TITLE: High Angiotensin Converting Enzyme Activity-Containing Plasma For The Treatment Of Angiotensin Converting Enzyme Inhibitor-Induced Angioedema

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High Angiotensin Converting Enzyme Activity-Containing Plasma for the Treatment of Angiotensin Converting Enzyme Inhibitor-Induced Angioedema

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A Introduction

A1 Study Abstract

Angiotensin Converting Enzyme (ACE) inhibitors are among the most important and widely prescribed drugs. Unfortunately, their use carries the risk of causing upper airway angioedema that can progress to life-threatening respiratory compromise, requiring intubation or a surgical airway. In some groups, e.g., African Americans, the incidence is > 2%[1]. Deaths occur due to the difficulty in securing a patent airway in patients with angioedema and from complications of mechanical ventilation. Indeed, in the year 2000, when ACE inhibitors were prescribed far less frequently than they are today, it was estimated that there were 1200 deaths worldwide due to ACE inhibitor-induced angioedema[2].

There is no definitive treatment for ACE-inhibitor induced angioedema. There was one small phase 2 trial in which icatibant, a bradykinin receptor antagonist, appeared to show benefit; however, it was ineffective in two subsequent larger trials[3-5].

Notably, there are several case reports and case series in which transfusion of fresh frozen plasma (FFP) was effective for treatment[6-10]. It is thought that the excessive levels of the normally ACE-degraded inflammatory mediators bradykinin, substance P, and des-Arg9-bradykinin that accumulate when ACE is inhibited in certain patients cause the angioedema[11,12] and that the ACE from the FFP degrades the mediators. Notably, the use of FFP for the treatment of ACE inhibitor-induced angioedema is described in the widely used clinical reference UpToDate.

However, FFP is not uniformly effective and it has even been reported that FFP can worsen ACE inhibitor-induced angioedema[13]. One possible explanation for the variability that had not been considered in the literature is the potential variation in ACE activity levels between units of FFP from different blood donors—indeed, we thought it likely that a number of donors take ACE inhibitors.

Therefore, to begin to understand the variability in the efficacy of FFP, we assessed ACE activity levels among 330 blood donors to the American Red Cross. We found that there was a greater than 20-fold range of ACE activity levels among the blood donors. Further, 11.8% of the donors had ACE activity levels of 20 U/L or less, levels that are 95% specific for full compliance with ACE inhibitor use [Chen, S. X., Hermelin, D., & Weintraub, S. J. (2019). Possible donor-dependent differences in efficacy of fresh frozen plasma for treatment of ACE inhibitor-induced angioedema. J. of Allergy and Clinical Immunology-in Practice, 7, 2087].

We therefore would like to perform a pilot study to begin to test the hypothesis that plasma that is pre-selected for high ACE activity content (HA-P) will be effective in treating ACE inhibitor-induced angioedema.

Note

Although the plasma product that is currently used clinically is typically referred to by non-blood bank clinicians as “fresh frozen plasma” or “FFP”, a term that should be reserved for plasma that is frozen within 8 hours after phlebotomy, most of the plasma currently in use is actually a different product, “plasma frozen within 24 hours after phlebotomy” or “PF24” (refrigerated within 8 hours and frozen within 24 hours after phlebotomy). In this protocol, we will use “FFP” when we refer to published literature in which the term “FFP” was used (although it is likely that most of what was used was actually PF24); however, for the sake of accuracy, we will use the terms “plasma” and “HA-P” (for high ACE activity-containing plasma) for the studies we propose. Importantly, ACE activity has been shown to be very stable[14], so the ACE activity in FFP and PF24 from the same donor would likely be similar.

A2 Primary Hypothesis

We hypothesize that transfusion of plasma pre-selected for high ACE content (HA-P) will increase the circulating levels of ACE in patients with ACE inhibitor-induced angioedema, that the increase in ACE will hasten the degradation of the inflammatory mediators that cause the angioedema, and that their degradation will slow or prevent the progression of the angioedema and/or result in its more rapid resolution.

A3 Purpose of the Study Protocol

This document is a protocol for a human research study. The purpose of this protocol is to ensure that this study is to be conducted according to applicable government regulations and Institutional research policies and procedures.

B Background

B1 Prior Literature and Studies

There is no data available on the total number of patients who suffer ACE inhibitor-induced angioedema. However, because of the widespread use of ACE inhibitors and the fact that the incidence is relatively high in certain groups, e.g., the incidence among black patients who take ACE inhibitors has routinely been found to be greater than 2%[1], it can be inferred that a large total number of patients suffer ACE inhibitor-induced angioedema. Indeed, it has been stated, "ACE inhibitor-induced angioedema is a relatively common life-threatening condition that is occurring in epidemic…proportions[15]."

At BJH, a search of the Epic EHR Clarity database revealed that between 7/1/18 and 6/30/19 there were 101 patients seen in the emergency department with likely ACE inhibitor-induced angioedema and, of these, 43 were admitted and there was 1 death. Of the admitted patients, 1 was sent directly to the OR. The number of patients admitted to an ICU, the number of patients requiring intubation, the reason for the surgery, and the cause of the death were not specified.

More detailed information is available from a published study. During a one-year period in two Philadelphia community hospitals that serve a predominantly black population, 91 patients presented with ACE inhibitor-induced angioedema. Among these patients, 60 were admitted to a monitored or intensive care setting, 6 required tracheal intubation, and there was 1 death due to an inability to secure the airway[15] .

Finally, in the year 2000, when ACE inhibitors were used much less frequently than they are today, it was estimated that there were approximately 1200 deaths worldwide annually due to ACE inhibitor-induced angioedema[2].

Hence, there is significant morbidity and mortality due to ACE inhibitor-induced angioedema.

Furthermore, the newer, increasingly prescribed drugs sacubitril/valsartan (for heart failure) and DPP-4 inhibitors (for type II diabetes) likely cause angioedema through mechanisms that are similar to the pathophysiologic mechanisms that underlie ACE inhibitor-induced angioedema[16].

It is therefore frustrating that no treatment has definitively been proven effective in reversing or even slowing progression of ACE inhibitor-induced angioedema and there are no listings of active trials evaluating potential treatments on clinicaltrials.gov. The importance of developing an effective treatment will further increase with the increasing use of ACE inhibitors and the new drugs mentioned above as metabolic syndrome continues to increase in prevalence and the population ages.

ACE is most well known for its role in the conversion of angiotensin I to angiotensin II. However, it is also the primary peptidase for the inactivating degradation of the vasoactive peptide bradykinin. In fact, ACE has a much higher affinity for bradykinin (Km = 0.18 μM) than it does for angiotensin I (Km = 16 μM)[17] and ACE inhibitors generally have a higher affinity for the bradykinin binding site than for the angiotensin I binding site on ACE[18]. Therefore, bradykinin levels are increased in patients who take ACE inhibitors[19,20]. The increased bradykinin activates the bradykinin B2 (B_2) receptor, a receptor that can induce vasodilation and increased microvascular permeability, effects that could lead to the development of angioedema.

