CLINICAL RESEARCH PROTOCOL

UNIVERSITY OF MARYLAND MEDICAL CENTER, DEPARTMENT OF MEDICINE, DIVISION OF PULMONARY AND CRITICAL CARE MEDICINE

DATE: 8/23/2024 **CLINICAL PROTOCOL NO**.: HP-00070208

ClinicalTrials.gov ID: NCT03126968

TITLE: Prophylactic topical epinephrine to reduce transbronchial lung biopsy-related hemorrhage in lung transplant recipients: a prospective double-blind placebo-controlled trial (PROPHylactic Epinephrine in Transbronchial biopsy [PROPHET] Trial)

SHORT TITLE: Prophylactic epinephrine and bleeding risk in transbronchial lung biopsies

IDENTIFYING WORDS: Lung transplantation; Fiberoptic bronchoscopy; Transbronchial lung biopsy; Topical epinephrine

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ESTIMATED DURATION OF STUDY: 24 months

TYPE OF PATIENTS: Post-single or double lung transplantation

SUBJECTS OF STUDY:
Number of participants to be screened: 200
Number of participants to be enrolled: 100
Sex: Male & Female
Age Range: 18 years and above

PROJECT USES IONIZING RADIATION: Yes, for medically indicated reasons only

PROJECT USES "DURABLE POWER OF ATTORNEY": No

OFF-SITE PROJECT: No

MULTI-INSTITUTIONAL PROJECT: No

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INTRODUCTION

Transbronchial Lung Biopsy

General Considerations

Transbronchial Lung Biopsy (TBLB) is one of the most important applications of flexible bronchoscopy. The procedure, initially introduced by Andersen in 1965 for use via a rigid bronchoscope (1), became more widely performed after it was adapted for use with the flexible bronchoscope in the early 1970s (2) for the evaluation of localized as well as diffuse pulmonary disease. Today, TBLB is regularly performed by 69% of practicing physicians documented in a survey of 1,700 North American pulmonary and critical care physicians (3). This indispensable procedure, which may be performed under fluoroscopic guidance, constitutes a diagnostic alternative to percutaneous transthoracic needle biopsy, thoracoscopy with forceps lung biopsy, and thoracotomy with open lung biopsy. Apart from mastering the technique, the bronchoscopist must have a thorough understanding of indications, limitations, and immediate complications of the TBLB. When properly performed, several studies have established the safety of TBLB in an outpatient setting under moderate sedation (4,5).

Biopsy forceps commonly used for TBLB via the flexible bronchoscope are generally of the order of 3 mm or smaller in any given dimension. Because of this restriction in size, tissue samples obtained via the transbronchial approach are generally 2–3 mm in any dimension. Despite the small size, TBLB provides information regarding pathology that is located beyond the cartilaginous airways that may include elements of the small airways of the distal bronchial tree, the alveolar space, the vasculature, and lymphatic structures immediately surrounding the alveoli (6). Pulmonary diseases that require examination of larger pieces of lung tissue to assess heterogeneity or homogeneity of different regions of the involved lung, such as many of the idiopathic interstitial lung diseases, are generally not amenable to diagnosis by TBLB and consideration of video-assisted thoracoscopic lung biopsy ,which has been shown to have a greater diagnostic yield (7,8), should be pursued. With these limitations in mind, TBLB is useful for the diagnosis of a variety of infectious, interstitial, and malignant pulmonary diseases, including pulmonary infiltrates in the immunocompromised host (9,10), sarcoidosis (11,12), eosinophilic lung disease (13,14), drug-induced pneumonitis (15), pulmonary alveolar proteinosis (16), lung cancer (17), pulmonary lymphangitic carcinomatosis (18,19), and acute allograft rejection in lung transplant recipients (20,21). Absolute contraindications for TBLB include inability to obtain informed consent, severe or refractory hypoxemia, uncontrolled bronchospasm, and unstable cardiovascular condition, including active ischemia and uncontrolled arrhythmia. Relative contraindications include uncorrected coagulopathy, platelet count of less than 50 K/µL, severe pulmonary hypertension, uncooperative patient, inability to control cough, and lack of resuscitation facilities (22,23).

Technique

A thorough airway examination usually precedes the TBLB because bleeding after lung biopsy may preclude its performance. Adequate control of cough with topical application of lidocaine

and systemic administration of opiates is recommended for optimal biopsy procedure and to reduce the risk of pneumothorax (24).

The choice of biopsy site depends on radiological and fluoroscopic findings. Generally, TBLB is not attempted from both lungs during the same bronchoscopic procedure on the same date due to risk of bilateral pneumothorax. For selection of the site for the TBLB in the case of diffuse lung disease, some providers prefer to perform the biopsy from the dependent parts of the lungs; right and left lower lobes (23). In an event of bleeding, the blood is more likely to remain contained in this area before spilling into the adjacent lobes (25). The procedure can be performed "blindly" or under fluoroscopic guidance when available.

There is limited information on the optimal number of transbronchial biopsies in various lung disease. In general, four to six biopsy specimens are adequate in a majority of patients with diffuse lung disease (26). For acute graft rejection surveillance in lung and lung-heart transplant recipients, most transplant centers will obtain at least 5 and up to 10 biopsies per procedure (20,27).

Hemorrhagic Complications

TBLB is generally safe, although severe and life threatening procedure-related complications are occasionally encountered, most common among them are pneumothorax and procedure-related hemorrhage. Significant bleeding related to TBLB has been traditionally attributed to laceration of a bronchial artery (28). Established risk factors for clinically significant bleeding following TBLB include bleeding diathesis including thrombocytopenia (29), use of antiplatelet and anticoagulant medications (30,31), pulmonary hypertension (32), advanced renal failure (33), and immune compromised state (34). The propensity to bleed following TBLB was also shown to be related to the type of forceps used with some evidence suggesting that bleeding complications are less common with alligator forceps as opposed to cup forceps (35,36). According to a recent retrospective population-based study from the USA incorporating data from almost 100,000 patient visits involving TBLB, the overall rate for procedure-related clinically significant hemorrhage was 0.58% (37). Another survey from Japan reviewing over 100,000 procedures, among them 57,199 forceps biopsies, recorded a bleeding rate of 0.73% (38). Death related to airway bleeding in association with bronchoscopic procedures is exceedingly rare (39,40).

Quantification of TBLB-related bleeding is difficult, subjective, and prone to observer bias. Although not formally adopted, several grading systems have been suggested for the purpose of quantification of procedure-related hemorrhage. Most published studies use grading systems that rely on the amount of mixed blood and bronchial wash fluid in the vacuum suction system at the end of the procedure (5,38,41,42). This method may be prone to measurement errors in estimation of the actual extent of blood loss during the procedure due to dilution of blood with other airway secretions as well as fluid administered via the bronchoscope working channel such as saline. A recently published study exploring the safety of percutaneous bronchoscopy-guided tracheostomy in thoracic transplantation patients suggested a grading system that classifies endobronchial hemorrhage as mild, moderate, or severe based on the most proximal bronchus in the bronchial tree obstructed by the bleeding (43). Among the other grading scales described in the literature thus far, the one suggested by Herth et al. (44) may have to most clinical relevance. This scale grades airway bleeding according to the action implemented by the

operator in order to achieve hemostasis. Despite being the only scale to be implemented in a subsequent study (45), it seems that a bias may be introduced into this system by the fact that different operators may have different thresholds for implementation of various interventions in order to control hemorrhage. Indeed, there is lack of agreement in currently available literature as to the best approach to achieve control over the bleeding lung. In general, one school of thought supports the wedging technique, first described by Zavala in 1975 (46), where the bronchoscope is wedged into the bleeding bronchus in effort to allow enough time for a clot to form and at the same time prevent spillage of blood into the rest of the bronchial tree (23). The other main school of thought argues for a "back and forth" or "in and out" maneuver that includes positioning of the bronchoscope proximal to the bleeding bronchus and suctioning of blood as it emerges from the airway in order to avoid coagulation in the airway, which can block the suctioning channel and also obscure vision (47).

