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Effect of the Vascular Endothelial Growth Factor Inhibitor Toceranib on Cardiac Function and Endothelial Dysfunction Biomarkers in Dogs With Cancer

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ABSTRACT

Background: Hypertension is documented in dogs with cancer receiving toceranib, but no studies have evaluated left ventricular (LV) systolic function and biomarkers of endothelial function.

Objectives: To characterize changes in echocardiographic variables and biomarkers of endothelial function in dogs treated with toceranib.

Animals: Twenty-six client-owned dogs with no evidence of pre-existing cardiac disease or systemic hypertension are receiving a single agent toceranib for cancer treatment.

Methods: Dogs were enrolled in this prospective observational study with study visits at baseline, 1, 3, and 5 months after starting toceranib for echocardiographic exams, blood and urine collection, and blood pressure measurements, with an additional blood pressure obtained 2 weeks after starting toceranib. Serum markers of vascular endothelial function (VEGF, endothelin-1, platelet derived growth factor [PDGF], prostacyclin, cyclic guanosine monophosphate [cGMP]) and urinary nitrate were evaluated with ELISA.

Results: Dogs were enrolled between 2019 and 2023. Systolic blood pressure increased 2 weeks after initiating toceranib treatment ($p = 0.009$). Serum prostacyclin concentration was lower after 1 month of treatment (mean 98.8 pg/mL vs. 140.0 pg/mL at baseline, $p = 0.03$), and serum VEGF concentration was higher after 3 months of treatment (mean of 247.8 pg/mL vs. 135.4 pg/mL at baseline, $p = 0.01$). Global longitudinal strain (GLS) decreased at the five-month time point (mean −14.5% vs. −15.7% at baseline, $p = 0.048$) with no significant change in LV fractional shortening by M-mode or ejection fraction by Simpson's method of discs.

Conclusions: Dogs treated with toceranib might have higher systemic blood pressure associated with changes in VEGF and prostacyclin and decreased systolic function.

Abbreviations: cGMP, cyclic guanosine monophosphate; GLS, global longitudinal strain; hs-cTnI, high sensitivity cardiac troponin I; IVSd, interventricular septum in diastole; LV, left ventricle; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

1 | Introduction

Toceranib is a tyrosine kinase inhibitor (TKI) that non-selectively inhibits vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR), among others. Toceranib is part of a class of VEGFR inhibitors that are widely used as anti-cancer agents in human medicine and is most closely related to sunitinib used in humans. Both compounds inhibit VEGFR-2, PDGFR, and c-KIT [1, 2]. In veterinary medicine, toceranib is labeled for the treatment of mast cell tumors [3, 4].

As VEGFR inhibitors become more widely used in human medicine, there is an increased recognition of their cardiovascular adverse effects, including hypertension and heart failure [1, 5–7]. Systemic hypertension is the most common adverse effect, reported in 47% of humans treated with sunitinib [8] and 80%–90% of patients treated with VEGFR inhibitors as a group [9, 10]. Increases in systemic blood pressure have similarly been reported in dogs with cancer treated with toceranib, with 37% of dogs developing a systolic blood pressure > 160 mmHg in one study [11]. Cardiotoxicity, manifested as decreased left ventricular (LV) systolic function and subsequent heart failure, is documented in human patients. In people receiving sunitinib, 10%–30% developed a decline in LV systolic function based on LV ejection fraction (LVEF) and global longitudinal strain (GLS), and 8% of those went on to develop clinical signs of heart failure [2, 6, 8, 12, 13]. Of these two echocardiographic variables, GLS is more sensitive and permits detection of early changes consistent with LV systolic dysfunction [12]. High-sensitivity cardiac troponin (hs-cTnI) levels have been evaluated as a biomarker for cardiotoxicity in human cancer patients; however, changes in troponin during treatment were not statistically significant [8, 12].

VEGF is necessary for the survival and normal function of the endothelial cells that line all blood vessels. Hence, VEGFR inhibitors were developed to treat solid tumors as they impair endothelial function and angiogenesis. Healthy endothelial cells produce nitric oxide and prostacyclin, vasodilators that act on underlying vascular smooth muscle via the production of cGMP, enhancing blood flow to downstream tissues, including the heart [14]. With VEGFR inhibitor, this normal endothelial function is disrupted [15–17]. In human patients, circulating serum levels of VEGF rise with VEGFR inhibition, demonstrating drug efficacy. On the other hand, vasodilators including serum prostacyclin and cGMP, as well as urinary nitric oxide metabolites decline, while serum endothelin-1 is expected to rise [16–19]. This endothelial dysfunction secondary to VEGFR inhibition might be especially important for those predisposed to adverse cardiovascular events [20, 21].

