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Project Title: Avastin, Sutent, and Votrient Induced Cardiotoxicity Study (ASICS): Early Detection of Bevacizumab, Sunitinib, and Pazopanib Mediated Cardiotoxicity in Cancer Patients

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Summary (250 words max single spaced):

BACKGROUND: Bevacizumab (BVZ), Sunitinib (SNT), and Pazopanib (PAZ) are novel targeted therapies which are used in the treatment of metastatic colorectal cancer (CRC) and renal cell carcinoma (RCC). Though these agents have demonstrated improvements in progression free survival, they have been associated with left ventricular (LV) systolic dysfunction. As such, early markers of cardiac dysfunction would be useful in the detection and prevention of cardiotoxicity seen in these patients.
OBJECTIVE: To determine whether cardiac biomarkers and/or novel echocardiographic techniques may allow for the early detection of BVZ, SNT, and PAZ mediated cardiac dysfunction in metastatic CRC and RCC patients.
METHODS: Cancer patients receiving BVZ, SNT, or PAZ were prospectively enrolled at two tertiary care centres and studied at 4 time points: i) baseline; ii) 1 month; iii) 3 months; and iv) 6 months after the initiation of therapy. At each visit echocardiography was performed and blood taken to evaluate serum levels of cardiac troponin I (TnI) and C-reactive protein (CRP).
RESULTS: Of the 40 patients (mean age 63 ± 9) prospectively followed in this study, 3 (8%) developed cancer therapeutic related cardiac dysfunction (CTRCD), identified by a reduced left ventricular ejection fraction (LVEF) at 3-month follow-up. In contrast, speckle tracking echocardiography (STE) parameters including global longitudinal strain (GLS) and global longitudinal strain rate early diastole (GLSRE) demonstrated changes in LV function at 1-month follow-up. Cardiac biomarkers including TnI and CRP did not change over time.
CONCLUSION: In a clinical study of metastatic CRC and RCC patients receiving BVZ, SNT, or PAZ, STE parameters were able to detect pre-clinical changes in LV function prior to a reduction in LVEF.

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INTRODUCTION

Cancer is a significant health concern in Canada, as an estimated 196,000 new cases were diagnosed in 2015.[1] Treatment strategies, which typically include a combination of surgical resection, radiation, chemotherapy, and novel targeted biological therapy, have led to improvements in overall cancer survival. Vascular endothelial growth factor (VEGF) inhibitors are effective novel targeted biological therapies which target tumor angiogenesis, an integral mechanism in the progression of metastatic cancers.[2] Unfortunately, cardiac toxicity including left ventricular (LV) systolic dysfunction, has been identified as a severe complication of this anti-cancer therapy.[3-4] The emerging interdisciplinary field of Cardio-Oncology seeks to improve the understanding, diagnosis, and prevention of cardiotoxicity resulting from cancer treatment.[5]

Colorectal cancer (CRC) accounts for approximately 13% of all new cancers diagnosed in Canada, and kills an estimated 9,300 Canadians annually.[1] In the treatment of metastatic CRC, Bevacizumab (Avastin) has proven to improve survival duration when combined with conventional chemotherapy, including 5-fluorouracil, leucovorin, oxaliplatin and irinotecan.[6] Bevacizumab (BVZ) is a recombinant humanized monoclonal antibody that targets the VEGF isoform A (VEGF-A). Through inhibition of VEGF-A, BVZ prevents tumor angiogenesis and has been shown to decrease tumor progression and improve overall survival in patients treated for metastatic CRC.[6-7] However, BVZ has been associated with adverse cardiovascular effects including LV systolic dysfunction and hypertension. Although the cardiotoxic mechanisms of BVZ are not completely understood, impaired nitric oxide (NO) production and increased vascular resistance from endothelial smooth muscle proliferation are believed to play an important role.[8] The incidence of BVZ-mediated heart failure ranges between 2-3% as reported in phase III clinical trials.[9] However, in a retrospective study evaluating CRC patients receiving BVZ at Cancer Care Manitoba between 2010 and 2011, approximately 1 in 4 developed LV systolic dysfunction.[10] As such, these findings support the need for early detection and prevention of BVZ-mediated cardiotoxicity.

It is estimated that in 2015, renal cell carcinoma (RCC) accounted for 6,200 new cancer cases and 1,800 related deaths in Canada.[1] Sunitinib (Sutent) is an oral receptor tyrosine kinase inhibitor (TKI) used in the first-line treatment of metastatic RCC.[11-12] A more recently developed TKI, Pazopanib (Votrient), is used in the second-line treatment of advanced RCC.[13] Both Sunitinib (SNT) and Pazopanib (PAZ) are multi-target inhibitors against several growth factor and cytokine receptors including VEGF receptors 1-3 and the platelet derived growth factor receptors (PDFGR). Through inhibition of angiogenic signaling pathways, both SNT and PAZ have proven to be effective therapies in suppressing tumor growth and metastasis.[11-14] Unfortunately, TKIs including SNT and PAZ, have been associated with several adverse cardiovascular effects including LV systolic dysfunction, hypertension, arrhythmias, and acute coronary syndrome (ACS).[8,11,15-16] Animal models suggest TKI-mediated cardiotoxicity involves a combination of impaired response to mechanical and oxidative stress (OS) and loss of cardiac myocytes from adenosine monophosphate-activated kinase (AMPK) inhibition.[8,15-16] In an observational study of RCC patients receiving TKIs, Schmidinger *et al.* found that over one-third of patients developed a cardiac event, defined as increased cardiac enzymes, symptomatic arrhythmia, new LV systolic dysfunction, or ACS.[11] It is clear that cardiotoxicity with TKIs necessitates a high degree of screening, prevention, and management.