ACE also has a critical role in the regulation of the levels of two other vasoactive peptides that have the potential to induce angioedema, substance P[19,20] and des-Arg9-bradykinin[21]. The increase in bradykinin brought about by ACE inhibition in turn stimulates the release of the substance P, which is also degraded primarily by ACE. Additionally, when ACE is inhibited, bradykinin is diverted to metabolism by carboxypeptidase N, which, instead of inactivating bradykinin, transforms it into another vasoactive peptide, des-Arg9-bradykinin, that has similar effects to bradykinin and can be degraded by ACE[22]. Elevated levels of substance P and des-Arg9-bradykinin activate the neurokinin 1 (NK_1) and bradykinin B1 (B_1) receptors, respectively, receptors that can induce vasodilation and increased microvascular permeability.

It is an excessive increase in the levels of the vasoactive peptides bradykinin, substance P, and des-Arg9-bradykinin that occurs due to environmental and genetic factors in some patients when ACE is inhibited that is thought to induce the vasodilation and increased microvascular permeability that results in the plasma extravasation underlying the pathophysiology of ACE inhibitor-induced angioedema[23-25].

Efforts at developing a definitive treatment first focused on bradykinin. However, the B_2 receptor antagonist icatibant was ineffective in the two largest randomized trials to date[4,5]. This, along with the finding that there are genetic and phenotypic differences in the secondary peptidases that regulate substance P and des-Arg9-bradykinin when ACE is inhibited in patients who develop ACE inhibitor-induced angioedema when compared with the same peptidases in patients who take ACE inhibitors without developing angioedema[11,12], suggests that a combination of bradykinin, substance P, and des-Arg9-bradykinin must be targeted for effective treatment.

Notably, the genetic changes in the secondary peptidases that degrade substance P and des-Arg9-bradykinin when ACE is inhibited are not uniform among patients who develop ACE inhibitor-induced angioedema[20,21], suggesting that the relative importance of each of the vasoactive peptides in the development of angioedema varies among the patients.

Because bradykinin, substance P, and des-Arg9-bradykinin act independently through the B_2, NK_1, and B_1 receptors, respectively, simultaneous targeted inhibition of all three peptides would be difficult. However, there are case reports and case series in which transfusion of fresh frozen plasma (FFP) was effective in treating ACE inhibitor-induced angioedema[6-10]. It is thought that the ACE from the FFP degrades and thereby decreases the levels of all three peptides, which results in more rapid improvement of the angioedema.

For example, in a retrospective cohort study of adults admitted to an ICU for ACE inhibitor-induced angioedema comparing 20 patients who were treated with FFP with 108 patients who were not treated with FFP, it was found that patients treated with FFP had significantly decreased ICU lengths of stay (1.5 days versus 3.5 days; $p < 0.001$) and were intubated less often (35% versus 60%; $p = 0.05$)[26]. There are also several case reports of dramatic improvement within 2 hours of administration of FFP when the angioedema had been resistant to all other attempts at treatment[6-9,27].

However, FFP has not been uniformly effective and it has even been reported that FFP can worsen ACE inhibitor-induced angioedema[13]. One possible explanation for this variability that had not been considered in the literature is the potential variation in ACE activity levels between FFP units from different blood donors—indeed, it is likely that a number of donors take ACE inhibitors. Therefore, to begin to attempt to improve outcomes when using FFP to treat ACE inhibitor-induced angioedema, we assessed the circulating ACE activity levels among a typical group of Midwestern US blood donors.

The frequency distribution of ACE activity levels among the 330 donors is shown in Figure 1 (median, 37.65 U/L; interquartile range, 27.1-48.8 U/L; range, <5-109.6 U/L). Notably, 39 (11.8%) donors had ACE activity levels of 20 U/L or less, levels that are 95% specific for full compliance with ACE inhibitor use[28].

These results demonstrate that there can be greater than a 20-fold difference in ACE activity levels between different units of FFP. This strongly suggests that the variability in the reported efficacy of FFP for the treatment of ACE inhibitor-induced angioedema is at least in part due to a wide variation in ACE activity levels between different units of FFP.

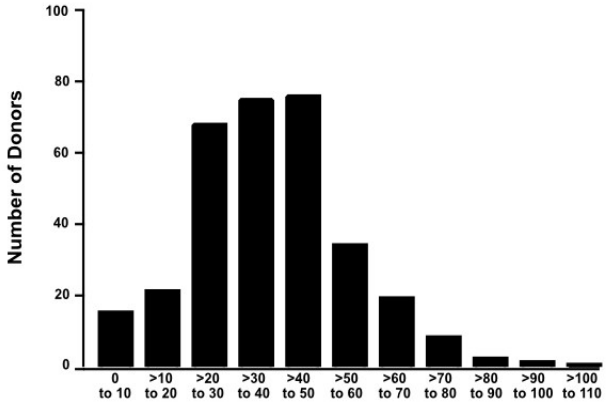


Figure 1. Frequency distribution of ACE activity levels among blood donors

B2 Rationale for this Study

There is no definitive therapy for ACE inhibitor-induced angioedema. The findings outlined above provide both biologic plausibility and preliminary clinical evidence for the potential effectiveness of HA-P* for treatment of ACE inhibitor-induced angioedema. We would now like to test this.

* For the following protocol, we will define HA-P as plasma from a donor with an ACE activity level ≥ 50 U/L, or approximately the highest quartile in the study outlined above.

C Study Objectives

C1 Primary Aim

- Determine if transfusion of HA-P increases circulating ACE activity levels in patients with ACE inhibitor-induced angioedema.
    - **Endpoint:** An increase in serum ACE activity compared with baseline when measured 30 minutes after transfusion of 2 units of HA-P in patients with ACE inhibitor-induced angioedema. The increase should be significantly greater than the increase observed in patients with ACE inhibitor-induced angioedema who are transfused with normal saline (N.S.) as a control.

C2 Secondary Aims

- Determine if transfusion of HA-P increases circulating ACE activity levels at 2 hours and 8 hours after the transfusion in patients with ACE inhibitor-induced angioedema.
    - **Endpoint:** An increase in serum ACE activity compared with baseline when measured 2 hours and 8 hours after transfusion of 2 units of HA-P in patients with ACE inhibitor-induced angioedema. Increases at these time points should be

significantly greater than increases observed in patients with ACE inhibitor-induced angioedema who are transfused with N.S. as a control.

