STUDY PROTOCOL

Title: Prevention of central venous catheter-associated thrombosis in critically ill children: A multicenter phase 2b trial.

NCT Number: 03003390

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STUDY TITLE: Prevention of central venous catheter-associated thrombosis in critically ill children: A multicenter phase 2b trial.

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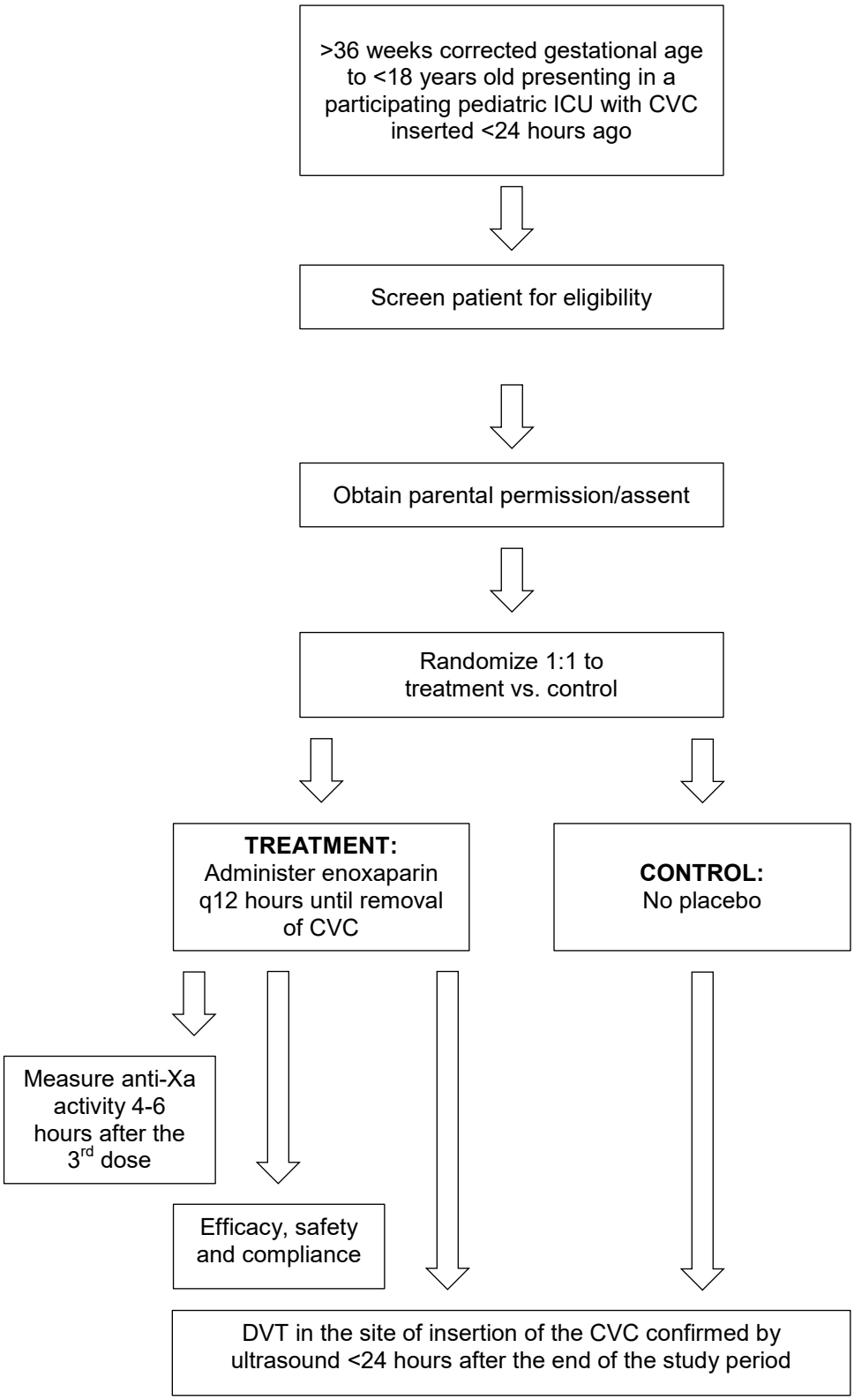
TRIAL SUMMARY

Abbreviated Title	Catheter-Related Early Thromboprophylaxis with Enoxaparin (CRETE) Trial
Trial Phase	2b
Indication	Critically ill children admitted to the pediatric ICU with a CVC
Trial Type	Double arm phase 2b
Control	Concurrent
Product	Enoxaparin
Number of Subjects	100
Estimated Enrollment Period	21 months
Aims	Aim 1 – To obtain preliminary evidence on the effect of early prophylaxis on the incidence of CADVT in critically ill children Aim 2 – To evaluate the effect of an anti-Xa activity-directed prophylactic strategy on thrombin generation in critically ill children
Inclusion Criteria	1. <24 hours after insertion of an untunneled CVC in the internal jugular or femoral vein 2. Admitted to the ICU with an anticipated stay of ≥48 hours 3. CVC required for ≥24 hours 4. >36 weeks corrected gestational age to <18 years old
Exclusion Criteria	1. Coagulopathy (i.e., international normalized ratio >2.0, activated partial thromboplastin time >50 seconds or platelet count <50,000/mm^3) 2. Known bleeding disorder 3. Clinically relevant bleeding as defined by the International Society on Thrombosis and Hemostasis (i.e., Hb decreased ≥2 g/dl in 24 hours, required medical or surgical intervention to restore hemostasis, or in a critical organ system [*i.e.*, retroperitoneum, pulmonary, intracranial or central nervous system]) 4. Admitted for trauma, or neurologic or spine surgery) 5. <60 days from a clinically relevant bleeding as defined above 6. <7 days after trauma or surgery 7. Anticipated surgery within 48 hours after insertion of the CVC 8. Renal failure (*i.e.*, creatinine clearance <30 mL/min) Presence of epidural catheter 9. Currently taking an antithrombotic agent (e.g., LMWH, UFH at therapeutic doses, Coumadin or aspirin) 10. Radiologically documented DVT at the site of insertion of the CVC in the previous 6 weeks 11. Known hypersensitivity to heparin or its components, including pork products 12. History of HIT (i.e., positive serotonin release assay)

	13. Currently pregnant 14. Currently lactating 15. Prior enrollment in the study 16. Limitation of care
Treatment Plan	Eligible subjects will be randomized 1:1 to treatment or control. In subjects randomized to treatment, noxaparin will be given within the first 24 hours after CVC insertion and then every 12 hours (+/-2 hours) thereafter until removal of the CVC. Dosing will start at 0.75 mg/kg for children ≤2 months old or 0.5 mg/kg (maximum of 30 mg) for older children and will be titrated to a target anti-Xa activity of 0.2-0.5 IU/mL. Safety assessments for bleeding will be completed daily. In subjects randomized to control, no placebo will be given.

STUDY SCHEMA



LIST OF ABBREVIATIONS

AE – adverse event
CADVT – catheter-associated deep venous thrombosis
CAT - Calibrated Automated Thrombogram
Co-I – co-investigator
CRF – case report form
CVC – central venous catheter
DSMB – data and safety monitoring board
DVT – deep venous thrombosis
EDTA –ethylenediaminetetraacetic acid
ETP – endogenous thrombin potential
FDA – Food and Drug Administration
Hb – hemoglobin
HIPAA – Health Insurance Portability and Accountability Act
HIT – heparin-induced thrombocytopenia
ICU – intensive care unit
IL – interleukin
IP – Investigational Product
IRB – institutional review board
LMWH – low molecular weight heparin
MCU – Multicenter Unit
NDA – new drug application
PI – principal investigator
PROTEKT – Prophylaxis of Thromboembolism in Kids Trial
RCT – randomized controlled trial
RLD – reference listed drug
UFH – unfractionated heparin
SAE – serious adverse event
SD – standard deviation
TFPI – tissue factor pathway inhibitor
UFH – unfractionated heparin
USP – U.S. Pharmacopeia
YCCI – Yale Center for Clinical Investigation

RATIONALE AND BACKGROUND

Disease

DVT, a potentially preventable pediatric condition, is associated with nearly $400 million of excess cost in children in the United States annually. Excess inpatient stay accounts for $200 million and another $200 million are needed to treat DVT and its complications outpatient.[1, 2] Co-I Raffini reported an annual 10% increase in the incidence of DVT in children suggesting that the cost of DVT is expected to increase if it is not prevented.[3]

Critical illness and CVC are the most important risk factors for DVT in children.[4] Each increases the risk of DVT at least 2-fold. In our recent meta-analysis of 11 studies in which 676 critically ill children with untunneled CVC, the most commonly used type, were actively surveilled radiologically, we showed that the frequency of CADVT was 0.18 (95% CI: 0.12-0.25).[5] In children, >85% of DVT and nearly all DVT-related deaths are related to CVC.[6, 7] PI Faustino has shown that children with symptomatic CADVT, on average, stay in the ICU in excess of 16 days, significantly increasing their cost of care.[8] Even when not symptomatic, DVT is associated with significant morbidity and mortality. Nearly 50% of cases of pulmonary embolism in children are from asymptomatic DVT and 83% of cases of pulmonary embolism in children that contributed to death did not have symptoms.[9, 10] CADVT also serves as a nidus of infection increasing the risk of catheter-associated bloodstream infection at least 3-fold.[11]

Data from adults should not be generalized to children. In the absence of well-conducted pediatric RCTs, prophylaxis against CADVT is not recommended in children.[12] The recommendation in critically ill adults, >85% of whom have CVC, to provide prophylaxis against DVT should not be applied to children because the hemostatic system evolves with age affecting the risk of DVT and anticoagulant effects.[12-14] Co-morbidities and medications, which differ between children and adults, affect hemostasis and the effects of prophylaxis. Smaller veins in children, vs. adults, likely increase the risk of CADVT. Well-conducted pivotal trials are urgently needed to determine whether prophylaxis can safely prevent CADVT in critically ill children. However, timing and extent of reduction in thrombin generation needed to safely prevent CADVT in children are unclear. The goal of this trial is to explore the efficacy of early prophylaxis against CADVT in

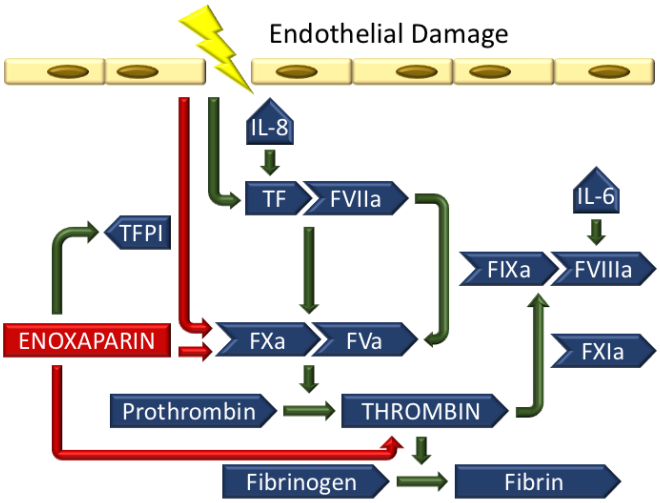


Figure 1. Central role of thrombin in coagulation. → stimulate; → inhibit.

critically ill children.