Serial monitoring of left ventricular ejection fraction (LVEF) with transthoracic echocardiography (TTE) and multiple-gated acquisition scanning (MUGA) continues to be the predominant non-invasive method for monitoring cardiac function in the cancer population.[17-19] According to expert consensus guidelines, cancer therapeutic related cardiac dysfunction

(CTRCD) is defined as a decrease from baseline LVEF of >10% to a value <53%.[20] Unfortunately, measurement of LVEF is not a very sensitive tool for the detection of subtle changes in LV systolic function.[16] As the heart has substantial contractile reserve, the LVEF parameter represents a late marker of cardiac disease. Once a decrease in LVEF has been observed, significant myocardial damage has already occured.[18-19] Alternatively, recent animal and clinical studies have suggested novel echocardiographic techniques and cardiac biomarkers as more sensitive indices of early CTRCD.[3,18-19]

In the early detection of CTRCD, novel techniques including tissue Doppler imaging (TDI) and speckle tracking echocardiography (STE) have emerged as useful and sensitive indicators of subtle changes in LV function. A modification of conventional Doppler ultrasound, TDI allows for the analysis of high amplitude, low velocity signals for tracking tissue velocities.[21] Accordingly, TDI has become a useful tool in the evaluation of myocardial tissue motion throughout the cardiac cycle.[22] Two indices of TDI include tissue velocity imaging (TVI) and strain imaging. TVI is typically used to assess peak myocardial velocities during systole (S'), early diastole (e'), and late diastole (a').[21-22] Strain imaging can be used to determine myocardial tissue deformation in longitudinal, radial, and circumferential dimensions throughout the cardiac cycle. Alternatively, myocardial strain can be measured using a technique known as STE. Standard echocardiographic images produce bright acoustic markers within the myocardium, known as speckles. STE analyzes the movement of these markers and allows for quantification of myocardial tissue deformation.[23]

Both TVI and strain imaging allow for an assessment of LV systolic and diastolic function making them useful potential markers for CTRCD.[19,24-25] The utility of TDI and STE has been studied previously in the *early detection* of CTRCD in the treatment of breast cancer patients. In animal models and the clinical breast cancer setting, TVI and strain imaging are accurate markers of early LV systolic dysfunction involving anthracycline-mediated cardiotoxicity.[18,23-26] Extending our findings from the breast cancer setting, we recently evaluated the utility of TDI as an early marker of cardiac dysfunction in an acute murine model of BVZ and SNT mediated cardiotoxicity. In mice receiving BVZ and SNT, TVI and strain imaging detected LV systolic dysfunction as early as day 8 of the study. In contrast, a decline in conventional LVEF values was not observed until day 13 of the study.[28] As little is known on the utility of TVI and strain imaging for the early detection of CRTCD in the clinical setting of CRC and RCC, further study is required.

In addition to novel imaging modalities, cardiac biomarkers have been extensively studied for their potential role in predicting CTRCD.[3] Cardiac biomarkers are serum molecules widely used in the early detection of a number of cardiac pathologies, including heart failure.[28-29] Considered the most sensitive and specific cardiac biomarkers, cardiac troponin isoforms I (TnI) and T (TnT) are proteins released into the blood following severe myocardial injury.[28,30] Their use as early markers of myocardial ischemia and heart failure are well documented throughout the literature.[28,30-31] As such, their utility as early markers of CTRCD has been previously investigated and validated in the breast cancer setting.[31-33] Our own basic science study recently demonstrated a rise in TnI levels for mice treated with BVZ/SNT.[27] C-reactive protein (CRP) is an acute phase reactant protein that is synthesized by hepatocytes in response to inflammation and trauma. Although CRP has traditionally been used as a biomarker for acute inflammation, infection, and tissue damage, in recent years, it has emerged as a novel marker of cardiovascular risk.[34] In the breast cancer setting, several studies have suggested CRP as a prognostic predictive marker of disease progression.[33-34] In contrast, investigations to date have failed to demonstrate a clear association between serum CRP and the development of cardiotoxicity from breast cancer treatment.[18,33-34] Whether cardiac biomarkers, including TnI and

CRP, can accurately predict CTRCD in a clinical study involving VEGF inhibitors remains to be seen.

OBJECTIVE

The objective of this study was to evaluate whether early indices of LV systolic dysfunction, specifically TVI, strain imaging, and cardiac biomarkers could detect ***early*** CTRCD prior to a reduction in LVEF in CRC and RCC patients receiving BVZ, SNT, or PAZ.

METHODS

A. STUDY POPULATION:

A total of 62 patients were prospectively enrolled at two tertiary care centres between 2013-2015 inclusive (Figure 1). Patients older than 18 years of age and receiving either BVZ, SNT, or PAZ for metastatic CRC or RCC were considered eligible for this study. Patients with a baseline LVEF less than 50% were excluded from this study. Baseline demographics including cardiovascular risk factors, cancer history, and medications were collected.

B. STUDY PROTOCOL:

Patients were studied at 4 time points: i) Baseline; ii) 1 month; iii) 3 months; and iv) 6 months after the initiation of VEGF inhibitor therapy (Figure 2). At each visit, a TTE was performed and blood taken to evaluate serum levels of cardiac biomarkers. In addition, blood pressure was measured and patients were interviewed for cardiovascular symptoms.

C. ECHOCARDIOGRAPHY:

A comprehensive TTE was performed using a GE Vivid 7 platform (General Electric, Milwaukee, Wisconsin) with a multi-frequency ultrasound probe. All images were acquired by an experienced sonographer following standard echocardiographic protocol as per the American Society of Echocardiography (ASE) and European Association of Echocardiography (EAE) guidelines.[20,35] Grayscale images including parasternal (short and long axis) and apical (four chamber, two chamber, and long axis) views were acquired sequentially for offline speckle-tracking analysis. All views consisted of three consecutive heart cycles acquired during a breath hold at 50-70 frames per second. Images were post processed offline using both the EchoPAC PC software (Vivid 7, version 11.2, GE Medical Systems, Milwaukee, WI, US) and Syngo-VVI clinical software (Syngo 3.5b and VVI 2.00, Siemens Medical Solutions, Erlangen, Germany).[3,18,32]

The LV cavity dimensions and LVEF were determined from 2-dimensional images according to the modified biplane Simpson's method.[35] A conventional Doppler assessment of the LV filling pressures was performed by measuring the peak early diastolic filling (E wave) and late diastolic filling (A wave) velocities as well as the E/A ratio. TDI values were recorded on the lateral, septal, anterior, and inferior walls of the LV at the levels of the mitral annuli and papillary muscles. These indices included systolic (S'), early diastolic (e'), and late diastolic (a') velocities.

