Title: Post-thrombotic Syndrome and Predictors of Recurrence in Catheter-Related Thrombosis

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Post-thrombotic Syndrome and Predictors of Recurrence in Catheter-Related Thrombosis

Protocol Version 10.0

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1. Background and Significance

Over 5 million central venous catheters (CVCs) are inserted annually in the United States.[1] CVCs improve quality of life in cancer patients by allowing safe administration of chemotherapy and easy access for phlebotomy. Despite these benefits, long-term presence of CVCs are associated with infectious and thrombotic risks.[1] The incidence of catheter-related thrombosis (CRT), or thrombosis in the vein housing the catheter, will occur in up to a third of patients with an indwelling catheter.[2] CRT account for approximately 80% of upper extremity thrombosis, and pulmonary emboli have been detected in 15% of patients with symptomatic CRT.[3,4] A systematic review and meta-analysis has reported rates of post-thrombotic syndrome (PTS) in an average of 15% of adults with upper extremity thrombosis. Retrospective studies of patients with upper extremity thrombosis report PTS rates between 7-47% and prospective studies report PTS rates of 15-25%. These studies contain a limited number of patients with catheter-related thrombosis.[5] Presence of a catheter as the etiology of the upper extremity thrombosis is suggested to decrease the risk of PTS, but the outcomes of patients with catheter-related thrombotic events alone have not been well defined. Residual thrombosis after treatment and thrombosis of the axillary and/or subclavian vein increase the risk of PTS development[5]; however, no prospective studies have evaluated if duration of anticoagulation in CRT influences the development of PTS. Patients with PTS have significantly worse quality of life and limb function;[6] thus PTS is a patient important outcome to evaluate.

Anticoagulant treatment of CRT is used to improve patient symptoms and decrease recurrent thrombosis and pulmonary embolism. Low molecular weight heparin (LMWH, enoxaparin) has been the anticoagulant of choice for cancer patients with catheter-related thrombosis.[7] Enoxaparin is the only LMWH FDA approved for treatment of acute venous thrombosis (enoxaparin product insert attached). However, in recent years, direct oral anticoagulants such as dabigatran, rivaroxaban, apixaban, and edoxaban have offered a more convenient alternative for anticoagulation. A randomized control trial and prospective cohort study of cancer patients with venous thromboembolism treated with a direct oral anticoagulant have been published.[8] Two small studies have also been published in using rivaroxaban to treat CRT in cancer patients.[9,10] Therefore direct oral anticoagulants have become part of clinical practice to treat cancer patients with thrombosis in addition to LMWH. The FDA package inserts can be found at the follow websites:

Rivaroxaban (Xarelto®): http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/202439s001lbl.pdf
Apixaban (Eliquis®): http://www.accessdata.fda.gov/drugsatfda_docs/label

/2012/202155s000lbl.pdf
Edoxaban (Savaysa®): http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/206316lbl.pdf
Dabigatran (Pradaxa®): http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/022512s007lbl.pdf

The catheter is removed if it is non-functioning or is no longer needed.[7] The duration of anticoagulation in patients with catheter-related thrombosis has not been studied in a systematic fashion and is not standardized in clinical practice. A delicate balance exists between thrombosis and bleeding in cancer patients because cancer patients have 3 times higher risk of recurrent thrombosis and 2.5 times higher risk of major hemorrhage compared to non-cancer patients.[11] Consensus guidelines have a weak recommendation for anticoagulation for 3 months after catheter removal in patients with upper extremity CRT (ACCP and ISTH guidelines attached).[12,13] These recommendations are extrapolated from data on provoked lower extremity thrombosis, retrospective case series and two prospective cohort studies.[12,13] Review of the literature reports treatment of catheter-related thrombosis ranging from catheter removal without anticoagulation to treatment with anticoagulation up to 6 months.[14-16] In cancer patients, upper extremity CRT are often treated with anticoagulation for 4-6 weeks to balance the risks of thrombotic recurrence with bleeding. Additionally, extraction of the catheter removes an inciting factor for thrombosis which may allow for shorter duration of therapy. In a survey of MCW Heme/Onc physicians, treatment of patients with CRT ranged from 4 weeks to 3 months. A retrospective review of patients with hematologic malignancies from 2009-2011 found 85 patients with catheter-related thrombosis treated at MCW. Three patients (4%) were not treated for their CRT and 20 patients (24%) had the catheter removed only. Of the patients that underwent anticoagulation, the median total treatment duration was 42 days (range 3-1485 days). Of patients who had their catheter removed, the median treatment duration after catheter removal was 44 days (range 4-453 days). The retrospective study was not able to assess development of PTS due to lack of assessment as a part of routine practice. Overall, there is no standard treatment duration for patients with catheter-related thrombosis and 1 month of anticoagulation after catheter removal is within clinical practice at both locations. This multicenter prospective cohort pilot study project defines the duration of anticoagulation after catheter removal at one month to balance the risk of thrombosis with the risk of bleeding, patient cost and discomfort of injecting enoxaparin twice a day. The project will assess feasibility and obtain estimates of clinical outcomes including PTS, thrombosis recurrence, and bleeding. The feasibility outcomes will assess accrual, enrollment, and retention procedures. The data obtained from the pilot study will be used to provide sample size estimates for a larger definitive study. Additionally the

estimates will allow an appropriate comparison of duration of anticoagulation to be selected for a future randomized trial.