As mentioned above, the main danger of TBLB-related bleeding is aspiration of the blood into the non-involved bronchial tree rather than exsanguination. Therefore, priority should be given to prevention of flooding of the airways with blood. Fortunately, most cases of major airway bleeding following TBLB can be managed in the bronchoscopy suite. Apart from positioning the bronchoscope in the airway as mentioned above, other measures to achieve hemostasis. These include placing the patient in a lateral decubitus position with the bleeding lung in the dependent position and application of topical application of 4°C saline (48) and/or vasopressors such as cocaine or diluted epinephrine (49,50). The dosing of topical epinephrine for the treatment of airway bleeding lacks standardization in terms of dilution and volume of instillation and is based mainly on individual operator experience and institutional protocols. Review of the literature reveals epinephrine dilution recommendations range from 1:10,000 to 1:100,000 and the volume of instillation ranges of 0.5 and up to 20 mL (22,50–52). The British Thoracic Society guidelines on diagnostic flexible bronchoscopy suggest small aliquots of 1:10,000 diluted epinephrine (49). Topical administration of tranexamic acid as a measure of achieving hemostasis was also previously described in case series (53,54). Bleeding refractory to conservative therapies may require balloon tamponade with application of an embolectomy balloon catheter via the bronchoscope's working channel (23,49). Inability to control bleeding with those interventions should prompt securing the airway with endotracheal intubation. Additionally, selective intubation of the non-bleeding lung and application of an endobronchial blocker may be considered along with advanced interventions such as selective bronchial artery embolization (55,56).

Local practices in University of Maryland Medical Center (UMMC) for the initial treatment of endobronchial bleeding in the bronchoscopy suite includes administration of iced saline in aliquots of 5-10 mL followed by 1-2 mL aliquots of 1:10,000 topical epinephrine (0.1-0.2 mg) to a maximum of 6 mL (0.6 mg). Additional suggested measures include positioning the patient with the bleeding lung in the dependent position and balloon tamponade of the bleeding airway with application of a 2-4 FR embolectomy balloon catheter (Edwards Lifesciences®) via the bronchoscope's working channel before attempting more advanced methods to allow hemostasis.

The Role of Transbronchial Lung Biopsies in Lung Transplant Recipients

Fiberoptic bronchoscopy with multiple TBLB has become the "gold standard" diagnostic modality for the assessment of lung allograft function and specifically acute cellular rejection (ACR) following lung transplantation. Most lung transplant programs now perform TBLB for graft function surveillance, new onset symptoms, ≥10% decrease in FEV1, assessment of a new lung infiltrate on chest imaging, or as a follow-up for acute rejection or CMV pneumonitis (57). Acute rejection is an established risk factor for the development of bronchiolitis obliterans syndrome (BOS), and therefore it has been proposed that early detection and treatment is of paramount importance (58). The relatively high incidence of silent rejection or infection in asymptomatic patients has lent support for routine surveillance protocols (59,60). Surveillance protocols vary widely between transplantation centers since there is little data to guide the frequency of surveillance biopsies. The ACR surveillance protocol in UMMC includes routine bronchoscopy with TBLB at one-month post-transplant and then every three months for one year. Additional bronchoscopies with TBLB are performed based on surveillance TBLB findings, symptoms, or results of pulmonary function tests (PFT) (61). As in the general population, the most common complications of TBLB in lung transplant recipients are bleeding and pneumothorax. Generally, there is evidence that shows a higher propensity for procedure-related bleeding in the post lung and heart-lung transplant population. Once again, the recorded rate of clinically significant lung hemorrhage is highly dependent of the system adopted for grading of the amount of bleeding. In an early study by Chan et al., bronchoscopy data from 83 lung transplant recipients was reviewed retrospectively. The rate of clinically significant procedure-related bleeding, defined arbitrarily as >50 mL blood loss was 1.9% (62). Scott et al. prospectively evaluated the yield and complications of TBLB in 219 procedures on combined heart-lung transplant patients. Blood was recovered by suctioning in approximately 44% of procedures, while the rate of >100 mL procedure related bleeding was recorded at 12.3% (20). Bjørtuft et al. studied the rate of TBLB-related bleeding in 104 procedures in 51 patients, 29 of whom were post lung or heart-lung transplantation. The rate of clinically significant bleeding, arbitrarily defined is more than 20 mL, was 7.7%. No difference in the rate or volume of clinically significant bleeding was observed between transplant and non-transplant patients. Interestingly, screening platelet count and coagulation tests did not predict bleeding risk in this study (63). A prospective cohort study performed at the Johns Hopkins Medical Institutions between 1996 and 1997 evaluated bleeding complications among 38 bronchoscopies in 15 lung transplant recipients and compared them to 659 bronchoscopies in 423 non-transplant patients. In this cohort, lung transplant recipients were shown to bleed significantly more, report post-procedure hemoptysis, and have their procedure terminated prematurely due to bleeding in comparison to the non-transplant patients. Bronchoscopy procedures, including those where TBLB was performed, were significantly longer in transplant recipients. In a logistic regression analysis, worse hemoptysis was associated with lung transplant status, older age, and longer procedure time. As in prior studies, bleeding risk was independent of platelet count, coagulation studies, use of aspirin, and use of immunosuppressive medications (64). The relation between risk of bleeding and procedure time might reflect a technically challenging procedure or in some cases prolongation of the procedure by attempt to control bleeding. Other possible explanations for the increased propensity to bleed among lung transplant recipients include an already inflamed lung tissue from infection, ACR, or BOS or an increase in blood flow to the transplanted lung, particularly in recipients of single lung

transplants. Evidence from transplantation centers outside the USA correlates with USA reported numbers. For example, a study in performed in a single institution in Japan recorded a bleeding rate of 11% among 206 procedures on 28 lung transplant recipients (65). Additional relevant studies include McWilliams, 2008 (includes additional citations: 1-5) and Smith, 2012.

Topical Epinephrine for Bleeding Prophylaxis

Epinephrine, also known as adrenalin or adrenaline, is a non-selective agonist of all adrenergic receptors, including the major subtypes α1, α2, β1, β2, and β3. β-Adrenergic effects are more pronounced at low doses and α1-adrenergic effects at higher doses. The rationale for the use of epinephrine for prevention, minimization, and control of superficial bleeding is derived from its vasoconstrictive effect on arterioles and capillaries, mediated via activation of α1 receptors on blood vessel smooth muscle (66).

Although adopted by some providers as means to reduced TBLB-related hemorrhage, the use of prophylactic instillation of diluted epinephrine into the target bronchus before attempting TBLB as means to reduce to occurrence of biopsy-associated bleeding is not established in the medical literature. Limited evidence from case series performed in a single institution suggest a beneficial effect of prophylactic injection of tranexamic acid into bleeding-prone endobronchial lesions such as tumors before attempting endobronchial biopsies (67,68).