- Determine if transfusion of HA-P slows or prevents the progression of ACE inhibitor-induced angioedema and/or results in its more rapid resolution.
    - **Endpoint:** Any improved clinical parameter of the angioedema in subjects who receive usual care and transfusion of 2 units of HA-P when compared with the subjects who receive usual care and transfusion of N.S. as assessed by a clinician from the BJH Emergency Care Research Core who is blinded to the treatment modality and results of assays described above (see section "E1 Design Summary" below for details of the clinical assessment of angioedema).

C3 Rationale for the Selection of Outcome Measures

Hypothesis: Angioedema is caused by ACE inhibitors because they block the degradation of inflammatory mediators that are normally degraded by ACE; therefore, increasing the circulating ACE levels through transfusion of HA-P should slow or prevent the progression of ACE inhibitor-induced angioedema and/or bring about its more rapid resolution.

Our outcome measures were selected to test this hypothesis.

D Investigational Agent

Preclinical Data

See section B1.

D1 Clinical Data to Date

See section B1.

D2 Dose Rationale and Risk/Benefits

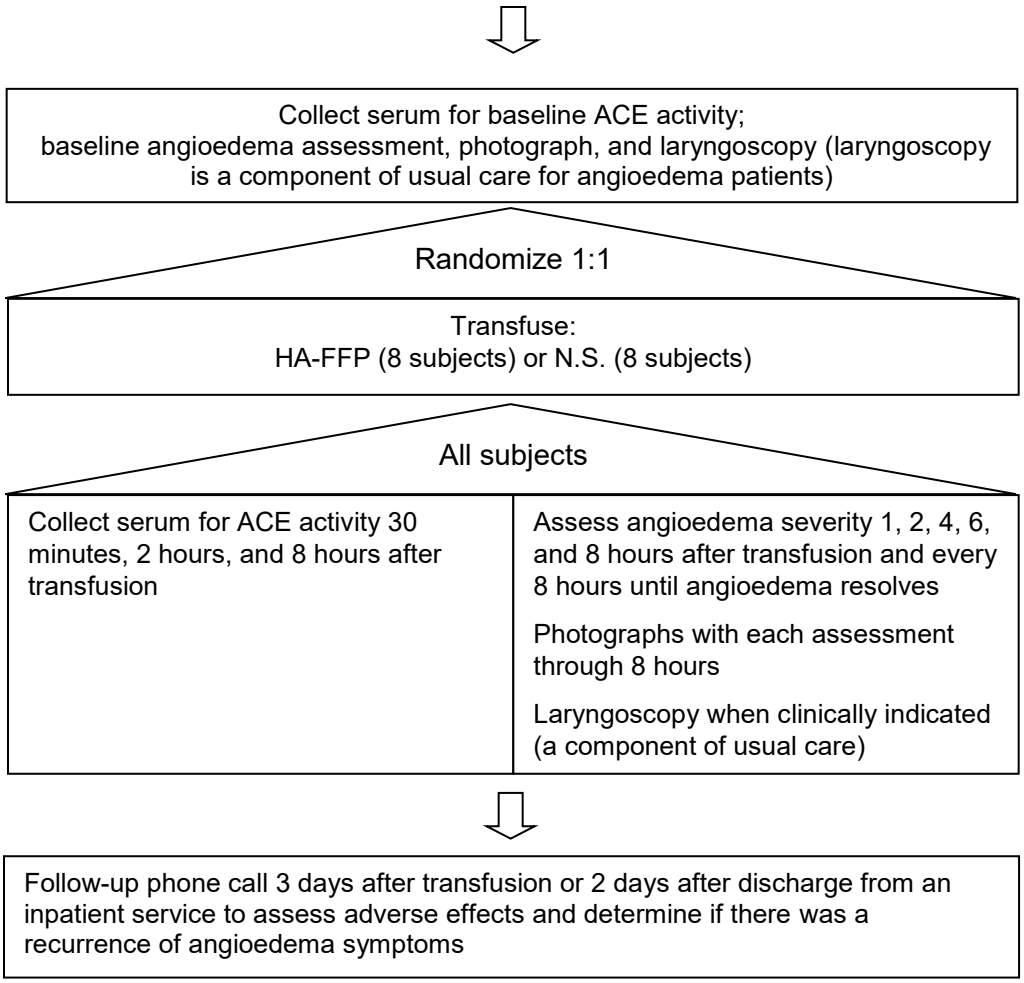
2 units of HA-P was chosen as the dose for this protocol because 2 units of FFP is the dose that has been most widely reported for treatment of ACE inhibitor-induced angioedema in the literature. An HA-P unit containing the highest ACE activity level available will be paired with an HA-P unit containing the lowest ACE activity level above a minimum threshold of 50 U/L. Control patients will receive 500 ml of N.S., which is approximately the same volume as 2 units of plasma. In the context of safety concerns, 2 units of plasma is less than is recommended for anticoagulant reversal (12-15 ml/kg is recommended).

We also note that in a recent study, the mean ACE activity level in patients with ACE inhibitor-induced angioedema was 15.1 U/L (control patients taking an ACE inhibitor without a history of angioedema had a mean ACE activity level of 20.7)[20]. Because one unit of plasma is approximately 250ml and the average person has 4.9L of blood, this suggests that the transfused plasma (2 units or 500 ml) will add a minimum of approximately 25 U of ACE activity to an approximate mean of 74 U of circulating ACE activity in the typical subject of the current study. This represents a 34% increase in total circulating ACE activity.

E Study Design

E1 Design Summary

History and physical; screen for potential participants by inclusion and exclusion criteria; obtain informed consent
Total 16 subjects



E2 Subject Selection and Withdrawal

2.a Inclusion Criteria

1. Males and females 18 years of age or older
2. Must currently be on ACE inhibitor therapy and have received a dose within 36 hours
3. Presenting with moderate to severe angioedema affecting the upper aerodigestive tract (face, lips, cheeks, tongue, soft palate/uvula, pharynx, and larynx). The severity of the angioedema attack will be determined by the subject's worst severity rating at baseline among 4 clinical domains (difficulty breathing, difficulty swallowing, voice changes, and tongue swelling) based on a clinically validated angioedema severity scale[29] (Table)
4. Presenting with ACE inhibitor-induced angioedema within 12 hours after onset
5. All females must have a locally obtained negative pregnancy test prior to administration of the study drug. Those who have had a total hysterectomy, bilateral oophorectomy, or are two years post-menopausal do not require a pregnancy test.
6. Must be able to provide written informed consent to participate in the study to fulfill all study requirements

Exclusion Criteria

1. Pregnancy and/or breast feeding
2. Patients with angioedema that is likely due to causes other than ACE inhibitors, including hereditary angioedema, acquired angioedema, and allergic angioedema (food, insect bite or sting with clear response to anti-allergy medications)
3. Patients exhibiting acute urticaria
4. Evident clinical response to glucocorticoids, antihistamines, or epinephrine
5. A family history of recurrent angioedema
6. Documented intolerance to plasma
7. Documented congenital deficiency of IgA in the presence of anti-IgA antibodies
8. Patients with heart failure of the severity that precludes safe transfusion of HA-P
9. Patients with acute pulmonary edema
10. Patients with morbid obesity as defined by BMI>40; morbidly obese patients have a higher volume of blood needing higher amounts of plasma and therefore will be excluded from this pilot study
11. Opinion of the investigator that the patient would not be a good candidate
12. Participation in a clinical study in the past 30 days

2.b Ethical Considerations

We will not proceed with this trial/study until we have written and dated approval from the IRB for this study protocol and for our informed consent document. The informed consent must be reviewed and signed by all study participants.