Table 1: Care of patients with CRT in practice vs. research protocol

	Typical Care Approach	Research protocol
Catheter removal	Not needed or malfunction	Not needed or malfunction
Anticoagulation Type	LMWH (enoxaparin or dalteparin) Direct oral anticoagulant	Enoxaparin or direct oral anticoagulant
Duration of anticoagulation	1-3 months after catheter removal	1 month after catheter removal

A clinical scoring system or biomarker to predict recurrent catheter-related thrombosis in cancer patients do not exist. Currently patients are treated with anticoagulation for varying amounts of time based on local practice and extrapolation from clinical trials in lower extremity thrombosis. Development of biomarkers predictive of thrombosis would allow targeting of anticoagulation strategies in cancer patients at highest risk of thrombosis. The Ottawa score allows prediction of recurrent thrombosis in cancer patients and is based upon gender, history of thrombosis, cancer type and stage.[17,18] The Khorana score integrates site of cancer, body mass index, and pre-chemotherapy platelet, leukocyte and hemoglobin levels to predict initial thrombosis.[19] The Khorana score has not been evaluated as a predictor of thrombotic recurrence and will be assessed from clinical information at the time of CRT diagnosis. Validations of the Khorana score have also added biomarkers including D-dimer to expand the ability to detect high risk groups.[20] Additionally, high levels of thrombin generation have been associated with increased risk of initial thrombosis in cancer patients.[21] Elevated D-dimer and peak thrombin generation have been shown to be risk factors for recurrent thrombosis in non-cancer patients, but the association has not been evaluated in cancer patients.[22,23] Tissue Factor Pathway Inhibitor (TFPI) functions to limit the activation of factor X by the tissue factor/Factor VIIa complex. TFPI has been shown to be a predictor of thrombosis with low levels of TFPI antigen associated with twice the risk of thrombosis.[24] Platelet TFPI may also play a role in thrombotic risk as platelets accumulate at the site of injury. Platelet TFPI has not been assessed as a risk factor for thrombosis. Lastly, extracellular DNA released from neutrophils (NETs-neutrophil

extracellular traps) has been shown to form a scaffold for venous thrombosis in animal models. Circulating plasma free DNA is a marker for NET formation and will be assessed in our patients.[25] Overall, the proposed project will evaluate novel assays (platelet TFPI, plasma free DNA, and thrombin generation with platelet-rich plasma) and apply techniques that predict incident thrombosis (Khorana score, D-dimer and thrombin generation with platelet-poor plasma) to predict the risk of recurrent thrombosis. Overall, identification of a biomarker to predict thrombosis would allow a personalized approach to anticoagulation that would avoid unnecessary bleeding risk, cost and lifestyle burdens in patients who do not require more prolonged courses of anticoagulation.

2. Study Objectives

2.1 Feasibility Objectives:

1) To determine the feasibility of a multicenter prospective cohort study of cancer patients with catheter-related thrombosis treated with 1 month of anticoagulation.

    a. Feasibility will be defined as accrual of 56 patients in 1 year and 80% completion of PTS assessments

2) To determine the feasibility of obtaining plasma samples for biomarker analysis to predict recurrent venous thrombosis

    a. Feasibility will be defined as obtaining 80% of plasma samples for biomarker analysis

2.2 Clinical Objectives:

3) To determine the incidence of post-thrombotic syndrome, thrombotic recurrence and bleeding in cancer patients with catheter-related thrombosis treated with 1 month of anticoagulation

    a. Success of the study will be defined as an incidence of post-thrombotic syndrome <20%.

3. Study Population

The intended study population is any adult with cancer and catheter-related thrombosis. Children will not be included as the involved institutions do not care for children. No gender or ethnic group will be excluded or targeted in the study.

3.1 Study Sites

1) BloodCenter of Wisconsin, Medical College of Wisconsin (MCW) and Froedtert Hospital, Milwaukee, Wisconsin

   The primary study site will be in Milwaukee. Patients will be recruited from MCW/Froedtert Hospital. Laboratory testing will be completed at the BloodCenter of Wisconsin in the laboratory of Dr. Alan Mast.

2) Ohio State University, Wexner Medical Center, Columbus, Ohio

   Ohio State University (OSU), Wexner Medical Center, will be a second site for the study. Patients from OSU will be followed with clinical assessments only.