The main body of evidence regarding the efficacy and safety of prophylactic topical epinephrine for the prevention of superficial hemorrhage is derived from the surgical literature, specifically endoscopic endonasal and sinus surgery, rhinoplasty, and surgical management of burn injuries. During functional endoscopic sinus surgery (FESS) and rhinoplasy, common practice involves administration of topical vasoconstrictor in the form of 1:1,000 epinephrine-soaked neuropatties as well as intranasal injection of a mixed solution of local anesthetic and 1:100,000 epinephrine (69). A recent randomized controlled trial comparing cocaine- and epinephrine-soaked neuropatties in 37 patients found no significant difference in blood loss with cocaine as compared to epinephrine. In this study, no adverse effects associated with cocaine or epinephrine were documented (70). A non-randomized trial comparing cocaine with epinephrine in 65 patients undergoing rhinoplasty showed similar results (71). The reported rate of cardiovascular complications associated with topical epinephrine in this patient population is 0.1% or less (72,73) with no deaths reported. In a series of 1,998 patients undergoing endoscopic sinus surgery in which 1:1000 diluted topical epinephrine was used for hemostatic purposes, Orlandi *et al.* reported two complications likely associated with the use of topical epinephrine (<0.1%). Once case involved ECG changes and the other case involved intraoperative hypertension due to inadvertent submucosal injection of the epinephrine solution (73). In a more recent prospective series of 19 patients undergoing the same surgery using epinephrine at the same dilution of 1:1000, there were no documented cases of altered hemodynamics related to topical application of epinephrine (74). A study in 10 burn patients undergoing burn wound excision using 1:10000 diluted epinephrine-soaked gauze wrappings, did not show a significant increase in intraoperative serum concentration of epinephrine to indicate systemic absorption (75). Nonetheless, a different study in burn patients has demonstrated that tachycardia was more common in burn patients undergoing surgery where 1:10,000 epinephrine-soaked gauzes were used (76). This finding can be related to the relatively large interface between the applied

epinephrine and inflamed and injured vasculature in the surgical field of burn patients. One study reported benefit and a favorable side-effect profile of 1:10,000 epinephrine-soaked gauzes applied prophylactically for hemostasis during laparoscopic cholecystectomy (77).

Potential Adverse effects of Endotracheally- and Endobronchially-Administered Topical Epinephrine

Data concerning the systemic effects of endobronchially administered epinephrine are limited. Adverse hemodynamic effects of topical epinephrine are attributed to systemic absorption of epinephrine from the airways into the pulmonary and bronchial circulatory systems. Two pharmacokinetic studies in a canine model explored blood concentrations of epinephrine after administration through the airways. Roberts et al. compared blood concentrations and hemodynamic effect of intravenous (IV) versus endotracheal (ET) instillation of epinephrine administered in doses of 0.005, 0.03, 0.06, and 0.09 mg/kg diluted in 5 mL of normal saline. The authors reported that following ET installation, maximum blood concentrations of epinephrine were approximately one-tenth of those achieved with an equal dose administered IV. Interestingly, while IV administered epinephrine was rapidly metabolized and cleared, ET administration was characterized by a more sustained blood concentration. The same relationship between IV and ET administration was observed when comparing pharmacological effects with the ET route requiring ten times the IV dose in order to result in a significant increase in blood pressure (78). Mazkereth et al. demonstrated significant measurable systemic levels when epinephrine in a dose of 0.02 mg/kg diluted in 2 mL of saline was administered into the airways using a catheter. More than a 4-fold increase in systemic levels was achieved when epinephrine was installed into the distal airways via a catheter as compared with when it was administered into the trachea (maximal concentration 8.9±3.2 vs. 2.0±0.4 ng/mL). However, neither group demonstrated any significant change in the heart rate, and both had similar, minor decreases in blood pressure for 2 to 5 minutes with no significant differences in arterial blood gases (79). It is noteworthy that the dose range of epinephrine utilized in the aforementioned studies exceeds the dose used for achievement of hemostasis in TBLB-related hemorrhage by a factor of 4 to 10.

The potential of endobronchially administered epinephrine to result in adverse hemodynamic effects is well-recognized and dosing recommendations have been made since the 1970s (80). Nevertheless, evidence of such adverse effects are scarce and the currently available data are mostly derived from cases where epinephrine was administered following iatrogenic bleeding, potentially resulting in direct contact of the administered epinephrine with injured blood vessels and hence introduction of epinephrine into the systemic circulation. Janjua et al. reported a case of coronary vasospasm manifesting as chest pain, ST-segment elevation, and ectopy induced by topical administration of 3 mL of 1:10,000 diluted epinephrine during bronchoscopy. It is noteworthy that epinephrine was administered into inflamed and friable airways of a lung cancer patient for the treatment of bleeding related to endobronchial brushing, potentially augmenting the surface area of contact between epinephrine and injured blood vessels. Left heart catheterization in this patient revealed mild coronary atherosclerosis and he was discharged 5 days later following an uneventful hospital course (81). A recent report of two cases implicating bronchoscopic administration of epinephrine for TBLB-associated bleeding as the trigger for

ventricular fibrillation has generated discussion regarding the safety of this technique (82–84). It is worth noting that these episodes of arrhythmias occurred with bronchoscopic instillation of epinephrine by a catheter which was positioned in close proximity to a bleeding vessel as well as the thin alveolar-capillary interface which allows more systemic absorption than the more proximal airways. It seems that the volume of diluents used has as much of a significant role on determining the magnitude of systemic absorption of epinephrine as the absolute dose administered. In one interesting canine study, 1 mg of epinephrine diluted in 10 mL of normal saline administered endotracheally, resulted in higher mean plasma epinephrine concentrations when compared with the same dose administered in 1 mL of saline (85). These results lend support to the theory maintaining that a more distal delivery of epinephrine results in more systemic absorption with a larger volume of instillation likely resulting in carriage of more epinephrine into the pulmonary absorption areas. Unfortunately, there is no study comparing different concentrations and/or volumes of installation for bronchoscopic administration of epinephrine for hemostatic control following TBLB, let alone prophylactic use of topical epinephrine for bleeding prevention or minimization. It is possible that bronchoscopically delivered epinephrine after the onset of bleeding would not reach the source of bleeding due to dilutional effect and therefore would be of limited or no benefit. However, as pulmonary organogenesis occurs by branching morphogenesis, proximal administration may vasoconstrict the vessels feeding the injured vasculature, thus decreasing flow and promoting hemostasis.

STUDY IMPLICATIONS

The role of prophylactic topical epinephrine in improving hemostasis and prevention of clinically-significant pulmonary hemorrhage has never been studied formally in the past, despite its common use by pulmonologists performing bronchoscopic TBLB. Lung transplant recipients undergo multiple bronchoscopies and TBLBs for surveillance and evaluation of ACR as well as lung infection, especially during the first year post-transplantation. This population may have a predilection to TBLB-related bleeding and may benefit from measures to reduce the frequency and magnitude of this relatively common complication.

In the PROPHET trial, we intend to assess the degree of biopsy-related bleeding, measures taken to control bleeding, and potential effects of bleeding on completion of the intended procedure in 100 bronchoscopy procedures performed on single and double lung transplant recipients, randomized to prophylactic topical epinephrine versus placebo (normal saline). The study participant, physician performing the TBLB, and independent observer reviewing the procedure recording will all be blinded to the randomization. Further use of measures to control bleeding that occurs during the procedure as well as the decision to complete the procedure as planned or to abort it prematurely will be left to the discretion of the performer. In this way, we aim to elucidate a potential role of topical epinephrine in the prevention of TBLB-related airway bleeding in lung transplant recipients and assess whether the dose and volume of instillation used in our institution comprises an effective means to prevent hemorrhagic complications of TBLB.

HYPOTHESIS

We hypothesize that prophylactic instillation of topical epinephrine prior to performance of TBLB will decrease the frequency and extent of biopsy-related hemorrhage as well as result in fewer procedures being aborted earlier than intended due to bleeding complications as well as shorter overall procedure time due to the preventive effect on bleeding. Concomitantly, we hypothesize that the instillation of topical epinephrine will not be associated with a serious adverse event profile in comparison to placebo.

SPECIFIC AIMS

In this randomized-controlled double blind placebo controlled clinical trial post-lung transplant patients scheduled to receive bronchoscopy with TBLB as part of their routine standard of care will be randomized to receive a fixed dose and volume of topical endobronchial epinephrine versus matching volume of placebo which will be instilled into the target biopsy airway prior to performance of TBLB. Our study will specifically aim at providing the following information:

Aim 1: Demonstrate the feasibility of assessments of bleeding related to TBLB, including:

1. The degree of TBLB-related bleeding using a standardized grading scale used by the physician performing the procedure to quantify the degree of procedure-related bleeding.
2. The degree of TBLB-related bleeding using a standardized grading scale used by two independent observers blinded to patient data and study drug assignment who will review a video recording of the procedure to quantify the degree of procedure-related bleeding.
3. The magnitude of inter-observer variability in grading TBLB-related hemorrhage based on review of video recording of the procedure.