Our control patients will not be treated with plasma, which may be a beneficial treatment; however, the use of plasma to treat ACE inhibitor-induced angioedema is not standard of care and it is rarely, if ever, used for this purpose in the BJH ED. Furthermore, our findings on the ACE activity in blood donors strongly suggests that many units of plasma would be ineffective because of low ACE activity content. In fact, our findings suggest that some plasma is potentially harmful to individuals with ACE inhibitor-induced angioedema because some donors are using ACE inhibitors. Therefore, it is unlikely that subjects who participate in this study will be denied a beneficial treatment that they would have received had they not participated. On the other hand, 15 subjects will be receiving a low risk but potentially effective treatment, 2 units of HA-P, that would not be available for their treatment if they did not participate in the study.

2.c Subject Recruitment Plans and Consent Process

Recruitment:

We will recruit 16 subjects who present to the BJH Emergency Department with moderate-to-severe ACE inhibitor-induced angioedema. Subjects will be paid $50 for their participation via gift cards if they complete the 30-minute post-transfusion phlebotomy. We note that all patients with moderate-to-severe angioedema are admitted while patients with mild angioedema are typically discharged from the ED. Between 7/1/18 and 6/30/19 there were 43 patients with ACE inhibitor-induced angioedema admitted to BJH, and recruitment in a previous study of ACE inhibitor-induced angioedema in which BJH was a center was near 100% of eligible patients, so we should meet our goal of 16 subjects during the study period.

Informed Consent Document Provided to Participants:

A consent form describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to administering study intervention. The Informed Consent Document has been submitted with this protocol. All participants will also be required to consent to being treated with plasma by reviewing and signing the standard BJH Blood Product consent form.

Consent Procedures and Documentation:

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. A member of the research team will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. All participants will also be required to consent to being treated with plasma by reviewing and signing the standard BJH Blood Product consent form. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

2.d Randomization Method and Blinding

Following enrollment subjects will be randomly assigned to one of two groups: HA-P or N.S. with a 1:1 allocation. The study will use the REDCap randomization module to create an online password-protected randomization system that will facilitate the random assignment of subjects. Through the REDCap project website, the user will provide information that establishes the eligibility of the individual. Group assignments will be revealed only if all eligibility criteria are satisfied. To avoid temporal bias, randomization will be blocked using random block sizes in order to preclude the possibility that investigators might know in advance the assignment of the last subject in a particular block.

This will be an open-label study; however, the study team members who assess the severity of each patient's angioedema will be blinded to the treatment the patient received.

2.e Risks and Benefits

Risks:

1. Phlebotomy and insertion of an intravenous catheter: Venipuncture may cause a slight pain and there is a very small risk of fainting during the procedure. An infection may arise at the site of the needle stick, but this is highly unlikely. Bruising may occur around the venipuncture site. An intravenous catheter will be inserted in an arm vein for purposes of administering plasma or normal saline. The subject may experience discomfort, bruising, dizziness, faintness and/or bleeding at the site of needle insertion, and rarely an infection may arise at the site of the catheter insertion. Sometimes multiple attempts are necessary to insert the catheter.
Of note is that non-study patients with ACE inhibitor-induced angioedema also routinely undergo phlebotomy and insertion of an intravenous catheter. Study subjects, however, will have three additional phlebotomies as described above. The number of venipunctures will be minimized by drawing blood from the IV if possible.
2. Plasma Transfusion: Reports of the incidence of adverse effects of plasma transfusion vary widely. In France, where reporting is mandatory, the incidence of adverse events to plasma was 1:1700 units in 2010. In a large U.S. hospital, in 31,329 plasma transfusions a reaction rate of 1:360 was reported. The most common risks associated with plasma transfusion are: (1) transfusion related acute lung injury; (2) transfusion associated circulatory overload, and (3) allergic/anaphylactic reactions[30]. The reactions to plasma transfusion are typically reversible.

3. There is one case report of worsening of angioedema with transfusion of FFP; however, it is not clear whether this was induced by the FFP or if it was just the natural course of the angioedema. If the worsening was truly induced by the FFP transfusion, a likely explanation is that the FFP was from a donor who was taking an ACE inhibitor. This will be avoided in our study by the use of only HA-P. Importantly, FFP transfusion is listed as a potential treatment of ACE inhibitor-induced angioedema in the widely used clinical reference UpToDate.
4. Laryngoscopy: All patients with moderate-to-severe ACE inhibitor-induced angioedema at BJH undergo laryngoscopy at clinically indicated intervals. Indications for laryngoscopy will be the same for study subjects as non-study patients, so there will not be an increased risk from laryngoscopy to study subjects.

Alternative treatments:

There are no effective alternative treatments for ACE inhibitor-induced angioedema. Many patients with ACE inhibitor-induced angioedema are treated with antihistamines and glucocorticoids for the rare instance in which they are misdiagnosed and are truly suffering a histamine-mediated form of angioedema. Antihistamines and glucocorticoids will be administered to study subjects at the treating physicians' discretion.

Benefits

There is no proven effective treatment for ACE inhibitor-induced angioedema. Patients with moderate-to-severe ACE inhibitor-induced angioedema are admitted for monitoring for the possibility of airway compromise and the consequent need for intubation or a surgical airway. There are case series and case reports of the effectiveness of FFP in treating ACE inhibitor-induced angioedema. Its effectiveness is thought to be a function of the ACE activity content of FFP. However, we found that FFP units frequently have low or even immeasurable ACE activity content. We hypothesize that ACE inhibitor-induced angioedema will resolve more rapidly in subjects treated with HA-P than patients who receive usual care due to an increase in ACE activity levels. This pilot will be part of a project that may identify HA-P as the first effective treatment for ACE inhibitor-induce angioedema—it may decrease the need for intubation or surgical airway and decrease admissions and length of stay for patients who have ACE inhibitor-induced angioedema.

Risks vs. Benefits

As noted above, adverse reactions to plasma transfusion rarely occur and are unlikely to occur on a statistical basis among the 15 subjects who will be treated with HA-P in this study. Therefore, the potential benefits to study subjects exceed the risks.