3.2 Inclusion Criteria:

1. Upper extremity venous thrombosis involving the axillary, subclavian, brachiocephalic, superior vena cava, and/or internal jugular veins associated with an indwelling catheter documented by ultrasound, CT or venography
2. Current hematologic or solid tumor malignancy undergoing chemotherapy, surgery, radiation or hormonal therapy for malignancy and bone marrow transplant if within 100 days of transplant
3. ≥18 years of age
4. Platelet count >50,000
5. Creatinine clearance >30 ml/min
6. Ability to provide informed consent

3.3 Exclusion Criteria:

1. Underlying medical condition or chemotherapy requiring long-term anticoagulation
2. Known underlying higher risk thrombophilias including antiphospholipid antibody syndrome, antithrombin, protein C or protein S deficiencies, or homozygosity or compound heterozygosity for prothrombin G20210A or Factor V R506Q mutations.
3. Inability to remove venous catheter
4. Anticipated replacement of central venous catheter within 3 months after enoxaparin or direct oral anticoagulant therapy has been discontinued
5. Personal history of deep vein thrombosis or pulmonary embolism
6. Concurrent acute deep vein thrombosis of the leg or pulmonary embolism
7. Major bleeding or clinically relevant non-major bleeding in the preceding 60 days
8. Participation in another clinical trial that requires anticoagulation
9. Treatment with thrombolysis
10. Catheter removal >1 month prior to enrollment

11. Use of anticoagulation >6 months prior to catheter removal

3.4 Study Discontinuation Criteria

Subjects may be removed from the study by the Investigator for any of the following reasons:

1. Subject's condition has changed after entering the study so that they no longer meet inclusion criteria, or develop any of the exclusion criteria.
2. Subject experiences an adverse event that warrants withdrawal from the study.
3. Principal investigator determines subject is non-compliant.
4. Subject requires placement of an alternate central venous catheter after discontinuation of enoxaparin or direct anticoagulant therapy.

3.5 Continuation of anticoagulation criteria

Participants will be continued on anticoagulation longer than one month after catheter removal if any of the following criteria exist:

1. Development of thrombosis other than the CRT
2. Extension of the catheter-related thrombosis (i.e. additional vein occlusion on imaging obtained for clinical reasons)

If an enrolled subject at MCW requires anticoagulation longer than 1 month after catheter removal, laboratory assessments will not be completed as the testing can be affected by the presence of an anticoagulant. The participant will not be removed from the study and will be followed for PTS outcomes.

4. Study Design

4.1 Study Overview

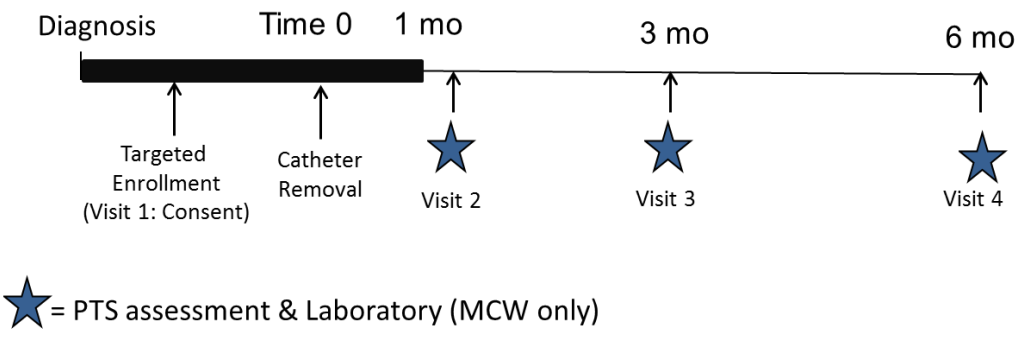

Figure 1: Study diagram. Thick boxes denote duration of anticoagulation. Stars indicate assessment for post-thrombotic syndrome & laboratory measurements (MCW only). mo=months

Figure 1 shows the overall study design of the multicenter prospective cohort of cancer patients with catheter-related thrombosis. Participants may be referred for screening at MCW in two possible mechanisms: participant contact study staff from information on CTSI FACT website or referral from treating Hematologist or Oncologist. At MCW, an EPIC report will be generated to identify people with CRT. The treating provider would then be contacted to determine if referral to the study was appropriate. Subjects at OSU will be referred from the treating Hematologist or Oncologist. A report generated in Epic will be used to identify people with CRT. The treating provider would then be contacted to determine if referral to the study was appropriate. Participants will be given printed information about the study (Appendix A) or verbal explanation using the informational flyer. Verbal consent will be obtained to be screened for participation and contacted by study personnel. Screening will be completed by BCW nurse coordinator (Script Appendix B; Screening form Appendix C). We aim to consent and enroll patients after diagnosis of catheter-related thrombosis and prior to catheter removal. Consent and enrollment (Visit 1) must occur prior to discontinuation of anticoagulation. Enrollment will be limited to patients undergoing anticoagulation with either enoxaparin or a direct oral anticoagulant at the discretion of the treating provider. The study will follow patients treated with anticoagulation for one month after catheter removal. Prior to discontinuation of anticoagulation, patients will be contacted via telephone to review study protocol, assess for bleeding, and arrange for Visit 2 in the following 1-2 weeks. Appendices D-H include the background information that will be collected. Visits 3 and 4 will occur 3 and 6 months after catheter removal, respectively. At visits 3 & 4, Medical history, cancer history, and medications will be updated (Appendices E-G). At each visit, participants will be assessed for post-thrombotic

syndrome using the modified Villalta scale (Appendix I) and functional limitation using the DASH questionnaire (Appendix J). At MCW, surveys will be administered by the TRU staff and physical examination will be completed by BCW nurse coordinator, Zora Jovanovic. At OSU, surveys will be administered by research coordinators trained to complete the assessment. Laboratory samples will be obtained for MCW participants only and processed as noted in section5.6 and section 6.