Reduction of thrombin generation is biochemically the goal of prophylaxis. All known drugs that reduce thrombin generation have an antithrombotic effect regardless of their mode of action.[15] Endothelial injury while inserting a CVC increases thrombin generation through factor Xa (Figure 1).[16] Thrombin produces fibrin, and activates platelets and other coagulation factors, particularly factor VIIIa, to amplify thrombin generation. The result is a mesh of fibrin and platelets than can lead to CADVT. It is logical that prophylaxis should be started soon after insertion of the CVC to maximize the likelihood of preventing CADVT because it would reduce thrombin generation.

Enoxaparin has become the "standard" anticoagulant for prophylaxis in children despite the lack of conclusive data.[12, 17] Like other LMWH, it reduces thrombin generation by inhibiting factor Xa (Figure 1).

Timing of prophylaxis against CADVT is unclear. The natural history of CADVT suggests that prophylaxis should be started <24 hours after insertion of the CVC because, although CADVT develops in <2.5% of critically ill children at this time, nearly all CADVT have developed by 4 days after insertion of the CVC.[18] The PROTEKT trial studied reviparin, a LMWH.[19] It is the largest trial of prophylaxis against CADVT in children (186 children from 20 centers) though it was stopped early due to poor enrollment. The risk ratio of CADVT with reviparin vs. placebo was 1.2 (95% CI: 0.4-3.2). Reviparin was started late at a mean of 2.6 days after insertion of the CVC when nearly 50% of the CADVT would have already developed.[19, 20] In contrast, a cohort study of children with leukemia showed that enoxaparin given before inserting a CVC might reduce the risk of CADVT (risk ratio: 0.2; 95% CI: 0.03-1.1).[21] This study supports the use of early prophylaxis though it may be biased because it only included symptomatic CADVT as outcome. Misclassification of cases may have occurred because symptoms of CADVT are difficult to detect in children.[22] Before conducting a pivotal trial, it is essential to establish whether early prophylaxis may be effective against CADVT in critically ill children.

Extent of reduction in thrombin generation needed to prevent CADVT is unknown. Based on limited evidence from adults, prophylaxis with LMWH is dosed to an anti-Xa activity of 0.2-0.5 IU/mL.[12, 23] Although anti-Xa activity is the current standard for monitoring the effect of LMWH, studies suggest that anti-Xa activity weakly correlates with clinical outcome. Anti-Xa activity does not account for LMWH's effect on thrombin generation through its actions on thrombin (i.e., anti-IIa activity) and TFPI, and the subject's coagulation status (Figure 1).[23-27]

ETP is the best measure of thrombin generation among all currently available measures.[27] Using a chromogenic or fluorogenic assay, thrombin generation is triggered *in vitro* in the patient's plasma by tissue factor.[28] ETP is a direct measure of the total amount of thrombin generated over time and includes all biologic effects on thrombin generation, *i.e.,* natural and pharmacologic pro- and anticoagulants, occurring in the

patient.[27] Other available markers of thrombin generation, *e.g.*, prothrombin fragment, thrombin-antithrombin complex, D-dimer and fibrin degradation products, do not accurately measure thrombin generation because their levels are influenced by factors other than thrombin generation, have very short half-lives, and provide limited assessment of thrombin generation.[28, 29]

Studies on ETP and prophylaxis with LMWH are limited to non-critically ill adults. At doses proven to prevent DVT without increasing the risk of bleeding in women after Caesarian delivery and adults after arthroplasty, enoxaparin and other LMWH reduce ETP by 10% of pre-operative levels at 24 hours after the 1st dose.[29, 30] The reduction in ETP is sustained for at least 4 days while on prophylaxis.[31] These findings are corroborated by *in vitro* studies showing similar reductions in ETP after LMWH is added to plasma of healthy volunteers.[32] In these non-critically ill adults, prophylactic doses of enoxaparin reduce ETP from 1630 nM·min to 543 nM·min.[26] This level of anticoagulation is considered effective in preventing DVT (<700 nM·min) and safe in avoiding clinically relevant bleeding (>300 nM·min).[15]

Although a reduction in ETP mediates the prevention of DVT in non-critically ill adults, it is unclear whether this is also true in critically ill children because thrombin generation increases with age and critical illness.[15, 33] High levels of factor VIIIa, IL-6 and IL-8 during critical illness increase thrombin generation and may explain the increased risk of DVT with trauma, cancer, cardiac disease, systemic infection, or after surgery (Figure 1).[34-38] Before a pivotal trial of prophylaxis against CADVT is conducted, it is essential to determine the extent to which current dosing of enoxaparin reduces thrombin generation.

Clinical Trial Data

Prophylaxis in critically ill children. PI Faustino, co-PI Spinella and Site PIs Higgerson, Hanson, Li and Cholette conducted a cross-sectional study of prophylaxis in critically ill children.[17, 39] Of 2,484 children from 59 pediatric ICUs in 7 countries, 53% had ≥1 CVC with the untunneled type being most common (45%). Nearly 90% of the untunneled CVC were inserted in the internal jugular or femoral vein. Only 27% of those with untunneled CVC received anticoagulation, with enoxaparin as the most commonly used agent.

Frequency of CADVT in critically ill children. In 2 prospective cohort studies, PI Faustino and co-PI Spinella enrolled 69% of 284 critically ill children <24 hours after insertion of an untunneled CVC over 34 months.[40, 41] The frequency of ultrasound-diagnosed CADVT was 0.30 (95% CI: 0.23-0.37). Due to unexpected death or transfer, 4% of children did not have ultrasound. Co-I Silva led a team of pediatric radiologists that centrally and blindly reviewed all images. Between radiologists, chance-corrected agreement was 0.77.[42] Mean catheterization was 6 days (standard deviation [SD]: 5 days), which was similar between those with and without CADVT.

Bleeding assessment tool. PI Faustino recently completed the development of a web-based bleeding assessment tool that operationalizes clinically relevant bleeding as defined by the International Society on Thrombosis and Hemostasis (*i.e.,* Hb decreased ≥2 g/dl in 24 hours, required medical or surgical intervention to restore hemostasis, or in a critical organ system; admitted for trauma, or neurologic or spine surgery).[22] Using medical records of 35 children admitted to the ICU with a diagnosis of bleeding, 4 physicians blindly and independently determined the presence of clinically relevant bleeding. Using the tool, the chance corrected agreement between physicians was 0.82.

ETP and anti-Xa activity in critically ill children. Co-PI Spinella analyzed ETP and anti-Xa activity in 9 samples of platelet-poor plasma from 4 critically ill children on heparin. ETP was measured with the CAT (Thrombinoscope, Netherlands) using 1 pM of tissue factor to trigger thrombin generation. Anti-Xa activity was measured using STA® Liquid Anti-Xa assay in the fully automated STA® analyzer (Diagnostica Stago S.A.S., France). The mean (SD) of ETP was 394 (326) nM·min and that for anti-Xa activity was 0.3 (0.2) IU/mL. The intra- and inter-individual correlation coefficients between ETP and anti-Xa activity were −0.59 and −0.51, respectively.

Biomarkers of inflammation in critically ill children. In a prospective cohort study, PI Faustino measured plasma factor VIIIa after insertion of an untunneled CVC in 85 critically ill children.[41] Factor VIIIa was measured at the Clinical Laboratory at Yale-New Haven Hospital with a 1-stage clotting assay (Dade® Actin® FSL, Siemens Healthcare) using the BCS XP System (Siemens Healthcare, Germany). Mean (SD) of factor VIIIa was 133 (61) IU/dL. The incidence of CADVT doubled (95% CI: 1.1-3.6) for every SD increase in factor VIIIa.

PI Faustino measured IL-6 and IL-8 in platelet-poor plasma from 10 critically ill adolescents using Milliplex® MAP kits and the Millipore Luminex 200 System (EMD Millipore Corp., Germany) located at the Immune Monitoring Core Facility at Yale. Mean (SD) of IL-6 was 129 (199) pg/mL and 97 (161) pg/mL for IL-8.

Rationale for Trial and Selected Patients

CADVT is a significant problem in critically ill children. It occurs in 18% of children with untunneled CVC and is associated with adverse outcomes.[5] These outcomes include prolonged stay in the ICU, increased risks of catheter-associated bloodstream infection, pulmonary embolism, and mortality, and increased costs of care.[1, 2, 6, 7, 9-11] Despite the adverse outcomes associated with CADVT, prophylaxis is not recommended in children because of the absence of data on its efficacy in preventing CADVT in children.[5] The recommendation to provide prophylaxis in critically ill adults should not be routinely applied to children because of differences in the hemostatic system between these age groups.[12] This trial will explore the efficacy of early prophylaxis against CADVT in critically ill children.

We will enroll children >36 weeks corrected gestational age to <18 years old. This age

range represents the patient population admitted to the pediatric ICU. PROTEKT enrolled children in the same age range.[19] We will only enroll children with untunneled CVC, the most common type used in critically ill children, to minimize confounding based on type of CVC.[5] Only children with CVC in the internal jugular or femoral veins will be eligible to minimize bias related to the accuracy of ultrasound in diagnosing CADVT.[22] We will exclude children at high risk of bleeding such as those with coagulopathy, known bleeding disorder, recent history of clinically relevant bleeding, surgery or trauma, in renal failure, or those on another antithrombotic agent, to minimize the risk of bleeding.

Bidlingmaier et al conducted the most comprehensive literature review of the safety and efficacy of LMWH in children up to 18 years old.[43] They reviewed 8 studies in which LWMH was used as prophylaxis against DVT. Of 305 children analyzed, only 7 (2.3%) developed DVT and 7 (2.3%) had clinically relevant bleeding while on the drug.

Recently, co-I Raffini studied enoxaparin as prophylaxis against DVT that was not associated with a CVC in critically ill adolescents as part of a quality improvement initiative.[6] Enoxaparin was given subcutaneously every 12 hours at 0.5 mg/kg (maximum of 30 mg). No DVT that was not associated with a CVC and no clinically relevant bleeding occurred in 90 adolescents in the study

STATEMENT OF PURPOSE/OBJECTIVES

Aim 1: To obtain preliminary evidence on the effect of early prophylaxis on the incidence of CADVT in critically ill children.