Segmental peak values of strain and strain rate (systolic and early diastolic) were calculated using an automated speckle tracking algorithm. Global longitudinal strain (GLS) and

strain rates (GLSR) were calculated by averaging peak strain in 18 LV segments for each subject. The global radial and circumferential strain and strain rates were calculated by averaging peak strain in six LV segments at the level of the papillary muscles.

Tracking integrity was confirmed visually and on the credibility of the strain curves. Image quality was classified as good, reasonable, or poor (and ultimately excluded) based on the number of segments which failed to track. To assess intra-observor and inter-observor variability within the strain measurements, 15 randomly selected patients were analyzed.

D. BIOMARKERS:

High sensitivity TnI (hsTnI) and CRP were evaluated at 4 separate time points. Quantitative measurements of serum hsTnI were performed using an Abbott Architect analyzer (Abbott Diagnostics, Abbott Park, Illinois). Serum CRP levels were measured with an Immage 800 (Beckman Coulter, Brea, California) antigen-antibody precipitant rate reaction.[18]

E. STUDY DEFINITIONS:

CTRCD was defined as a decrease in the LVEF of at least 10% to below 53%.[20]

F. STATISTICAL ANALYSIS:

Data are presented as mean ± standard deviation (SD) for continuous variables or as percentage for categorical variables. Baseline characteristics between the CTRCD and normal populations were compared using a Wilcoxon test for continuous variables and Fisher's exact test for categorical variables. An analysis comparing predictors of cardiotoxicity between the CTRCD and normal populations was performed using the Wilcoxon test for continuous variables. The intraclass and interclass correlation coefficient was calculated to quantify intra-observer and inter-observer reliability and variability over 15 randomly selected patients. A p value of <0.05 was considered statistically significant. All statistical calculations were performed by JMP (version 10.1) statistical software (SAS Institute Inc., Cary, NC).

RESULTS

A. PATIENT POPULATION:

Overall, 62 patients were prospectively enrolled in this study (Figure 1). Of the total population, 1 patient was excluded for a baseline LVEF <50% while 3 patients declined to participate. Ten patients were only available at baseline and therefore not included in this study. In total, 48 patients completed the entire study protocol. Following echocardiographic analyses, a further 8 patients were excluded from the study due to poor image quality. Consequently, a total of 40 patients (mean age 63 ± 9, 85% male) were ultimately included in the final study results. Baseline clinical characteristics and cardiovascular risk factors for the entire cohort are summarized in Table 1. A total of 34 (85%) patients were treated for metastatic RCC while 6 (15%) were treated for metastatic CRC. A total of 26 (65%) patients received treatment with SNT, 8 (20%) with PAZ, and 6 (15%) with BVZ.

B. VEGF INHIBITOR-MEDIATED CARDIOTOXICITY:

Of the total study population, 3 (7.5%) patients were identified as developing CTRCD.[20] Among these, 2 patients who received PAZ developed CTRCD at 3 and 6 months, respectively. Both patients had pre-existing cardiovascular risk factors and a history of chest radiation or conventional chemotherapy. The third patient, who had pre-existing coronary artery disease, developed CTRCD at 3 months following treatment with BVZ. Only 2 (67%) of the 3 patients experienced symptoms of heart failure during treatment. Baseline clinical characteristics and cardiovascular risk factors for these 3 patients are summarized in Table 1.

Two other cardiovascular side effects observed in this study were hypertension and QT prolongation. Baseline hemodynamic measurements including systolic (SBP) and diastolic (DBP) blood pressure for the entire cohort are described in Table 1. Out of 40 patients, 14 (35%) developed new (n=3, 7.5%) or worsening (n=11, 28%) hypertension. The mean absolute maximum increase in SBP and DBP from baseline was 20 ± 12 mm Hg and 12 ± 7 mm Hg, respectively. The majority of these cases were secondary to therapy with SNT (n=8, 57%) and occurred most frequently at 1 month of follow-up (n=9, 64%). Among the 11 patients with prior hypertension, all were receiving anti-hypertensive medication at baseline and 8 (73%) had adjustments in management including increased dose of prior medication (n=4, 36%), new drug added (n=3, 27%), or both (n=1, 90%). Among patients who developed new hypertension, 2 (67%) were initiated on anti-hypertensive therapy. Additionally, one patient receiving treatment with SNT (with pre-existing cardiovascular risk factors) developed significant QT prolongation at 1 month (390 msec to 470 msec).

C. ECHOCARDIOGRAPHY:

Baseline conventional echocardiographic parameters for all patients (n=40) are summarized in Table 2. At baseline, the mean LVEF for the entire cohort was 63 ± 3%. Mean LVEF decreased at 1, 3, and 6 months following treatment with VEGF inhibitor ($p<0.0001$ at 1 and 3 months; $p<0.001$ at 6 months). However, the mean LVEF remained within normal reference range (>60%) at all time points.

A comparison of baseline conventional echocardiographic measurements between CTRCD (n=3) and no-CTRCD (n=37) groups can be seen in Table 2. Baseline LVEF values were within normal limits and were not statistically different between the two groups. Figure 3 illustrates the temporal changes in LVEF between the CTRCD and no-CTRCD subgroups across all time points. Of the 3 patients who developed CTRCD, LVEF significantly decreased at 3 months as compared to baseline (62 ± 1.2% to 51 ± 1.7%; $p<0.05$).