4.2 Selection of Subjects

Subjects will be recruited at MCW and OSU through referral from their treating Hematologist or Oncologist. The patient's treating physician will give potential participants the informational flyer (Appendix A) or use the flyer to verbally describe the study and receive permission for the patient to be screened for eligibility and contacted by the research coordinator. Additionally at Milwaukee and Columbus, an electronic alert will be created that identifies a person with catheter-related thrombosis through the results of their radiology report. The research coordinator will review the report and then contact the treating physician to inform them of the study and receive permission to contact the subject. In 2012, 2350 new patients were seen in the Heme/Onc and BMT clinics at Froedtert Hospital and 1003 had a Port-A-Cath placed. Estimating a catheter-associated thrombotic rate of 15%,[5,26] this predicts approximately 150 patients annually with CRT due to a Port-A-Cath. Additional patients are anticipated due to CRT from peripherally inserted central catheters (PICC). A survey was conducted among the Heme/Onc and BMT physicians at MCW with a 60% response rate (21/35). The physicians estimated that 63 patients had been seen with CRT in the last 3 months (252 annual). 86% of physicians reported that they would refer patients to a study of CRT treated with 4 weeks of anticoagulation; 14% were uncertain. MCW physicians also noted a variety of treatment practice from 4 weeks to 3 months of anticoagulation after catheter removal. We estimate a 10% drop out rate due to reinsertion of a catheter or inability to remove a catheter. We also estimate that we will need to screen and approach 3 patients for every participant. Therefore, we will plan to screen and approach 250 patients to enroll 56 patients (36 BCW and, OSU 20,) and have 50 complete the study as the overall target.

4.3 Sample Size Estimates

Sample size estimates were based on the confidence interval method and were based upon the feasibility endpoints. With 50 patients completing the study, we would have 95% confidence that completion of 80% of PTS assessments in the pilot study would correspond to 66-90% completion in another study. Completion of 90% of assessments

would correspond to between 78-96% of assessments. We estimate a 10% drop-out rate so will plan to enroll 56 patients over 1 year.

Evaluation of 50 patients would provide 95% confidence that if the feasibility study found a PTS incidence of 10% that the true proportion in a larger study would not be greater than 20%.

5. Clinical Procedures

5.1 Enrollment and Consent

We aim to consent and enroll participants after diagnosis of catheter-related thrombosis and prior to catheter removal. Participants must be consented and enrolled prior to anticoagulation discontinuation. Screening will be completed using Appendix B (Script Appendix B; Screening form Appendix C). Medical history, family history, and medications will be reviewed after consent is obtained (Appendices D-H).Consent for medical record release will be obtained to determine cancer staging and laboratory information from the time of CRT diagnosis.

5.2 Anticoagulation

Participants will be treated with either enoxaparin for one month following catheter removal, or direct oral anticoagulant treatment (apixaban, rivaroxaban, dabigatran, or edoxaban). Dosing an alternate enoxaparin dose or interval based on anti-factor Xa testing that was obtained by clinical team, at standard dosing per FDA package insert. Anticoagulation will be continued for 1 month after catheter removal. The minimum duration of anticoagulation will be one month and maximum duration is 7 months. The cost of enoxaparin or direct oral anticoagulant treatment will be the responsibility of the participant as use of the medication is part of routine care. Subjects will be contacted by telephone prior to discontinuation of anticoagulation to review if any continuation rules apply and arrange for research visit 2.

5.3 Concomitant medications

The decision of which anticoagulant to prescribe for the thrombotic event will be made by the patient's physician per standard of care. The patient's prescribing physician will be responsible for assessing the potential drug interactions between anticoagulant therapy and other medications as he or she would in their normal medical practice. For example, direct oral anticoagulant therapy medications have drug interactions with CYP3A4 inhibitors and inducers. Use of anti-platelet agents will be allowed and recorded. We anticipate the use of antiplatelet agents to be low because of the risk of thrombocytopenia associated with many chemotherapeutic agents.

5.4 Catheter removal

The timing and mechanism of catheter or port removal will be directed by the subjects' treating physician. The practitioner type (ex. MD Interventional Radiologist vs RN PICC

Team) will be recorded. Additionally, the reason for catheter removal will be recorded. In a patient that requires catheter or port re-insertion while on enoxaparin or direct oral coagulant therapy, the enoxaparin or direct oral coagulant therapy will be continued for 30 days following removal of all catheters or ports provided the time of anticoagulation does not exceed 6 months duration. If a patient requires re-insertion of a catheter or port after discontinuation of enoxaparin or direct oral anticoagulant therapy, they will be removed from the study at that time because a new catheter would be an additional thrombotic inciting factor.

