-related Organ Failure Assessment, Simplified Acute Physiology II.[15,25,45–47] Given its good overall performance, plasma Ang-2 concentration may be a promising prognostic biomarker in dogs with SIRS and sepsis. In the human medical literature, the Ang-2 to Ang-1 ratio sometimes is reported to be of

TABLE 4 Sensitivities, specificities, likelihood ratios, and areas under the curve for optimum cutoff concentrations to predict illness (SIRS, sepsis) and a negative outcome in dogs with SIRS and sepsis

Outcome variable	AUC (95% CI)	Cutoff	Sensitivity (95% CI)	Specificity (95% CI)	Positive likelihood ratio	Negative likelihood ratio
Healthy versus diseased						
Ang-2 (ng/mL)	0.83 (0.72-0.9)	13.7	0.61 (0.47-0.73)	1.0 (0.81-1)	61	0.4
VEGF(pg/mL)	0.70 (0.57-0.81)	6.6	0.51 (0.36-0.66)	0.9 (0.59-0.99)	5.1	0.5
CRP (mg/mL)	0.96 (0.88-0.97)	16.7	0.93 (0.84-0.98)	0.95 (0.75-0.99)	18.6	0.1
Survivors versus non-survivors						
Ang-2 (ng/mL)	0.75 (0.59–0.85)	25.3	0.5 (0.29–0.71)	0.87 (0.75-0.95)	3.8	0.6
VEGF(pg/mL)	0.68 (0.5-0.8)	59.8	0.38 (0.18-0.62)	0.84 (0.68-0.94)	2.4	0.7
CRP (mg/L)	0.72 (0.6-0.82)	169.7	0.33 (0.16-0.55)	0.93 (0.82-0.98)	4.7	0.7
$APPLE_{fast}$	0.75 (0.57-0.86)	29	0.63 (0.41-0.81)	0.89 (0.73-0.97)	5.7	0.4
	0.75 (0.57–0.86)	25	0.75 (0.53-0.9)	0.57 (0.39-0.74)	1.7	0.4
SIRS versus sepsis						
Ang-2 (ng/mL)	0.58 (0.42-0.72)	10.6	0.84 (0.64-0.95)	0.35 (0.2-0.54)	1.3	0.5
VEGF(pg/mL)	0.67 (0.49-0.8)	52.3	0.50 (0.26-0.74)	0.76 (0.56-0.9)	2.1	0.7
CRP (mg/L)	0.54 (0.4-0.7)	143.6	0.44 (0.24-0.65)	0.77 (0.6-0.9)	1.9	0.7

Abbreviations: Ang-2, angiopoietin-2; $APPLE_{fast}$ score, five-parameter acute physiologic and laboratory evaluation score (0–50); AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; VEGF, vascular endothelial growth factor.

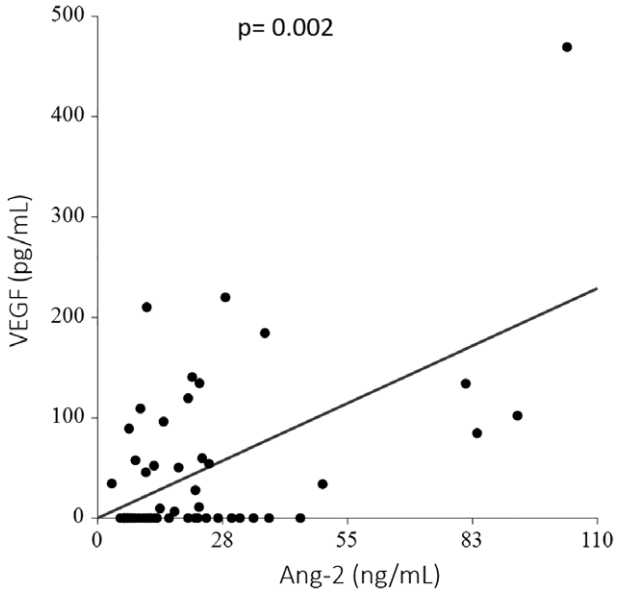

FIGURE 3 Weak positive correlation between plasma concentrations of VEGF and Ang-2 in dogs with SIRS and sepsis. Abbreviations: Ang-2, angiopoietin-2; SIRS: systemic inflammatory response syndrome; VEGF, vascular endothelial growth factor

even higher prognostic value than Ang-2 alone.[48,49] However, there is currently is no validated assay for measurement of Ang-1 in dogs. Therefore, the performance of the Ang-2 to Ang-1 ratio for outcome prediction could not be assessed.

