PARTNERS HUMAN RESEARCH COMMITTEE DETAILED PROTOCOL

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Protocol Title: PET fibrin imaging of DVT and PE

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I. BACKGROUND AND SIGNIFICANCE

Venous thromboembolism (VTE), comprising deep venous thrombosis (DVT) and pulmonary embolism (PE), is a major public health problem. The estimated average annual incidence rate of overall VTE among persons of European ancestry ranges from 104 to 183 per 100,000 person-years and is similar to that of stroke,[1] the fifth leading cause of death in 2013.[2] For those of African descent, the incidence may be higher.[3–5] Incidence rates for PE ± DVT are 29 to 78 per 100,000 person-years and for leg DVT alone are 45 to 117 per 100,000 person-years.[1] Over the decade from 2002 to 2012, the incidence of VTE has been rising.[6] This has raised the question of whether this increase represents a true rise in incidence or potentially over-diagnosis with more available, but not necessarily more accurate, imaging techniques. Whatever the reason, it is clear from this data that it is vitally important to improve the accuracy of identifying VTE with targeted diagnostic tools.

The tools for diagnosis of VTE have evolved from contrast venography, ventilation-perfusion (V-Q) scans and pulmonary angiograms to D-dimer testing, venous duplex ultrasound and CT-angiograms. While these newer modalities are less invasive and more available; none is perfectly accurate and must rely on indirect measurements of clot, whether that is non-compressibility with ultrasound or filling defects with CT angiograms. Because of these limitations, it is recommended to improve the performance of the test through the use of Bayes' theorem, which incorporates pre-test probability to enrich the population for those that might or might not have VTE.[7] Despite the high value of this recommendation when there is concordance between the pre-test probability and the test result, problems arise when there is discordance. For CT-angiograms, if the pre-test probability of PE is high, the negative predictive value is 60%.[8] Conversely, if the pre-test probability of PE is low, the positive predictive value is 58%.[8] In contrast, if pre- and post-test results are concordant, the positive and negative predictive value is 96%.[8] The discordant situations lead to both under- and overdiagnosis if pulmonary artery angiogram is not pursued, and represent an area where targeted diagnostics utilizing a highly specific probe for newly cross-linked fibrin could help.

There are many other groups of patients at high risk for VTE that need targeted diagnostics. Venous thromboembolism is a disease of older age, with VTE virtually non-existent in adolescence but increasing in incidence rapidly with older age. Diagnosis becomes more difficult in older patients because of the diminished discriminatory power of D-dimer,[9,10] the high prevalence of renal dysfunction that increases the risk of contrast dye nephropathy and precludes the use of contrast-enhanced CT angiography, and/or the high prevalence of lung disease that renders interpretation of V-Q scans difficult or impossible. Patients with prior DVT or PE present another diagnostic challenge. When the imaging method relies on filling defects to diagnose VTE, it is nearly impossible to distinguish old versus new clot. Here again is an area where targeted diagnostics utilizing a highly specific PET probe for newly cross-linked fibrin could help distinguish old clot from new, throughout the body.

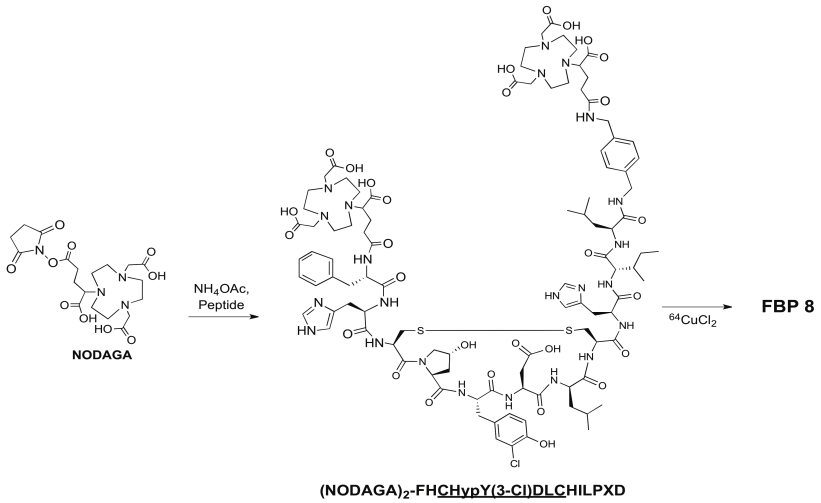
Furthermore, there are other patient groups that represent a challenge to our current diagnostic tools. Patients with contrast dye allergy may need premedication that delays or sometimes even prevents those patients from receiving intravenous contrast. Typically, these patients would receive corticosteroids and antihistamines starting 13 hours prior to injection.[11,12] However, even those that receive premedication can have contrast reactions.[13] Patients with PE who have negative venous duplex ultrasound exams are sometimes suspected to have pelvic vein thrombi, or those with suspected chronic thromboembolic pulmonary hypertension or angiosarcomas of the

pulmonary arteries could benefit from a diagnostic tool that can image fresh intravascular thrombi and distinguish it from tumor or organized, endothelialized old thrombus.