Even less is known about the cardiovascular effect of toceranib on dogs. To date, only the response of serum VEGF concentrations and the development of systemic hypertension have been investigated [22, 23]. To better understand the mechanism of hypertension development and effects on endothelial function, we investigated how toceranib changes circulating levels of PDGF, cGMP, endothelin-1, prostacyclin, and nitric oxide metabolites (i.e., urine nitrate and nitrite). We hypothesized that serum levels of biomarkers of vasodilators, including prostacyclin and cGMP, as well as urinary nitric oxide metabolites will decline with treatment, while serum levels of endothelin-1 will rise. Furthermore, we evaluated the LV systolic function of dogs receiving toceranib, as measured with echocardiography and cardiac biomarkers.

2 | Materials and Methods

2.1 | Study Design

This was a prospective observational study approved by the Institutional Animal Use and Care Committee at the Cummings School of Veterinary Medicine at Tufts University. Client-owned animals were recruited for enrollment by the Tufts Cummings School Clinical Trials Office or by a member of the veterinary cardiology or medical oncology services between 2019 and 2023. Dogs were required to be at least 1 year of age and treated with single agent toceranib for cancer diagnosis. For nearly all dogs, cytological or histopathologic confirmation of cancer type was undertaken. However, cytological sampling for heart base tumors was not possible. Diagnosis of heart base tumors (chemodectomas) was based on echocardiographic features of the mass. Exclusion criteria included previous or concurrent treatment with any other chemotherapeutic agent, evidence of significant abnormalities on the baseline complete blood count, biochemistry panel, or urinalysis, or pre-existing cardiac disease. The initial toceranib dose was determined by the attending oncologist and was within the accepted published dose range for each dog based on body weight.

Dogs had echocardiogram, electrocardiogram (ECG), blood pressure measurement, and blood and urine collection for biomarker measurement performed at baseline (Day 0, before toceranib treatment initiation), 1, 3, and 5 months after starting treatment. A five-month study duration was selected as newly developed hypertension occurs as soon as 2 weeks after starting toceranib [11], and possible cardiac structural changes secondary to hypertension would be detectable by 5 months. At each visit, a complete blood count, chemistry panel, urinalysis (including creatinine) and urine protein creatinine ratio were performed as a part of the oncology standard of care for dogs receiving toceranib. In addition, a visit at 2 weeks included a blood pressure measurement and blood and urine collection for biomarker measurement. Study endpoints included the development of systemic hypertension that required medical management, requiring cardiac medications (e.g., antiarrhythmics) or other anti-cancer treatments, reaching the 5-month timepoint, or death.

2.2 | Echocardiogram

Echocardiographic images (GE Vivid E95 or the GE Vivid E9) were obtained by a board-certified cardiologist (VKY) or by a cardiology resident (KEL) under the supervision of a board-certified cardiologist. Echocardiograms were performed on awake animals in all cases, but in the event the dog required mild sedation for their oncology visit (trazodone or gabapentin), the dose was standardized across all exams for that specific dog. Echocardiographic exams included standard 2-dimensional and M-mode images, and all echocardiographic measurements were performed by the same observer (KEL). M-mode measurements were normalized to body weight using previously published formulas [24]. LVEF was obtained via Simpson's method of discs in the right

parasternal long axis. Global longitudinal strain (GLS) measurements were performed using a series of three, 2-dimensional left apical images, which included a 2-chamber view, a 3-chamber/APLAX view, and a 4-chamber view. Post-processing strain measurements were performed using GE EchoPAC v.23 once all study echocardiograms for the dog were completed. All measurements were made by a single, blinded investigator (KEL).

2.3 | Electrocardiogram

A 6-lead ECG was performed after each echocardiogram. The ECG was monitored for ~2 min.

2.4 | Blood Pressure

Three consecutive blood pressure measurements were obtained via Doppler (Parks Medical 811-BTS), and the mean value was reported. Blood pressure was obtained at Day 0, 2 weeks, 1, and 3 months per the study schedule. In the event the dog required therapy for systemic hypertension, the blood pressure was rechecked 2 weeks after initiating therapy. Treatment was initiated when blood pressure was measured at ≥ 170 mmHg, which is within the range of hypertension with moderate target organ damage [25].