Hypothesis – Among critically ill children, prophylaxis administered <24 hours after insertion of the CVC decreases the incidence of ultrasound-diagnosed CADVT compared with no prophylaxis. The natural history of CADVT in children suggest that prophylaxis needs to be administered <24 hours after the insertion of the CVC to maximize the likelihood of preventing CADVT.[18, 19, 21]

Aim 2: To evaluate the effect of an anti-Xa activity-directed prophylactic strategy on thrombin generation in critically ill children

Hypothesis –Among critically ill children, standard prophylactic dose of enoxaparin adjusted by anti-Xa activity reduces thrombin generation to <700 nM·min, as measured by ETP. In non-critically ill adults, prophylactic dose of enoxaparin proven to prevent DVT reduces ETP to <700 nM·min.[15, 26]

PHARMACEUTICAL AND THERAPEUTIC BACKGROUND

Product Description

Enoxaparin is a LMWH produced from UFH that exerts its anticoagulant effects by binding to and inducing a conformational change in antithrombin to accelerate the inactivation of factor Xa and thrombin.[12, 44] Enoxaparin is the most commonly used thromboprophylactic agent in adults and children. It is equipotent with other LMWH. In contrast to UFH, enoxaparin has significantly greater inhibitory activity against factor Xa and has lower risk of bleeding and HIT. It has good bioavailability with predictable pharmacokinetics requiring less monitoring compared with UFH.

Lovenox® (enoxaparin sodium injection, Aventis Pharmaceuticals Inc.) was initially approved in the United States in 1993 for prophylaxis of DVT, and inpatient and outpatient treatment of acute DVT.[45] FDA approved the generic version of Lovenox® in July 2010. Both approvals were for use in adults only. In double blind multicenter RCTs, enoxaparin was shown to be superior to placebo in preventing DVT in adults recovering from hip or knee replacement surgery (10-11% with enoxaparin vs. 46-62% with placebo) and in acutely ill medical patients (4.4% with enoxaparin vs. 11.3% with placebo).[45] The drug was not inferior to UFH in preventing DVT in adults recovering from abdominal surgery (7.0-9.7% with enoxaparin vs. 6.5-10.9% with UFH).[45] Similar efficacy data is not available in children although the incidence of DVT of 4.7-5.2% in children receiving prophylactic doses of LMWH suggests that the drug is also efficacious in children.[43]

Enoxaparin sodium is obtained by alkaline depolymerization of heparin benzyl ester derived from porcine intestinal mucosa.[45] Its structure is characterized by a 2-O-sulfo-4 enepyranosuronic acid group at the non-reducing end and a 2-N,6-O-disulfo-D-glucosamine at the reducing end of the chain. About 20% (ranging between 15% and 25%) of the enoxaparin structure contains an 1,6 anhydro derivative on the reducing end of the polysaccharide chain. The drug substance is the sodium salt. The average molecular weight is about 4.5 kDa. The molecular weight distribution is <2 kDa (20%), 2 kDa to 8 kDa (68%), and >8 kDa (18%).

Pharmacokinetics

Maximum anti-Xa and anti-IIa activities occur 3 to 5 hours after subcutaneous injection of enoxaparin in adults.[45] Mean peak anti-Xa activity was 0.16 IU/mL (1.58 µg/mL) and 0.38 IU/mL (3.83 µg/mL) after the 20 mg and the 40 mg clinically tested subcutaneous doses, respectively. Mean (n = 46) peak anti-Xa activity was 1.1 IU/mL at steady state in patients with unstable angina receiving 1 mg/kg subcutaneous every 12 hours for 14 days. Mean absolute bioavailability of enoxaparin, after 1.5 mg/kg given subcutaneously, based on anti-Xa activity is approximately 100% in healthy adult volunteers. After repeated subcutaneous administration of 40 mg once daily and 1.5 mg/kg once-daily regimens in healthy adult volunteers, the steady state is reached on

day 2 with an average exposure ratio of about 15% higher than after a single dose. Steady-state enoxaparin activity levels are well predicted by single-dose pharmacokinetics. After repeated subcutaneous administration of the 1 mg/kg twice daily regimen, the steady state is reached from day 4 with mean exposure about 65% higher than after a single dose and mean peak and trough levels of about 1.2 and 0.52 IU/mL, respectively. Based on enoxaparin sodium pharmacokinetics, this difference in steady state is expected and within the therapeutic range.

Metabolism

Enoxaparin sodium is primarily metabolized in the liver by desulfation and/or depolymerization to lower molecular weight species with much reduced biological potency.[45] Renal clearance of active fragments represents about 10% of the administered dose and total renal excretion of active and non-active fragments 40% of the dose.

Excretion

Following intravenous dosing, the total body clearance of enoxaparin is 26 mL/min.[45] After intravenous dosing of enoxaparin labeled with the gamma-emitter, 99mTc, 40% of radioactivity and 8 to 20% of anti-Xa activity were recovered in urine in 24 hours. Elimination half-life based on anti-Xa activity was 4.5 hours after a single subcutaneous dose to about 7 hours after repeated dosing. Following a 40 mg subcutaneously once a day dose, significant anti-Xa activity persists in plasma for about 12 hours. Following subcutaneous dosing, the apparent clearance of enoxaparin is approximately 15 mL/min

Drug Interactions

Unless really needed, agents which may enhance the risk of hemorrhage should be discontinued prior to initiation of Lovenox Injection therapy. These agents include medications such as: anticoagulants, platelet inhibitors including acetylsalicylic acid, salicylates. If co-administration is essential, conduct close clinical and laboratory monitoring.

Drug Supply

Specific manufacturer/supplier of Enoxaparin Sodium Injection, USP is up to the discretion of the participating site as long as the product's FDA application has NDA 020164 as the RLD.

Drug Dose

For prophylaxis, enoxaparin is started at 0.75 mg/kg subcutaneously every 12 hours for children ≤2 months old or 0.5 mg/kg (maximum dose of 30 mg) subcutaneously every

12 hours for children >2 months old.[12, 23] The dose is adjusted to a target anti-Xa activity of 0.2-0.5 IU/mL.[23, 46, 47] In the absence of prior trials in children, the same target anti-Xa activity recommended in adults will be used in this trial.[23] Among post-operative orthopedic patients on prophylactic dose of LMWH, the mean peak anti-Xa activity on Days 1, 3, 4 and 10 were 0.29, 0.25, 0.33 and 0.37 IU/mL. In acutely ill medical patients, the prophylactic dose of LMWH achieved anti-Xa activity of 0.21 to 0.41 IU/mL. Dose calculations are completed per local standards. An example dose calculation work sheet is provided in Appendix 2.

Rationale for Dose Selection/Regimen

The selection of doses to be used in this trial is based on commonly used doses for the drug.[17] When prescribing enoxaparin in children as prophylaxis against DVT, the starting dose is typically 0.75 mg/kg for children ≤2 months old or 0.5 mg/kg (maximum of 30 mg) for older children. The present trial will utilize this dose as a starting point because of the proven tolerability in clinical care. The maximum dose is based on FDA-approved doses in adults. At these doses, enoxaparin has been shown to be safe without any significant increase in the risk of clinically relevant bleeding.[43]

Reconstitution

Dilute Enoxaparin Sodium Injection, USP with sterile water for injection or normal saline. Draw up the appropriate dose in a polypropylene plastic tuberculin syringe sealed with a rubber tip cap.

Storage and Stability Requirements

Enoxaparin should not be stored above 25^{o}C and should not be frozen.[45] When stored at 20^{o}C to 22^{o}C, enoxaparin, 1.2 mg/ml in normal saline, was stable for up to 48 hours. Enoxaparin when repackaged from commercial 0.3 mg syringes of enoxaparin, 100 mg/mL, into tuberculin syringes retained activity and sterility and remained pyrogen free for up to 10 days at 3^{o}C. Undiluted enoxaparin lost activity within 2 days at 22^{o}C. Enoxaparin diluted with sterile water for injection to 20 mg/ml, and stored at room temperature or under refrigeration, was stable for up to 4 weeks in glass or prefilled syringes, as measured by anti-Xa activity.

Handling

Enoxaparin must be handled and administered according to the local study site's regulations for the handling and administration of these products. The product storage manager should ensure that the study drug is stored in accordance with the environmental conditions (temperature, light, and humidity) as determined by the sponsor. If concerns regarding the quality or appearance of the study drug arise, do not dispense the study drug and contact the sponsor immediately. Used (and/or empty) syringes of enoxaparin will be destroyed locally. The method of destruction will be

reported on the Drug Destruction Form filed in the pharmacy/investigator site file. Unused syringes will be destroyed locally per local policy.

Availability

Enoxaparin will be ordered directly by each participating site's investigational pharmacy and shipped directly to each investigational pharmacy.

Ordering

Commercially available product will be shipped to the investigational pharmacies and over labeled as an IP. The label will include sponsor name, address and telephone number, the protocol number, IP name, dosage form and strength (where applicable), amount of IP per container, lot number, expiration date (where applicable), medication identification/kit number, dosage instructions, storage conditions, and required caution statements and/or regulatory statements as applicable, as per local regulations. Additional information may be included on the label as applicable per local regulations.

Accountability

Only the pharmacist or designee will dispose study drug. A record of drug dispensed and administered to each patient must be maintained. The pharmacist will document the dose dispensed in the appropriate study records which will be reviewed during monitoring visits. In addition, the pharmacist needs to maintain an inventory log of all IP delivered, stored, dispensed and destroyed at the site (including expiry date and batch number).

The investigational drug accountability record (see Pharmacy Manual) must be completed with permanent ink. Errors cannot be covered up with scratch outs or correction fluid. Cross through any errors with a single pen stroke. Initial and date the crossed-out errors

Destruction

The sponsor (or designee) will review with the investigator and relevant site personnel the procedures for documenting receipt of IP, as well as the procedures for counting, reconciling IP, and documenting this process. Used (and/or empty) syringes of enoxaparin will be destroyed locally. Unused syringes will be destroyed locally after sponsor's approval for destruction. Documentation of all used and unused drug disposal must be maintained on site for review during monitoring visits.

Contraindications

Enoxaparin is contraindicated in patients with active major bleeding, in patients with thrombocytopenia associated with a positive in vitro test for anti-platelet antibody in the

presence of enoxaparin sodium, or in patients with hypersensitivity to enoxaparin sodium. Patients with known hypersensitivity to heparin or pork products should not be treated with enoxaparin or any of its constituents.

Warnings and Precautions

Enoxaparin is not intended for intramuscular administration. Enoxaparin cannot be used interchangeably (unit for unit) with UFH or other LMWH as they differ in manufacturing process, molecular weight distribution, anti-Xa and anti-IIa activities, units, and dosage. Each of these medicines has its own instructions for use. Enoxaparin should be used with extreme caution in patients with a history of HIT. Please refer to package insert for additional information.