5.5 Post-thrombotic syndrome assessments

Visit 2 will occur within 2 weeks of discontinuation of anticoagulation. Visit 3 will be at 3 months after catheter removal plus or minus 2 weeks and Visit 4 within 6 months plus or minus 2 weeks after catheter removal. At each visit, medical history and medication will be reviewed (Appendices E-G). The subjective assessment of the modified Villalta (page 1 and 2 of Appendix I) and the DASH questionnaire (Appendix J) will be administered by the Translational Research Unit (TRU) staff or the clinical nurse coordinator after completing the objective assessment. The objective assessment of the modified Villalta score will be completed by trained clinical nurse coordinators blinded to the subjective assessment (page 3 and 4 of Appendix I). The subjective assessment of the modified Villalta (page 1 and 2 of Appendix I) and the DASH questionnaire (Appendix J), will be administered by the research coordinator at OSU.

5.6 Blood measurements

Blood will be collected by venipuncture using a 20G needle or larger from patients at the BCW site at Visit 2, 3 and 4. A 21G needle may be used for collection of blood via venipuncture if blood cannot be obtained with a 20G needle or larger. The first 2 ml of blood will be discarded due to tissue factor activation with the venipuncture. Three BD Vacutainer citrated tubes (0.105M sodium citrate) with 4.5 ml blood will be collected. Thrombin generation, D-dimer, plasma total TFPI and TFPIα, and platelet TFPIα, will be assayed using platelet-poor plasma (PPP). Thrombin generation will also be assayed using platelet rich plasma (PRP). Laboratory procedures as noted in section 6.

Table 2. Study Schedule of Procedures

Procedure	Screening	Visit 1 (Consent)	Visit 2	Visit 3	Visit 4
Review Eligibility criteria	X	X			
Consent for research participation		X			
Documentation-medical history, medications, &		X	X	X	X

clinical data					
Physical Examination			**X**	**X**	**X**
PTS Assessment: modified Villalta, DASH			**X**	**X**	**X**
Blood draw- D-dimer, thrombin generation, TFPI, plasma free DNA (MCW Only)			**X**	**X**	**X**

6. Laboratory Procedures

6.1 Plasma preparation

Tube 1 of citrated blood will be used to prepare PRP and tubes 2 and 3 of citrated blood will be used to prepare PPP. Tube 1 will be centrifuged at 130 g at room temperature for 10 minutes within 30 minutes of blood collection.[27] After centrifugation, the PRP will be pipetted off without disturbance of the white blood cell layer (buffy coat). 200 ul of PRP will be used to assess the platelet count via a Coulter counter. Approximately half of the PRP will be placed into centrifuge tubes for preparation of PPP by centrifugation at 2000g for an additional 10 minutes. Tubes 2 and 3 will undergo centrifugation at 2000g for 10 minutes and centrifugation at 14,000 g for 5 minutes at room temperature.[27] PPP will be obtained from the upper half volume of plasma supernatant. PPP will be used to adjust the platelet count of the PRP to 150,000/uL. The unused PPP will be frozen to allow batched processing of thrombin generation, D-dimer, plasma free DNA and TFPI testing.

6.2 Thrombin Generation

Thrombin generation will be measured through a calibrated automated thrombogram system (CAT, Thrombinoscope bv, Maastricht, The Netherlands) with and without tissue factor activation using both PRP and PPP. Each well of the 96-well plate will contain 20 ul of reagent (PPP-Reagent or PRP-Reagent), 20 ul of thrombin calibrator, and 80 ul of plasma. Plasma samples will be measured in duplicate.

6.3 D-Dimer

The Stago Asserachrom® D-Di ELISA assay will be used to determine D-dimer levels. The Asserachrome ELISA has similar sensitivity and specificity as other D-dimer assays in clinical use.[28] Batched laboratory testing will prevent clinical decisions from being altered by the D-dimer results.

6.4 TFPI Assay

Levels of platelet TFPI (TFPIα), plasma total TFPI and TFPIα will be assessed through TFPI AlphaLISA assays. AlphaLISAs are a washless, bead based assay system. When the two beads are brought into close proximity by antigen binding, a singlet oxygen is released from the donor bead to the acceptor bead, resulting in light emission. Two AlphaLISAs are established in the lab to measure TFPI, one for total TFPI (full length TFPIα, truncated TFPIα, and TFPIβ) and one specifically for TFPIα. Both assays use a common acceptor bead to which mouse anti-human TFPI antibody (directed toward the second Kunitz domain) is conjugated. The total TFPI AlphaLISA uses biotinylated 2H8 (directed toward the first Kunitz domain) to detect all forms of TFPI, while the TFPIα AlphaLISA uses a biotinylated anti-K3 antibody to detect only TFPIα. A streptavidin-conjugated donor bead is added to bind these antibodies. Each well of the 96-well plate will contain 5ul of plasma and 20 ul mixture of anti-K2 conjugated acceptor bead and either the biotinylated 2H8 to measure total TFPI or biotinylated anti-K3 to detect TFPIα. Plasma samples will be measured in duplicate.

6.5 Plasma Free DNA

Levels of plasma free DNA will be assessed using the Quant-iT PicoGreen dsDNA kit and the Sytox Green fluorescent dye.[25] The Victor microplate reader will be used to determine the fluorescence omitted from the samples. The DNA concentration will be determined through comparison to a standard curve.