2.f Early Withdrawal of Subjects

The investigator may withdraw a patient from the trial for any of the following reasons:

- A significant protocol violation occurs.
- A serious or intolerable adverse transfusion-related event occurs before the HA-P or N.S. is completely infused such that the transfusion cannot be completed.
- A clinical adverse event, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- A change in the clinical status of the patient occurs that precludes further study assessments.
- The study is terminated.
- The patient requests to be discontinued from the study.

The reason for participant withdrawal from the study will be recorded on the electronic case report form (eCRF).

- Patients who are consented but withdraw from the trial before the 30-minute post-transfusion phlebotomy will be replaced.
- Patients who withdraw from the trial after the 30-minute post-transfusion phlebotomy will not be replaced (the data from such patients can be included to determine if the Primary Endpoint was met).

2.g When and How to Withdraw Subjects

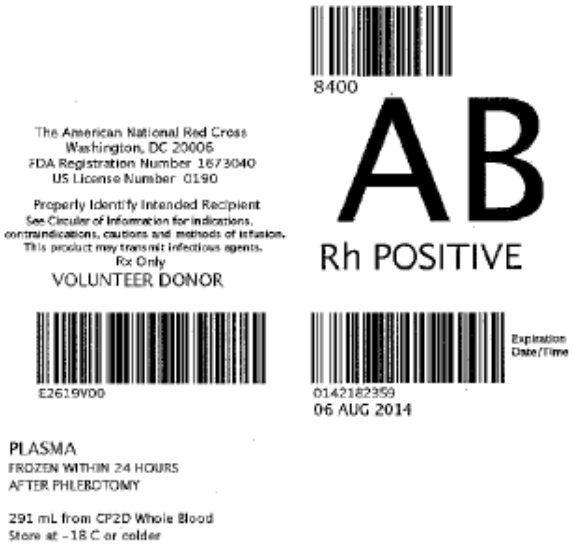
Subjects withdrawn from the study at any point for any reason will continue to receive the usual care, assessments, and follow-up that would be afforded patients with ACE inhibitor-induced angioedema who are not participants in the study.

2.h Data Collection and Follow-up for Withdrawn Subjects

All available study data from subjects who are enrolled in the study will be included in the final analysis of the data (intent-to-treat).

E3 Study Drug

3.a Description



PF24, defined as plasma refrigerated within 8 hours and then frozen within 24 hours after phlebotomy, colloquially referred to by non-blood bank clinicians as FFP, will be used off-label for this study. American Red Cross Missouri/Illinois Blood Services Region will provide units of plasma to the BJH blood bank. The use of FFP/HA-P for this study has been declared IND-exempt by the FDA (see Appendix).

Placebo Description

A volume of 500 ml of N.S. will be administered to the control participants. This volume is roughly equivalent to the volume of 2 units of plasma.

3.b Treatment Regimen

Either two units of HA-P or an equal volume of N.S. (500 ml) will be transfused. Plasma will be thawed according to the institution's approved standard blood bank protocol. The infusion will be administered at the standard rate in accordance with the institution's approved standard blood bank protocol.

3.c Method for Assigning Subjects to Treatment Groups

Following enrollment subjects will be randomly assigned to one of two groups: HA-P or N.S. with a 1:1 allocation. The study will use the REDCap randomization module to create an online password-protected randomization system that will facilitate the random assignment of subjects. Through the REDCap project website, the user will provide information that establishes the eligibility of the individual. Group assignments will be revealed only if all eligibility criteria are satisfied. To avoid temporal bias, randomization will be blocked using random block sizes in order to preclude the possibility that investigators might know in advance the assignment of the last subject in a particular block.

3.d Preparation and Administration of Study Drug

If the subject through randomization is assigned to receive HA-P, a member of the research team will call the BJH blood bank's 24/7 call center to notify the blood bank that HA-P is needed. A research team member will then order HA-P units through a designated study order set on Epic, which will include a type and screen as well as nursing order specifying that the HA-P is for a research study and will be delivered via a research coordinator instead of the usual method. The blood bank will prepare two ABO-compatible HA-P units and affix labeled tie tags (Fig. 2). A research coordinator from the Emergency Center Research Core (ECRC) will retrieve and hand-deliver the HA-P units from the blood bank to the subject's bedside. A health care provider will administer two units of HA-P at a rate in accordance to the BJH blood bank administration protocol.

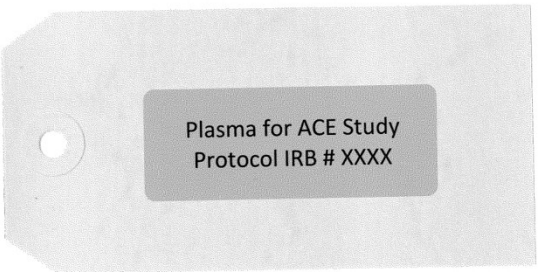


Figure 2. Tie tag for study plasma (HA-P)

If the subject through randomization is assigned to receive N.S., a member of the research team will order the normal saline through a designated study order set on Epic.

3.e Subject Compliance Monitoring

Not applicable (study treatment will be administered one time only in the Emergency Department under the supervision of qualified site personnel)

3.f Prior and Concomitant Therapy

All prescription, over-the-counter medications, herbals, and supplements which are being taken or have been taken by participants within 30 days before study entry and during the study including the ACE inhibitor that the patient is currently taking are regarded as concomitant medications and must be documented on the source document and eCRF following informed consent.

There are no non-investigational concomitant therapies excluded during the patient's participation in this study. Investigational therapies are not permitted 30 days prior to enrollment through the 2-day follow-up telephone visit.

3.g Packaging

The study product will be labeled according to FDA-approved ISBT labels. A tie tag specific to the study (Fig. 2) will be attached to each unit of plasma once the product is ordered from the blood bank

3.h Blinding of Study Drug

This is a randomized, open-label trial. All patients will be assigned 1:1 to receive either HA-P or N.S. There is currently no available placebo that looks like plasma and although the subject will not be notified of treatment assignment, the subject may become aware of the treatment assignment. Members of the research team grading the severity of edema will be blinded to the treatment administered to the patient until enrollment is complete and the study database is locked.

3.i Receiving, Storage, Dispensing and Return

American Red Cross Missouri/Illinois Blood Services Region will provide units of plasma along with corresponding tubes of serum from each donor to the BJH blood bank. The serum tubes will be sent to the ICTS Core Laboratory for Clinical Studies to assay for ACE activity level. The Core Laboratory for Clinical Studies will identify the serum samples with ACE activity levels ≥ 50 U/L. The corresponding plasma units will be designated as high ACE activity-containing plasma (HA-P) and allocated for the study. The remaining units of plasma (those with <50 U/L of ACE activity) will be separated from the study pool and designated for routine clinical use by the BJH blood bank.