Aim 2: Evaluate the hypothesis that prophylactic administration of topical epinephrine results in reductions in TBLB-related hemorrhage in lung transplant recipients, including:

1. The frequency of active measures taken to control pulmonary hemorrhage once it occurred.
2. The proportion of procedures completed as planned in terms of obtaining a predefined target number of biopsy specimens.
3. Comparison between single- and double-lung transplant recipients in terms of prevalence and degree of TBLB-related hemorrhage.
4. Identification of clinical factors associated with an increased or decreased risk of procedure-related hemorrhage.

Aim 3: Evaluate the hypothesis that prophylactic administration of topical epinephrine affects the overall efficiency of bronchoscopy with TBLB performance, including:

1. Overall duration of the bronchoscopic procedure.

2. The proportion of procedures completed as planned in terms of number of adequate biopsies obtained as assessed by the physician performing the bronchoscopy.
3. The proportion of procedures resulting in acquisition of adequate biopsy samples that allows proper pathologic evaluation of assignment of a pathologic diagnosis.

Aim 4: Explore the hypothesis that instillation of our prespecified dose and volume of topical epinephrine into the target biopsy airway is not associated with an adverse event profile that is significantly different from placebo, including:

1. The prevalence of clinically significant hemodynamic changes.
2. The prevalence of cardiac adverse events, including conduction abnormalities, arrhythmia, and myocardial ischemia.
3. The prevalence of other vascular adverse events, including stroke, mesenteric ischemia, and critical limb ischemia.
4. Identification of clinical factors associated with an increased or decreased risk of drug-related adverse events.

STUDY PROTOCOL

Overview

The PROPHET trial is a prospective randomized double-blind placebo-controlled pilot study. During the study period, we intend to recruit post single or double lung transplantation patients expected to undergo bronchoscopy with TBLB as a part of their routine post-transplantation care. Study participants who meet inclusion and exclusion criteria will be randomized to receive prophylactic endobronchial topical epinephrine or placebo in a blinded manner prior to performance of TBLB. Bleeding complications of TBLB will then be assessed by the bronchoscopist at the time of the procedure and subsequently by two independents reviewers who will observe a video recording of the procedure. The bronchoscopist as well as the reviewers will be blinded to the study drug assignment. Additionally, patients will be monitored for potential adverse events associated with topical epinephrine instillation. In this trial, we intend to include a total of 100 procedures in the final analysis. The first 40 procedures will serve as a pilot for proof of concept, refinement of the protocol, and interim analysis of data for safety outcomes.

Eligibility Criteria

Inclusion Criteria

1. Male and female subjects, ≥18 years of age.
2. Single- or double-lung transplant recipients scheduled for bronchoscopy with TBLB.
3. Willingness to sign an informed consent for study participation.

Exclusion Criteria

1. Age <18 years.
2. Pregnancy.
3. Inability to understand and provide a written informed consent.
4. Exclusion criteria for TBLB
    - 4.1. Platelet count <50 K/microL.
    - 4.2. International normalized ratio (INR) >1.5.
    - 4.3. Known bleeding diathesis.
    - 4.4. Use of prophylactic or therapeutic dose of unfractionated heparin within 6 hours of the procedure.
    - 4.5. Use of prophylactic dose of low molecular weight heparin within 12 hours of the procedure.
    - 4.6. Use of therapeutic dose of low molecular weight heparin within 24 hours of the procedure.
    - 4.7. Use of oral direct thrombin inhibitors or oral factor 10a inhibitors within 48 hours of the procedure.
    - 4.8. Use of clopidogrel, ticlopidine, ticagrelor, or prasugrel within 5 days of the procedure.
    - 4.9. Uremia, defined as estimated glomerular filtration rate (eGFR) ≤30 mL/min.
    - 4.10. Moderate to severe pulmonary hypertension as defined by a mean pulmonary artery pressure of >40 mm Hg on right heart catheterization or an estimated pulmonary artery systolic pressure of >62 mm Hg on transthoracic echocardiography, both performed within 1 year of the procedure.
    - 4.11. An additional synchronous procedure with possible bleeding (bronchoalveolar lavage and endobronchial biopsy allowed), unless the additional procedure is to be performed following TBLB.
    - 4.12. Decompensated liver cirrhosis, defined as the presence of clinically significant ascites, clinical evidence of esophageal or gastric varices, or history of bleeding from gastric or esophageal varices.
    - 4.13. Prior history of TBLB-related airway bleeding requiring admission to the hospital or advanced measures to achieve hemostasis, including endotracheal intubation, bronchial blocker application, bronchial artery embolization, or surgical intervention.
5. Exclusion criteria for application of topical epinephrine:
    - 5.1. Systolic heart failure with an ejection fraction (EF) of <35% as assess by echocardiography performed within one year prior to the procedure.
    - 5.2. Myocardial infarction, acute coronary syndrome, percutaneous coronary intervention, or coronary artery bypass surgery within 6 months prior to the procedure.
    - 5.3. Symptoms and/or ECG findings suggestive of ongoing cardiac ischemia on the day of the procedure.
    - 5.4. Moderate- to severe-grade cardiac valvulopathy as assessed by echocardiography performed within one year prior to the procedure.

5.5. Inadequately controlled supraventricular arrhythmia, including atrial fibrillation, atrial flutter, and atrio-ventricular node re-entrant tachycardia (AVNRT) as revealed by ECG or cardiac monitoring at the time of the procedure.
5.6. Presence of an internal cardioverter/defibrillator.
5.7. History of second or third degree (complete) heart block or sick sinus syndrome.
5.8. Baseline ECG or cardiac monitoring revealing frequent occurrence (≥10 events per minute) of atrial or ventricular ectopy documented prior to or at the time of the procedure.
5.9. History of ventricular arrhythmias requiring pharmacologic or electrical cardioversion within the 60 days preceding the procedure.
5.10. Serum potassium of <3.0 mmol/L within the week prior to the procedure.
5.11. Serum glucose level of ≥300 mg/dL within the week prior to the procedure.
5.12. Any history of critical ischemia related to peripheral arterial disease.
5.13. Persistent resting heart rate (HR) measurement of ≥120 beats per minute prior to or at the time of the procedure.
5.14. Persistent resting systolic blood pressure (SBP) measurement of ≥180 mm Hg prior to or at the time of the procedure.
5.15. Persistent resting diastolic blood pressure (DBP) measurement of ≥110 mm Hg prior to or at the time of the procedure.
5.16. History of acute closed-angle glaucoma within one year of the procedure.
5.17. Diagnosis of pheochromocytoma requiring pharmacologic therapy with an alpha adrenoreceptor blocker at the time of the procedure.
5.18. Diagnosis of thyrotoxicosis requiring pharmacologic therapy with an anti-thyroid agent at the time of the procedure.

Recruitment Strategy

Recruitment for the proposed clinical trial will follow the well-established procedures at UMMC. Patients will be recruited from the daily clinical practice of the study investigators as well as the patient cohort being followed and treated by the lung transplantation service in our institution. As a referral center for lung transplantation in the state of Maryland as well as the surrounding states, UMMC care for a large number of post-lung transplantation patients who are likely to meet inclusion and exclusion criteria. Based on our experience with regard to our local practice over past years, we are fairly confident we have access to adequate numbers of patients and procedures in order to complete recruitment. This provides reasonable assurance that a sufficient number of participants should be able to be recruited and a sufficient number of procedures should be able to be recorded for this trial as well as speaks to its clinical and scientific relevance as its conduct will provide important information in the role of prophylactic epinephrine as means to prevent pulmonary and airway bleeding in lung transplant recipients undergoing bronchoscopic TBLB.