Baseline 2-dimensional STE parameters are summarized in Table 2. A comparison of these parameters between the CTRCD and no-CTRCD groups identified both GLS and early diastolic GLSR ($GLSR_E$) as early predictive markers of cardiotoxicity. At baseline, GLS and $GLSR_E$ were significantly lower ($p<0.05$) in the CTRCD group than the no-CTRCD group (Table 2). Of the 3 patients who developed LV systolic dysfunction, GLS decreased by 13% at 1 month and 24% at 3 months when compared to baseline measurements (Figure 4A). Likewise, these patients experienced declines in $GLSR_E$ by 31% at 1 month and 38% at 3 months relative to baseline (Figure 4B). Comparing these predictive markers at the 1 month follow up, both GLS and $GLSR_E$ were significantly lower ($p<0.05$) in the CTRCD than the no-CTRCD group (Table 3).

D. BIOMARKERS:

Serial measurements of cardiac biomarkers, including hsTnI and CRP, are shown in Table 4. At baseline, both markers were within normal limits for the entire population. No significant changes were detected in these biomarkers at each time point and no significant differences were seen between the CTRCD and no-CTRCD subgroups.

DISCUSSION

A refined understanding of cancer pathology has led to significant advancements in the treatment of cancer patients. Novel targeted therapies, including the VEGF inhibitors BVZ, SNT, and PAZ, have nearly doubled progression-free survival in metastatic CRC and RCC patients.[7,12-14] Unfortunately, cardiotoxicity resulting in LV systolic dysfunction, has emerged as a critical side effect of these novel therapies.[8,36] In our clinical study following metastatic CRC and RCC patients receiving BVZ, SNT, or PAZ, we made several novel findings in regards to CTRCD. First, we demonstrated that in a cohort of CRC and RCC patients receiving VEGF inhibitor therapy, 8% developed CTRCD. Second, STE indices appeared to predict early LV systolic dysfunction prior to changes in conventional LVEF values. In contrast, serum levels of cardiac biomarkers, including hsTnI and CRP, did not change across time points. Finally, new or worsening hypertension (35%) was the most common adverse cardiovascular event in patients treated with VEGF inhibitors for metastatic CRC and RCC.

Due to its widespread availability, portability, and lack of radiation exposure, TTE has become an indispensable tool in the evaluation of cardiovascular disease.[19] In the surveillance of patients at risk for cardiotoxicity, serial assessment of LVEF with TTE is the predominant method for detecting changes in cardiac function.[18] However, LVEF is influenced by several hemodynamic conditions including heart rate, preload, and afterload, and fails to detect early subclinical changes in myocardial contractility.[18-19] As significant myocardial damage is required to cause a decrease in LVEF, detection of LV systolic dysfunction is often delayed. Ultimately this may lead to irreversible cardiac damage, late initiation of heart failure treatment, and poor recovery.[37] In contrast, basic science and clinical studies have suggested novel echocardiographic indices and cardiac biomarkers as more reliable and sensitive markers of early CTRCD. While most of these studies were performed in the setting of anthracycline-mediated cardiotoxicity, our study is the *first* to investigate the utility of these markers in a clinical setting involving VEGF inhibitor-mediated cardiotoxicity.[20,36]

In the setting of CTRCD, novel echocardiographic techniques which include TDI and STE have demonstrated the potential to detect early changes in LV function prior to a decrease in conventional LVEF.[18-20] The ability to track myocardial tissue velocities with TDI has proven to be a more sensitive and early marker for LV dysfunction.[22] Two major indices that can be analyzed with TDI are TVI and strain. Systolic markers of LV function including S' measured with TVI, and GLS measured with strain imaging, have been previously studied as early markers of anthracycline-mediated cardiotoxicity.[18,22] We previously validated the utility of TDI in an acute murine model of BVZ and SNT mediated cardiotoxicity.[27] In mice receiving BVZ or SNT, LVEF decreased from 75% at baseline to 52% and 49% at day 13, respectively. In contrast, TDI indices including S' and peak systolic strain rate (SR) decreased significantly as early as day 8 of treatment with BVZ/SNT.[27] Although TDI was able to detect early LV systolic dysfunction in our murine model of BVZ and SNT mediated cardiotoxicity, this finding was not supported in our current clinical study. A major limitation of TDI is its angle dependency, as velocities may be underestimated if the angle of interrogation is greater than 20 degrees.[38] Accordingly, if TDI was

not acquired at a consistent and appropriate angle in our study population, it might explain why TDI failed to predict early cardiotoxicity in this study.

Strain and strain rate may be measured using a technique known as STE.[20,36] Compared to TDI, STE uses lower frame rates, is not angle dependent, and demonstrates superior reproducibility when used for strain-based analysis.[36,39] In a systematic review examining strain imaging as an early marker of anthracycline-mediated cardiotoxicity, Thavendiranathan et al. identified a 10-15% early reduction in GLS by STE as the most useful parameter for the early prediction of subsequent CTRCD.[39] Likewise, Negishi et al. found that GLS at 6 months compared to baseline was the best predictor of trastuzumab-mediated CTRCD at 1 year in breast cancer patients. They identified an 11% reduction in GLS as the optimal predictor of early cardiotoxicity, with sensitivity of 65% and specificity of 94%. At present, GLS as characterized by STE appears to be a promising method for predicting future reductions in LVEF due to CTRCD.[20,36] Our clinical study is the first to investigate the role of STE in the clinical setting of BVZ, SNT, and PAZ mediated cardiotoxicity.