All these shortcomings of current diagnostic strategies mean that physicians often are either uncertain whether a patient truly has an acute VTE or feels certain when none is present. These situations invariably lead to errors in diagnosis and lead to two dangerous problems. Either patients have an acute thrombosis and do not receive anticoagulation, putting them at risk for a deadly pulmonary embolus, or, they are subjected to anticoagulation unnecessarily and the significant risk of life-threatening hemorrhage.

The development of a fibrin-specific targeted diagnostic that identifies fresh thrombotic foci anywhere in the body has the potential to be a transformative tool for VTE assessment that would greatly enhance our diagnostic accuracy and capability for an extremely common and deadly disease. ^{64}Cu-FBP8 (Copper-64 labeled fibrin binding probe 8, Scheme 1) is a molecular imaging probe which selectively binds the main constituent of human thrombi: fibrin. Thrombus formation and fibrin deposition are pivotal events in several human conditions including major cardiovascular diseases, cancer, and inflammation. ^{64}Cu-FBP8 combines the high specificity of a fibrin-binding peptide and the great sensitivity and potential full-body applications of nuclear imaging. Studies in rats showed that ^{64}Cu-FBP8 has excellent pharmacological and pharmacokinetic profile, with high target uptake and low retention in background tissues and organs.[14]

Our long-term research objective of this translational study is to establish a novel thrombus body scan (TBS) using a new fibrin-specific probe for positron emission tomography (PET) imaging to overcome the limitations of indirect VTE diagnosis, and, with one single test, provide a total-body assessment of fresh intravascular clot.



Scheme 1. Simplified synthetic route and chemical structure of ^{64}Cu-FBP8. The sequence of the core fibrin-binding peptide is reported on the bottom.

II. SPECIFIC AIMS

This proposal aims to generate strong evidence for VTE diagnosis using the thrombus body scan (TBS) from an initial small translational study that will justify a larger clinical study to obtain clinical approval for the TBS. We hypothesize that TBS using our new PET fibrin probe that specifically binds to fresh polymerized fibrin clot (< 1 week old), can directly detect thrombus anywhere in the body similar to ^{18}F-FDG PET scans for cancer nodules, will have better diagnostic accuracy for deep venous thrombosis and pulmonary embolus (DVT-PE) compared to current imaging modalities, can quantify the total body burden of fresh clot and can be performed in patients with renal dysfunction. Before we can fully test this hypothesis in an appropriately-powered clinical study, we propose a proof-of-concept study in a smaller cohort of patients to test the following specific aims and hypotheses:

1. Compare the presence, location and extent of PE diagnosed with the PET ^{64}Cu-FBP8 probe to conventional imaging. *Hypothesis:* The PET fibrin probe will allow direct imaging of acute thrombi causing PE, which is currently only indirectly diagnosed by filling defects on CT-angiogram or mismatched perfusion defects on V-Q scans.

2. Compare the presence, location and extent of DVT diagnosed with the PET ^{64}Cu-FBP8 probe to conventional imaging. *Hypothesis:* The PET fibrin probe will allow direct imaging of acute thrombi causing DVT diagnosed indirectly by incompressibility and loss of flow on lower extremity venous Doppler ultrasound.

3. In addition to PE and lower extremity DVT, assess for upper extremity and pelvic clot and compare the extent of total body clot burden imaged with the ^{64}Cu-FBP8 probe to the plasma D-dimer level. *Hypothesis:* The PET fibrin probe will discover acute thrombi including areas not assessed by current imaging methods and the total clot burden will correlate with the plasma D-dimer.

Successful execution of this study will lay the groundwork for a clinical trial to compare the efficacy of a clot-specific imaging modality (**thrombus body scan or TBS**) that has the potential to be more accurate for the diagnosis of VTE, better able to establish the total clot burden in the body with a single test and more widely applicable to patients with renal failure and lung disease.