Potential PROPHET Trial participants will be screened from the pool of post-lung transplantation patients followed in UMMC and requiring TBLB, based on standards of care at our institution. Common indications for bronchoscopy with random TBLB sampling of the lung allograft include surveillance for acute graft rejection over the first post-transplantation year; evaluation of potential graft dysfunction based on clinical judgment (increased shortness of breath, worsening cough, decline in pulse oximetry), decline in the forced expiratory volume in one second (FEV1) or average forced expiratory flow at 25-75 percent of the forced vital capacity (FEF25-75%) as demonstrated by serial standardized spirometry, or radiological evidence; and tissue acquisition for suspected chronic graft rejection or bronchiolitis obliterans syndrome when the patient is a non-surgical candidate or a less invasive modality for tissue acquisition is desired before the decision regarding more invasive surgical modalities is made. A common indication for bronchoscopy with targeted TBLB sampling of the lung allograft or the native lung in single-lung transplant recipients is evaluation of focal parenchymal lung findings observed in radiologic studies and suspected for infectious, inflammatory, toxic, or a malignant process.

Patient Evaluation

Patients will undergo all study related procedures, including screening, informed consent, initial evaluation, and study intervention, during the hospital encounter scheduled for their bronchoscopy and TBLB.

Process of Obtaining Informed Consent

Informed Consent will be performed on the day of study recruitment. Eligible PROPHET Study patients will be discretely approached by one of the study investigators who will explain in layman's language the aims, methods, potential benefits, and hazards associated with participation in the PROPHET trial. Details of the protocol will be discussed with the potential participant at the time of scheduling the bronchoscopy procedure, ensuring adequate time for consideration of the protocol (>24 hours from initial discussion). These individuals will then be approached with the informed consent form. Whenever possible, the informed consent form will be given to the potential participants in advance. The risks, benefits, and alternative will be discussed so that the decision to participate in the study will be made independently by the candidate. Only a well-trained clinical coordinator, the principal investigator, or an associate investigator from the Division of Pulmonary and Critical Care Medicine in UMMC will obtain consent. The consent will be obtained using and Institutional Review Board (IRB) approved consent document.

Any potential participants who cannot consent for themselves will be excluded from the study, as defined by the exclusion criteria (see above). This includes anyone who will be, or will have been, in a stressful, painful, or drugged condition before or during the consent process or otherwise lacks the mental capacity to consent for themselves. We will not obtain consent from Legally Authorized Representatives (LARs). We will not enroll minors.

Lung transplantation recipients undergo multiple bronchoscopic procedures in the post-transplantation period. Hence, it is very likely that a patient who is eligible for participation in the

study will undergo more than one bronchoscopy with TBLB during the study period. In this instance, a separate informed consent will be obtained for each individual procedure.

Initial Evaluation

Any potential PROPHET participants who meet initial screening criteria and are anticipated to undergo bronchoscopy with TBLB will undergo a thorough chart review conducted by a member of the research team. Demographics, past medical history and comorbid conditions, indication for lung transplant, indication for bronchoscopy with TBLB and possible concurrent procedure(s), prior complications related to bronchoscopy or TBLB, current and active medications, most recent pulmonary function tests, and most recent report of echocardiogram will be reviewed to ensure inclusion and exclusion criteria are met. Recent laboratory data including complete blood count, comprehensive metabolic panel, coagulation studies, and 12-lead electrocardiogram will be reviewed following our institutional standard protocol for bronchoscopy.
On the day of procedure, as per our institutional procedural protocol, participants will have intake vital signs obtained. Recent history and physical examinations dated within 30 days of procedure will be reviewed or obtained if not available. Participants will be monitored under telemetry starting with this intake period, during and approximately an hour after conclusion of procedure unless procedural complication merit additional closer monitoring.

Monitoring Subjects and Criteria for Withdrawal of Subjects from the Study

Before and after the procedure, on the procedure day, study participants will be monitored in the UMMC endoscopy peri-procedure suite (for outpatients) or in the admitting unit (for inpatients). An on-call pulmonary physician will be available to assist with any immediate issues. During the procedure, study participants will be monitored by the UMMC bronchoscopy suite nursing staff, the performing bronchoscopist, and any additional physicians participating in the procedure. We do not anticipate problems requiring withdrawal of patients from the PROPHET study once enrolled, other than by the patient's request. In the event a study subject elects to be withdrawn from the study and requests their data to be withdrawn as well, we will comply.

Study Drug and Placebo Preparation, Packaging, and Randomized Assignment

The dosing of topical epinephrine for the purpose of airway bleeding prophylaxis is not standardized and based mainly on operator preference and in some cases local policies. The UMMC does not have a formal policy regarding the prophylactic use of epinephrine in TBLB. Treatment of airway bleeding with topical epinephrine once it has occurred is also controversial in terms of dilution and volume of instillation and, again, is based mainly on individual operator practice and institutional protocols. Dilution ratio ranges from 1:1,000 to 1:100,000 and volume of instillation ranges from 1 to 20 mL in different reports published across the medical literature (22,50–52). The only guidelines addressing this practice are the British Thoracic Society (BTS) guidelines on Diagnostic Flexible Bronchoscopy, which suggest using "small aliquots of 1:10,000 diluted epinephrine" (49).

For the PROPHET study, we decided to adopt the recommendation made by the BTS regarding the dilution. Regarding the volume of instillation, we chose a volume of 2 mL based on the common local practice in UMMC. It is standard practice during a bronchoscopy for a cup of saline and a cup of 1:10,000 diluted epinephrine to be prepared by the bronchoscopy suite support staff. Syringes for administration of both are included with the cups so to permit administration. Our plan for randomization simply takes these syringes that are already available and on the bronchoscopy cart as standard practice and applies a two-step randomization process to ensure team blinding.

On day of procedure, a diluted 1:10,000 epinephrine syringe will be prepared by a member of the research team who will not be involved in the allocation of the study drug. To allow for the possibility that biopsies possibly will be performed from separate airways, two milliliters of 1:10,000 concentration of epinephrine along with 8 ml of ambient air will be drawn into a two syringes ("study drug"). Two separate syringes will be made with 2 ml of sterile saline and 8 ml of ambient air ("placebo"). The two sets of syringes will be labeled "A" or "B" based on an electric coin toss for randomization. A record of the coded syringes and their content will be documented in a unique database separate from the study database. In order not to breach blinding, lot numbers for both Epinephrine and saline aliquots will be documented in each study participant's CRF without disclosing which letter was assigned to each aliquot.

As described below, the next step is for a second team member to randomly select one set of labeled syringes without the first study team member knowing which was selected, and then log the selection. As such, the team remains blinded and only when the two logs are later merged will drug assignment be unblinded.

Study Group Randomized Assignment

To eliminate bias, two randomization and blinding processes will be employed: in preparing and labeling the sets syringes (as described above) and in choosing of the set of syringes at the time of the procedure. Those two separate processes will be performed by different members of the research team, each of them blinded to the results of the other procedure:

1. Two syringes will be prepared with the study drug (1:10,000 dilution of epinephrine) and two will be prepared with placebo (sterile saline). The syringes will be randomly labeled "A" and "B" based on a computerized electric coin toss and documented in a separate database as described above. This will be done by a member of the research team who will not be participating in the procedure and therefore blinded to the second randomization (syringe selection).
2. The syringes will be delivered to the procedure room prior to the procedure. At this time, the research team member who is responsible for the syringes will leave the room prior to the second randomization point.
3. The bronchoscopist or his/her assistant who is blinded to the drug and placebo preparation process will randomly choose "A" or "B" based again on a computerized electric coin toss.
4. The chosen set of syringes will be recorded as part of the study database.