Current ASE/EAE guidelines recommend that systolic parameters of STE be used in the early detection of CTRCD.[20] In our study, indices of STE including GLS and $GLSR_E$ emerged as more accurate predictive markers of early CTRCD. In the 3 patients who developed CTRCD, GLS decreased 13% and 24% at 1 month and 3 month follow-up, respectively. These findings coincide with the recommendations by Negishi et al., who suggested at least an 11% reduction in GLS as an early marker of LV systolic dysfunction.[40] In our patients, CTRCD developed much faster than was demonstrated in previous studies with anthracycline and trastuzumab-mediated cardiotoxicity.[20] We observed reductions in GLS only 1 month into VEGF inhibitor therapy. This is in contrast to investigations by Negishi et al. who identified reductions in GLS 6 months into therapy for breast cancer patients receiving trastuzumab.[40] Additionally, our study suggests $GLSR_E$, a diastolic measurement, may be an early predictive marker of CTRCD. Our study demonstrated a statistically significant reduction in $GLSR_E$ at 3 months. This appears to be the first study to suggest diastolic strain measurements, specifically $GLSR_E$, as early markers of cardiotoxicity in the cancer setting. Interestingly, both GLS and $GLSR_E$ at baseline were significantly lower in patients who developed CTRCD when compared to patients that did not develop CTRCD. This may suggest these patients had an increased susceptibility to VEGF inhibitor-mediated cardiotoxicity. Whether baseline STE can identify patients at greatest risk for developing CTRCD, requires further investigation.

The role of cardiac biomarkers in chemotherapy-mediated cardiotoxicity has been investigated extensively in recent years. The most studied biomarkers include cardiac troponin release following myocardial injury and natriuretic peptides associated with elevations in LV filling pressure.[37] Other markers that have previously been studied include those reflecting inflammation, endothelial dysfunction, and myocardial ischemia.[31,33]

In studies examining cardiac biomarkers in breast cancer patients receiving anthracyclines, serum rises in cardiac troponins have consistently been associated with cardiotoxicity.[32-33] Furthermore, Cardinale et al. has suggested that cardiac troponins may accurately identify patients less likely to recover from CTRCD, despite optimal treatment with ACE inhibition and beta blockade.[31] Unfortunately, in the setting of VEGF inhibitor therapies, the relationship between cardiac troponins and cardiotoxicity has been much less clear. Chen et al. demonstrated significant elevations in serum TnI in mice treated with BVZ.[41] Comparatively, a clinical study by Schmidinger et al. identified rises in serum TnT in 10% of patients receiving SNT for metastatic RCC. These findings, however, did not correlate with an increased risk of cardiotoxicity.[42] In our murine model, BVZ and SNT treated mice demonstrated a significant

increase in serum TnI at 14 days, confirming cardiotoxicity had occurred. Unfortunately, there were no early changes in serum TnI when it was measured at day 7.[27] Clearly, the utility of cardiac troponins in VEGF inhibitor-mediated cardiotoxicity requires further investigation.

Studies investigating the role of CRP in the breast cancer setting have yet to show any clear association between serum concentration and the risk of developing CTRCD.[18,33] In breast cancer patients receiving anthracyclines, Ky et al. demonstrated elevations in serum CRP at 3 months as compared to baseline. However, these elevations were not associated with an increased risk of cardiotoxicity.[33] More recent studies have suggested CRP as a potential predictive marker of cancer prognosis. In a study of metastatic CRC patients receiving conventional chemotherapy, increased serum CRP concentration was found to be an independent prognostic factor for reduced survival.[43] Similarly, Teishima et al. demonstrated that a decrease in CRP was associated with improved anti-tumor effects in metastatic RCC patients treated with molecular targeted therapy.[44] Our study is the first to investigate the role of CRP as an early marker of cardiotoxicity in metastatic CRC and RCC patients receiving VEGF inhibitor therapy.

In our clinical study, neither TnI nor CRP levels demonstrated an association with VEGF inhibitor-mediated cardiotoxicity. Furthermore, both markers remained within normal limits at all time points. These findings are in line with results from Fallah-Rad et al. who found that TnT and CRP did not change over 12 months or predict cardiotoxicity in breast cancer patients treated with trastuzumab.[18] The small number of patients (n = 3) who developed CTRCD in this study may explain for the lack of detecting significant changes in these cardiac biomarker levels.

Hypertension is recognized as a common adverse effect of VEGF inhibitors. Hypertension develops in as many as 47%, 49% and 40% of patients receiving BVZ, SNT, and PAZ, respectively.[7-9,13] In most cases, hypertension was observed within the first 4 weeks following initiation of therapy.[9] It has been suggested by Rixe et al. and others that the appearance of hypertension is associated with an improvement in treatment response.[9,45] The mechanism of hypertension related to anti-angiogenic therapy is not fully understood. However, the predominant theory suggests VEGF inhibition decreases endothelial NO production in arterial walls. As NO is a vasodilator, its decline would enhance vasoconstriction leading to an elevation in peripheral vascular resistance and mean arterial pressure.[8-9] Accordingly, we recently demonstrated that mice treated with BVZ or SNT had a >50% increase in mean arterial pressure at 14 days as compared to baseline.[27] In our current clinical study, 35% of patients receiving BVZ, SNT, or PAZ developed new or worsening hypertension. In most cases, the hypertensive changes occurred within 1 month of starting treatment. These findings are in line with previous studies investigating the incidence of hypertension from VEGF inhibitors.

All patients who developed worsening hypertension were receiving anti-hypertensive medications at baseline, and most (73%) had compensatory adjustments in management throughout treatment. Furthermore, at baseline, 28% and 30% of all patients were receiving angiotensin-converting enzyme inhibitors (ACEI) and beta blockers, respectively. These observations are noteworthy, given recent investigations examining the cardioprotective effects of these agents in CTRCD. While beta blockers and ACEI are commonly used in the treatment of established heart failure, their utility in the prevention of cardiotoxicity is currently a topic of interest.[46] It has been suggested that renin angiotensin system (RAS) antagonists, including ACEI, may prevent oxidative stress, apoptosis, and the development of heart failure in the setting of VEGF inhibitor-mediated cardiotoxicity.[27,46-47] We recently initiated a new basic science study examining the cardioprotective role of RAS antagonists in a murine model of BVZ and

SNT induced cardiotoxicity. Whether patients in our clinical study benefited from baseline ACEIs or beta blockers necessitates further investigation.