III. SUBJECT SELECTION

A total of eighty (80) subjects will be recruited for this study, which will be performed at the Massachusetts General Hospital (MGH). All eighty (80) subjects will be studied with the same protocol: a single injection of ^{64}Cu-FBP8 and PET-CT imaging at least three hours after injection at four bed positions, which will provide the imaging data for fresh blood clot at the three locations covering all three specific aims.

Inclusion Criteria:
Each subject must meet the following criteria to be enrolled in the study:

- 18 years of age or older with a clinically significant pulmonary embolus (PE) confirmed by a filling defect in CT angiography (CTA), and
- Subjects must receive the radiotracer injection within 72 hours of their diagnosis.

Exclusion Criteria:

- Subjects < 18 years of age
- Time of expected radiotracer injection > 72 hours from the time of a positive venous duplex ultrasound or CT- angiogram,
- Women subjects of childbearing potential who are pregnant, seeking to become pregnant, or have a positive serum pregnancy (beta-HCG) test,
- Unable to lie flat for 45 minutes as assessed by physical examination and medical history (e.g. back pain, arthritis, dyspnea),
- Weight that exceeds the PET camera table limit (300 kg)
- The subject will not be enrolled in the study if the radiation exposure for research studies during the prior 12 months, combined with the exposure from this study would exceed 50 mSv.
- Due to the radiation exposure from imaging studies, all women of childbearing potential will be required to have a negative serum pregnancy test performed prior to any imaging procedures on the same day (if not already done that day). Patients with a positive serum pregnancy test will be excluded. Breast feeding women will also be excluded.
- A pre-existing condition or use of a medication including vasopressors and tPA (due to its effects on clot) that in the opinion of the investigator may place the subject at a substantially increased risk
- Hemodynamic instability, including requiring escalating doses of vasopressor medication.
- No groups designated as “special vulnerable populations” will be studied.
- No exclusions will be made based on race, sex, or ethnic origin.
- 64Cu-FBP8 (Copper-64 labeled fibrin binding probe 8) is cleared by the kidneys, patients with eGFR < 30 will be excluded.

IV. SUBJECT ENROLLMENT

Identification of eligible subjects will be done from 1) admissions to the Massachusetts General Hospital for treatment of pulmonary embolus (PE) 2) admissions to MGH for other indications but with an acute PE at admission or during hospitalization, 3) patients who present to the Emergency Department with an acute PE, but will not be admitted to the hospital. The subjects will be identified through the MGH Pulmonary Embolus Response Team (PERT), radiology notifications or running PeRC Epic team report, which could identify inpatients who were not picked by PERT or radiology notifications, or through patients physician investigators may be aware of that have an acute PE. PERT is a well-established group of cardiologists, pulmonologists, interventional radiologists, emergency room physicians, and cardiac surgeons who are "on-call" when a clinician anywhere in the hospital suspects a patient has a clinically significant pulmonary embolus. When PERT is activated, a fellow organizes a web-based meeting within minutes through a page and email to the participating physicians. The data is reviewed via "go-to-meeting" conference and a treatment plan is decided upon by consensus. An email will be sent to Dr. Witkin, and Mamary Kone when PERT is activated and Dr. Witkin will assess whether the patient is appropriate and potentially eligible for the study. Also, since the fibrin probe must be manufactured at MGH Navy Yard and sent to MGH main campus, only patients diagnosed at a time when they can receive the radiotracer within 72 hours of their diagnosis will be included. If the patient meets eligibility criteria, the patient's treating physician will be contacted to ask the patient for permission to be contacted by the study staff. During the first contact, the physician co-investigator will screen the subject for eligibility and describe the study and its implications, risks and benefits. If the subject is interested, the consent form will be given to the subject to read. The subject will be given as much time as necessary to decide, however if a decision cannot be made within the 72-hour limit from initial diagnostic imaging to the fibrin probe injection, or not before a scheduling time window for fibrin injection, the subject will be excluded. We will allow as much time as necessary for the subject's questions to be answered or for the subject to consult family members or treating doctors before the consent form is signed. After the subject signs the consent form, the original signed consent form will be kept on file, a copy will be given to the subject, and a copy will be scanned to the subject EMR. No study procedures will be performed prior to the subject signing the informed consent document. The informed consent process will be accurately documented in the subject medical records.