2.5 | Cardiac and Vascular Biomarkers

Blood and urine samples were obtained from each dog at the baseline, 1 week, 1, 3, and 5-month visits. The blood was centrifuged at 845 × g for 15 min at 4°C after each appointment, and serum was stored at −80°C. Urine samples were also stored at −80°C. Serum samples were submitted for batch processing for N-terminal pro-B-type natriuretic peptide (NT-proBNP IDEXX) and hs-cTnI (Texas A&M GI Lab) analyses.

Measurement of each vascular biomarker was performed using commercially available ELISA assays that have been previously validated in dogs following manufacturers' instructions, including serum VEGF (Canine VEGF Quantikine ELISA, R&D systems—cat: CAVE00), serum PDGF (Canine PDGF-BB ELISA Kit, Abcam—cat: ab273171), serum cGMP (Canine Cyclic Guanosine Monophosphate (cGMP) ELISA kit, mybiosource—cat: MBS016147), urine nitric oxide metabolites (Parameter Total Nitric Oxide and nitrate/nitrite Assay, R&D systems—cat: KGE001), serum prostacyclin (Canine Prostacylin (PGI2) ELISA kit, mybiosource—cat: MBS736213), serum endothelin-1 (Endothelin-1 ELISA Kit, Abcam—cat: ab133030). All ELISA plates were analyzed with a Biotek Synergy H1 plate reader.

2.6 | Statistical Analysis

Based on a power calculation from published human LVEF data, a sample size of at least 25 dogs was needed to obtain a power = 0.80 and $\alpha = 0.05$ [8]. Statistics were performed using GraphPad Prism v10. A Shapiro–Wilk test was used to test data normality. Outlier data points were assessed using Grubb's test ($\alpha = 0.05$) and removed before to further statistical analysis. A repeated mixed effect analysis was used to analyze change over time for each variable in addition to Dunnett's multiple comparison test. For normally distributed data, a one-sample *t* test was additionally used to compare between two timepoints, and a Wilcoxon test was used for non-normally distributed data. Pearson's test was used to analyze correlation between two parameters.

3 | Results

3.1 | Study Animals

There were 26 dogs recruited into the study. Study subjects ranged from 5 to 12 years, and several different breeds and tumor types were represented (Table 1). All 26 animals were included in the blood pressure and biomarker analysis, while only those who had reached the endpoint (obtained echocardiographic examination at 5-month time point or dropped out of study earlier because of systemic hypertension, requiring

TABLE 1 | Demographic data for canine study participants.

Sex		Number
	Spayed female	11
	Castrated male	13
	Intact male	2
Breed		
	Mixed breed	12
	Labrador retriever	6
	Golden retriever	1
	Pit bull	1
	Australian shepherd	1
	Dogue de bordeaux	1
	Boxer	1
	Bernese mountain dog	1
	Dachshund	1
	French bulldog	1
Tumor type		
	Mast cell tumor	10
	Anal sac adenocarcinoma	7
	Thyroid carcinoma	3
	Heart base tumor	3
	Mesothelioma	1
	Nasal adenocarcinoma	1
	Multiple tumor types	1
Weight (kg)	Mean ± STD: 22.3 ± 14.1 Median: 29.8	Min: 6.4 Max: 66.2
Age	Mean ± STD: 10.0 ± 2.0 Median: 10.5	Min: 6 Max: 13

cardiac medication or additional cancer treatment, or death) of the study at the time of manuscript preparation were included in the echocardiographic analysis (15 of the 26). Eight out of 26 dogs received sedation for their examination, with gabapentin being the most common. None of the dogs developed any clinical signs associated with cardiac disease during the five-month study period. One dog developed worsening of ventricular arrhythmia 3 months after starting treatment requiring sotalol therapy. This dog was later determined to have arrhythmogenic right ventricular cardiomyopathy. The increase in arrhythmia might be part of cardiomyopathy disease progression, although an adverse event associated with toceranib cannot be ruled out.