Hemorrhage. Enoxaparin, like other anticoagulants, should be used with extreme caution in conditions with increased risk of hemorrhage. Cases of epidural or spinal hematomas have been reported with the associated use of enoxaparin and spinal/epidural anesthesia or spinal puncture resulting in long-term or permanent paralysis.

Major hemorrhages including retroperitoneal and intracranial bleeding have been reported. Some of these cases have been fatal. Bleeding can occur at any site during therapy with enoxaparin. An unexplained fall in hematocrit or blood pressure should lead to a search for a bleeding site.

Thrombocytopenia. Thrombocytopenia can occur with the administration of enoxaparin in adults. Moderate thrombocytopenia (platelet counts between 100,000/mm^3 and 50,000/mm^3) occurred at a rate of 1.3% in patients given enoxaparin, 1.2% in adults given UFH, and 0.7% in adults given placebo in clinical trials.[45] Platelet counts less than 50,000/mm^3 occurred at a rate of 0.1% in adults given enoxaparin, in 0.2% of adults given UFH, and 0.4% of adults given placebo in the same trials. Thrombocytopenia of any degree should be monitored closely. Cases of HIT with thrombosis have also been observed in clinical practice.

Elevations of serum aminotransferases. Asymptomatic increase in aspartate and alanine aminotransferase levels greater than 3x the upper limit of normal of the laboratory reference range have been reported in up to 6.1% and 5.9% of patients, respectively, during treatment with enoxaparin.

Adverse Reactions

Hemorrhage. The incidence of major hemorrhagic complications with enoxaparin treatment has been low.

Local Reactions. Mild local irritation, pain, hematoma, ecchymosis, and erythema may follow subcutaneous injection of enoxaparin.

Other Reactions. Diarrhea and nausea have also been reported with enoxaparin treatment.

STUDY PLAN AND PROCEDURES

Study Population

We will enroll 100 critically ill children between the ages >36 weeks corrected gestation age and <18 years admitted to the pediatric ICUs of the six participating sites and with a CVC.

Screening Procedures

Subjects will be identified through regular clinical care in the pediatric ICU by the participating investigators and research staff. All children who had a CVC inserted within the prior 24 hours will be included in a screening log maintained in the pediatric ICU at each site. The screening log will be used to identify potential study participants to be screened for eligibility, record the number of eligible, and ineligible children and reason for exclusion. Eligible children who were not enrolled will also be captured in this log and the reason they were not enrolled (parental refusal, time, etc.). It will also track those children who were eligible and consented but not treated. Treatment with enoxaparin needs to be started within 24 hours after insertion of the CVC, therefore children can be screened for study if CVC insertion occurred within 24 hours of admission to the pediatric ICU or during their stay in the ICU. If a child meets all eligibility criteria, parental permission/assent will be obtained.

Inclusion Criteria

1. Untunneled CVC inserted in the internal jugular or femoral vein within the past 24 hours
2. Child anticipated to stay in the pediatric ICU ≥48 hours
3. CVC anticipated to be required for ≥24 hours
4. >36 weeks corrected gestational age to <18 years old

Exclusion Criteria

1. Coagulopathy (i.e., international normalized ratio >2.0, activated partial thromboplastin time >50 seconds or platelet count <50,000/mm^3)
2. Known bleeding disorder
3. Clinically relevant bleeding as defined by the International Society on Thrombosis and Hemostasis (i.e., Hb decreased ≥2 g/dl in 24 hours, required medical or surgical intervention to restore hemostasis, or in a critical organ system [*i.e.*, retroperitoneum, pulmonary, intracranial or central nervous system])
4. <60 days from a clinically relevant bleeding as defined above
5. <7 days after trauma or surgery
6. Anticipated surgery within 48 hours after insertion of the CVC
7. Renal failure (*i.e.*, creatinine clearance <30 mL/min)

8. Presence of epidural catheter
9. Currently taking an antithrombotic agent (e.g., LMWH, UFH at therapeutic doses, Coumadin and aspirin)
10. Radiologically documented DVT at the site of insertion of the CVC in the previous 6 weeks
11. Known hypersensitivity to heparin or its components, including pork products
12. History of HIT (i.e., positive serotonin release assay)
13. Currently pregnant
14. Currently lactating
15. Prior enrollment in the study
16. Limitation of care

Parental Permission and Assent Process

Parents or guardians of eligible children will be approached for permission to participate in the trial. Assent from children aged 7-17 years old, when feasible will be obtained as required per local site IRB policy. Research staff and investigators permitted to consent parents will describe the trial in detail including the data collection requirements, risks, procedures, and alternative treatments. Parents will be made aware that they are under no obligation to allow their child to participate, that failure to participate will not adversely affect their child's care, and that they may withdraw permission for participation at any time. Data will continue to be collected until the parents withdraw permission to use it. They will be given an opportunity to ask questions and have these answered to their satisfaction. The parental permission form will then be signed and dated by both the parent and the study personnel obtaining consent. If there are any questions regarding parental/guardian competence, the trial will not be offered to the patient. The Consent and Assent process will be documented in the medical record per local site policy.

Study Entry/Registration

Eligibility will be determined locally by the site PI and/or research team using an eligibility checklist. Once a child is confirmed eligible and parental permission is obtained, the child will be assigned a unique study ID. A list of study IDs will be provided to each site at start-up and assigned to each enrolled child consecutively. The study ID will be used when the child is registered into OnCore, Yale's Clinical Trials Management System and included on all correlative lab samples and related study documents. Subjects will be entered into OnCore by the local site data manager.

Randomization

Eligible subjects will be randomized 1:1 to treatment vs. control using a permuted block design with variable block sizes that will be developed by the data coordinating center. Randomization will be stratified by ICU and age (i.e., <1 year, 1-13 years and >13 years) to reflect the known epidemiology of DVT in children.[3, 48] Randomization will

occur at the time of registration into OnCore. The computerized randomization scheme will be entered in OnCore, such that allocation will only be known after the subject is registered.

Subjects randomized to the treatment arm will receive enoxaparin adjusted to the target anti-Xa activity. Subjects randomized to the control arm will not receive placebo because of ethical concerns of inducing pain with subcutaneous administration without any potential for direct benefit. All subjects in the study will undergo the remaining study procedures.

TREATMENT REGIMEN WITH ENOXAPARIN

The 1st dose of enoxaparin be given <24 hours after insertion of the CVC. The initial dose will be 0.75 mg/kg for children ≤2 months old or 0.5 mg/kg (maximum of 30 mg) for older children (Table 1). The initial dose will be doubled in obese children (refer to Appendix I and II) and halved in children with renal insufficiency (estimated creatinine clearance calculated by the Schwartz equation <75 mL/min/1.73 m^2).[49]. The clinical nurse will give enoxaparin subcutaneously every 12±2 hours after the first dose until removal of the CVC, or earlier upon discharge from the ICU, radiologic diagnosis of CADVT, start of therapeutic anticoagulation, clinically relevant bleeding develops, HIT is diagnosed, or 28 days after insertion of the CVC.

Administration

Route. Enoxaparin is to be administered by subcutaneous route per local site clinical procedures.

Site. In children <10 kg or <1 year of age, the thigh is the preferred site of administration. Alternate between the left and right thigh. In children ≥10 kg or ≥1 year of age, the abdomen is the preferred site. For children who do not have sufficient subcutaneous tissue in the abdomen, the anterolateral thigh may be used as secondary site for administration.

Table 1: Initial dose

Age	Drug	Dose/ Potency*	Dose Frequency	Route	Regimen/ Treatment Period†	Use
Children ≤2 mos.	**Enoxaparin**	0.75 mg/kg	Q12 hours	Subcutaneous	Until CVC is removed	Experimental
Children >2 mos.	**Enoxaparin**	0.5 mg/kg (maximum of 30 mg)	Q12 hours	Subcutaneous	Until CVC is removed	Experimental

*The initial dose will be doubled in obese children (refer to Appendix I and II) and halved in children with renal insufficiency (estimated creatinine clearance calculated by the Schwartz equation <75 mL/min/1.73 m^2).[49] †Enoxaparin treatment will stop before the CVC is removed upon discharge from the ICU, radiologic diagnosis of CADVT, start of therapeutic anticoagulation, clinically relevant bleed develops, HIT is diagnosed, or 28 days after insertion of the CVC, whichever comes first.

Titrating dose

The dose of enoxaparin will be adjusted to a target anti-Xa activity of 0.2-0.5 IU/mL.[23, 46, 47] The target anti-Xa activity is a peak level and, therefore, timing is extremely important. The peak level must be drawn 4-6 hours after the dose is actually administered. If the child has other labs ordered which are not time dependent, the other labs should be drawn at the same time the enoxaparin peak level is needed.

Using standard methods, anti-Xa activity will be measured 4-6 hours after a dose at the following time points:

1. After the first 3 doses. If needed, the dose will be adjusted to achieve the target anti-Xa activity. See Table 2 for dose titration.
2. After the 3rd dose of a dose titration until the target anti-Xa activity is reached. Once the anti-Xa activity is within target, the dose will be maintained. See Table 2 for dose titration.
3. Weekly to ensure adequate dosing. Similar dose titration as listed in Table 2 will be done to achieve adequate dosing.

Table 2: Enoxaparin Dosage Titration

Anti-Xa Activity	Enoxaparin Dose Titration	Next Anti-Xa Activity Check
<0.10 IU/mL	Increase dose by 20%	After 3rd dose.
0.10-0.19 IU/mL	Increase dose by 10%	After 3rd dose.
0.20-0.50 IU/mL	Keep same dosage	Weekly
0.51-1.00 IU/mL	Decrease dose by 10%	After 3rd dose.
>1.00 IU/mL	Hold all doses until anti-Xa is 0.3, IU/mL, then decrease dose by 20%	Before next dose and every 12 hours, until anti-Xa <0.3 IU/mL

(Modified from Monagle P, et al. *Chest*, 2001, 119:344S)[50]

Discontinuing enoxaparin

The child will be removed from the study and Enoxaparin will be discontinued for the purposes of the study if ANY the following occur:

1. A clinically relevant bleeding event
    a. Drop in Hb of ≥2 g/dl in 24 hours
    b. Blood product transfused for the bleeding event
    c. Required medical or surgical intervention to restore hemostasis
    d. In a critical organ (*i.e.*, retroperitoneum, pulmonary, intracranial or central nervous system)
2. HIT is diagnosed (*i.e.,* positive serotonin release assay)

Holding the Dose

Enoxaparin will be stopped temporarily if ANY the following occur

1. Coagulopathy (*i.e.,* international normalized ratio >2.0, activated partial thromboplastin time >50 seconds or platelet count <50,000/mm^3) develops

2. 12 hours before surgery or invasive procedure
3. In cases of suspected HIT (*i.e.,* an unexplained decrease in platelet count <50,000/mm^3 or by 50% of baseline value in the ICU).