There were a number of limitations to this study which must be acknowledged. In our study population, only 3 (8%) patients receiving VEGF inhibitors developed LV systolic dysfunction. This makes it challenging to draw definitive conclusions about early indices of cardiotoxicity in this setting with a small population. Future studies with a larger patient population investigating VEGF inhibitor-mediated cardiotoxicity should be investigated to more accurately evaluate early indices of CTRCD. Furthermore, CTRCD, developed rather quickly in this study, with decreases in LVEF occurring as early as 3 months into therapy. As such, only two time points (baseline and 1 month) were able to be completed before a reduction in LVEF was detected. It may be beneficial to increase the number of follow up visits prior to 3 months, so that early indices of LV dysfunction may be more accurately delineated. Many patients were receiving ACEIs and beta blockers throughout their cancer treatment. As these medications show some promise as cardioprotective agents, it is possible they influenced the frequency of CTRCD in this study. We are currently performing an animal study examining the cardioprotective role of RAS inhibitors in a murine model of BVZ and SNT mediated cardiotoxicity.

CONCLUSION

In metastatic CRC and RCC patients, we demonstrated that VEGF inhibitors, including BVZ, SNT, and PAZ, are associated with adverse cardiovascular effects including LV systolic dysfunction and hypertension. Our data suggests that systolic and diastolic indices of strain and strain rate may be more sensitive markers of early LV dysfunction when assessed with STE. It is our goal that the application of these screening techniques will allow physicians to prevent CTRCD by way of treatment modifications and/or administration of cardioprotective agents.

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Characteristics	Entire Cohort (n=40)	CTRCD (n=3)	No CTRCD (n=37)	p Value
Age (yrs)	63 ± 9	63 ± 9	63 ± 8	0.87
Males	34 (85%)	3 (100%)	31 (84%)	<0.05
CV risk factors				
Hypertension	27 (68%)	3 (100%)	24 (65%)	<0.05
Diabetes	5 (13%)	0 (0%)	5 (13%)	1.0
Dyslipidemia	26 (54%)	2 (67%)	24 (65%)	0.85
Smoking	18 (45%)	1 (33%)	17 (46%)	0.63
Known CAD	2 (5%)	1 (33%)	1 (3%)	<0.05
BMI (kg/m^2)	29 ± 5	30 ± 6	29 ± 6	0.79
SBP (mm Hg)	132 ± 16	135 ± 15	132 ± 16	0.65
DBP (mm Hg)	80 ± 12	77 ± 3	80 ± 12	0.71
Heart rate (bpm)	73 ± 14	87 ± 9	72 ± 14	0.45
CV medications				
ACEI	11 (28%)	1 (33%)	10 (27%)	0.83
Beta blocker	12 (30%)	0 (0%)	12 (32%)	1.0
Type of cancer				
mCRC	6 (15%)	1 (33%)	5 (14%)	<0.05
mRCC	34 (85%)	2 (67%)	32 (86%)	<0.05
Cancer treatment				
BVZ	6 (15%)	1 (33%)	5 (14%)	<0.05
SNT	26 (65%)	0 (0%)	26 (70%)	1.0
PAZ	8 (20%)	2 (67%)	6 (16%)	<0.05
Chest radiation	6 (15%)	2 (67%)	5 (18%)	<0.05
Prior anthracycline	0 (0%)	0 (0%)	0 (0%)	1.00

Table 1: Baseline characteristics of cancer patients treated with VEGF inhibitors. Values are mean ± SD or number (percentage). Continuous data reported as mean (SD). *CTRCD, cancer therapeutic related cardiac dysfunction; CV,* cardiovascular; *CAD,* coronary artery disease; *BMI,* body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *ACEI,* angiotensin-converting-enzyme inhibitor; m*CRC*, metastatic colorectal cancer; m*RCC*, metastatic renal cell carcinoma; *BVZ*, bevacizumab; *SNT*, sunitinib; *PAZ*, pazopanib. p value <0.05 comparing CRTCD vs. no CRTCD.

Baseline Parameter	CTRCD (n=3)	No CTRCD (n=37)	p Value
Conventional echocardiography			
LVEF (%)	62 ± 2	63 ± 4	0.489
LAVI (mL/m^2)	20 ± 3	28 ± 5	**0.033**
E/A	0.86 ± 0.30	0.97 ± 0.28	0.589
e' (cm/s)	0.077 ± 0.015	0.078 ± 0.019	0.979
E/e'	8.5 ± 1.2	8.9 ± 2.4	0.678
2D speckle tracking			
GLS (%)	-14.7 ± 2.7	-17.9 ± 1.7	**0.048**
GLSR (1/s)	-0.86 ± 0.19	-1.01 ± 0.19	0.194
$GLSR_E$ (1/s)	0.74 ± 0.04	0.91 ± 0.17	**0.048**

Table 2. Baseline echocardiographic indices in patients with and without VEGF inhibitor mediated cancer therapeutic-related cardiac dysfunction (CTRCD). Values are mean ± SE. *LVEF*, left ventricular ejection fraction; *LAVI,* left atrial volume indexed to body surface area; *E/A,* peak early filling to late diastolic filling velocities ratio; *e'*, early diastolic annular velocity at septal mitral annulus; *E/e',* mitral inflow E velocity to tissue Doppler e' ratio; *GLS,* global longitudinal peak systolic strain; *GLSR*, global longitudinal peak systolic strain rate; $GLSR_E$, global longitudinal strain rate early diastole.

Echocardiographic Predictor at 1 Month	CTRCD (n=37)	No CTRCD (n=3)	p Value
LVEF (%)	62 ± 3	60 ± 1.2	0.65
GLS (%)	-18.1 ± 1.5	-12.8 ± 1.54	**<0.05**
$GLSR_E$ (1/s)	0.88 ± 0.02	0.51 ± 0.07	**<0.05**

Table 3. Univariate analysis of predictors of cardiotoxicity. Values are mean ± SE. *LVEF*, left ventricular ejection fraction; *GLS*, global longitudinal strain; $GLSR_E$, global longitudinal strain rate early diastole.