3.2 | Blood Pressure, Serum VEGF, PDGF

Thirty percent of the study dogs developed systemic hypertension during the study requiring medical management. There was an overall increase in blood pressure over the course of the study ($p = 0.02$ mixed effect analysis), even in those that did not develop criteria for a new diagnosis of hypertension that would require medical management (Figure 1A). Compared with baseline, a statistically significant increase in blood pressure was detected after 2 weeks of treatment ($p = 0.03$, Dunnett's comparison $p = 0.009$, one-sample *t*-test), and 3 months of toceranib therapy ($p = 0.06$, Dunnett's comparison $p = 0.02$, one-sample *t*-test). A statistically significant increase in serum VEGF with toceranib therapy (Table 3 and Figure 1B) was detected after 3 months of treatment (mean of 247.8 pg/mL at three-month vs. 135.4 pg/mL at baseline $p = 0.01$, one-sample Wilcoxon). The increase in blood pressure was positively correlated with the increase in VEGF (Spearman $r = 0.33$, $p = 0.013$ Figure 1C). Both the increase and the correlation with systemic blood pressure are expected as these changes are indicators of appropriate VEGFR inhibition [4, 11, 26]. However, a similar increase in PDGF with toceranib treatment was not seen in our study.

3.3 | Vasodilators—Prostacyclin, Nitric Oxide Metabolites, cGMP

To determine if the elevation in systemic blood pressure is a result of decreased concentrations of vasodilators, we measured the concentration of serum prostacyclin and cGMP in addition to urine nitric oxide metabolites (i.e., nitrate and nitrite). Of the vasodilators measured, only serum prostacyclin showed a change with toceranib treatment (Figure 1D). A decrease was detected after 1 month of toceranib treatment (mean 98.8 pg/mL at one-month vs. 140.0 pg/mL at baseline $p = 0.03$, one-sample *t*-test). However, this decrease did not correlate with the increase in systemic blood pressure (Spearman $r = 0.04$, $p = 0.76$). None of the other vasodilators tested—urine nitrate or serum cGMP—changed with treatment, and urine nitrite was undetectable within this cohort.

3.4 | Vasoconstrictor—Endothelin-1

Similarly, we evaluated if increased concentrations of vasoconstrictors might play a role in the elevated blood pressure. Serum endothelin-1 concentrations did not change with toceranib treatment, despite increases in systemic blood pressure seen during the treatment period.

3.5 | Renal Function

To determine if altered renal function might play a role in blood pressure elevation, we analyzed variables associated with renal function. There was no significant change in renal function as measured by blood urea nitrogen, creatinine, or the urine protein: creatinine (UPC) ratio in the study cohort through the toceranib treatment period. Consequently, no correlation between the increase in systemic blood pressure and renal function was found.

3.6 | Echocardiogram and Electrocardiogram

There was no significant change in LVEF or fractional shortening over the 5-month treatment period (Figure 2A,B). The percent change in interventricular septal thickness in end diastole significantly increased at the three-month timepoint ($p = 0.0006$ Dunnett's comparison) but not at the five-month timepoint compared with baseline (Figure 1C). Despite no change in ejection fraction, GLS, a marker of systolic dysfunction, was significantly decreased after five-months of toceranib treatment compared with baseline measurements ($p = 0.03$ Dunnett's comparison Figure 1D). Overall, 58% of dogs had a decrease in GLS by 1 month (mean −15.1%, $p = 0.8$), 70% by 3 months (mean −13.6%, $p = 0.32$), and 85.7% by the 5-month (mean −14.5%, $p = 0.048$) time points compared with baseline (−15.7%), suggesting a time-dependent decline. Although these changes in GLS followed the rise in blood pressure, there was no correlation between the blood pressure and GLS (Pearson $r = 0.07$, $p = 0.73$) nor between the change in blood pressure and change in GLS compared with baseline (Pearson $r = 0.05$, $p = 0.76$). Additionally, no difference was found in the GLS value for those with a corresponding blood pressure below 160 mmHg and greater than 160 mmHg ($p = 0.6$, unpaired *t*-test). The remainder of the two-dimensional echocardiographic parameters were not found to change over time (Table 2).

3.7 | Cardiac Biomarkers

There were no statistically significant changes in either hs-cTnI or NT-proBNP seen during the 5-month study period (Table 2).