Another agent will not be used while enoxaparin is held. Activities related to the management of the dose of enoxaparin will be the purview of the study. However, the clinical care of the child remains with the treating physician.

Restarting the Dose

Timing for restarting treatment

1. 24 hours after coagulopathy is corrected
2. 24 hours after surgery or procedure
3. After exclusion of HIT (*i.e.*, negative serotonin release assay)

Safety Laboratory Procedures and Assessments

Complete blood count, renal function tests (BUN and creatinine), and serum aminotransferases will be done within 48 hours of enrollment and at least weekly±2 days, thereafter. An unexplained decrease in platelet count <50,000/mm^3 or by 50% of baseline value in the ICU will trigger a work up for HIT (i.e., send blood for serotonin release assay). Stool occult bleed will be done if clinically indicated as determined by the local investigator.

Bleeding Assessment

Bleeding assessments will be completed by the site study team on a daily basis. The team will interview the clinical care team and review the medical records for the presence of a bleed. The Bleeding Assessment Tool (Appendix III) will be completed for each episode of bleeding. This tool contains questions regarding the site, severity and timing of bleeding.

Concomitant Medications

Antithrombotic agents, including other LMWH, UFH at therapeutic doses, Coumadin and aspirin, are not allowed during the trial.

Diet and Other Considerations

There are no dietary or food restrictions with enoxaparin

Rescue Medication and Supportive Case

In case of a clinically relevant bleed, the clinical team will decide whether blood products should be administered. Protamine may also be used to control the bleeding. For suspected HIT, serotonin release assay will be done. Aside from stopping all

heparin or heparin-related products, no other therapies are needed for HIT.

Duration of Therapy

Enoxaparin will be administered every 12±2 hours until CVC removal, or earlier if the following occur:

1. Discharge from the ICU
2. Radiologic diagnosis of CADVT
3. Start of therapeutic anticoagulation
4. Clinically relevant bleed develops
5. HIT is diagnosed
6. 28 days after insertion of the CVC

The child will be off treatment once any of these events occur. Children will be followed for adverse events for 30 hours after the last dose of enoxaparin in the trial. This time period corresponds to 5 half-lives of the drug.[44] The child will be considered off study after this time period or after the study ultrasound (see below), whichever is later.

OTHER STUDY PROCEDURES

Physical Exam and Medical History

A physical exam of the arms or legs will be performed on all patients at time of enrollment and at the end of treatment. If patient has a CVC in the internal jugular vein, only arms need to be examined. If patient has a CVC in the femoral vein, only legs need to be examined.

The following data collected at the time of admission to the PICU will be extracted from the patient chart and entered into OnCore;

1. CVC characteristics such as time of insertion, length, post insertion interventions and vasopressors administered
2. Components of the Pediatric Risk of Mortality 3 (PRISM 3) score
3. Components of the Pediatric Index of Mortality 2 (PIM) score
4. Patient characteristics including diagnosis, age weight, height, laboratory values, and personal or family history of venous thromboembolism

Ultrasound

Children in the trial will have an ultrasound of the site of insertion of the CVC within 24 hours of removal of the CVC, or earlier upon

1. Discharge from the ICU
2. Radiologic diagnosis of CADVT
3. Start of therapeutic anticoagulation
4. Clinically relevant bleed develops
5. HIT is diagnosed
6. 28 days after insertion of the CVC

The vein will be scanned proximal and distal to the site of insertion. Images in the transverse and longitudinal planes, with and without compression, and with and without color Doppler will be acquired.[42, 51] Co-I Silva will collaborate with certified sonographers from each ICU to maintain consistent imaging of the central veins. They will be blinded to study-related and clinical information for the trial.

ASSESSING AND REPORTING ADVERSE EVENTS

Definition

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (*i.e.,* any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of enoxaparin is also an adverse event.

Adverse events may occur during the course of the use of the IP in clinical trials or within the follow-up period specified by the protocol, or prescribed in clinical practice, from overdose (whether accidental or intentional), from abuse and from withdrawal.

Serious adverse event. A serious adverse event is an AE occurring during any study phase (i.e., treatment, follow-up), that fulfills one or more of the following criteria:

1. Results in death
2. Is immediately life-threatening
3. Requires in-patient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant disability or incapacity
5. Results in a congenital abnormality or birth defect
6. Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

Each AE will be graded for severity per the NCI CTCAE version 4.0, and these criteria must be used in grading the severity of AEs. The criteria can be found at: http://ctep.cancer.gov/reporting/ctc.html

For those AEs, which are not listed as part of the NCI CTCAE version 4.0, the same grading system should be used, where:

- **Mild** corresponds to an event not resulting in disability or incapacity and which resolves without intervention
- **Moderate** corresponds to an event not resulting in disability or incapacity but which requires intervention
- **Severe** corresponds to an event resulting in temporary disability or incapacity and which requires intervention
- **Life-threatening** corresponds to an event in which the patient was at risk of death at the time of the event
- **Fatal** corresponds to an event that results in the death of the patient

AE Expectedness

AEs can be 'Unexpected' or 'Expected'. Expected AEs include Bleeding development of HIT, pain from subcutaneous administration of the drug, and anemia from blood draws.

Unexpected AEs are those AEs occurring in one or more patients participating in the research protocol, the nature, severity, or frequency of which is not consistent with either:

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

AE Attribution

The participating site investigator must attempt to determine if an AE is in some way related to the use of the study drug and define an attribution category. This relationship should be described as follows:

- Definite – The AE *is clearly related* to the study treatment.

  The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug and based on the known pharmacology of the study drug, the event is clearly related to the effect of the study drug. The AE improves upon discontinuation of the study drug and reappears upon repeat exposure.

- Probable – The AE *is likely related* to the study treatment.

  The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug AND the event cannot have been reasonably explained by an intercurrent medical condition OR the event cannot be the effect of a concomitant medication.

- Possible – The AE *may be related* to the study treatment.

  The event follows a reasonable temporal sequence from administration of the study drug and the event follows a known response pattern to the study drug BUT the event could have been produced by an intercurrent medical

condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug OR the event could be the effect of a concomitant medication.

- Unlikely – The AE *is doubtfully related* to the study treatment.

  AE does not have temporal relationship to intervention, could readily have been produced by the patient's clinical state, could have been due to environmental or other interventions, does not follow known pattern of response to intervention, does not reappear or worsen with reintroduction of intervention

- Unrelated – The AE *is clearly NOT related* to the study treatment.

The event is clearly due to causes distinct from the use of the study drug, such as a documented preexisting condition, the effect of a concomitant medication, or a new condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unrelated to the use of the study. All SAEs will be reported to the project manager within 24 hours of the site becoming aware of the event.

Procedure for Reporting Adverse Events

All AEs will be recorded from the time the permission form is signed through 30 hours after the last dose of enoxaparin in the trial. AEs will be recorded on an Adverse Event log at each site and entered in OnCore within 72 hours of the site becoming aware of the event.

All AEs will be reported to regulatory authorities, IRB and investigators in accordance with all local applicable laws and regulations.

AE reporting must also be carried out by each participating site PI, according to their local policy and procedures, to the IRB responsible for oversight of their patients. A copy of the local IRB's acknowledgement should be forwarded to the project manager. All SAEs must be followed until resolution or stabilization. Unanticipated problems involving risk to patients or others and all SAEs related to participation in the study should be promptly reported to the project manager. The project manager shall report all SAEs to the FDA.

All SAEs that are unexpected, which occur any time after the patient has consented up to 30 hours after the last dose of enoxaparin in the trial and are possibly, probably, or definitely related to the research must be reported by the participating site to the project manager within 24 hours of becoming aware of the event. The project manager is responsible for reporting to the FDA (via MedWatch report) according to FDA 21CFR312.32 within 15 calendar days of the event occurring. The project manager will also notify the Yale Human Investigation Committee (within 5 days) and all participating

site investigators within 15 calendar days.

A safety monitor is responsible for ongoing timely monitoring of reports of all SAEs submitted by site PIs, or other sources to ensure high-quality reporting, good clinical care and to quickly identify safety concerns. The identification of SAEs will begin when a member of the local research team reports an adverse event. The local research team will monitor each child daily for pre-defined adverse events, particularly clinically relevant bleeding. The research team will interview the clinical team and review the medical records daily for these events. The site PI or his/her designee will conduct prompt investigations of all reported adverse events, especially bleeding. All SAEs will be documented on a SAE form, entered in OnCore and communicated to the PM as soon as possible and <24 hours of the site PI knowing the event.

SAEs will be reported to the Yale Human Investigation Committee and the IRBs of the participating sites per policy.

Adverse Event Committee (AEC)

PI Faustino will assemble an independent 3-member AEC composed of a pediatric hematologist (chair), a pediatric intensivist and a nurse. The responsibility of the AEC is to classify all AEs for publication and presentation purposes.

Minimizing Risks

To minimize the risk of bleeding, we will exclude children at high risk of bleeding. Enoxaparin will be held immediately and permanently after a clinically relevant bleeding event as defined by the International Society on Thrombosis and Hemostasis.[52] Bleeding from enoxaparin can be reversed with protamine or administration of fresh frozen plasma. See section on Holding the Dose, which contains criteria for holding enoxaparin temporarily and when to restart the drug. Enoxaparin will not be replaced with another agent while the drug is on hold.

Platelet count will be monitored for the development of HIT. Enoxaparin and all heparin-related products will be held temporarily for unexplained decrease in platelet count.[14, 53] Enoxaparin will not be restarted until the child tests negative for HIT.

Subcutaneous administration of enoxaparin may cause pain. As per local ICU practice, enoxaparin may be given through a subcutaneous catheter.

It is unlikely that the blood draws will result in anemia. Packed red blood cells can be transfused for anemia per the direction of the clinical team.

The frequency of clinically relevant bleeding in critically ill children in the absence of anticoagulation is 0%-3% while that of children on prophylaxis with LMWH is 2.3%.[43, 54-57] If ≥5 clinically relevant bleeding events (100 children x 5.3% ≈ 5 events) occur, the

safety monitor will notify the chair of the data DSMB. The DSMB chair may convene a meeting or teleconference of the DSMB to consider the concerns and plan appropriate action, including temporarily putting the trial on hold. Because of the difficulty in determining the relationship between bleeding events and enoxaparin in critically ill children, all bleeding events will be conservatively attributed to the drug unless the child has not received a dose for ≥30 hours, which corresponds to 5 half-lives of the drug.