Procedure Performance

1. Bronchoscopy will be performed in the controlled environment of the UMMC dedicated bronchoscopy suite under moderate sedation. The bronchoscopy suite in UMMC is routinely staffed by a nurse and a respiratory therapist in addition to the bronchoscopist and any trainees assisting with the procedure.
2. In addition to informed consent for participation in the study protocol, a formal UMMC informed consent for the performance of fiberoptic bronchoscopy, bronchoscopy-related procedures, and use of moderate sedation will be obtained.
3. Per local protocol, peri-procedurally and during the procedure, the patient's heart rate, electrocardiography rhythm strip, respiratory rate, and oxygen saturation will be monitored continuously. Blood pressure will be repeatedly measured every 3 minutes. Up to 6 liters of supplemental oxygen via nasal cannula will be given in order to maintain an oxygen saturation of >92%. A defibrillator as well as a resuscitation drugs and equipment are available at all times in the bronchoscopy suite.
4. Patient will undergo the procedure in the supine position on an adjustable bed. Premedication will be performed per local protocol, including inhalation of 5 mL of 4% lidocaine via a nebulizer. Gurgling of 2 to 4 mL of 2% liquid lidocaine (20-40 mg) for upper airway anesthesia may also be applied depending on the performing bronchoscopist's preference. Moderate sedation will be achieved with a combination of midazolam (maximal dose of 6 mg per procedure) and fentanyl (maximal dose of 300 mcg per procedure). Laryngeal and lower airway anesthesia during the procedure will be obtained with the use of 2 mL aliquots of 2% liquid lidocaine up to a cumulative liquid lidocaine dose of 400 mg. A bite block will be used for procedures performed through the transoral approach. For the transnasal approach, 2% viscous lidocaine will be applied with a cotton swab to the chosen nostril and nasal passage to allow easy advancement of the bronchoscope.
5. Bronchoscopy will be performed with an Olympus BF-1T160 or BF-1TH190 (Olympus, Tokyo, Japan) video bronchoscope under fluoroscopic guidance (OEC 9900 Elite Mobile C-arm, GE Healthcare, Chicago, Illinois, United States).
6. For the purpose of independent post-procedure analysis, the entire procedure from insertion of the bronchoscope into the airways until its final retraction will be video-recorded on DVD media and individually labeled without disclosing study arm assignment. DVDs will be stored with other individual patient study documents for later review. Access to the stored DVDs will be provided only to the PROPHET study research team.
7. Prior to biopsy performance 2 mL of 1:10,000 diluted epinephrine followed by 8 mL of ambient room air or 2 mL of sterile saline solution followed by 8 mL of ambient room air as described in "Study Drug and Placebo Preparation" will be instilled into the target airway by the bronchoscopist. This process may be repeated once, should the performing bronchoscopist choose to perform additional biopsy passes through a different airway. If choose to do so, the same study drug will be instilled into both airways. Any additional biopsies performed, will be done without airway instillation of study drug.
8. Biopsies will be performed using brand bronchoscopic biopsy forceps as preferred by the procedure performer. For ACR, aim will be to obtain 5 to 8 adequate biopsies, preferably from

the lateral subsegment of either the right or left lower lobes to allow optimal visualization and minimize forshortening under fluoroscopy as well as to avoid as much as possible spillage of blood into uninvolved lung segments in the event of biopsy-related hemorrhage as described above. For the evaluation of radiological findings, aim will be to obtain 4 to 8 adequate biopsy specimens from an involved segment in one of the lungs based on imaging. All bronchoscopies will be performed by an experienced bronchoscopist.

9. The following data will be recorded with regard to the performance of the procedure:
   9.1. Patient study-arm assignment – recorded in a separate database.
   9.2. Indication for bronchoscopy with TBLB.
   9.3. Target number of TBLB specimens and target lobe and segment.
   9.4. Name and dosages of all medications administered to the patient in the hospital during the day of the procedure, including medications frequently administered during bronchoscopy such as topical anesthetic agents, sedation and analgesia agents, and hemostatic agents. Study drug vs. placebo administration will be recorded as described previously under "*Randomization*" and "*Study Drug and Placebo Preparation and Packaging*".
   9.5. Supplemental oxygen requirements which exceed patient's baseline requirements during or following the procedure.
   9.6. Any hemodynamic, cardiac, or respiratory events as recorded by telemetry in the procedure suite and/or in the recovery suite following the procedure.
   9.7. Bronchoscope model, including outer diameter and inner diameter of working channel.
   9.8. Date and time of scope insertion and completion of the procedure as defined by the performing bronchoscopist.
   9.9. Biopsy forceps brand, including outer diameter, open forceps diameter, and type of forceps (round cup, oval cup, alligator, impaling needle).
   9.10. Fluoroscopy time.
   9.11. Number of forceps passes.
   9.12. Total number of acquired biopsy specimens; Specimen distribution across laboratories (anatomic pathology, microbiology, other laboratory).
   9.13. Lobe and segment of each biopsy specimen.
   9.14. Any medical intervention performed in the bronchoscopy suite or in the recovery suite for stabilization of the patient's medical condition during or following the procedure, including hemostasis, management of cardiac events, and management of respiratory events.

Outcome Measures

Primary Efficacy Outcome

The primary efficacy outcome of the PROPHET Study is the prevalence of major hemorrhage as defined by two independent reviewers who will review the procedure video while blinded to the individual patient information, study drug assignment, and any procedure related data not recorded on video. Hemorrhage will be graded according to a grading system suggested by

Pilarczyk *et al.* (43) and modified in accordance with the PROPHET Study scientific question as follows:

Grade	Description
No hemorrhage	No hemorrhage
Minor	Endobronchial hemorrhage resulting in segmental or more distal bronchus blood spillage
Intermediate	Endobronchial hemorrhage resulting in lobar bronchus blood spillage
Major	Endobronchial hemorrhage resulting in mainstem bronchus, more proximal blood spillage, or spillage into the uninvolved lung

Secondary Efficacy Outcomes

1. Occurrence of no, minor, or intermediate bleeding as defined by the independent reviewer.
2. Likert scale grading of bleeding by independent observer on a scale of 1 to 5:

1	2	3	4	5
Much less than usual for TBLB	Less than usual for TBLB	Usual for TBLB	More than usual for TBLB	Much more than usual for TBLB

3. Intra-procedural grading by the performing bronchoscopist according to procedure-related outcome as described by Ernst *et al.* and Herth *et al.* (31,44):

Grade	Description
No hemorrhage	No hemorrhage
Mild	Any bleeding originating from the biopsy target airway requiring wedging of the bronchoscope or “in and out” motion in order to achieve hemostasis
Moderate	Any bleeding originating from the biopsy target airway requiring in addition to maneuvering the bronchoscope application of iced saline or topical epinephrine or placing the patient with the bleeding lung in the dependent position
Severe	Any bleeding originating from the biopsy target airway requiring, in addition to the above-mentioned maneuvers, early termination of the procedure or necessitating application of balloon tamponade, endotracheal intubation, application of a bronchial blocker, or use of other invasive measures to achieve hemostasis, such as bronchial artery embolization or surgical intervention

4. Number of forceps passes performed.
5. Number of adequate tissue samples obtained.

6. Early termination of the procedure due to bleeding complication, defined as non-achievement of the target number of tissue samples as designated by the performing bronchoscopist prior to the procedure related to hemorrhage.
7. Duration of the procedure from first bronchoscope insertion to last bronchoscope withdrawal.
8. Total volume of iced saline used during the procedure.
9. Total dose of unblended topical epinephrine used during the procedure.
10. Any use of hemostatic procedures including balloon tamponade, endobronchial blocker, bronchial artery embolization, emergency surgery.
11. Any use of devices or procedures with intention of securing the airway including oral airway, nasal airway, laryngeal mask, endotracheal intubation, emergency cricothyroidotomy, and emergency tracheostomy.
12. Unplanned admission to the hospital or escalation in level of care within 48 hours of the procedure.
13. Pathologic diagnosis achieved.