V. STUDY PROCEDURES

Subject Screening: Subjects will be studied while admitted in the hospital and followed up as an outpatient (via phone or after clinic follow up visit) for 1 year at 3, 6 and 12 months after their imaging. Additionally, subjects recently presenting to the MGH and / or admitted but discharged before enrolling into the study may return to MGH for the purpose of participating in this study. Initially, they will be screened only after the patient's treating physician has ask the patient for permission to be contacted by the study staff. The screening will consist of an in-depth review of the medical records (notes, labs, imaging tests, medications, etc.) to ensure that the subjects meet the inclusion and exclusion criteria for the study. After the informed consent is obtained, a physician investigator will take a detailed history, perform a physical exam and assess the subject's capacity to lie flat for imaging. Once inclusion criteria have been met and the subject consents to the study procedures, labs will be drawn daily (D-Dimer, PT, PTT, INR, platelets and fibrinogen) if not done so clinically for a maximum of 3 days from the day of initial CTA to the day of PET-CT.

On the day of PET-CT imaging, an intravenous catheter will be placed if there is not one already in place that can be used in the case of subjects admitted to the hospital. All women of childbearing potential will be tested for pregnancy unless a test result is available in the medical record that is valid for the day of PET-CT imaging. Women who are documented by medical history of not having child-bearing potential (i.e. tubal ligation or hysterectomy) or are post-menopausal with a minimum one year without menses will not need to undergo pregnancy testing. A STAT quantitative serum hCG pregnancy test will be performed on the day of the scan. Blood samples (up to 8 ml total) will be drawn for pregnancy test, coagulation panel (PT, PTT, INR), platelets and D-dimer if not already done that day by the clinical team. If the test performed comes back negative for pregnancy (<6 IU/L), the subject will be enrolled. If the test performed comes back positive for pregnancy the subject will not be enrolled in the study. Blood samples will be taken via indwelling catheters already present for hospitalized patients. If the subject's intravenous access is unable to be used, a new peripheral intravenous catheter will be placed by study staff to draw the required blood for beta-HCG (if required), D-dimer and

administer the radiopharmaceutical. For subjects coming for PET-CT as an outpatient an intravenous catheter will be placed to allow for obtaining blood samples and radiopharmaceutical administration.

Once finished, arrangements will be made to conduct the PET-CT imaging in a way that does not interfere with treatment for subjects admitted to the hospital.

Prior to radiotracer injection for PET imaging:

- The procedure will be explained to the subject and any questions he/she has about the procedure will be answered.
- Subject information such as height and weight will be obtained if not available.
- Vitals and ECG will be obtained prior to radiotracer injection.

Radiotracer injection:

- Up to 10 mCi ^{64}Cu-FBP8 will be injected at least 3 hours before the scheduled time for PET-CT imaging. The injected dose and time of injection will be recorded. The injection will take place at the bed side.
- The catheter will be flushed post-injection of ^{64}Cu-FBP8 with 10ml 0.9% saline solution.
- Vitals will be obtained immediately after radiotracer injection.

PET-CT imaging:

- For subjects admitted to the hospital close to the scheduled time for imaging, which is at least 3 hours after injection, the subject will be escorted (transported) in the wheelchair or stretcher/bed by hospital transport and/or study staff depending on the subject conditions to the PET-CT suite; we will follow the hospital standard procedures for patient transport. The PET-CT technician will submit a patient transport order through Epic. When the PET suite becomes available, the hospital transport team will transport to the PET-CT location. If the subjects need to be monitored during the transport, then one of the study physicians will be present during the transport.
- Subjects coming as outpatient will come to the hospital (Anesthesia or Pulmonary Research area) at least 4 hours prior to the imaging where they will be injected and monitored before taking them to the PET-CT imaging suite.

- Prior to the scan subjects will be asked to urinate to minimize the possibility that they will need to move during the scan.
- The subjects will be positioned on the scanner table; support devices under the back and/or legs will be used as needed to enable the patient to comfortably maintain his/her position throughout the scan.
- A scout image (Topogram) which takes about 10 seconds will be obtained to locate the proper fields for imaging.
- Afterwards, CT and PET scans will be obtained for four bed positions: thorax, pelvis and thighs (2 positions). The CT scan for each position of the PET scans is necessary to correct for the attenuation of the ^{64}Cu-FBP8 activity caused by body tissue. We will perform an additional PET/CT scan of the lower portion of the thighs in order to image the full/whole thighs to the knees. Consequently, a total of 4 CT and 4 PET scans will be performed in four different positions. Imaging duration will be less than 10 seconds for each CT, and 10 minutes for each PET scan. The total time of imaging is expected to be approximately 45 minutes.
- We may have to repeat a PET-CT scan if we have an unexpected problem (for example, if subjects need to change position during a scan).
- Subjects will be monitored throughout the imaging session, and measurements including heart rate, blood pressure, and pulse oximetry will be recorded.