4 | Discussion

This study evaluates changes in cardiac function, including GLS, in addition to biomarkers for endothelial function in dogs with cancer receiving toceranib, a VEGFR inhibitor. The results demonstrate an increase in circulating VEGF concentration, a marker of effective VEGFR inhibition. VEGF levels have been well documented to increase in both human and veterinary patients receiving VEGF receptor inhibitor medications, and the rise of circulating VEGF is an anticipated

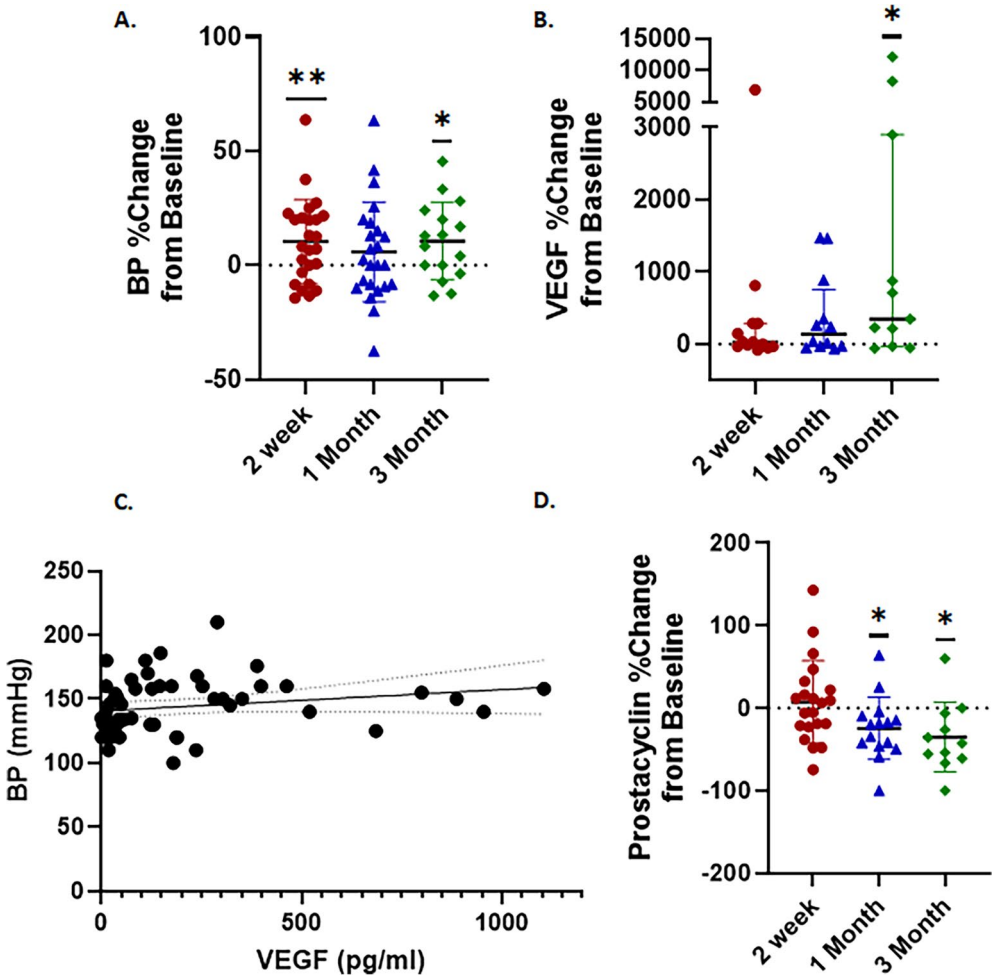

FIGURE 1 | Increased blood pressure and circulating serum VEGF with decreased circulating serum prostacyclin are associated with toceranib treatment. Both systemic blood pressure (BP) [$n = 25$ (2-week), 24 (1-month), 16 (3-month) one-sample t-test] (A) and serum vascular endothelial growth factor (VEGF) [$n = 13$ (2-week), 12 (1-month), 11 (3-month) one-sample Wilcoxon] (B) increased with toceranib treatment. These changes are positively correlated (Spearman $r = 0.33$, $p = 0.013$). Solid line shows the linear regression, and the dotted lines are the 95% confidence bands (C). At the same time, serum prostacyclin, a vasodilator, decreased [$n = 21$ (2-week), 15 (1-month),11 (3-month) one-sample t-test] with treatment. (D) $^*P < 0.05$.

result of VEGFR inhibitor due to blockade of the VEGF receptors [18, 22, 27]. A concurrent increase in systemic blood pressure was also seen, consistent with previous observations [11]. Our study also found that the level of one vasodilator, prostacyclin, decreased with toceranib treatment, while no significant changes were noted in the concentration of other vasodilators, including nitric oxide and cGMP. Neither was the level of endothelin-1, a vasoconstrictor, affected by toceranib. Interestingly, LV systolic function changes were not detected based on traditional echocardiographic parameters (LVEF and fractional shortening), but a decrease in systolic function measured by GLS was documented.