The study product, HA-P, will be stored frozen on a separate and labeled shelf in a continuously monitored blood bank freezer at -18°C or colder according to FDA 21 CFR 640.34 as per the BJH blood bank protocol. At this temperature, the study product has a shelf-life of one year, the duration of this study. The study product will be thawed in accordance with the BJH blood bank protocol. Once the study product is thawed, all unused product must be returned for regulatory tracking. All units of the product that are not used by the end of this study will be distributed for routine clinical use by the BJH blood bank.

F Study Procedures

F1 Screening for Eligibility

1.a Informed Consent

The informed consent form must be executed prior to performing any study related activities. Informed consent will be obtained for all participants in the study. Participants may withdraw consent at any time. Participation in the study may be terminated at any time without the participant's consent as determined by the primary investigator.

1.b Inclusion/Exclusion Criteria

Patients must meet all of the inclusion criteria and none of the exclusion criteria in Section 2a in order to participate in this trial.

1.c Physical Examination

A complete physical examination will be performed.
The physical examination will include:

- Pulse, temperature, respiration rate, oxygenation saturation, and systolic and diastolic blood pressure
- Height and weight
- General appearance
- Head, Eyes, Ears, Nose, and Throat
- Respiratory
- Cardiovascular
- Abdomen
- Neurologic
- Extremities
- Dermatologic
- Lymphatic

1.d Clinical Laboratory Tests

Laboratory testing will be performed using standard methods. Prior to study drug administration, blood samples and urine will be collected for laboratory testing. Study-specific blood samples will be collected again at 30 minutes, 2 hours, and 8 hours following study drug administration. Participants will be in a seated or supine position during blood collection. Clinical laboratory tests will include the following:

- Hematology panel (CBC)
- Serum chemistry panel (BMP)
- Coagulation panel (PT/INR/PTT)
- ACE activity level
- Urine human chorionic gonadotropin (hCG) for females who require test as described in inclusion criteria
- Blood samples for a hematology panel (CBC), serum chemistry panel (BMP), and coagulation panel (PT/INR/PTT) are routinely drawn as standard of care when a patient presents to the ED for ACE inhibitor-induced angioedema. Although these will not be assessed as a component of this study, we may use the de-identified results of these tests in publications, presentations, or grant applications

1.e ER Presentation and Symptom Onset

The following information will be captured and recorded in the source documents and eCRF:

- ER presentation date and time
- Length of time participant has been on an ACE inhibitor
- Date and time participant began experiencing angioedema symptoms for this attack
- Reason for taking an ACE inhibitor
- Time of most recent ACE inhibitor dose

The specific ACE inhibitor that the patient is currently taking along with the dose information is captured in the concomitant therapy section.

1.f Photograph of Subject

Photographs of each subject who consents to being photographed will be taken prior to study drug administration and at 1, 2, 4, and 8 hours after study drug administration and then every 8 hours until the angioedema has improved enough that it is safe to discharge the patient. Consent to have photographs taken is not a requirement for participation in the study. Prior to photography, subjects will be asked to remove or hide identifying jewelry or clothing. Photos will be taken with a digital camera and both camera and photos will be stored behind 2 locked doors. All photos will be carefully cropped to remove identifying features.

1.g Laryngoscopy

Visualization of a subject's airway through direct laryngoscopy is a component of usual care in patients with angioedema in the area of tongue, throat, larynx or in the case of the following symptoms: respiratory distress, hoarseness, foreign body sensation in the throat, dysphagia, change of voice. Laryngoscopy will be performed by a qualified provider when clinically indicated. It will not be performed solely for the purposes of this study; however, findings from laryngoscopy will be recorded for study purposes.

1.h Provider Assessment of Angioedema

A study provider blinded to the treatment assignment will assess the severity of angioedema at baseline, at 1, 2, 4, 8 hours post study treatment administration and then every 8 hours until the angioedema has improved enough so that it is safe to discharge the patient. Assessment will use a clinically validated rating scale[29] (Table). The severity of the angioedema attack will be determined by the subject's worst severity rating at baseline among 4 clinical domains (difficulty breathing, difficulty swallowing, voice changes, and tongue swelling) and the scores will be recorded in the eCRF.

F2 Schedule of Assessments

2.a Screening Visit (Prior to study drug administration)

- Informed Consent
- Confirmation of inclusion/exclusion criteria
- Medical History
- Physical examination
- Clinical laboratory tests
- Photograph of subject
- Provider assessment of angioedema using angioedema severity scale
- Baseline Laryngoscopy (performed on presentation of all angioedema patients)

2.b Administration of study drug (Time = 0)

See Section E.3.d. Time = 0 will be end time of administration recorded on source document.

F3 Treatment Period (Up to 8 hours from administration of study drug, or until discharged from ER or hospital)

3.a 15-30 minutes post administration

- ACE activity blood draw
- Adverse event record

3.b 1 hour post administration (+/- 15 minutes)

- Vital signs
- Photograph of subject
- Provider assessment of angioedema using angioedema severity scale
- Adverse event record

3.c 2 hours post administration (+/- 15 minutes)

- Vital signs

- Photograph of subject
- Provider assessment of angioedema using angioedema severity scale
- ACE activity blood draw
- Adverse event record

3.d 4 hours post administration (+/- 30 minutes)

- Vital signs
- Photograph of subject
- Provider assessment of angioedema using angioedema severity scale
- Adverse event record

3.e 6 hours post administration (+/- 30 minutes)

- Vital signs
- Photograph of subject
- Provider assessment of angioedema using angioedema severity scale
- Adverse event record

3.f 8 hours post administration (+/- 30 minutes)

- Vital signs
- Photograph of subject
- Laryngoscopy if clinically indicated
- Provider assessment of angioedema using angioedema severity scale
- ACE activity level blood draw
- Adverse event record

3.g Then every 8 hours (+/- 30 minutes) until the angioedema has improved enough so that it is safe to discharge the participant

- Vital signs
- Laryngoscopy if clinically indicated
- Provider assessment of angioedema using angioedema severity scale
- Adverse event record

F4 Follow-up Phone Call

A member of the research team will call the subject either 3 days after transfusion or 2 days after discharge from an inpatient service, whichever comes first, to assess adverse effects and determine if there was a recurrence of angioedema symptoms or any other problems.

F5 Safety and Adverse Events

5.a Safety and Compliance Monitoring

Dr. Steven Weintraub, the PI, will be responsible for assuring patient safety and compliance with the protocol.

5.b Protocol Deviations

Study personnel should notify the study PI of any suspected protocol deviations within 1 working day of awareness by email, phone, or submitting the eCRF. After review, the study PI will categorize as either major (has the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study), or minor (does not have the potential

to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study). All deviations will be entered into REDCap using the eCRF.