CORRELATIVE SAMPLES

Blood

Blood for correlative studies will be drawn from the CVC into two 2.7 mL citrated tubes at the following four time points

1. Draw 1 (D1): <24 hours after insertion of CVC (and before enoxaparin is administered for subjects in the control arm)
2. Draw 2 (D2): 24±6 hours after D1 (or together with the blood draw for the anti-Xa activity for subjects in the treatment arm)
3. Draw 3 (D3): 4 days after insertion of CVC (if the child is still in the study at that time)
4. Draw 4 (D4): At the end of the study period

Blood will be processed locally using standard procedures for obtaining platelet-poor plasma. It will then be frozen at −70^{o}C.[58] All specimens will be shipped to Yale for banking and will be analyzed every 6 months.

At any **one** of the time points specified above, additional blood will be drawn into **one** 0.5 mL EDTA tube for future genetic analysis. No processing is needed prior to freezing this sample.

Urine

At time points (D1-4) specified above for blood collection, 2-5 mL of urine will also be collected in urine collection tubes for correlative studies. No processing will be needed prior to freezing the urine locally at −70^{o}C. Administration of enoxaparin should not be delayed if urine is not available before first dose of the drug is administered.

Please refer to laboratory manual for specific instructions on processing, storage and shipment of blood and urine samples to Central Laboratories for analyses and storage. Blinded personnel at each Central Laboratory will perform all assays in duplicate on platelet-poor plasma collected at all time points. Results will be averaged for analysis.

CORRELATIVE STUDIES

ETP. This will be measured at the Spinella Laboratory using CAT, a fully automated, computer-controlled fluorometric microplate reader and its specified software (Thrombogram Thrombinoscope Synapse, Netherlands). Calibration will be conducted with each test to guarantee correction for color of the tested plasma sample. Reagents containing 1 pM and 5 pM of tissue factor (to account for the presence of enoxaparin) plus 4 μM of phospholipid will be used to trigger the thrombin generation reaction.[59]

Factor VIIIa and anti-Xa activities. These will be measured at the Clinical Laboratories at Yale-New Haven Hospital, except for anti-Xa activity that is used to titrate the enoxaparin dose in subjects in the treatment arm, which is measured locally. Factor VIIIa will be measured using a 1-stage clotting assay with the BCS XP System following the manufacturer's recommendations. To minimize variability in its measurement in our analysis for this Aim, we will centrally measure anti-Xa activity using STA® Liquid Anti-Xa assay with the fully automated STA® line analyzer.[60]

IL-6 and IL-8. These will be measured at the Immune Monitoring Core Facility at Yale University using Milliplex® MAP assay kits and the Millipore Luminex 200 System. This is a bead-based system that allows analysis of up to 100 analytes per well. Samples will be analyzed using Milliplex® Analyst software (EMD Millipore Corp., Germany).

Anti-IIa and TFPI. These will be measured at the Yale Center for Clinical Investigation Core Laboratory at Yale University using commercially available ELISA kits.

OUTCOME MEASURES

The primary outcome is the presence of CADVT, defined as DVT in the site of insertion of the CVC confirmed by ultrasound <24 hours after the end of the study period.[19, 61] Co-I Silva will lead a 3-member outcomes adjudication committee that will blindly and independently diagnose CADVT.[40, 41] CADVT will be diagnosed if at least 2 are present: (1) intravenous echogenic material adherent to the venous wall, (2) non-compressibility of the vein, or (3) abnormal venous Doppler.[18, 42, 62]

Secondary outcomes include measures of efficacy (other thromboembolic events including pulmonary embolism, and length and cost of stay in the ICU and the hospital), safety (clinically relevant bleeding, confirmed HIT and mortality), feasibility (proportion of enrolled eligible children, time to 1st dose of enoxaparin, and time to target anti-Xa activity), and compliance (proportion of missed doses, and proportion of children with ultrasound). Outcomes will be measured from insertion of the CVC. Imaging for other thromboembolic events will depend on the clinical team but all images will be centrally adjudicated for the trial. Bleeding will be monitored, recorded and classified using our bleeding assessment tool. Costs will be estimated from inpatient charges using each hospital's cost-to-charge ratio.

STATISTICAL CONSIDERATIONS

This trial is designed to inform: (1) beliefs regarding the posterior probability distribution for the prophylaxis treatment effect size, defined as the difference ($pS - pE$) of the proportion of subjects developing CADVT among those receiving the current standard of no prophylaxis (pS) vs. the proportion among those receiving the early prophylaxis (pE), (2) the decision whether to proceed to a phase 3 trial, and (3) appropriate and necessary design features of a phase 3 trial.

For Aim 1, we will use a Bayesian statistical approach for modeling and data analysis, for formulating decision rules, and for interpreting results. We will also provide analogous frequentist results for reporting purposes only. We will use our empirical results to decide between 2 hypotheses regarding the difference of proportions $\delta = pS - pE$ of subjects who develop CADVT: $\delta \leq 0$ (prophylaxis is not efficacious) vs. $\delta \geq 0.06$ (prophylaxis is efficacious).

We define clinically significant efficacy of prophylaxis as $\delta \geq 0.06$. The absolute proportion reduction ≥ 0.06 (i.e., relative risk reduction ≥ 33%, relative to 0.18) is considered clinically significant in prior pediatric RCTs of prophylaxis against CADVT and is consistent with that achieved in RCTs of prophylaxis in critically ill adults.[5, 63] In our recent meta-analysis, we showed that the proportion of children with no enoxaparin prophylaxis who developed CADVT was $pS = 0.18$ (95% CI: 0.12-0.25).[5] Based on the finding from this meta-analysis, we will use an informative prior of $pS \sim B(18,82)$. Because we know very little about pE, a non-informative prior of $pE \sim B(1,1)$ will be used. We will assess the robustness of our results via sensitivity analyses that are based on other choices for hyper-parameters α, β for $pE \sim B(\alpha,\beta)$ and $pS \sim B(\alpha,\beta)$ that may have implications for salient results.

The accepted threshold of posterior probability for not proceeding with a phase 3 trial after a phase 2 trial for success is >0.90, while the threshold for not proceeding for futility is unique to each study.[64, 65] If our results suggest that the posterior probability of $\delta \geq 0.06$ is less than 0.60, then we will not proceed with a phase 3 trial using the same dose of enoxaparin. We will determine whether a higher dose is appropriate from the results of Aim 2.

Subjects will be randomized 1:1 to the 2 treatment arms. An interim analysis of the binary outcome CADVT status consisting of the first 50 subjects enrolled will be performed to assess if the futility boundary has been crossed. Futility in the interim analysis will be defined as a predictive probability <0.10 for $\delta \geq 0.06$. The same primary analysis priors for pS and pE mentioned above will be used for the interim analysis. There will be no interim stopping or adaptation based on efficacy.

For Aim 2, we will calculate the posterior probability that the ETP with enoxaparin is <700 nM·min for difference of means of ETP at D2 after 100 subjects complete the trial. We chose D2 because anticoagulation should be achieved as soon as possible. We

used an informative prior of ETP without enoxaparin ~N(1443, 356) from healthy children and a non-informative prior ~N(0, ∞) for ETP without enoxaparin due to lack of data in critically ill children.[48,94] The posterior probability that ETP with enoxaparin <700 nM·min is ≥ 0.60 will be used to decide on the dose for a phase 3 trial. For secondary analysis, we will analyze the primary outcome of ETP over time (D1-D4) using intent-to-treat principle and Bayesian linear mixed effects model with treatment arm as the main predictor of interest, adjusted for the stratifying variables of age and ICU. We will analyze the relationship of ETP with anti-Xa level using linear mixed effects model, then identify explanatory variables from the coagulation-related markers. From the clinical variables, we will identify those that are associated with achieving the target ETP and anti-Xa level using generalized estimating equations. Using a joint model of linear mixed effects model for ETP or anti-Xa level, and generalized linear model for CADVT or bleeding, we will examine the relationship of ETP and anti-Xa level with CADVT and clinically relevant bleeding. We will use SAS® v9.4, WinBUGS, or R packages to perform Bayesian modeling and data analysis.

DATA MANAGEMENT AND RECORD KEEPING

The investigators are required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated in the investigation. Data that are derived from source documents and reported on the CRF must be consistent with the source documents or the discrepancies must be explained. OnCore will be the designated electronic data capture tool. All data should be entered onto OnCore within 1 week of study entry. AEs need to be entered within 72 hours and SAEs need to be entered within 24 hours of the site becoming aware of the event.

ADMINISTRATIVE AND REGULATORY

Multicenter Management and Coordination

The YCCI MCU will support PI Faustino with the multi-site management aspects of this trial. This includes, but is not limited to, study start-up, regulatory assistance (IRB submissions, amendments, and renewals), provision of template study forms (tracking and eligibility checklists, etc.), site qualification and activation, training, and overall project management. The MCU will provide tools to assist the study teams in conducting the trial appropriately and according to Good Clinical Practice Standards.

The MCU will ensure that all participating sites undergo remote training in which all key study personnel will be instructed study procedures, informed consent, source documentation requirements, safety and adverse event reporting, Good Clinical Practice guidelines and additional study related topics. This training will be conducted by the YCCI Study Monitor and/or MCU staff and documentation of completion will be required for all personnel. Co-I Silva will remotely collaborate with certified sonographers from each ICU to maintain consistent imaging of the central veins. PI Faustino will be available to site investigators for questions and re-training (if necessary).

Data safety and monitoring plan

Authority for monitoring data and safety will reside exclusively with the DSMB. The DSMB will hold the investigative sites responsible for data quality and completeness, and ensure the safety of children. The DSMB will be composed of a pediatric intensivist (chair), a pediatric hematologist, a biostatistician and a patient advocate who are not involved in the trial. The DSMB will review this protocol at a minimum of once every six months. Information to be provided to the DSMB includes: a study narrative by the Sponsor PI, a summary DSMB report produced by OnCore (which includes participant accrual, response, trial status history, SAEs, AEs, deviations and survival); and audit results and monitoring reports as applicable. Other information (e.g. scans, laboratory values) will be provided upon request. At the conclusion of each DSMB meeting, the chair will provide a written summary of the recommendations to PI Faustino. PI Faustino will forward this promptly to participating sites for submission to their local IRBs. The DSMB has the authority to recommend that the study protocol be terminated, temporarily suspended, or amended.

Responsibilities of the DSMB. The DSMB is responsible for assuring that children in the study are not exposed to unnecessary or unreasonable risks and that the study is being conducted according to high scientific and ethical standards. Specifically, the DSMB will:

1. Assess the performance of the study with respect to recruitment, retention, follow-up, protocol adherence, and data quality and completeness.
2. Monitor interim data regarding the safety of the study regimen.

3. Review and consider protocol modifications or ancillary studies proposed by the investigators after the study begins to ensure that these do not negatively impact the trial
4. Advise the investigators as to whether the protocol should continue as scheduled or undergo modification due to a finding from the monitoring process.