7. Subject Remuneration

For their time and efforts in study activities, research subjects will receive $25 for each of the following patient visits:

- Study visit 2, 1 month after catheter removal
- Study visit 3, 3 months after catheter removal
- Study visit 4, 6 month after catheter removal

A total of $75 will be paid to subjects completing all study visits to cover travel to the research site, parking, and time of the visits. The subject will be paid $25 for each completed study visit whether they withdraw, the investigator withdraws them or they complete all study activities. Payment will occur when they are no longer an active participant in the study. Remuneration for study visits will only take place at the BCW site.

7.1 Potential benefit to subjects

Our protocol will treat participants with anticoagulation for 1 month after catheter removal. There are no definite direct benefits to subject participation. Because the

duration of anticoagulation varies in clinical practice, treatment with one month of anticoagulation could have lower risk of hemorrhage in comparison to 3 or 6 months of anticoagulation. Additionally, the cost and discomfort associated with use of injected anticoagulant could be lower. The study may also benefit others with CRT if the study is able to identify biomarkers that will predict thrombosis to allow a tailored approach to treatment of CRT.

8. Human Subjects Protection

8.1 Informed Consent:

Subjects are required to sign an informed consent prior to enrollment, and before undergoing any study procedures or assessments. When substantial modifications are made to the informed consent, the IRB may require that all subjects currently enrolled in the study will be re-consented.

Subjects will be provided with a copy of the signed informed consent form used in the study, procedures, and assessments. Subjects will also be provided with the contact telephone numbers of the investigator and qualified personnel who can assist with their questions and concerns.

8.2 Protected Health Information

Protected health information that will be collected as part of this study includes name, date of birth, date of catheter placement, date of thrombosis diagnosis, date of bleeding event, and date of thrombosis recurrence. This information will be stored for 10 years beyond the completion of the study. After blood is used for study testing it will be discarded.

8.3 Confidentiality

Loss of confidentiality is always a potential risk when collecting protected health information. Research records will remain confidential. Subject's records will be available to the study staff and to each site's IRB and for audit purposes. Only authorized personnel will have access to the protected health information and research records. The investigators, clinical research nurses, and TRU nurses will have access to the patient identifying code. In order that confidentiality can be maintained, the PI/study staff will keep records in locked cabinets/room and results of tests will be coded to prevent association with volunteers' names. All electronic data will be entered into a secured, website data management system, REDCap, that requires training and is password protected. Identifying information will not be included on laboratory samples,

faxes, or correspondence. Laboratory samples and printed surveys will only have participant number and not identifying information.

All study team members are trained in HIPAA privacy regulations and other applicable site privacy policies. No information will be released, nor will participation in the research be acknowledged, to any party except where compulsory according to law or intuitional policy. The results of the research study may be published, but volunteers' names or identities will not be revealed.

9. Adverse Event Monitoring

9.1 Definitions

Adverse Event (AE) **–** Any untoward or unfavorable medical occurrence in a human subject related to bleeding or thrombosis, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in research.

Serious Adverse Event (SAE) – Any adverse event temporally associated with the subject's participation in research and related to bleeding or thrombosis that meets any of the following criteria:

- Results in death;
- Is life-threatening (places subject at immediate risk of death from the event as it occurs);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in a persistent significant/incapacity;
- Results in a congenital anomaly/birth defect; or
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent on the other outcomes listed in this definition

Note that seriousness and severity are separate concepts. The term "severe" refers to the intensity of a specific event; a severe event may be of minor medical significance (e.g., a severe leg cramp). The term "serious" is based on an outcome or action criteria that are usually associated with events that pose a threat to the patient's life or functioning. An event that is mild in severity is serious if it leads to one of the outcomes defined above.

Grade 4 and 5 events will always be considered Serious Adverse Events. Many Grade 3 and some Grade 1 and 2 events may meet the definition of a Serious Adverse Event.

Unexpected Adverse Event- Any adverse event occurring in one or more subjects in a research protocol, the nature, the severity, or frequency of which is not consistent with either:

- The known of foreseeable risk of adverse events associated with the procedures involved in the research that are described in;
    - the protocol related documents or the current IRB-approved informed consent document and;
    - other relevant sources of information, such as a product labeling or package insert or;
- The expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Unanticipated problem involving risks to subjects or others (UP) - Any incident, experience or outcome that meets all the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
    - The research procedures that are described in the protocol related-documents, such the IRB-approved research protocol and the informed consent form document and,
    - The characteristics of the subject population being studied;
- Related or possible related to the subject participation in research; and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

Grading of Adverse Events – Events will be graded by using the Common Terminology Criteria for Adverse Events (CTCAE) Criteria version 4.0.

Attribution – the determination and documentation of whether an adverse event is related to a medical procedure.

Attribution Categories:

1. Not Related –Event clearly related to other factors (e.g., clinical state, other therapies; concomitant drugs)

2. Possible Related – Sequence of event is compatible with study drug, device, or procedure, but could have been produced by other factors
3. Probably Related - Sequence of event is compatible with study drug, or procedure and cannot be explained by other factors without much doubt
4. Definitely Related - Sequence of event is compatible with study drug, or procedure and beyond doubt cannot be explained by other factors

9.2 Site Monitor Physician

A site monitor physician will be identified at each site. Lisa Baumann Kreuziger is the site monitor physician at MCW. Tzu-Fei Wang will be the site monitor physician at OSU.