Safety Outcomes

The safety of topical epinephrine administration and potential instillation-related adverse events will be derived from clinical monitoring, vital signs, and ECG recordings during and immediately following the procedure as well as from any clinical, laboratory, or radiologic investigations performed during or following the procedure. Complications deemed related to topical epinephrine administration and comprise the study safety outcomes include:

1. All-cause mortality within 3 hours of instillation of the study drug.
2. Acute cardiovascular event, including cardiac arrest, new onset chest pain, acute myocardial ischemia, pulmonary edema, acute ischemic stroke, critical limb ischemia, acute mesenteric ischemia, occurring within 30 minutes of instillation of the study drug.
3. Acute closed angle glaucoma within 30 minutes of the instillation of the study drug.
4. New onset ECG changes suggestive of myocardial ischemia, including ST segment changes (defined: ≥1 mm ST segment elevation or ≥3 mm ST segment depression in ≥2 precordial or limb ECG leads that correspond together to one of the cardiac walls), new onset ventricular tachycardia, significant increase (≥10 per minute) in occurrence of ventricular ectopic beats, new onset supraventricular arrhythmia, new onset bundle branch block, and new onset 2nd or 3rd degree atrioventricular block, all occurring within 30 minutes of the instillation of the study drug.
5. Significant change in heart rate (defined as heart rate of >120 bpm and/or increase by ≥30 bpm above the baseline or heart rate <60 bpm and/or ≥30 bpm below the baseline) as recorded within 30 minutes of the instillation of the study drug.
6. Significant change in systolic blood pressure (defined as increase in systolic blood pressure >180 mm Hg and/or ≥40 mm Hg above the baseline; increase in diastolic blood pressure >110 mm Hg and/or ≥20 mm Hg above the baseline; or fall in systolic blood pressure <90 mm Hg and/or ≥20 mm Hg below the baseline) as recorded within 30 minutes of the instillation of the study drug.

Please refer to the section titled “Adverse Events Reporting and Data Monitoring” for further elaboration regarding reporting adverse events to the study monitoring committee. Please refer to the section titled “Adverse Events Reporting to the IRB” for further elaboration regarding reporting adverse events to the IRB.

Immediate Post-Procedure Surveillance

Following retraction of the bronchoscope, fluoroscopy will be performed while the patient is still on the bronchoscopy bed in order to try and identify early pneumothorax. The patient will then be transferred for recovery in the UMMC endoscopy peri-procedure suite or in the admitting unit. A chest radiograph will be obtained to identify late pneumothorax within two hours of the termination of the procedure or later if late symptoms suggestive of pneumothorax appear. Also, a repeat ECG will be obtained within two hours of the termination of the procedure or earlier if symptoms suggestive of cardiac arrhythmia, ischemia, or conduction abnormality appear. The patient will also be assessed by a clinician to evaluate for signs and symptoms of pneumothorax or significant pulmonary hemorrhage on the same day of the procedure. Patients will be instructed not the drink or eat in the 2 hours following the procedure and if stable for discharge from the hospital on the same day of the procedure, to be driven to their place of residence by an escort. Patients will also be instructed to call if they had any hemoptysis, dyspnea, chest pain, or wished to ask questions.

Long-Term Post-Procedure Surveillance

A follow-up telephone call will be performed by one of the study investigators 24 to 48 hours following the bronchoscopic procedure. Patients will be requested to report any ill-effects of the procedure and will be specifically asked about sore throat, fever, chills, hemoptysis, dyspnea, and chest pain. Symptomatic patients will be managed as deemed appropriate according to clinical judgment and the UMMC bronchoscopy suite guidelines. Management options include reassurance, prescription of appropriate medication, referral to the primary care physician, referral to the lung transplantation clinic, or referral to a local or the UMMC emergency room.
Emergency situations may arise in which it is necessary to unmask treating physicians, emergency department personnel, clinic personnel, or the patient to the assigned treatment. Situations that require unmasking are expected to be rare. Since epinephrine has a short half-life it will probably be already eliminated from the study participant’s circulation by the time they are seen by a provider for possible study drug-related side effect. It is possible, however, that emergency personnel or treating physicians might feel it is necessary to know the medication that participant was taking to decide upon a rational course of treatment, or to ensure that other medications are not given that might adversely interact with epinephrine. In such cases the treating medical personnel should call the PI for study unmasking. In that event, an Unmasking Report for the event must be completed.

Study Flow (Outpatients)

Time Frame	Study Procedure(s)

Days -30 to -1	1. Identification of potential study participants from the pool of lung transplant recipients considered for TBLB within the coming month in UMMC according to inclusion and exclusion criteria. 2. Description of study rational, aims, methods, potential benefits, and potential hazards by one of the study investigators during the routine phone call performed for the purpose of procedure coordination, allowing time for consideration.
Day 0, time -2 to -1 hours	1. Patient admission to UMMC endoscopy peri-procedure suite. 2. Anthropometric data, vital signs, baseline ECG, and routine laboratory tests will be obtained per local protocol. 3. Patient's medical record including past medical history, comorbidities, indication for current bronchoscopic TBLB, and ancillary studies (labs, ECG, echocardiography, imaging) will be again reviewed by one of the study investigators to confirm that the potential participant meets inclusion and exclusion criteria. 4. Recent (previous 30 days) history and physical exam will be reviewed and/or performed and recorded if absent. 5. Participant related data will be documented in the CRF. 6. Study protocol will be again described in detail by one of the study investigators and informed consent will be obtained.
Day 0, Time -1 to 0 hours	1. Study drug preparation and randomization will be performed in a blinded manner.
Day 0, time 0	1. Study procedure will be performed in the UMMC bronchoscopy suite as described above. 2. Procedure related data will be documented in the CRF.
Day 0, time 0 to +3	1. Upon completion of the procedure, the study participant will return to the UMMC endoscopy peri-procedure suite. 2. Vital signs will be obtained and patient will be monitored. 3. Chest radiograph will be obtained within 2 hours of study completion. 4. Additional studies (laboratory, imaging, etc.) will be obtained on an individual basis based to emerging need. 5. Any therapeutic measures, including hospital admission, will be performed according to emerging needs. 6. Study participant will be interviewed and examined by one of the study investigators who participated in the procedure. Initial outcomes and results will be reported to the participant. 7. Study participant will be discharged home according to local policy unless emerging conditions require their admission to the hospital.

Day +1 to +2	1. Study participant will be contacted by phone by one of the study investigators to inquire regarding any ill-effects of the procedure.
Day 0 to +30	1. 30-day all-cause mortality will be recorded. 2. Pathologic diagnosis will be recorded.
Day 0 and forth	1. Study video DVD will be reviewed and graded by two blinded independent observers.

Study Confidentiality and Privacy

Data Documentation

1. All study participants' data will be anonymized and assigned a unique 5-character study identification number consisting of the first letter of the participants' last name, the first digit, and the last 3 digits of their UMMC medical record number. In a potential case where two different patients will be assigned the same study identification number, the first and last 4 digits of the UMMC medical record number will be assigned to both patients.
2. Individual PROPHET Study participant data will be collected in a designated study paper Case Report Form (CRF). Any information identified as Protected Health Information (PHI) will be kept separate from the participants CRF in a secure environment at UMMC accessible only to study staff allowed access to PHI. Each study participant CRF will also include the DVD media used for recording the bronchoscopy procedure.
3. All study collected data will be incorporated into computerized databases. Databases will be located behind UMMC network firewall on a UMMC server and accessible only under the following conditions:
    - 3.1 A workstation physically present in UMMC and physically connected to the network.
    - 3.2 Granted system access to database server.
    - 3.3 Granted password to the database folder(s).
    - 3.4 Granted password to the database item(s).
4. Computerized databases in use for the PROPHET study will include:
    - 4.1 Patient identifier and study drug assignment. This database will be accessible to investigators involved in study drug marking and blinded to the procedure and outcomes.
    - 4.2 All other study participant data collected as described above. This database will be accessible to investigators blinded to study drug assignment.
5. All study investigators will be trained and instructed not to export data to a standalone file, whether on laptops, hard drives, thumb drives, CDs, or any other media.