Plasma VEGF concentration was below the level of detection in all healthy dogs and in the majority of dogs with SIRS. Although some dogs with sepsis also had very low plasma VEGF concentrations, median VEGF was higher in this group compared to healthy controls, but this difference did not reach statistical significance after Bonferroni correction. This result was unexpected but may be linked to differences in the pathogenesis between SIRS and sepsis. Microbial mediators such as bacterial lipopolysaccharide are important triggers for the production of VEGF and may have more potent effects on VEGF expression than other nonmicrobial triggers.[50]

Median VEGF plasma concentrations were not significantly higher in non-survivors than in survivors, and ROC analysis indicated that its power to differentiate between survivors and non-survivors was poor. Plasma VEGF concentration also had low sensitivity and specificity to differentiate between dogs with SIRS and sepsis and was not correlated with disease severity ($APPLE_{fast}$ score). Plasma VEGF concentration therefore represents a less useful biomarker than Ang-2 in dogs with systemic inflammation. These findings align with reports from human patients where the use of VEGF as an outcome predictor in patients with systemic inflammation has yielded conflicting results and its performance was very poor in some studies.[35–37] One possible explanation could be the high biological variability reported for VEGF in human patients, regardless of the use of standardized methods.[51] Similarly, no significant relationship could be identified between blood VEGF concentrations and a survival prediction index in dogs with SIRS in a previous study.[52]

In this cohort, plasma concentrations of Ang-2 and VEGF were weakly positively correlated with each other. Both also were significantly positively correlated with band neutrophilia and negatively correlated with plasma albumin concentration which is considered a negative acute phase protein. These associations support the current evidence that both VEGF and Ang-2 are induced by inflammation and act synergistically. Ang-2 counteracts the stabilizing action of Ang-1 by exposing the endothelium to pro-inflammatory factors such as VEGF.[53] In the presence of VEGF, Ang-2 promotes endothelial activation and induction of permeability, and in the absence of VEGF, it destabilizes the existing vessels and leads to vascular regression.[54] Furthermore, neutrophil activation by Ang-2 leads to VEGF secretion, which increases neutrophil adhesion.[44]

In agreement with previous studies, plasma concentrations of CRP, a marker of systemic inflammation, were significantly higher in dogs with SIRS and sepsis compared to healthy controls but did not discriminate between SIRS and sepsis.[55,56] Although Ang-2 was significantly positively correlated with CRP, no correlation was found between CRP and VEGF. Because both CRP and VEGF are well-known pro-inflammatory factors, this lack of correlation was unexpected. One explanation might be a lower sensitivity of VEGF compared to CRP as a marker of inflammation. Also, the previously described biological variability of VEGF could have had an impact.

In this cohort, Ang-2 was a good variable to predict the outcome using a cutoff plasma concentration of 25.3 ng/mL (sensitivity 50%; specificity 87%). Therefore, Ang-2 may have utility as a biomarker and could be used to increase the diagnostic performance of the $APPLE_{fast}$ score for outcome prediction.

Our study had several limitations: Firstly, because of the relatively small sample size of 77 dogs, type I or type II errors cannot be excluded. Secondly, dogs were classified in the SIRS or sepsis group based on published SIRS criteria.[38] Although these criteria have been widely used by researchers,[57] rectal temperature, heart and respiratory rate are influenced by pain, stress, and anxiety leading to low specificity (74%-83%) of these critera.[38] Although all cases were carefully reviewed for evidence of sepsis, it can be very difficult to definitively prove or rule out an infectious cause of SIRS. In our study, dogs with gastrointestinal disease including intestinal obstruction (n = 2), AHDS (n = 2), GDV (n = 1), and ulcerative gastritis (n = 1) without evidence of gastric or intestinal perforation, were included in the SIRS group. However, bacterial translocation leading to sepsis cannot completely be ruled out in these animals. Eight dogs with confirmed hemangiosarcoma fulfilled the criteria for SIRS and were included in the study. However, it is well known that Ang-2 production can be increased in humans with neoplasia.[58,59] Our study design does not allow differentiation of whether Ang-2 was increased in these dogs because of SIRS or neoplasia, and further studies are underway to examine Ang-2 dynamics in dogs with neoplasia in more detail. Furthermore, Ang-2 and VEGF only were assessed at admission, which does not take into account the effect of progression of the underlying disease and potential complications during treatment. A study investigating the kinetics of serum Ang-2 over several days in patients with multi-trauma found that Ang-2 on days 4 and 7 after trauma was increased compared to day 1.[31] Similarly, CRP was found in several studies of dogs to be of greater value in outcome prediction if serial measurements were performed.[60]

Finally, although an effort was made to exclude dogs that were euthanized for financial reasons rather than terminal illness, we cannot fully exclude an effect of euthanasia bias on the outcome analysis of our study.