The site monitor physician will:

1) Assess all adverse events and the frequency of adverse events
2) Report directly to the IRB in the event of a serious adverse event

9.3 Study Staff

Information on all adverse events, whether reported by the subject, directly observed, or detected by physical examination, laboratory test or other means, will be collected, recorded, followed, and reported as described in the following sections. If follow-up or clinical intervention is required, the treating provider will be informed and will provide treatment or follow-up as needed.

The PI will monitor and review adverse events monthly or more often as needed.

9.4 Timeline for Reporting of Adverse events, Serious Adverse Events, Unexpected Adverse Events and Unanticipated Problems

All serious adverse events, all unexpected adverse events, and all unanticipated problems will be reported to the IRB and DSMC as per standard operating procedure by phone, email or SAE form regardless of attribution.

9.5 Reporting Events to Local Institutional Review Boards

All serious adverse events will be reported to the institutional review board (IRB). Each site will comply with their institution's standard operating procedures for reporting adverse events. Subjects will be instructed to report any adverse event(s) during the study and post-study .The investigator will

notify the IRB and any other applicable regulatory agency of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

9.6 Data Safety Monitoring Committee (DSMC)

This study will be reviewed by the Medical College of Wisconsin Cancer Center Data Safety Monitoring Committee (MCW CC DSMC). A summary of the MCW CC DSMC activities are as follows:

- Review the clinical trials for data integrity and safety
- Review all adverse events requiring expedited reporting as defined per protocol
- Review all DSM reports
- Submit a summary of any recommendations related to study conduct
- Terminate the study if deemed unsafe for patients

A copy of the MCW CC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI twice annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary. Any available DSMC letters will be submitted to the IRB of record as required.

9.7 Potential Risks to Volunteer

Likely:

Blood Sampling: Venipuncture can cause pain and discomfort at the needle puncture site

Physical Exam*:* Pain associated with movement of arm and assessment of swelling.

Anticoagulation: Use of injected anticoagulation can cause pain and bruising at the injection site. Cost associated with anticoagulation will be the responsibility of the participants and/or their insurance companies. The cost of enoxaparin (lovenox) will range in price from $24 to $119 a dose depending on the dosage necessary for each person. The cost of direct oral anticoagulant treatment averages $11-15 a dose. The amount of

insurance copays will depend on the insurance plan. Some insurance companies do not cover costs associated with research studies.

Use of aspirin, clopidogrel (Plavix) and NSAIDs [ex. ibuprofen (Motrin), naproxen (Aleve)], while using anticoagulation will increase your risk of bleeding. The physician prescribing aspirin, clopidogrel or NSAIDs will determine if these medications should be continued while you receive enoxaparin or direct oral anticoagulant therapy. If needed, aspirin, clopidogrel, and NSAIDs can be safely resumed the day after anticoagulation is discontinued.

Due to risk of bleeding, spinal procedures should be avoided while using anticoagulation.

Less Likely:

Blood Sampling: Venipuncture can cause, bruising, fainting or local infection at the needle site.

Survey: Filling out the surveys could cause stress or anxiety to the participant

Physical Exam: *E*xamining the chest cause participant embarrassment

Anticoagulation: Use of anticoagulation is known to cause an increased risk of bleeding that can occur at the injection site or systemically. Use of anticoagulation is known to cause an increased risk of bleeding that can occur at the injection site or systemically. Participants will have had anticoagulation for 0-6 months prior to catheter removal and one month after catheter removal. Usual care would include anticoagulation between 1-3 months after catheter removal. With a shortened course of anticoagulation, an increased risk of thrombosis or post-thrombotic syndrome could occur.

Potential side effects of enoxaparin include fever, nausea, diarrhea, fluid retention, elevated LFTs, decreased platelets, decreased RBCs, local irritation, hematoma and redness.

A potential side effect of all direct oral anticoagulant treatment includes bleeding. Edoxaban also reported a rash, abnormal liver function tests, and anemia. Dabigatran is also associated with gastritis-like symptoms in 15% of patients.

Rare:

Blood Sampling: In rare circumstances venipuncture can cause blood clots, peripheral nerve injury or an arterial puncture.

Anticoagulation: The anticoagulation medication prescribed from the treating provider as planned treatment for catheter-related thrombosis can rarely cause bleeding requiring hospital admission, transfusion, or death.

Breach of confidentiality: personal health information will be collected and rarely a breach of confidentiality may lead to disclosure of that information.

In addition to the above possible risks, there is always the risk of developing previously unknown side effects.

10. Data

10.1 Data Management

The data gathered will be entered into REDCap data system with each ID having a unique identifier for each participant. REDCap is a secure, web-based application for building and managing online databases. All data will be de-identified and samples will only have a unique identifier that will link the unique identifier with the patient's name.