Studies performed in human patients receiving analogous VEGFR inhibitors demonstrate decreases in systolic function detected by GLS, a marker of early subclinical cardiac dysfunction [13]. In human patients treated with VEGFR inhibitors, 8% develop clinically asymptomatic cardiac dysfunction, while 30% develop clinically significant decreases in GLS [13]. Changes in GLS might be more sensitive and often precede decreases on standard 2-dimensional echocardiographic images (fractional shortening and LVEF) [13, 28]. Similarly, in our study, changes consistent with early subclinical dysfunction were only detected by GLS and not by LVEF or fractional shortening. However, the clinical relevance of these changes in GLS is uncertain as none of the dogs developed signs consistent with heart failure and no significant change in LVEF was detected during the five-month study period. In human patients, the time to development of symptomatic LV systolic dysfunction after initiation of sunitinib therapy ranges from 22 to 435 days [6]. Therefore, our sample size and duration of study limit our ability to accurately assess the risk of heart failure development with toceranib treatment. In addition, once dogs developed systemic hypertension requiring treatment, their participation in this study ended; we therefore cannot assess the combined effects of severe hypertension and toceranib treatment on cardiac systolic dysfunction.

The underlying mechanism for cardiotoxicity that could lead to systolic dysfunction in dogs receiving VEGFR inhibitors is

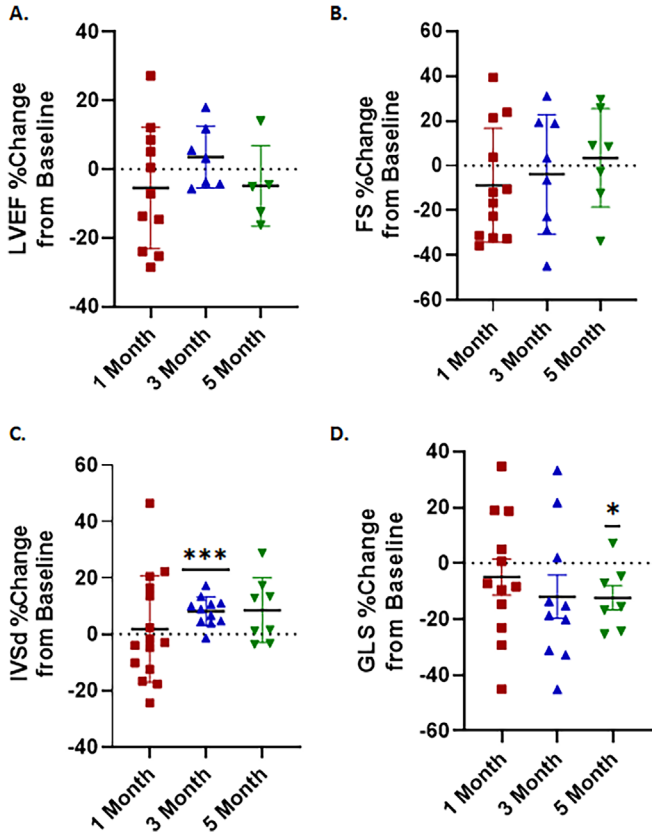

FIGURE 2 | Increased left ventricular wall thickness and decreased global longitudinal strain are associated with toceranib treatment. After starting toceranib treatment, systolic function as measured by either left ventricular ejection fraction (LVEF) measured by the Simpson's method [$n = 14$ (1-month), 10 (3-month), 8 (5-month)] (A) or fractional shortening by M-mode [$n = 12$ (1-month), 8 (3-month), 7 (5-month)] (B) did not change. However, an increase in interventricular septal thickness in end diastole (IVSd) was noted 3 months later but not after 5 months. [$n = 15$ (1-month), 11 (3-month), 8 (5-month)] (C), and systolic function as measured by Global Longitudinal Strain (GLS) indicated a decrease in left ventricular contractile function [$n = 12$ (1-month), 10 (3-month), 7 (5-month)] (D). Mixed effect analysis with Dunnett's multiple comparisons test. $^{*}P < 0.05$, $^{***}P < 0.001$.

not fully understood. One proposed mechanism for the cardiotoxic effects of sunitinib is its inhibition of PDGFR-β [20, 21]. PDGFR-β in murine models protects against afterload stress, which would be important especially in the event of systemic hypertension, thus preventing cardiomyocyte apoptosis. Inhibition of PDGFR-β leads to ventricular hypertrophy and dilation, and subsequent cardiac failure [20]. Evaluation of the PDGF concentrations in our cohort did not reveal any significant changes over the course of the study; however, changing circulating levels of PDGF have not been confirmed as a marker of PDGFR-β blockade. Hence, we cannot rule out the possibility that the decline in PDGFR-β signaling in the heart due to kinase inhibition by toceranib could contribute to cardiac dysfunction in dogs, and further studies are warranted.