In conclusion, Ang-2 is a promising diagnostic and prognostic biomarker in dogs with SIRS and sepsis and might be useful to better understand the pathophysiology of vascular leakage syndromes. Admission Ang-2 correlated with other known biomarkers (CRP, albumin, and bilirubin) that previously have been studied in dogs with SIRS and sepsis, whereas VEGF did not. Based on our results, plasma VEGF showed less promise as a useful clinical biomarker compared to Ang-2, but larger studies in more homogenous populations are needed to prove this hypothesis.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The study protocol was approved by the Swiss Federal Food Safety and Veterinary Office (BLV 38/15).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

REFERENCES

1. Esper AM, Martin GS. Extending international sepsis epidemiology: the impact of organ dysfunction. *Crit Care*. 2009;13:120.
2. Otto CM, Boller EM. *Sepsis and Septic Shock. In Small Animal Critical Care Medicine*. 2nd ed. Philadelphia: Elsevier; 2014:472-480.
3. de Laforcade AM, Freeman LM, Shaw SP, et al. Hemostatic changes in dogs with naturally occurring sepsis. *J Vet Intern Med*. 2003;17:674-679.
4. DeClue AE, Sharp CR, Harmon M. Plasma inflammatory mediator concentrations at ICU admission in dogs with naturally developing sepsis. *J Vet Intern Med*. 2012;26:624-630.
5. Rivers E, Nguyen B, Havstad S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med*. 2001;345:1368-1377.
6. Lukasz A, Kumpers P, David S. Role of angiopoietin/tie2 in critical illness: promising biomarker, disease mediator, and therapeutic target? *Scientifica (Cairo)*. 2012;2012:160174.
7. Fiedler U, Scharpfenecker M, Koidl S, et al. The tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. *Blood*. 2004;103:4150-4156.
8. Fiedler U, Reiss Y, Scharpfenecker M, et al. Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. *Nat Med*. 2006;12:235-239.
9. Witzenbichler B, Westermann D, Knueppel S, Schultheiss HP, Tschope C. Protective role of angiopoietin-1 in endotoxic shock. *Circulation*. 2005;111:97-105.
10. Parikh SM, Mammoto T, Schultz A, et al. Excess circulating angiopoietin-2 may contribute to pulmonary vascular leak in sepsis in humans. *PLoS Med*. 2006;3:e46.
11. Ricciuto DR, dos Santos CC, Hawkes M, et al. Angiopoietin-1 and angiopoietin-2 as clinically informative prognostic biomarkers of morbidity and mortality in severe sepsis. *Crit Care Med*. 2011;39:702-710.
12. David S, John SG, Jefferies HJ, et al. Angiopoietin-2 levels predict mortality in CKD patients. *Nephrol Dial Transplant*. 2012;27:1867-1872.
13. Thurston G, Rudge JS, Ioffe E, et al. Angiopoietin-1 protects the adult vasculature against plasma leakage. *Nat Med*. 2000;6:460-463.
14. Gamble JR, Drew J, Trezise L, et al. Angiopoietin-1 is an antipermeability and anti-inflammatory agent in vitro and targets cell junctions. *Circ Res*. 2000;87:603-607.
15. Giuliano JS Jr, Lahni PM, Harmon K, et al. Admission angiopoietin levels in children with septic shock. *Shock*. 2007;28:650-654.
16. Lukasz A, Hellpap J, Horn R, et al. Circulating angiopoietin-1 and angiopoietin-2 in critically ill patients: development and clinical application of two new immunoassays. *Crit Care*. 2008;12:R94.
17. Samraj RS, Zingarelli B, Wong HR. Role of biomarkers in sepsis care. *Shock*. 2013;40:358-365.
18. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med*. 2003;9:669-676.
19. Paleolog EM. The vasculature in rheumatoid arthritis: cause or consequence? *Int J Exp Pathol*. 2009;90:249-261.
20. Benest AV, Kruse K, Savant S, et al. Angiopoietin-2 is critical for cytokine-induced vascular leakage. *PLoS One*. 2013;8:e70459.
21. Siner JM, Bhandari V, Engle KM, Elias JA, Siegel MD. Elevated serum angiopoietin 2 levels are associated with increased mortality in sepsis. *Shock*. 2009;31:348-353.
22. Lymperopoulou K, Velissaris D, Kotsaki A, et al. Angiopoietin-2 associations with the underlying infection and sepsis severity. *Cytokine*. 2015;73:163-168.
23. Orfanos SE, Kotanidou A, Glynos C, et al. Angiopoietin-2 is increased in severe sepsis: correlation with inflammatory mediators. *Crit Care Med*. 2007;35:199-206.
24. David S, Mukherjee A, Ghosh CC, et al. Angiopoietin-2 may contribute to multiple organ dysfunction and death in sepsis. *Crit Care Med*. 2012;40:3034-3041.
25. Kumpers P, Lukasz A, David S, et al. Excess circulating angiopoietin-2 is a strong predictor of mortality in critically ill medical patients. *Crit Care*. 2008;12:R147.
26. Calfee CS, Gallagher D, Abbott J, Thompson BT, Matthay MA, NHLBI ARDS Network. Plasma angiopoietin-2 in clinical acute lung injury: prognostic and pathogenetic significance. *Crit Care Med*. 2012;40:1731-1737.
27. Davis JS, Yeo TW, Piera KA, et al. Angiopoietin-2 is increased in sepsis and inversely associated with nitric oxide-dependent microvascular reactivity. *Crit Care*. 2010;14:R89.
28. Kranidioti H, Orfanos SE, Vaki I, et al. Angiopoietin-2 is increased in septic shock: evidence for the existence of a circulating factor stimulating its release from human monocytes. *Immunol Lett*. 2009;125:65-71.