5.c Medical Monitoring

i Investigator only

Dr. Weintraub, study PI, will be responsible for reviewing adverse events, ensuring participants' safety, and for reporting Unanticipated Problems, Serious Adverse Events, and Unexpected Adverse Drug Events to the IRB according to their policies.

5.d Definitions of Adverse Events

Adverse Event (AE): Any untoward medical occurrence in a subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. The failure of the anticipated pharmaceutical action of an investigational drug does not constitute a related AE.

Adverse Drug Event (ADE): All noxious and unintended responses to a medicinal product, including HA-P, related to any dose should be considered adverse drug events. The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

Serious AE (SAE): Any untoward medical occurrence that results in any of the following outcomes:

- death,
- is life threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect

In addition, important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Life-Threatening Experience: An adverse event is life threatening if the patient is at immediate risk of death from the event as it occurs, i.e., it does not include a reaction that, if it had occurred in a more serious form, might have caused death. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not have been considered life threatening, even though drug-induced hepatitis can be fatal.

Disability/Incapacitating Experience: An adverse event is incapacitating or disabling if the experience resulted in a substantial and/or permanent disruption of the subject's ability to carry out normal life functions.

An Unanticipated Problem (UP) is defined as being any incident, experience, or outcome that meets **all** of the following criteria:

1) it is unexpected (in terms of nature, severity, or frequency) given the research procedures that are described in the protocol-related documents, such as the IRB- approved research protocol and informed consent document, and the characteristics of the subject population being studied;

2) it is related or possibly related to participation in the research (meaning that there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research; and

3) it suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

Classification of Events

i Relationship

The investigator must use his/her medical judgment to assess the relationship of the AE to plasma transfusion. Even if the investigator feels there is no relationship to plasma transfusion, the AE is to be reported.

- **Definitely Related** – The AE is known to occur during or secondary to plasma transfusion and there is an appropriate temporal relationship between the plasma transfusion and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the plasma transfusion and the AE.
- **Probably Related –** An AE has a strong temporal relationship to the plasma transfusion and another etiology is significantly less likely.
- **Possibly Related –** An AE has a strong temporal relationship to the plasma transfusion and an alternative etiology is equally or less likely.
- **Not Related** – There is not a reasonable possibility that the plasma transfusion caused the event, there is no temporal relationship between the plasma transfusion and event onset, or an alternate etiology has been established.

ii Severity

The investigator will assess the severity of the AE based on the criteria below:

- **Mild** - Awareness of a sign or symptom that does not interfere with the patient's usual activity or is transient, resolved without treatment and with no sequelae
- **Moderate** - Interferes with the patient's usual activity, but he/she is still able to function
- **Severe** - Interrupts a patient's usual daily activity and generally requires a systemic drug therapy or other treatment

iii Expectedness

The PI, Dr. Weintraub, or another delegated study investigator, will be responsible for determining whether an AE is expected or unexpected.

Each AE should be evaluated as to whether it was expected or unexpected. An unexpected AE is defined as any AE for which the nature, severity, or frequency is **not** consistent with either:

1) the known or foreseeable risk of AEs associated with the procedures involved in the research that are described in (a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document; and b) other relevant sources of information, such as product labeling and package inserts; or

2) the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the AE and the subject's predisposing risk factor profile for the AE.

AEs that do not meet the above criteria of an unexpected AE should be graded as expected.

Potential SAEs specific to this study

The only potential anticipated SAEs due to a study intervention are those associated routine transfusion of plasma, which are mostly (1) transfusion-related acute lung injury; (2) transfusion-associated circulatory overload, and (3) allergic/anaphylactic reactions. As noted in section E2, however, because these occur only infrequently as a result of plasma transfusion, it is statistically unlikely that any of these will occur among the 8 patients who randomize to HA-P transfusion in this study and if they do occur, they are typically completely reversible.

5.e Data Collection Procedures for Adverse Events

All AEs occurring from time of consent until final follow-up phone call, regardless of seriousness, severity, or presumed relationship to study therapy, must be recorded using medical terminology in the source document. Whenever possible, the diagnosis should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection" instead of identifying and listing the individual signs and symptoms). The investigator must record, on the AE source document, his or her medical assessment of the severity of the event and the relationship of the AE to Study Treatment. For events that meet criteria as a SAE, UP, or ADE, additional event details will be submitted using the eCRF and PI Dr. Weintraub will be notified. All AEs must be followed until they have resolved or until a stable clinical endpoint is reached. All measures required for managing the AE and the ultimate outcome of the AE must be recorded in the source document and reported in the eCRF for SAEs, UPs, and ADEs.

5.f Reporting Procedures

All AEs will be reported to Dr. Weintraub, the PI responsible for safety reporting. Dr. Weintraub will report AEs as described below in 5.f.

5.g Adverse Event Reporting Period

An individual adverse event that meets the definition of an unanticipated problem involving risk to participants or others: such events will be described in a reportable event form in myIRB within 10 working days or 1 day if they result in the death of a participant in the study.

Individual serious adverse events that do not meet the definition of an unanticipated problem involving risks to participants or others: such events will be included in the summary of information at the time of continuing review.

G Statistical Plan

G1 Sample Size Determination and Power

The HA-P that will be used for this study will have a minimum of 50 U/L of ACE activity and in a recent study, the mean ACE activity level in patients with ACE inhibitor-induced angioedema was 15.1 U/L (control patients taking an ACE inhibitor without a history of angioedema had a mean ACE activity level of 20.7)[20]. Of the 2 units of HA-P, an HA-P unit containing the highest ACE activity level available will be paired with an HA-P unit containing the lowest ACE activity level above a minimum threshold of 50 U/L. Because one unit of HA-P is approximately 250 ml and the average person has 4.9 L of blood, this suggests that the transfused HA-P (2 units or 500 ml) will add at least 25 U (0.5L x 50U/L) of ACE activity to an approximate mean of 74 U (4.9L x 15.1 U/L) of circulating ACE activity in the typical subject of the current study. This represents a 34% increase in total circulating ACE activity.

The primary end point of the study will be the change in serum ACE activity level from baseline to 30 minutes after treatment. Based on the figures above, we estimate that the HA-P group should see an increase of at least 3.5 U/L while the N.S. group should have almost no change in serum ACE activity, therefore expecting a difference in the change of serum ACE activity levels of 3.0 U/L.

We will not be able to complete a sufficiently powered study as originally proposed:

1. The start of the study was delayed by the pandemic and, unexpectedly, there were hardly any angioedema cases during the pandemic (we plan to study if there is a transmissible agent or allergen that is involved in angioedema that was blocked by social distancing and/or masking in the future). Because of these two factors, the FFP we originally screened and the assays we purchased all expired. Therefore, we will need to complete the study with only ~60% of the funds we originally were granted. Thus, we will not be able to include as many subjects in the study as we originally proposed.
2. When we originally screened FFP for ACE activity, approximately 12% of the units had >60U/L of ACE activity. Our original proposal was based on that finding. However, in the two subsequent screens we performed, the ACE-activity values were significantly lower. For example, we just screened 60 units, and only one of them had >60 U/L of ACE activity. Therefore, instead of the threshold of 60 U/L for useable units that we originally proposed, we will now use units that contain >50 U/L of ACE activity.