Cardiac Biomarker	CTRCD (n=3)	No CTRCD (n=37)	p Value
hsTnI (ng/L)			
Baseline	15 ± 1.3	14 ± 1.4	0.98
3 months	20 ± 1.4	17 ± 1.5	0.86
6 months	18 ± 1.2	15 ± 1.5	0.76
CRP (mg/L)			
Baseline	4.6 ± 1.1	4.4 ± 1.1	0.89
3 months	5.1 ± 1.3	4.6 ± 1.2	0.78
6 months	4.9 ± 1.2	4.5 ± 1.3	0.76

Table 4. Summary of serial cardiac biomarker results. Values are mean ± SE. *hsTnI*, high sensitivity troponin I (normal reference: <16 ng/L for females, <34 ng/L for males); *CRP,* C-reactive protein (normal reference: <8 mg/L).

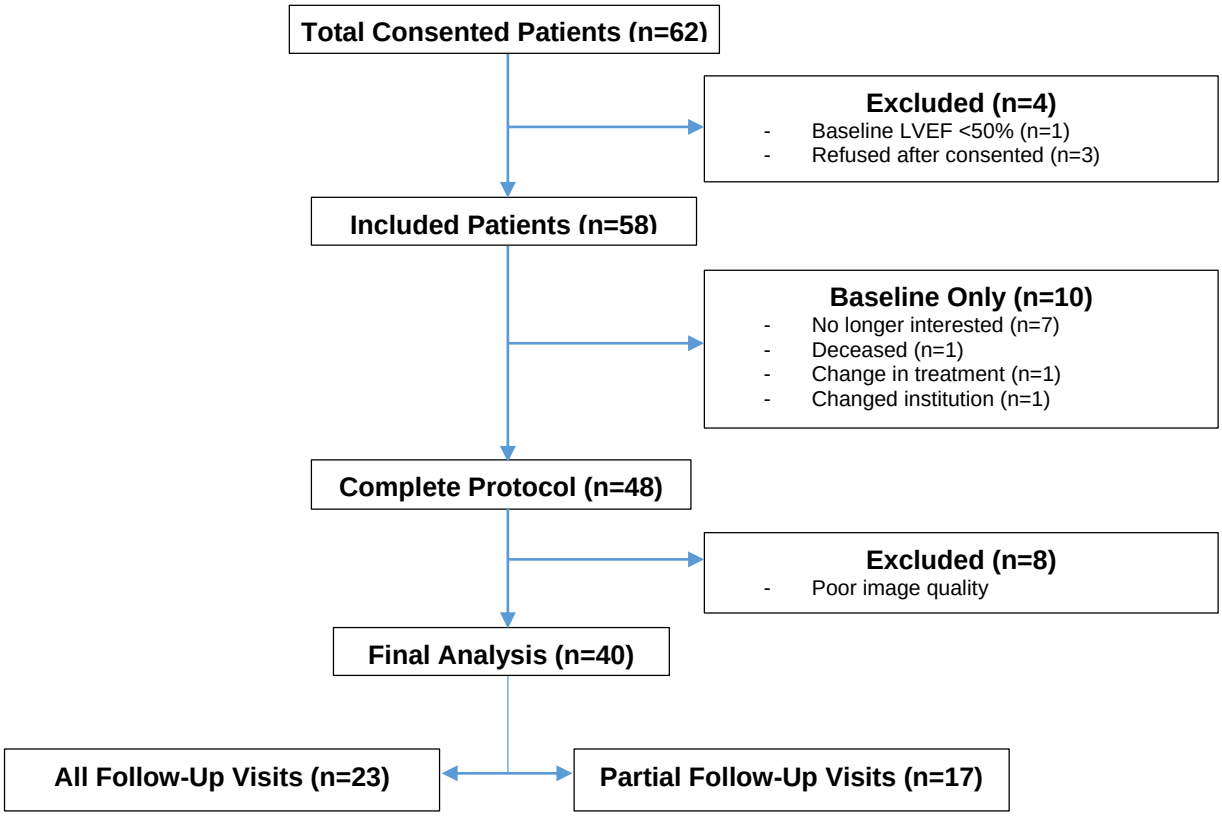


Figure 1: Clinical study flow chart.

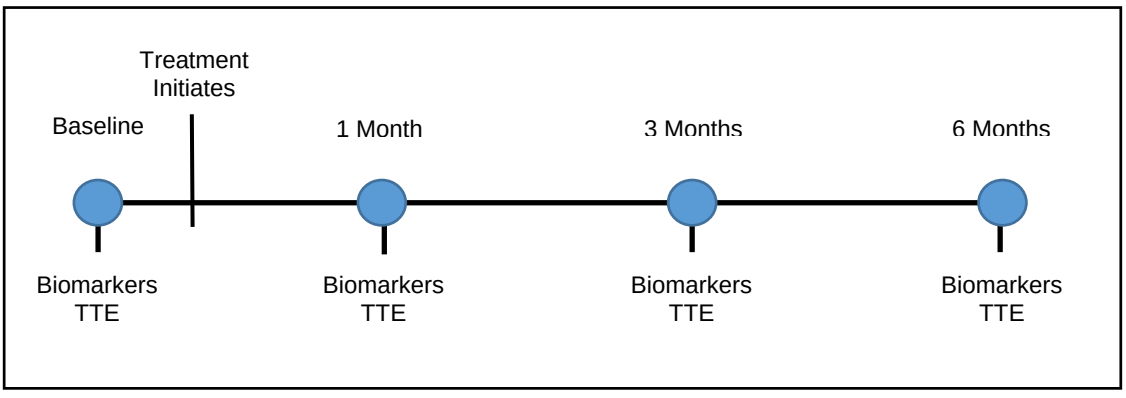


Figure 2: Clinical protocol: A total of 62 patients were prospectively enrolled at two tertiary care centres between 2013 and 2015 inclusive. Patients were studied at 4 time points: i) Baseline; ii) 1 month; iii) 3 months; and iv) 6 months after the initiation VEGF inhibitor therapy. At each visit a TTE was performed and blood taken to evaluate serum levels of cardiac biomarkers. In addition, blood pressure was measured and patients were interviewed for cardiovascular symptoms (dyspnea, peripheral edema, angina, syncope, palpitations).