10.2 Data Collection

Research information, including consent forms and PTS assessments will be maintained secure as described in the Confidentiality section 8.3.

Each participant will be identified with a unique identifier number in REDCap. This unique identifier will be linked to the same unique identifier for a biological specimen sample stored in the Dr Alan Mast's laboratory or the TRU to allow batched testing.

10.3 Data Monitoring

The PI or study staff will review all data collection forms on an ongoing basis for data completeness, accuracy, and protocol compliance. The data will be continuously monitored by the principal investigator of the study. The regulatory officer that is a part of the BCW Clinical Trials Office will assist with internal and external audits as appropriate. If protocol compliance or data accuracy comes into question study PI will request a study review.

Subject accrual, compliance with the protocol, status of enrolled subjects, adherence data regarding study visits, and adverse events will be summarized on a quarterly basis (or more frequently if needed) and reviewed by the PI. Serious adverse events will be reviewed at the time of the

occurrence by the PI and reported to the DSMC and IRB as per standard operating procedure.

A biannual safety and progress report will be submitted to the DSMC. The report will include the number of patients entered, number of patients treated, summary of all adverse events reported to date using standardized grading, a specific list of all serious adverse events [SAEs].

Data safety and monitoring activities for each study will continue until all patients have completed their treatment and the 6 months of follow-up. In the event that data and safety monitoring results in the suspension or termination of enrollment and/or treatment of patients, the principal investigator, the MCW CCC Director, the MCW Associate Director for Clinical Medical College of Wisconsin Cancer Center Research, the MCW IRB will be notified per MCW Office of Research Standard Operating Procedure.

The PI is responsible for the overall conduct of the project. Prior to start of the project at OSU, the PI will visit the site to ensure staff training, data management, and monitoring procedures are in place. Weekly or as needed teleconferences will be arranged between the study staff and investigators between OSU and WI to discuss enrollment, monitoring, concerns and trouble shoot any problems that arise. Audits of study procedures will be completed through WI institutional review boards and BCW clinical trials offices.

10.4 Stopping Rules

The DSMC can consider stopping the study prior to completion if:

1. The intervention is associated with adverse events that call into question the safety of the intervention

    a. Interim analysis after 20 patients shows an incidence of thrombotic recurrence >40% (8/20 patients)

        i. Rationale: A retrospective review of CRT in patients with hematologic malignancy at MCW found development of thrombosis other than CRT or extension of the catheter-related thrombosis in 20/141 (14%) of patients. Recurrent thrombosis has been reported in other studies in 20% of cancer patients.[1] If after half of patients are recruited, the thrombotic incidence has doubled over the reported incidence in the literature, the study will be stopped. This level was chosen because it would be unlikely that if 8 events in the first 20 patients occur that no thrombotic events would occur in the next 20 patients to have an overall 20% (8/40) recurrent thrombosis incidence.

2. Difficulty in study recruitment or retention will significantly impact the ability to evaluate the study endpoints

3. New information becomes available that necessitates stopping the trial

11. Statistical Considerations

11.1 Description of Endpoints:

1. **11.1.1 Feasibility Endpoints:**

    a. Recruitment of 56 patients in 1 year and 80% completion of PTS assessments by enrolled patients.

    b. Feasibility of obtaining plasma samples for biomarker analysis as determined by obtaining 80% of samples from enrolled patients.

11.1.2 : Clinical Endpoint

2. Incidence of post-thrombotic syndrome <20% at 6 months.

11.1.3: Secondary Endpoints

1. Recurrent thrombosis

2. Incidence of major and clinically relevant non-major bleeding

3. Reliability of QuickDASH questionnaire in patients with catheter-related thrombosis

11.2 Statistical Analysis

The incidence of PTS, thrombotic recurrence, major bleeding and clinically relevant non-major bleeding will be reported as proportions. These outcomes will be compared at 3 and 6 months using Chi-square or Fisher's exact tests. A Cox-proportional hazards model will be utilized to calculate the risk of PTS and thrombosis recurrence. Chi-square or Fisher's exact tests will be used to determine if clot presentation or location predicts PTS or thrombotic recurrence. Agreement between the DASH and QuickDASH scales will be assessed using the intraclass correlation coefficient. To compare the discriminating ability of the DASH and QuickDASH on the level of PTS, receiver operating curves will be developed using the scale scores at 6 months as the test variable and dichotomized PTS assessment as the classifying variable. Levels of D-dimer, thrombin generation, and TFPI will be reported as means. Logistic regression will evaluate if D-dimer, thrombin generation or TFPI levels at 1 month or % change from baseline predict thrombotic recurrence. Where necessary, for parametric assumption, we will employ appropriate transformations with justifications. For example, the log of some of the biomarkers data may be used if the data range is >1000. Statistical

significance will be determined with $p<0.05$. SAS version 9.13 and JMP 10 Statistical software will be employed for data analysis.

An interim analysis will be completed after 20 patients complete 6 months of follow-up. The interim analysis will allow assessment of thrombotic recurrence by the DSMC.

11.3 Follow-up and Record Retention

The current proposal involves on-going data collection for the duration of the study at each site. The records will be maintained per site policy.

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