Membership. The DSMB will be composed of a pediatric intensivist (chair), a pediatric hematologist a biostatistician and a patient advocate. Members will not be involved in the trial, and will have no vested interest in its outcome.

Initial meeting: Before the study opens to enrollment, the DSMB will meet with PI Faustino and other key investigators to review the research protocol. Particular attention will be paid to the outcome definitions, analysis plan, procedures for recording and reporting serious adverse events, the interim monitoring plan, and the informed consent documents. At this meeting, the DSMB may recommend amendments or clarification of the protocol, and it will formulate its operating procedures (*e.g.*, meeting schedule, reports due from the study statistician, and what interim data may be released to the investigators).

Meeting format. The DSMB will meet as often as needed but the minimum will be at least once before the start of enrollment, after 10, 25 and 50 children have been enrolled, and after enrollment is completed (100 children enrolled). The DSMB will monitor the study according to guidelines specified in the research protocol and the operating procedures established at the initial meeting, unless the DSMB determines during the course of the study that modification of the guidelines is in the best interest of the study or the children in the study. The DSMB chair will set the meeting agenda. The DSMB members, the study statistician, project manager and the Sponsor PI will attend the session, at which time data concerning study conduct and aggregate safety data are discussed. Minutes of the meeting will be taken by staff of the DSMB.

Interim data summary reports. The study statistician will prepare data summary reports and send these directly to the DSMB members and the study team ≥7 days prior to the meeting. The report will focus on patient enrollment and demographics, data completeness, other study performance measures, and rates of serious adverse events. In addition to reports prepared by the statistician, PI Faustino may prepare a report addressing concerns he anticipates the DSMB will have regarding the conduct of the study.

Communication of DSMB recommendations. At the conclusion of each DSMB meeting, the DSMB will provide a verbal report to PI Faustino indicating areas of concern regarding performance and safety. Soon after the meeting, the chair will provide a written report to PI Faustino, including a summary of the DSMB's recommendations. In addition, the chair will provide a memorandum to PI Faustino (including date of the meeting, a listing of data reviewed, recommendations, and date of

next review). PI Faustino will forward this promptly to each participating site for submission to their IRBs, including the Yale Human Investigation Committee and the Project Management team.

The DSMB has the authority to recommend that the study protocol be terminated, temporarily suspended, or amended. This recommendation will be discussed with PI Faustino before any action is taken. If the DSMB recommends a protocol amendment, the Yale Human Investigation Committee and site IRBs must approve the amendment before it is implemented.

Quality Management / Monitoring

PI Faustino and YCCI are responsible for monitoring the performance of all of the participating sites. This will be performed by conducting a study site initiation visit, as well as regularly scheduled monitoring visits throughout the life of the protocol. At the end of the trial, the monitor will then perform study site close-outs for all participating sites.

YCCI will utilize their institution's initiation, monitoring and close-out visit reports. Following monitoring, a monitoring report will be generated containing information on site activities, and a summary of pertinent points and action items together with a copy of the follow-up letter will be sent to each investigative site.

During these monitoring visits, some of the items that will be reviewed are the following:

1. Training of the sites
2. Site personnel qualifications to participate in the trial
3. That study related documents are current
4. That regulatory compliance is accomplished
5. That a parent has signed the permission form
6. That the child has signed the assent form (if applicable)
7. That the protocol is complied with (including reporting and logging of all protocol deviations)
8. That all SAEs and AEs have been reported to the local regulatory committees, IRB, YCCI and the FDA, as applicable
9. That source documentation matches CRFs
10. That required procedures for study drug accountability, distribution, and storage are followed

YCCI will document the required study monitoring activities in a Study Monitoring Plan.

Project Team Meetings

Scheduled meetings will be held and will include the site investigators and research staff involved with the conduct of the protocol.

During these meetings, the investigators will discuss:

1. Safety of protocol participants (adverse events and reporting)
2. Validity and integrity of the data (data completeness on CRFs and complete source documentation)
3. Enrollment rate relative to expectation of target accrual (eligible and ineligible participants)
4. Retention of participants, adherence to the protocol and protocol violations
5. Protocol amendments

CONFIDENTIALITY & SECURITY OF DATA

Data will be entered into OnCore. De-identified data will then be downloaded in Excel format for statistical analysis, which will be done on a HIPAA compatible, password protected encrypted laptop computer by biostatistician co-I Stolar for use. All data entry will be performed by the local study site personnel. All source documentation will be retained at the local study site per local site regulations and monitored during regularly scheduled monitoring visits.

TRIAL FLOW CHART

Procedure	Screening Period	Treatment Period	End of Study CVC Removal
Parental permission/assent	X		
Review of eligibility criteria	X		
Randomization	X		
Medical record review and data collection from ICU admission		X	
Physical exam of arms or legs, and for sexual maturity rating [a]		X	X
Administration of enoxaparin [b]		X	
Measure Anti-Xa activity [c]		X	
Ultrasound [d]			X
Bleeding assessment [e]		X	X
Assess for AEs [e, f]	X	X	X
CBC with differential [g]		X	
Renal function tests (BUN, creatinine) [g]		X	
Serum aminotransferases [g]		X	
Stool occult bleed [h]		X	
Blood collection for correlatives		X [i]	X
Urine collection for correlatives		X [i]	X

a: At time of enrollment for physical exam of arms or legs (if patient has a CVC in the internal jugular vein, only arms need to be examined; if the CVC is inserted in the femoral vein, only legs need to be examined), and at any time during the admission for the sexual maturity rating.
b: 1st dose <24 hours after insertion of the CVC, then every 12±2 hours until CVC removal. Only for subjects randomized to the treatment arm.
c: Anti-Xa activity will be measured 4-6 hours after the 3rd dose, then every 3rd dose until target is achieved. After target achieved, measurement is weekly.
d: Within 24 hours of removal of the CVC, or earlier upon discharge from the ICU, radiologic diagnosis of CADVT, start of therapeutic anticoagulation, clinically relevant bleed develops, HIT is diagnosed, or 28 days after insertion of the CVC
e: Daily.
f: Starting from time permission form is signed until 30 hours after the last dose of enoxaparin in the trial.
g: Within 48 hours of enrollment and at least weekly ± 2 days, thereafter.
h: If clinically indicated as determined by the site PI.
i: Collected <24 hours after insertion of CVC (and before enoxaparin is administered for subjects in the treatment arm); D1), 24±6 hours after D1 (or together with the blood draw for the anti-Xa activity for subjects in the treatment arm; D2), 4 days after insertion of CVC (if the child is still in the study at that time; D3), and at the end of the study period (D4).

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ACKNOWLEDGMENT OF THE INVESTIGATORS

PROTOCOL TITLE: Prevention of central venous catheter-associated thrombosis in critically ill children: A multicenter phase 2b trial.

Version Date: V5 August 22, 2017

Acknowledgement of the Investigator:

1.) I have read this protocol and agree that the study is ethical

2.) I agree to conduct the study as outlined and in accordance with all applicable regulations and guidelines

3.) I agree to maintain the confidentiality of all information received or developed in connection with this protocol

_____ _____

Signature of Investigator: Date:

_____

Name of Investigator (Printed or Typed)

APPENDIX I

Infants and Children 0 to 2 Years of Age
Weight-for-length 95th Percentiles, 45 cm to 110 cm

Length (cm)	**Male** (kg)	**Female** (kg)
45	2.85	2.87
46	3.04	3.07
47	3.23	3.26
48	3.43	3.47
49	3.64	3.68
50	3.87	3.91
81	12.23	11.98
82	12.45	12.23
83	12.69	12.49
84	12.94	12.76
85	13.20	13.04
86	13.47	13.33
87	13.75	13.62
88	14.02	13.92
89	14.30	14.21
90	14.57	14.50
91	14.83	14.79
92	15.10	15.08
93	15.37	15.37
94	15.64	15.67
95	15.91	15.96
96	16.19	16.26
97	16.48	16.57
98	16.78	16.88
99	17.09	17.19
100	17.41	17.52
101	17.74	17.86
102	18.09	18.21
103	18.44	18.58
104	18.80	18.96
105	19.17	19.34
106	19.55	19.75
107	19.95	20.16
108	20.35	20.60
109	20.76	21.04
110	21.18	21.50

51	4.11	4.16
52	4.36	4.42
53	4.64	4.70
54	4.94	5.00
55	5.26	5.31
56	5.58	5.62
57	5.91	5.93
58	6.25	6.25
59	6.57	6.55
60	6.90	6.86
61	7.20	7.15
62	7.50	7.44
63	7.79	7.72
64	8.07	8.00
65	8.35	8.26
66	8.62	8.53
67	8.89	8.78
68	9.15	9.03
69	9.41	9.28
70	9.68	9.52
71	9.94	9.75
72	10.19	9.99
73	10.45	10.21
74	10.69	10.44
75	10.93	10.66
76	11.16	10.87
77	11.39	11.08
78	11.60	11.29
79	11.81	11.51
80	12.02	11.74

Adopted from: *Centers for Disease Control and Prevention, National Center for Health Statistics, 2010*

(http://www.cdc.gov/growthcharts/html_charts/wtleninf.htm)

Male and Female 2-20 Years Old
Data Table of 95th Percentile BMI Value for Age

Body Mass Index, or BMI, is calculated using the following formula:
BMI = Weight (in kilograms)/Height (in meters)^2

Age	Male (BMI)	Female (BMI)
2	19.34	19.11
2.5	18.67	18.58
3	18.24	18.25
3.5	17.97	18.08
4	17.84	18.03
4.5	17.83	18.09
5	17.94	18.26
5.5	18.14	18.51
6	18.41	18.84
6.5	18.76	19.23
7	19.15	19.68
7.5	19.59	20.17
8	20.07	20.70
8.5	20.57	21.25
9	21.09	21.82
9.5	21.62	22.40
10	22.15	22.98
10.5	22.69	23.57
11	23.21	24.14

11.5	23.73	24.71
12	24.23	25.26

Age	Male (BMI)	Female (BMI)
12.5	24.71	25.79
13	25.18	26.30
13.5	25.62	26.79
14	26.05	27.26
14.5	26.45	27.70
15	26.84	28.12
15.5	27.21	28.53
16	27.56	28.91
16.5	27.91	29.28
17	28.26	29.63
17.5	28.60	29.98
18	28.96	30.33
18.5	29.33	30.67
19	29.73	31.03
19.5	30.16	31.40
20	30.59	31.76

Adopted from: *Centers for Disease Control and Prevention, National Center for Health Statistics, 2001 (http://www.cdc.gov/growthcharts/html_charts/bmiagerev.htm)*

APPENDIX II

DOSE CALCULATION WORKSHEET

Subject #:	Subject Name:	Subject MRN:
Age:	**Weight (kg):**	**Height (cm):**
	kg	cm
Date:	**Dose Sequence:** (e.g. 1-3, 4-6, etc)	**BMI (only applicable for age 2-18)**
		kg/m^2