Post-scan procedures:

- After the scans are completed and last vital signs collected, the subject will be escorted back to his/her room on the ward if applicable
- The subject will be asked to void again immediately after the scan, and he/she will be counseled on the importance of continuing to drink fluids for several more hours. This will increase urine flow rate, which will help minimize the radiation dose to the bladder wall.

Data acquisition and image reconstruction:
PET-CT images will be acquired using commercial, FDA-approved scanners using conventional sequences for image acquisition. Images will be reconstructed using the algorithms provided by the scanner manufacturer. All images will be completely anonymized and de-identified and labeled only with a study number. No personal information whatsoever will be contained in the images or header files. The images will be transferred electronically over a secure internal network to the storage site at the Pulmonary and Bioengineering Laboratory, where they are securely stored and backed-up. Only study staff will have the required permissions and ability to access these files.

A separate database file, linking the study number to the relevant clinical data on each subject will be generated. These data will be stored in a password-protected Redcap (Research Electronic Data Capture) database supported by the Massachusetts General Hospital and Partners Healthcare. Only study staff involved in data collection and analysis will have access to this database. For this study, we will record basic demographic data (age, gender, race/ethnicity), and clinical data (results of laboratory values such as serum creatinine, D-dimer and NT-pro-BNP, results of CT-angiogram, including de-identified DICOM files, results of venous duplex ultrasound, echocardiographic parameters of cardiac size/function/hemodynamics, and treatment, height, weight and baseline hemodynamics and hospital mortality), as available. Additionally, images will be stored for data analysis on a password protected file server at the Pulmonary Imaging and Bioengineering Laboratory at Massachusetts General Hospital, and access will be restricted to study staff involved in data analysis.

Sources of research material will include a) results of history and physical examination, b) medical record review (EPIC, Epic Systems, Verona, Wisconsin, USA) for pertinent clinical and laboratory results, and c) review of clinically relevant imaging studies including CT-angiograms, venous ultrasound with Doppler, echocardiograms, and drug and/or mechanical treatment for VTE. In the case of women of childbearing potential, a serum sample will be collected for the purposes of determining pregnancy status. The data will be used only for research purposes and will be kept anonymous (by using a number without names) and confidential. In addition to PET-CT data acquired as above,

Study Events

PRE-IMAGING			IMAGING			Follow up			
Events	Pre-dosing	Dosing (at least 3 hours before imaging)	CTs	PET		3 months	6 months	12 months	
PERT or Radiology Notifications	X								
Eligibility checklist	X								
Informed consent	X								
Medical History	X								
Physical exam	X								
Vital Signs[a]	X								
IV placement	X								
Pregnancy Test[b]	X								
D-dimer	X								
^{64}Cu-FBP8 injection		X							
PET-CT			X	X					

Hemodynamic measures[c]	X	X	X	X					
ECG[f]	X	X							
Adverse events	X	X	X	X					
VEINES -QOL	X					X	X	X	
6 MWT[g]	X					X	X	X	
EMR Review	X					X	X	X	

a. Vital signs: respiratory rate, pulse, blood pressure, and temperature.
b. Pregnancy serum HCG test: Women of childbearing potential.
c. Hemodynamic measures: During the scan heart rate, respiration rate, heart rhythm, pulse oximetry will be measured. Values will be recorded before probe injection, immediately after probe injection and throughout the scan.
d. Blood draws: If not done for clinical purposes, blood will be drawn prior to radiotracer injection - blood will be sent for a coagulation panel [Fibrinogen, PTT (PTT, ARPT), PT/INR (INR/ARINR)], platelets and D-dimer.
e. Anticoagulants used as well as timing and dosing will be recorded.
f. ECG: All subjects will have ECGs for monitoring before and after the scans if available.

Follow up:
Patients will be contacted at 3, 6 and 12 months following the diagnosis of PE. Death and recurrence of DVT-PE will be assessed. Results of any imaging studies (CT angiograms, echocardiograms, or venous duplex ultrasound studies) and serologic studies (follow up d-dimer) will be collected and patients will undergo a survey to collect information about symptoms attributable to PE-DVT as well as quality of life using Standard Form 36,[28] VEINES QOL29 and the patient's self-assessment of their overall health status on a 1 – 10 scale, with 1 indicating the worst possible and 10 the best possible health. For patients followed at MGH this will be done in person at the end of their PERT or pulmonary clinic follow up clinic visit. For those followed elsewhere, this will be done by telephone with subjects and their treating physicians. NYHA functional class, 6-min walk distance, and change in pulse oximetry with walking will be assessed.