Another possible cause for systolic dysfunction could be a result of hypertension from toceranib treatment. Hypertension is the most commonly reported adverse effect of VEGFR inhibitors in both humans and veterinary patients is vascular

TABLE 2 | Echocardiographic markers of systolic function and cardiac biomarkers.

	Baseline (±Std)	1 month (±Std)	% Change compared with baseline *p*-value	3 month (±Std)	% change compared with baseline *p*-value	5 month (±Std)	% change compared with baseline *p*-value	% change mixed effect *p*-value
% FS	30.8 (±9) $n = 15$	28.7 (±11) $n = 15$	0.59	30.7 (±9) $n = 11$	0.97	0.69 $n = 9$	0.96	0.42
LVEF	57.2 (±10) $n = 15$	55.4 (±12) $n = 15$	0.23	58.1 (±9) $n = 10$	0.98	57.0 (±8) $n = 8$	0.15	0.23
GLS	−15.7 (±3) $n = 12$	−15.1 (±3.2) $n = 12$	0.80	−13.6 (±2) $n = 10$	0.32	−14.5 (±3) $n = 7$	0.048	0.35
IVSd	1.20 (±0.23) $n = 15$	1.19 (±0.18) $n = 15$	0.97	1.24 (±0.22) $n = 11$	0.0006	1.21 (±0.18) $n = 9$	0.11	0.21
cTnI (pg/mL)	88.3 (±80.5) $n = 26$	95.8 (±71.1) $n = 25$	0.54	131.7 (±241.1) $n = 18$	0.57	79.3 (±48.4) $n = 11$	0.35	0.40
NT-proBNP (pg/mL)	939 (±1031) $n = 26$	723 (±675) $n = 26$	0.05	800 (±1028) $n = 21$	0.94	640 (±253) $n = 12$	0.94	0.08

Note: Dunnett's test used for multiple comparison to baseline.

TABLE 3 | Blood pressure and vascular biomarkers.

	Baseline (±Std)	2 week (±Std)	% change compared with baseline *p*-value	1 month (±Std)	% change compared with baseline *p*-value	3 month (±Std)	% change compared with baseline *p*-value	% change mixed effect *p*-value
BP (mmHg)	135 (±20.2) *n* = 26	146 (±23.2) *n* = 25	0.03 (0.009)*	137 (±20.1) *n* = 24	0.44 (0.20)*	148 (±22) *n* = 16	0.06 (0.02)*	0.02
VEGF (pg/mL)	135.4 (±215.9) *n* = 14	199.3 (±299.5) *n* = 13	0.51 (0.23)&	161.7 (±133.8) *n* = 12	0.10 (0.08)&	247.8 (±282.3) *n* = 11	0.20 (0.01)&	0.09
Prostacyclin (pg/mL)	140.0 (±128.9) *n* = 22	158.2 (±179.0) *n* = 21	0.84 (0.50)*	98.8 (±131.1) *n* = 15	0.06 (0.03)*	99.6 (±197.6) *n* = 11	0.05 (0.02)*	0.03
Endothelin-1 (pg/mL)	1.90 (±1.95) *n* = 15	2.03 (±1.87) *n* = 15	0.39 (0.39)&	1.96 (±1.77) *n* = 15	0.38 (0.25)&	2.47 (±1.81) *n* = 11	0.57 (0.37)&	0.23
Nitrate (μM)	213.5 (±244.7) *n* = 14	198.0 (±281.7) *n* = 11	0.36 (0.37)&	97.7 (±82.9) *n* = 14	0.86 (0.14)&	163.8 (±237.1) *n* = 10	0.28 (0.42)&	0.21
PDGF (pg/mL)	879.5 (±507.2) *n* = 15	825.9 (±444.2) *n* = 15	0.92 (0.93)&	1055.4 (±434.8) *n* = 14	0.1 (0.08)&	882.9 (±414.9) *n* = 11	0.37 (0.24)&	0.04