SECTION I

Instructions for **initial dose** (#1-3), otherwise skip to **Section II**

1. Using the table below, select dose for patient based on patient's age: _____mg/kg

Age	Drug	Dose/Potency	Dose Frequency	Route	Regimen/ Treatment Period
Children ≤2 months	**Enoxaparin**	0.75 mg/kg	Every 12 hours	Subcutaneous	Until CVC is removed
Children > 2 months	**Enoxaparin**	0.5 mg/kg (maximum of 30 mg)	Every 12 hours	Subcutaneous	Until CVC is removed

2. Evaluate patient's characteristics for dose modification.
   a. For subject between the **ages of 0 to 2 years old**, does the **weight** for the corresponding **height** exceed the 95th percentiles weight provided in ***Appendix II?*** _____Yes _____No
      - If yes, double the dose due to obesity
   b. For subject between the age of **2 to 18 years old**, does the **BMI** for the corresponding **age** exceed the 95th percentiles BMI provided in ***Appendix III?*** _____Yes _____No
      - BMI = (Weight (in kilograms)/Height (in meters^2))

        _____kg/m^2 = (_____kg / (_____m)^2)
      - If yes, dose is doubled due to obesity.
3. Based on patient's age and obesity status, input dose into Section II under "mg/ kg Dose Selected"

SECTION II

mg/kg Dose Selected	Serum Creatinine	Anti-Xa Activity
mg/kg	mg/dL	IU/mL

<u>Step 1:</u> Renal function

- Determine the subject's k constant:
  - k is 0.33 in premature infants through the first year of life
  - k is 0.45 for term infants through the first year of life
  - k is 0.55 in boys age 2-12 years old and girls of any age
  - k is 0.70 in adolescent boys over the age of 12
- Calculate Glomerular Filtration Rate using Schwartz Equation: GFR = k * Height (cm) / SCr (mg/dL)

  _____ ml/min/1.73m^2 = (_____x _____cm)/(_____mg/dL)
- Using the Schwartz equation, is GFR **less than 75 mL/min/1.73 m2?** _____Yes _____No
  - If yes, halved the dose selected from Section I due to renal insufficiency.
- What is the current dose based on renal function? _____mg/kg

<u>Step 2:</u> **Dose Calculation (1st Dose)**

- Take the dose (mg/kg) determined in step I and multiply it by the patient's weight (kg)

  _____mg (maximum of 30mg) = _____mg/kg (dose) x _____kg (weight)

<u>Step 3:</u> Dose Titration **(Subsequent Doses)**

- Select the appropriate titration factor corresponding to the anti-Xa activity.

Anti-Xa Activity	Enoxaparin Dose Titration	Titration Factor (TF)	Next Anti-Xa Activity Check
<0.1 IU/mL	Increase dose by 20%	1.2	After 3rd dose
0.1-0.19 IU/mL	Increase dose by 10%	1.1	
0.2-0.5 IU/mL	Keep same dosage	1	Check in 1 week
0.51-1 IU/mL	Decrease dose by 10%	0.9	After 3rd dose
>1 IU/mL	Hold all doses until anti-Xa is 0.3, IU/mL, then decrease dose by 20%	0.8	Before next dose and every 12 hours, until anti- Xa <0.3 IU/mL

- **Subsequent Dose**

  _____mg (maximum of 30mg) = _____mg (previous dose) x _____ **(TF)**

<u>Step 4:</u> **Volume Calculation**

- Take the dose (mg) calculated in Step 2 or Step 3 and divide it by 20 mg/ml concentration for doses <10mg. Use 100mg/mL concentration for doses ≥10mg to obtain the volume that needs to be dispensed in a Vanish Point or another suitable syringe.

  _____mL = _____mg / ___ mg/mL (******Round the dose volume to the nearest hundredth******)

Step 5: Dispensing

- Dispensed _____mL (step 4) of enoxaparin in a Vanish Point or other suitable syringe. Dose is stable for 24 hours. Discard any unused drug.

Pharmacist's or Designee's Signature/Print Name: _____ **Date:** _____

APPENDIX III

Bleeding in the PICU

Instructions for Assessors: Complete this form daily while the patient was admitted to the PICU. USE A SEPARATE FORM FOR EACH UNIQUE SITE OF BLEEDING.

* Required

1. **Patient Study ID** *

2. **Study Collaborator** *

3. **Day of Admission in the PICU** *
   Day of admission to the PICU = Day 1. Please do NOT include days admitted to other units in the hospital.

4. **Bleeding Site** *
   Choose the site of the bleeding event on this day. If the patient did not have a bleeding event on this day, select "No Bleeding Event."
   Mark only one oval.

- ◯ No Bleeding Event *Stop filling out this form.*
- ◯ Vascular Catheter or Insertion Site *Skip to question 5.*
- ◯ Other Skin Bleeding (NOT CVC-Related) *Skip to question 7.*
- ◯ Gastrointestinal *Skip to question 8.*
- ◯ Respiratory (Including the Thoracic Cavity) *Skip to question 9.*
- ◯ Surgical Site *Skip to question 10.*
- ◯ Genitourinary *Skip to question 11.*
- ◯ Intra/Retroperitoneal *Skip to question 12.*
- ◯ Central Nervous System *Skip to question 13.*
- ◯ Eye, Ear, Nose, or Mouth *Skip to question 14.*
- ◯ Pericardium *Skip to question 17.*
- ◯ Joint *Skip to question 15.*
- ◯ Other (Describe) *Skip to question 16.*

Bleeding Site - Vascular or Catheter Insertion Site

5. **Vascular or Catheter Insertion Site** *
Mark only one oval.

- ◯ Peripheral Vein - Arm
- ◯ Peripheral Vein - Leg
- ◯ Femoral Vein
- ◯ Internal Jugular Vein
- ◯ Subclavian Vein
- ◯ Other: _____

6. **Specify Side** *
Mark only one oval.

- ◯ Left
- ◯ Right

Skip to question 17.

Bleeding Site - Other Skin Bleeding (NOT CVC-Related)

7. **Other Skin Bleeding Site(s) - NOT CVC-Related** *
Please select all that apply.
Check all that apply.

- ☐ Bruising
- ☐ Petechiae
- ☐ Non-Surgical Wound
- ☐ Other: _____

Skip to question 17.

Bleeding Site - Gastrointestinal

8. **Gastrointestinal Bleeding Site** *
Please select all that apply.
Check all that apply.

- ☐ Hematemesis
- ☐ Melena
- ☐ Hematochezia
- ☐ Other: _____

Skip to question 17.

Bleeding Site - Respiratory (Including the Thoracic Cavity)

9. **Respiratory/Thoracic Cavity Bleeding Site** *
   Please select all that apply.
   Check all that apply.

   - [ ] ETT/Tracheostomy Aspirate
   - [ ] Hemoptysis
   - [ ] Hemothorax
   - [ ] Other: _____

Skip to question 17.

Bleeding Site - Surgical Site

10. **Surgical Bleeding Site** *
    Please select all that apply.
    Check all that apply.

    - [ ] Incision
    - [ ] Drain/Tube
    - [ ] Other: _____

Skip to question 17.

Bleeding Site - Genitourinary

11. **Genitourinary Bleeding Site** *
    Please select all that apply.
    Check all that apply.

    - [ ] Gross Hematuria
    - [ ] Bleeding Around a Urinary Catheter
    - [ ] Menstrual Bleed (that required medical consultation or intervention)
    - [ ] Other: _____

Skip to question 17.

Bleeding Site - Intra/Retroperitoneal

12. **Intra/Retroperitoneal Bleed** *
Please describe the location of the bleed.

Skip to question 17.

Bleeding Site - Central Nervous System

13. **Central Nervous System Bleed** *
Please select all that apply.
Check all that apply.

- [ ] Epidural Bleed
- [ ] Subdural Bleed
- [ ] Subarachnoid Bleed
- [ ] Intraparenchymal Bleed
- [ ] Intraventricular Bleed
- [ ] Intraspinal Bleed (any site within the spinal column)
- [ ] Other:

Skip to question 17.

Bleeding Site - Eye, Ear, Nose, or Mouth

14. **Eye, Ear, Nose, or Mouth Bleed** *
Please select all that apply.
Check all that apply.

- [ ] Subconjunctival Bleed
- [ ] Retinal Bleed
- [ ] Hemotympanum
- [ ] Epistaxis
- [ ] Oropharyngeal Bleed
- [ ] Other:

Skip to question 17.

Bleeding Site - Joint

15. **Bleeding in the Joint** *

Please specify which joint(s) is/are involved.
Check all that apply.

- [ ] Shoulder
- [ ] Elbow
- [ ] Wrist
- [ ] Finger
- [ ] Hip
- [ ] Knee
- [ ] Ankle
- [ ] Toe
- [ ] Other: _____

Skip to question 17.

Bleeding Site - Other (Describe)

16. **Other Bleeding Site** *

Please describe the location of the bleed (if possible).

_____

Skip to question 17.

Bleeding Severity

Please select AT LEAST one item from ONLY ONE of the bleeding severity categories below. Please select the most severe category as it applies to the bleeding event documented on this current day of admission. Remember to use a different form for another bleeding event on this same day but at a different site.

17. **Major Bleeding**

Please check all that apply.
Check all that apply.

- [ ] Fatal Bleeding
- [ ] Clinically Overt Bleeding with Decrease in Hgb of >= 2g/dL in a 24-hr Period
- [ ] Bleed that is Intra/Retroperitoneal, Pulmonary, or Involves the CNS
- [ ] Bleeding Requiring Surgical Intervention in the Operating Room

18. **Clinically Relevant Non-Major Bleeding**
Please check all that apply.
Check all that apply.

- [ ] Overt Bleeding Requiring Blood Product Administration
- [ ] Bleeding that Requires Medical or Surgical Intervention NOT in the Operating Room (and Does NOT Include Menstrual Bleeding)

19. **Minor Bleeding**
Please check all that apply.
Check all that apply.

- [ ] Overt or Macroscopic Evidence of Bleeding that Does NOT Fulfill Above Criteria (for Major Bleeding or Clinically Relevant Non-Major Bleeding)
- [ ] Menstrual Bleeding Resulting in Medical Consult and/or Intervention

Skip to question 20.

Timing

20. **Type of Bleed** *
Mark only one oval.

- ◯ New Bleed
- ◯ Ongoing Bleed
- ◯ Recurrent Bleed at the Same Site

21. **Bleeding Start Date**
Please type which day of admission the bleed started.

_____

22. **Bleeding End Date**
Please type which day of admission the bleed ended.

_____

23. **Started Prior to Today's Study Day?** *
Mark only one oval.

- ◯ Yes
- ◯ No