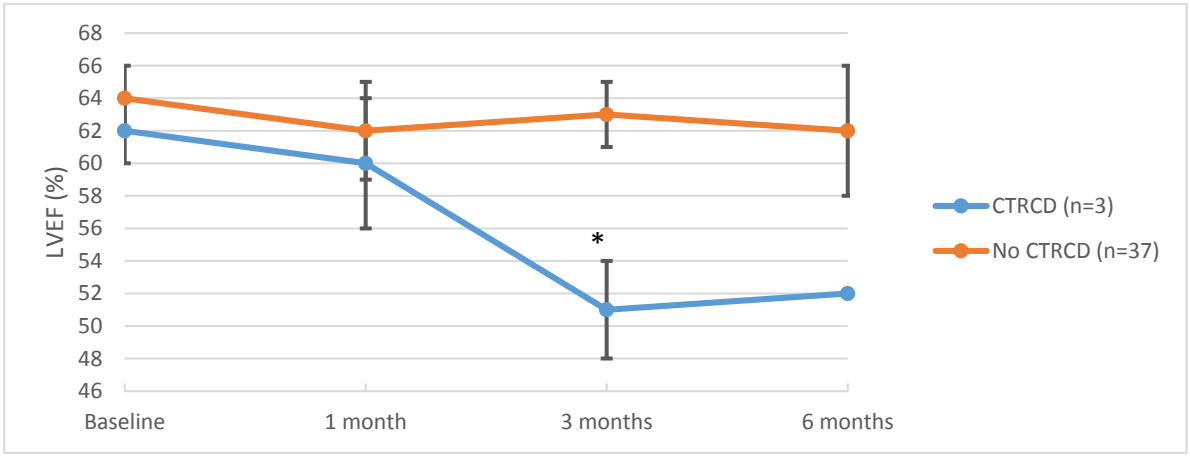
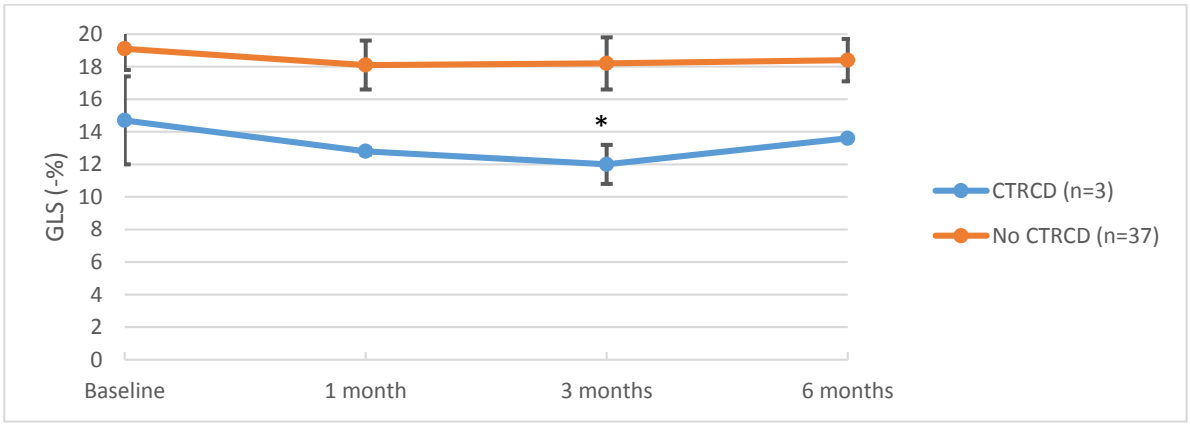


Figure 3. Temporal changes in LVEF for patients with and without VEGF inhibitor-mediated CTRCD. Error bars represent ± SD. *LVEF,* left ventricular ejection fraction. * p<0.05 CTRCD compared to baseline.

A.



B.

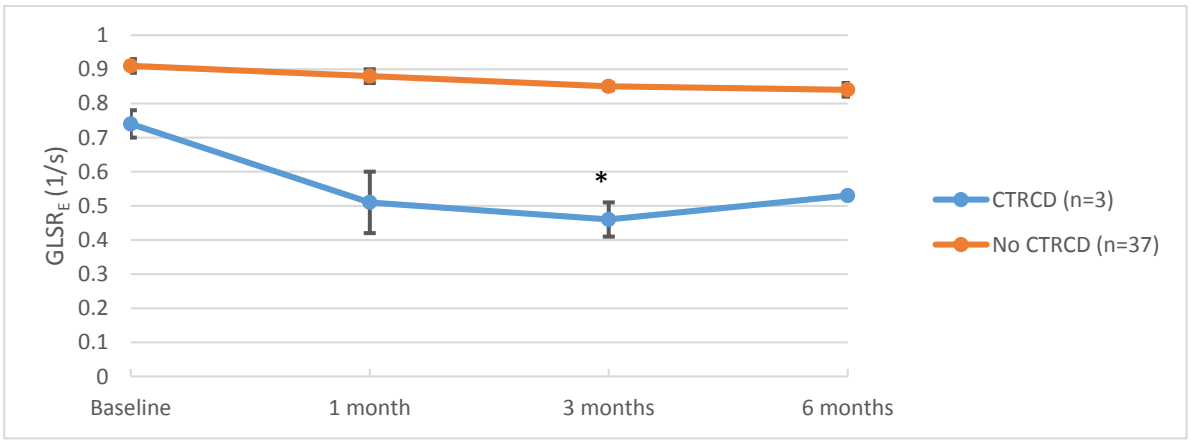


Figure 4. Temporal changes in GLS and $GLSR_E$ for patients with and without VEGF inhibitor-mediated CTRCD. Error bars represent ± SD. *GLS,* global longitudinal peak systolic strain; *$GLSR_E$,* global longitudinal strain rate early diastole. * p<0.05 CTRCD compared to baseline.