VI. BIOSTATISTICAL ANALYSIS

Once a patient is enrolled, the subjects will receive an injection of up to 10 mCi ^{64}Cu-FBP8 and imaged with CT-PET approximately at least 3 hours after injection, when the "target-to-background" ratio (TBR) of activity is expected to be high based on previous studies.[27] With CT-PET the attenuation correction is done using a low-dose CT volumetric scan. Afterwards, PET acquisitions of 10 min duration each are performed in four positions, thorax, pelvis and thighs (2 positions), which are used to assess regional ^{64}Cu-FBP8 activity.

Specific data variables:
The standardized uptake value (SUV) of ^{64}Cu-FBP8 uptake in the lungs, pelvis and thighs will be calculated. The target to background ratio (TBR) for ^{64}Cu-FBP8 activity will be calculated using as background a region of interest (ROI) created from large cardiac and vascular structures. This corrects the ^{64}Cu-FBP8 activity for differences in injected dose and timing of imaging and allows for flexibility in the image timing. One member of our group (T. Winkler) and another from Nuclear Medicine (U. Mahmood) will be blinded to the clinical results and will identify the presence or absence of clot in the three imaged locations as well as the anatomic location(s) and extent as best as possible from the overlay of the fibrin activity on the attenuation correction CT scans. Additionally, we will perform quantitative image analyses including the volume of the thrombus ROI, activity of imaged clot in each imaged section and clot burden. The thrombus ROI is defined by a threshold of 50% between peak activity of the ROI and background activity. Multiple ROIs will be used in the case of separated thrombi. Clot burden will be estimated using the sum of ^{64}Cu-FBP8 activity within a thrombus ROI and then normalized by the injected dose.

Outcome Measures:

Specific Aim 1: The location and extent of the thrombus region of interest (ROI), its volume and clot burden within the lung ROI will be compared with the ROI of filling defects on CT-angiogram. We will determine the number and location of the thrombus ROI(s) within the lungs, and the overall clot burden as sum of ^{64}Cu-FBP8 activity within a thrombus ROI(s) within the lungs. Additionally, we will calculate the intersection of the thrombus ROI and filling defects ROI relative to volume of the thrombus ROI. That will quantify the fraction of thrombus ROI(s) using a scale from being are completely outside (0) to completely inside (1). Also, we will perform a lung segmentation using the software APOLLO (VIDA Diagnostics) and determine in which lung lobe the thrombus ROI(s) are located.

Specific Aim 2: The thrombus ROI, its volume and clot burden within the thigh ROI will be compared with the location and extent of the clot on venous duplex ultrasound. We will determine the number and location of the thrombus ROI(s) within the thigh ROI, and the overall clot burden as sum of ^{64}Cu-FBP8 activity within a thrombus ROI(s) within the thigh ROI. Additionally, we will quantify the volumes and locations of the thrombus ROIs of the PET scan and the venous duplex ultrasound relative to each other. Also, we will perform a vessel segmentation using the 3D Slicer (open source software) and determine the location of thrombus ROI(s) with the blood vessel.

Specific Aim 3: The prevalence of high ^{64}Cu-FBP8 fibrin activity in the pelvic ROI and upper extremity ROI's will be reported. Additionally, as a secondary analysis, both the total fibrin activity in the imaged fields, excluding the liver, gallbladder, kidneys and urinary bladder normalized by injected dose for all patients will be correlated with the plasma D-dimer drawn at the time of imaging. D-dimer levels will be drawn daily from the time of first diagnostic imaging to the fibrin probe imaging (up to 3 days maximum in total) as well as PT, PTT, platelets and fibrinogen. Anticoagulants used, as well as timing and dose will be carefully recorded during this period.

For all Specific Aims, death, VTE recurrence, and quality of life measures will be compared with the clot burden measured by PET and conventional imaging modalities, stratified by treatment (systemic thrombolysis, catheter thrombolysis, or heparin alone) using multiple correlation, ANOVA, and t-tests for pairwise comparisons.