29. Gallagher DC, Parikh SM, Balonov K, et al. Circulating angiopoietin 2 correlates with mortality in a surgical population with acute lung injury/adult respiratory distress syndrome. *Shock*. 2008;29:656-661.
30. van der Heijden M, van Nieuw Amerongen GP, Koolwijk P, van Hinsbergh VWM, Groeneveld ABJ. Angiopoietin-2, permeability oedema, occurrence and severity of ALI/ARDS in septic and non-septic critically ill patients. *Thorax*. 2008;63:903-909.
31. Giamarellos-Bourboulis EJ, Kanellakopoulou K, Pelekanou A, Tsaganos T, Kotzampassi K. Kinetics of angiopoietin-2 in serum of multi-trauma patients: correlation with patient severity. *Cytokine*. 2008;44:310-313.
32. Ganter MT, Cohen MJ, Brohi K, et al. Angiopoietin-2, marker and mediator of endothelial activation with prognostic significance early after trauma? *Ann Surg*. 2008;247:320-326.
33. Canadas I, Taus A, Villanueva X, et al. Angiopoietin-2 is a negative prognostic marker in small cell lung cancer. *Lung Cancer*. 2015;90:302-306.
34. Shao YY, Hsu CH, Cheng AL. Predictive biomarkers of sorafenib efficacy in advanced hepatocellular carcinoma: are we getting there? *World J Gastroenterol*. 2015;21:10336-10347.
35. Alves BE, Montalvao SA, Aranha FJ, et al. Time-course of sFlt-1 and VEGF-A release in neutropenic patients with sepsis and septic shock: a prospective study. *J Transl Med*. 2011;9:23.
36. van der Flier M, van Leeuwen HJ, van Kessel KP, Kimpen JL, Hoepelman AI, Geelen SP. Plasma vascular endothelial growth factor in severe sepsis. *Shock*. 2005;23:35-38.
37. Karlsson S, Pettila V, Tenhunen J, et al. Vascular endothelial growth factor in severe sepsis and septic shock. *Anesth Analg*. 2008;106:1820-1826.
38. Hauptman JG, Walshaw R, Olivier NB. Evaluation of the sensitivity and specificity of diagnostic criteria for sepsis in dogs. *Vet Surg*. 1997;26:393-397.
39. Hayes G, Mathews K, Doig G, et al. The acute patient physiologic and laboratory evaluation (APPLE) score: a severity of illness stratification system for hospitalized dogs. *J Vet Intern Med*. 2010;24:1034-1047.
40. König ML, Marti S, Mirkovitch E, Wyder J, Giger M, Schuller U. Validation of a human angiopoietin-2 ELISA kit for measurement of canine angiopoietin-2 concentrations in plasma and supernatant of primary canine aortic endothelial cell cultures. *Am J Vet Res*. 2018;79:803-810.
41. Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. *Blood*. 2003;101:3765-3777.
42. Lui-Roberts WW, Ferraro F, Nightingale TD, et al. Aftiphilin and gamma-synergin are required for secretagogue sensitivity of Weibel-Palade bodies in endothelial cells. *Mol Biol Cell*. 2008;19:5072-5081.
43. Giuliano JS Jr, Lahni PM, Bigham MT, et al. Plasma angiopoietin-2 levels increase in children following cardiopulmonary bypass. *Intensive Care Med*. 2008;34:1851-1857.