Data Analysis

Sample Size and Power Calculations

Formal power calculations are not possible as studies of this kind have not been previously performed and an aim of the study is to develop new bleeding assessment tools. As such, the first 40 procedures will provide data intended to show feasibility as well as to inform more refined power calculations. Numbers are based primarily on feasibility.

Limitation of study participants to transplant recipients is anticipated to improve study power. In a relatively recent population-based study performed in the USA, Tukey et al. reviewed almost 100,000 patient visits involving TBLB and reported an overall rate for procedure-related clinically significant hemorrhage of 0.58% (37). In a second large survey from Japan, Asano et al. reviewed over 100,000 procedures, among them 57,199 forceps biopsies, and recorded a bleeding rate of 0.73% (38). These low rates of bleeding likely reflect both insensitive thresholds to define bleeding as well as a lower risk population than that of lung transplant recipients.

Bleeding risk in lung transplantation patients seems to be higher than that of the general population, although one must bear in mind the following caveats: (1) most of the currently published literature is retrospective and observational in nature; (2) lung transplant recipients studies mostly involve small patient populations; and (3) in most studies to date, the definition of clinically-significant bleeding was based on the amount of bloody fluid collected in the suction container during the procedure. This quantification method is prone to much bias since this fluid is a mixture of blood, airway secretions, and lavage fluid, and thus may over-estimated the true volume of blood loss. Also, the volume of blood loss considered clinically relevant varies across published studies and affects to a great extent on the reported rate of clinically-significant bleeding. The most prominent lung transplant studies examining the prevalence of TBLB-related bleeding are summarized in the table below:

Study	Number of procedures	Prevalence of clinically significant hemorrhage (%)	Definition of clinically significant hemorrhage	Reference
Scott *et al.*	219	12.3	>100 mL blood loss	(20)
Chan *et al.*	83	1.9	>50 mL blood loss	(62)
Bjørtuft *et al.*	29	7.7	>20 mL blood loss	(63)
Inoue *et al.*	206	11	Not defined	(65)
Chhajed *et al.*	363	4	≥100 mL blood loss	(86)
Diette *et al.*	15	44.5	>25 mL blood loss	(87)
Hopkins *et al.*	1,235	4	>100 mL blood loss	(88)
McWilliams *et al.*	353	13	>50 mL blood loss	
Smith *et al.*	2,111	0.5	>30 mL blood loss	

Data are expressed as means with standard deviations, medians with interquartile range, percentages, and proportions as appropriate. Continuous outcome variables were visually examined for normality and outliers using histograms and scatter plots. The normal distribution was assessed with the Shapiro-Wilk test. Continuous variables were compared with the Student's t-test for normally distributed data with similar variance and the Mann-Whitney tests for non-normally distributed data. Proportions were compared using Chi-squared or Fisher's exact tests as appropriate. Statistical significance was set at $P<0.05$. All statistical analyses were performed based on original assigned groups and using Stata software, version 14 (Stata Corp LLC).

Potential Benefits

We hypothesize that prophylactic instillation of topical epinephrine prior to TBLB will reduced procedure-related hemorrhage, leading to lower rate of prematurely aborted procedure due to bleeding complications and higher yield of intended biopsies. The participants may, therefore, have direct benefit from participation if prophylactic topical epinephrine reduces the rate of procedure related hemorrhage in TBLB therefore avoiding early termination of procedure and more intended biopsies taken providing a higher diagnostic yield.
No financial reimbursements or incentive for participating in the PROPHET study will be provided.

Potential Risks and Discomforts

The decision to perform bronchoscopy with TBLB will be based on clinical indications as a part of each study participant standard of care and not as a part of the research. Potential risks and discomfort of the bronchoscopy and TBLB themselves are no more than what the participant would undergo as a part of their post-transplantation bronchoscopy. These risks include hemorrhage related to the procedural maneuvers, respiratory distress, pneumothorax (i.e. collapsed lung). These complications are rare (less than <1% of all bronchoscopies) and can be expected in any bronchoscopy and TBLB. An experienced bronchoscopist and a well-trained staff such as ours are well trained to handle these potential complications in the controlled environment of the bronchoscopy suite. Bronchoscopic procedures performed in the UMMC bronchoscopy suite are usually performed under conscious sedation. Potential risks and discomforts of conscious sedation for our study participants are no more than what the participant would undergo for their post-transplantation bronchoscopy. Conscious sedation is generally safe, but can cause nausea and vomiting, ineffective ventilation resulting from airway obstruction or respiratory depression, causing hypoxia, hypercarbia, or aspiration associated with loss of protective airway reflexes, as well as cardiovascular complications including hypotension and arrhythmia. Drug overdose and drug reactions are a constant possibility in patients undergoing conscious sedation.
The potential risks of epinephrine are well described in cases of sufficient systemic absorption of the drug. Most serious complications are arrhythmias and hemodynamic compromise and tend to occur in a dose dependent manner. Most complications seen are in the setting of existing bleeding from procedural maneuvers. Topical epinephrine, in these situations, is instilled in airways with damaged mucosa and blood vessels. There is direct communication between the airway and blood vessels allowing for more direct absorption of epinephrine leading to higher concentration of epinephrine in the blood stream and therefore systemic complications. There is no reported data of the incidence rate of complications arising from epinephrine in the setting of topical prophylactic use, but it is expected to have lower rate of systemic absorption. In rare

cases of complications, our staff is well trained and the procedure room is equipped to treat these potential complications of epinephrine.
Research related risk to the patient will also be loss of confidentiality. Loss of confidentiality is unlikely to occur as all data will be accessed and stored securely only by members of the research team. All patient data will be incorporated into a central database. Patients will be assigned with a unique identification number. The database will be maintained behind University of Maryland firewall in a password-protected folder on the I: drive. The database file will also be password protected. Any limited physical data will be kept in a locked cabinet with only keys available to research team members. Confidential data will not be shared.

Adverse Events Reporting to the IRB

All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems, defined as complications thought to be likely related to the study drug administration and require some intervention, will be reported to the IRB as soon as possible but not more than 5 business days after the PI first learns of the event. For example, a brief arrhythmia that requires no intervention will not be reported within 5 business days, while an arrhythmia requiring drug or electrical shock administration will be reported within 5 business days. Likewise, any serious protocol deviations will be reported to the IRB as soon as possible but not more than 7 business days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB as soon as possible but not more than 14 business days after the PI first learns of the event. Not serious protocol deviations will only be reported to the IRB (within 14 business days after the PI first learns of the deviation) if they represent a departure from UMMC policies for the conduct of human subject research, adversely affect the health care of subject(s), or compromise the interpretation or integrity of the research. Not serious protocol deviations that result from normal subject scheduling variations or technical issues associated with sampling that do not impact the health of the subject or interpretation of the study data will not be reported.
Adverse events that are clearly not related to the study procedures, such as those that occur prior to initiation of study-related procedures, will not be reported to the IRB. Adverse events that are expected and thought to be related to the natural history of lung transplantation and its comorbidities will not be reported to the IRB.
Expected adverse events (sore throat, cough, mild hemoptysis, low-grade fever, non-life-threatening allergic reactions) that do not require intervention will not be reported to the IRB. All other adverse events will be reported in aggregate at the time of continuing review.
Deaths will be reported to the IRB within 7 business days after the PI first learns of the event.

Data Monitoring

There will be interim analysis of data for safety and all adverse events. These analyses will be done by an independent physician reviewer after a pilot period of 40 procedures or 10 major bleeding events, whichever occurs first. Thereafter, safety analysis will be performed and reviewed at a minimum of every 6 months.

All adverse events including known complications and unexpected adverse events of the bronchoscopy and TBLB and medications administered will be recorded as part of our data monitoring. Reportable event including but not limited to unanticipated problems, protocol deviation, any change in subject's status will generate a report. The report will include subject identification, date of event, date the PI became aware of the event, description of event, date the event resolved if applicable, type of harm if any experienced by the participant, relationship harm to the event and date the report was submitted to the IRB and other regulatory authorities.

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