Note: Dunnett's test used for multiple comparison to baseline, in addition to further comparison with one-sample *t* test (*), or one-sample Wilcoxon test (&).

toxicity, manifested by an increase in the systemic blood pressure. Our study showed that 30% of the enrolled dogs developed systemic hypertension as defined by the study guidelines (BP > 170 mmHg), and this percentage is similar to a report in dogs receiving toceranib [11]. It is suggested that hypertension might exacerbate or accelerate the other cardiotoxic effects of VEGFR inhibitor [10]. However, while there was a decrease in systolic function suggested by GLS as well as an increase in the blood pressure over time, there was no correlation between these two findings in our study.

Previous studies have speculated the mechanism of hypertension development in these patients, including glomerular endotheliosis and thrombotic microangiopathy leading to proteinuria [29]. There was no evidence of protein losing nephropathy or increased creatinine or urea in our cohort as a cause for the hypertension. While changes in endothelin-1 and nitric oxide levels have been previously suggested as causes, this study did not find any significant change in either serum endothelin-1 or urine nitric oxide metabolite (nitrate and nitrite) levels [5, 16, 18, 30, 31]. Larger studies would be needed to confirm the lack of effect of toceranib on endothelin and nitric oxide pathways. However, prostacyclin levels did decrease in this cohort over time. Prostacyclin (a vasodilator) release from the endothelial cell is activated by VEGF signaling [18, 19]. Therefore, the reduction in prostacyclin level in dogs receiving VEGFR inhibitors might play a role in the development of hypertension in dogs receiving toceranib [18, 19].

This study encountered several limitations. First, while the selected study period was long enough to detect early changes in systolic function, a longer follow-up period is needed to determine if these changes resulted in any clinically significant cardiac disease. Due to adverse effects of toceranib that were unrelated to the cardiovascular system, mainly gastrointestinal adverse effects, several dogs had dose adjustments throughout the course of their treatment based on the severity of the side effects, ranging from dose reduction to frequency change from every other day to three times or twice a week. Therefore, the dose of toceranib being administered was not the same for every dog throughout their entire course of treatment. In addition, 8 out of 26 dogs received sedation for their examination, with gabapentin being the most common. While the dogs receiving sedation had consistent doses and medication types throughout the study to minimize changes in blood pressure or echocardiographic findings attributable to sedation, the medication type and dose were not standardized across the entire study cohort. Similarly, if the attending oncologist deemed it necessary to start a dog on other medications such as NSAIDs or corticosteroids during the study period, the dog was allowed to continue in the study, and the effects of these drugs on blood pressure cannot be excluded. Lastly, the sample size of the study was small. Most notably, by the five-month time point, we had many fewer samples for biomarker analysis compared with the earlier time points and were therefore not included in the statistical analysis. With this attrition, we were not able to meet the minimal sample size of 25; especially for the five-month time point, as a result, differences in biomarkers or echocardiographic parameters might not be appreciated with our final sample size. Furthermore, a larger study size will be needed to validate the echocardiographic and vascular biomarker findings.

5 | Conclusion

Dogs with cancer undergoing treatment with toceranib have a higher risk of developing systemic hypertension. As our data showed, serum VEGF concentration correlates with blood pressure in dogs treated with toceranib; therefore, VEGF might serve as a biomarker to identify those at higher risk of developing hypertension requiring medical management. In addition, dogs developed early signs of cardiac systolic dysfunction after months of treatment, based on declines in GLS. Hence, serial cardiac monitoring with quantification of GLS might help identify those with early signs of systolic dysfunction. To mitigate the potential of clinically important changes associated with systolic dysfunction, an optimal monitoring strategy should be developed for dogs undergoing cancer treatment with toceranib by the oncologist and cardiologist as we continue to improve our understanding of the long-term effects of toceranib on the cardiovascular system. For dogs which do not have access to an oncologist or cardiologist, serial blood pressure monitoring should be part of the routine exam. Monitoring of respiratory rate and effort should be emphasized as well, as changes in these variables might be associated with cardiovascular dysfunction and indication of when the involvement of a cardiologist becomes beneficial.

Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

Institutional Animal Care and Use Committee approval from Tufts University was granted for this study (#2022–15). Authors declare human ethics approval was not needed.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.