44. Lemieux C, Maliba R, Favier J, Théorêt JF, Merhi Y, Sirois MG. Angiopoietins can directly activate endothelial cells and neutrophils to promote proinflammatory responses. *Blood*. 2005;105:1523-1530.
45. Minne L, Abu-Hanna A, de Jonge E. Evaluation of SOFA-based models for predicting mortality in the ICU: a systematic review. *Crit Care*. 2008;12:R161.
46. Knaus WA, Draper EA, Wagner DP, et al. APACHE II: a severity of disease classification system. *Crit Care Med*. 1985;13:818-829.
47. Desai S, Lakhani JD. Utility of SOFA and APACHE II score in sepsis in rural set up MICU. *J Assoc Physicians India*. 2013;61:608-611.
48. Fang Y, Li C, Shao R, Yu H, Zhang Q, Zhao L. Prognostic significance of the angiopoietin-2/angiopoietin-1 and angiopoietin-1/tie-2 ratios for early sepsis in an emergency department. *Crit Care*. 2015;19:367.
49. Zonneveld R, Jongman R, Juliana A, et al. Low serum angiopoietin-1, high serum angiopoietin-2, and high Ang-2/Ang-1 protein ratio are associated with early onset sepsis in Surinamese newborns. *Shock*. 2017;48:638-643.
50. Jeong SJ, Han SH, Kim CO, Choi JY, Kim JM. Anti-vascular endothelial growth factor antibody attenuates inflammation and decreases mortality in an experimental model of severe sepsis. *Crit Care*. 2013;17:R97.
51. Meo S, Dittadi R, Gion M, et al. Biological variation of vascular endothelial growth factor. *Clin Chem Lab Med*. 2005;43:342-343.
52. Silverstein DC, Montealegre C, Shofer FS, Otto CM. The association between vascular endothelial growth factor levels and clinically evident peripheral edema in dogs with systemic inflammatory response syndrome. *J Vet Emerg Crit Care (San Antonio)*. 2009;19:459-466.
53. Fiedler U, Augustin HG. Angiopoietins: a link between angiogenesis and inflammation. *Trends Immunol*. 2006;27:552-558.
54. Scholz A, Plate KH, Reiss Y. Angiopoietin-2: a multifaceted cytokine that functions in both angiogenesis and inflammation. *Ann N Y Acad Sci*. 2015;1347:45-51.
55. Kent J. Acute phase proteins: their use in veterinary diagnosis. *Br Vet J*. 1992;148:279-282.
56. Povoa P, Teixeira-Pinto AM, Carneiro AH, et al. C-reactive protein, an early marker of community-acquired sepsis resolution: a multi-center prospective observational study. *Crit Care*. 2011;15:R169.
57. Gebhardt C, Hirschberger J, Rau S, et al. Use of C-reactive protein to predict outcome in dogs with systemic inflammatory response syndrome or sepsis. *J Vet Emerg Crit Care (San Antonio)*. 2009;19:450-458.
58. Buehler D, Rush P, Hasenstein JR, et al. Expression of angiopoietin-TIE system components in angiosarcoma. *Mod Pathol*. 2013;26:1032-1040.
59. Hong S, Jung HI, Ahn TS, et al. Expressions and clinical significances of angiopoietin-1, angiopoietin-2, and tie-2 receptor in patients with colorectal cancer. *Ann Coloproctol*. 2017;33:9-15.
60. Lobo SM. Sequential C-reactive protein measurements in patients with serious infections: does it help? *Crit Care*. 2012;16:130.

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