VII. RISKS AND DISCOMFORTS

^{64}Cu-FBP8 PET probe:

All subjects will undergo PET-CT imaging with the fibrin-targeting PET probe ^{64}Cu-FBP8, which is covered by IND128547. 37 research subjects have been imaged to date with ^{64}Cu-FBP8 without adverse effects. Also, the safety of ^{64}Cu-FBP8 is being further evaluated in a separate study by a Co-Investigator of the grant (P. Caravan) in human volunteers. However, we expect this probe to be extremely well tolerated. A GLP toxicity study in rats, using the non-radioactive form of ^{64}Cu-FBP8, indicated no observable adverse effects in rats at 0.3 mg/kg (>200 times the anticipated dose in humans). ^{64}Cu-FBP8 is similar to a gadolinium-based MRI contrast agent EP-2104R. Both compounds comprise an eleven-amino acid peptide, of which there is a six-amino acid disulfide-bridged cyclic portion, and these peptides bind to the same site on fibrin. Although the peptides in the two compounds are not identical, they share the same seven key amino acids necessary for fibrin recognition. Both ^{64}Cu-FBP8 and EP-2104R have metal chelators to bind copper and gadolinium, respectively, to generate signal in PET and MRI. EP-2104R underwent phase 1 and phase 2 clinical trials to assess its safety and feasibility as a thrombus imaging agent in patients with thrombosis.[27] The results of the 52 patient phase 2 trial were published, and none of the 52 subjects enrolled were removed from the study because the appearance of adverse effects after probe administration. Mild adverse effects were present in 27% of patients (14 out of 52), but none were considered to be related to the study drug.[27] In that study, EP-2104R was administered at the dose of 4 μmol/kg, which corresponds to 17.3 mg/kg or 1.2 grams for a 70 kg adult. The EP-2104R dose is greater than 10,000 times higher than the dose of ^{64}Cu-FBP8 proposed in this application (90 micrograms per subject). Therefore, we do not anticipate any adverse effects related to ^{64}Cu-FBP8 probe administration. ^{64}Cu-FBP8 is cleared by the kidneys, but it does not contain any gadolinium. There is no risk of NSF. The reference to EP-2104R was to demonstrate that the fibrin binding peptide that is common to both ^{64}Cu-FBP8 and EP-2104R had been previously administered to human subjects at a dose more than 10,000 times the current chemical dose of ^{64}Cu-FBP8. This was meant to convey that ^{64}Cu-FBP8 should be safe and well tolerated

Radiation:

Radiation exposure has been assessed for ^{64}Cu-FBP8 using human time-activity curves extrapolated from the time-dependent rat biodistribution data. The cumulative activity (Bq-h/Bq) for each source organ was determined by integrating the area under the time-activity curves by using the least-squares analysis, and organ absorbed doses were estimated by using the software program OLINDA/EXM (version 1.0, Vanderbilt University, 2003). Based on these data, the kidneys are the primary critical organs, followed by urinary bladder and liver. The estimated effective dose for humans was 0.017 mSv/MBq.[23] Initial data in human volunteers confirmed this effective dose estimate. We plan to inject no more than 370 MBq (=10 millicuries) per subject, which would cause a maximal effective dose of approximately 6.2 milliSieverts (mSv), and is less than the proposed up to 15 mCi in the original IND. The radiation exposure in this study will be small and there is no evidence that it represents a major health risk. If subjects have participated in other research studies in the past 12 months that have involved radiation exposure, they will be asked to inform the investigators or study staff (by placing a check mark on the consent form verifying that they have or have not been exposed to other radiation in the past 12 months). If it is determined that the prior radiation exposure exceeds our current guidelines (i.e. 50 mSv/year) the subject will not be enrolled in this study. We estimate that the total exposure of each subject to ionizing radiation includes:

1. Topogram 0.5 mSv (for identifying scanning region for CT)
2. Low-Resolution CT scans in four positions (thorax, pelvis and thigh x2): 0.95 mSv x 4 (for attenuation-correction of PET), which 3.8 mSv.
3. ^{64}Cu-FBP8 Injection (10 mCi x 0.62 mSv/mCi): 6.2 mSv

The estimated total radiation dose for a subject is 10.5 mSv. However, if we repeat a PET-CT scan for an unexpected problem (for example, if subjects need to change position during a scan), the total radiation dose for will be 11.45 mSv.

Discomfort
The most common discomforts that we can anticipate are soreness and bruising at the intravenous placement site (if not already in place) and back pain from lying on the PET scanner table for a prolonged period of time. Both should be mild and short-lasting. We will warn subjects of these discomforts as well as assess by physical exam whether patients can tolerate lying flat for 45 minutes. This may require simulating this position and assessing the patient's response. Those patients with severe back pain, symptomatic arthritis, or dyspnea when lying flat will be excluded.

Drug side effects and toxicities
Lidocaine: The small amount that may be used (1-3cc, 1%) to numb the skin is such that there are almost no risks associated at this dose. Unlikely but serious side effects include nausea, drowsiness, mental/mood changes, ringing in the ears, dizziness, vision changes, tremors, numbness, headache, or backache.

Psychosocial risks
Since the PET scanner, like the CT scanner and in contrast to the MRI scanner, is not a closed tunnel but an open "donut" there should be no problems with claustrophobia. However, if for any reason, a subject is uncomfortable or anxious while in the PET scanner he or she is free to withdraw from the study at any point. We will be monitoring blood pressure, heart rate and oxygen saturation throughout the study and if the subject is noted to appear anxious or demonstrate sustained increase in systolic blood pressure or heart rate (> 25% above baseline), we will remove the subject from the scanner and assess.

Intravenous Catheter
In the unlikely event that a subject does not have intravenous access or has access that we cannot use for any reason, we will place a peripheral catheter for blood draws and injecting ^{64}Cu-FBP8. Risks of intravenous catheters include pain and bruising at the placement/injection site; infection; bleeding; injury to vessels, surrounding nerves, and tissues; air embolism, catheter fracture and embolism; hematoma; phlebitis; infiltration; vein thrombosis; catheter malfunction.

VIII. POTENTIAL BENEFITS

All of the CT scans will be read by a member of MGH's Thoracic Radiology staff as part the institution's policy

for identification of incidental findings. If incidental findings are identified and the subject gives consent, we will provide this information to the subject's primary care physician. If clot is identified by the fibrin scan in places not seen or not evaluated by the treating team (e.g. the pelvis), this information will be conveyed to the treating team and appropriate clinically-approved testing can be done to assess this if the treating team deems it necessary.

Venous thromboembolism (VTE), comprising deep venous thrombosis and pulmonary embolism, is a major public health problem. The estimated average annual incidence rate of overall VTE among persons of European ancestry ranges from 104 to 183 per 100,000 person-years and is similar to that of stroke,[1] the fifth leading cause of death in 2013.[2] Incidence rates for PE±DVT are 29 to 78 per 100,000 person-years and for leg DVT alone are 45 to 117 per 100,000 person-years.[1] For those of African descent, the incidence may be higher.[3–5] Over the decade from 2002 to 2012, the incidence of VTE has been rising.[6] This has raised the question of whether this rise represents a true increase in incidence or potentially over diagnosis with more available imaging techniques. Whatever the reason, it is clear from this data that it is vitally important to improve the accuracy of identifying VTE with targeted diagnostics. The knowledge gained from this study will allow an assessment of whether or not a fibrin-specific PET probe has the potential to improve our diagnostic accuracy of VTE, diagnose VTE in previously difficult areas (pelvis) and broaden the patient population on which specific diagnostic testing can be performed, such as those with renal failure.

IX. MONITORING AND QUALITY ASSURANCE

This is an interventional study. Therefore, the safety monitoring will be conducted by an Independent Safety Monitor, Rahimi,Rod A, MD, PHD, Pulmonologist at MGH. Monitoring: Subjects will be monitored for any adverse health effects during the study by the PI and staff pulmonologists (Dr. Witkin or Dr. Montesi). A physician investigator will perform a physical exam prior to, and after the study and will also assess the subjects at times during the study as the subject's symptoms and vital signs warrant. If the subject develops severe coughing, has a respiratory rate > 35/min for > 5 min, SpO2 < 88% for 5 min and not corrected by administering extra O2 or if SpO2 < 80% at any time, a HR sustained < 50/min or > 140/min, or BP falls below 90/60 or rises above 160/90 with no history of hypertension at baseline the study will be terminated.

An Independent Safety Monitor with expertise consonant with the nature of risks involved with the study will oversee the study safety. Specifically, he will review the safety of the data after the first subject completed the study. He will be responsible for this review and decide whether the research should be altered or stopped. After this initial review, the Independent Safety Monitor will continue to monitor the study along with the investigator each time any adverse event or serious adverse event occurs regardless of whether such an event is study related or not.

The independent research monitor shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report. The independent research monitor shall have the responsibility to promptly report their observations and findings to the IRB.

However, if any adverse effect occurs, the principal investigator will send, as immediately as possible, a report to the IRB for analysis. At least one physician investigator will be present throughout the entire study. Adverse events will be discussed with all research staff. All adverse effects associated with the use of the radioactive drug must will be reported directly to the RDRC and FDA.

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