Because of the above, the study will not be sufficiently powered to reach a statistically significant conclusion. However, I discussed this project and these problems with a program officer at NIH. She suggested that we study at least 5-10 subjects to generate preliminary data for an NIH grant. This will also allow us to show feasibility.

G2 Interim Monitoring and Early Stopping

An interim analysis is planned after data are available for approximately 40% of patients.

G3 Analysis Plan

3.a Primary Endpoint

The primary end point will be the change in serum ACE activity level from baseline to 30 minutes after transfusion of HA-P. Differences between the change in serum ACE activity levels between the HA-P and N.S. groups will be evaluated with a two-sample t-test. The average change in serum ACE activity with the administration of HA-P and the variability of that change is important, but unknown. Based on the figures above, we estimate that the HA-P group should see an increase of 3.5 U/L while the saline group should have almost no change in serum ACE activity, therefore expecting a difference in the change of serum ACE activity levels of 3 U/L.

3.b Secondary Endpoints

Secondary endpoints include change in serum ACE activity level from baseline to 2 hours and 8 hours. The serum activity levels of ACE over time will be summarized using descriptive statistics and graphically. Differences in secondary endpoints from baseline will be conducted using a separate two-sample t-test. Assumptions of the test will be verified and transformations or non-parametric alternatives used if violations are detected. All statistical tests will be two-sided and a significance level of 0.05 will be used.

A clinician from the Emergency Care Research Core blinded to the treatment modality and the results of the assays will assess severity of angioedema using a published validated clinical rating

scale[29] (Table). Differences in the time to resolution will be described using the Kaplan-Meier method and evaluated using the log rank test.

The secondary efficacy endpoints and adverse effects will be summarized using frequency and proportions and the proportions compared between the groups for each time point using a chi-square test or Fisher's exact test, as appropriate.

G4 Statistical Methods

Statistical analysis will be conducted on an intent-to-treat basis. Data will be described and analyzed using the SAS System. Individual subject data will be presented in subject data listings. Descriptive statistics (number of subjects [n], mean, standard deviation [SD], median, interquartile range, minimum and maximum) will be presented for continuous data. For categorical data, frequency and percentage of subjects in each category will be presented. Descriptive data will be tabulated to best represent the baseline characteristics of the study groups

G5 Missing Outcome Data

Missing data is expected to be low due to the acute nature of the condition and short duration of follow-up. Completion of all eCRF fields is mandatory, with queries being issued for missing or inconsistent data. Further, the database cannot be closed with any open queries. An overview of missing values via absolute and relative number of missing data (by treatment; by treatment and data time point) will be given.

G6 Unblinding Procedures

Our pilot study is an open-label trial and therefore unblinding procedures are not applicable.

H Data Handling and Record Keeping

H1 Confidentiality and Security

Participant confidentiality and privacy is strictly held in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, hospital medical records and pharmacy records for the participants in this study.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to REDCap and/or stored in a secure location behind 2 locked doors

in the Emergency Care Research Core. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by Emergency Care Research Core research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived on REDCap.

Each patient must give written informed consent as well as any authorizations required by local law (e.g., authorizations related to Protected Health Information). The Investigator agrees to not use or disclose Protected Health Information other than as permitted or required by the patient authorization or as required by law.

H2 Training

All research staff that will have subject contact or access to subject medical records have completed and passed the Collaborative Institutional Training Initiative Human Research Biomedical Research Investigators and Key Personnel basic course. All research personnel will receive protocol-specific training.

H3 Case Report Forms and Source Documents

Data collection is the responsibility of the principal investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the eCRF derived from source documents should be consistent with the data recorded on the source documents. Only the investigator or personnel authorized by him are authorized to enter data into the eCRF. All the authorized persons must be documented.

Clinical data (including SAEs, UPs, ADEs, and concomitant medications) and clinical laboratory data will be entered into REDCap. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

H4 Records Retention

All study documentation and materials will be retained for 7 years after the close of the study.

I Study Monitoring

The investigator will monitor data generated throughout the study and the eCRFs will be checked against the subject records for completeness and accuracy. Following completion of eCRF pages and entry of the data into a database, the data will be checked electronically for consistency and plausibility. Queries will be generated for questionable data and clarification sought from the applicable personnel. These data queries must be resolved in a timely manner by the investigator (or delegate).

J Study Administration

J1 Organization and Participating Centers

This is a single-center pilot study at Barnes-Jewish Hospital.

J2 Funding Source and Conflicts of Interest

The study is supported financially by the BJH Foundation. There are no conflicts of interest.

J3 Subject Stipends or Payments

Subjects will be paid $50 for their participation via gift cards after completing phlebotomy 30 minutes after transfusion.

K Publication Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

L Attachments

L1 Tables

TABLE Symptom severity ratings: Difficulty breathing, difficulty swallowing, voice changes, and tongue swelling*

Symptom	Rating	Description of rating
Difficulty breathing	0 = absence of symptoms	Normal breathing
	1 = mild	Mild additional effort required for breathing by subject, but no audible wheezing or no stridor heard with stethoscope
	2 = moderate	Audible wheezing and/or stridor heard with stethoscope only, with uncomfortable breathing and moderate additional effort required for breathing by subject
	3 = severe	Audible wheezing and/or stridor audible without stethoscope, with subject in moderate distress
	4 = very severe	Audible severe wheezing and audible marked stridor, with subject in severe distress and tripod posturing (sitting or standing, leaning forward and supporting the upper body with hands on the knees or on another surface)
Difficulty swallowing	0 = absence of symptoms	Normal swallowing
	1 = mild	Mild sensation of difficulty swallowing (fullness in throat), but can swallow solids and liquids
	2 = moderate	Marked difficulty or unable to swallow solids, but can swallow liquids
	3 = severe	Unable to swallow solids or liquids, but can swallow saliva
	4 = very severe	Unable to swallow solids, liquids, or saliva (drooling)
Voice changes	0 = absence of symptoms	Normal voice
	1 = mild	Audible speech, but mild disruption of normal voice (hoarseness)
	2 = moderate	Audible speech, but moderate disruption of normal voice (muffled voice)
	3 = severe	Very difficult to hear speech or for subject to articulate
	4 = very severe	Unable to speak at all
Tongue swelling	0 = absence of symptoms	No swelling
	1 = mild	Mild anterior or lateral tongue swelling, uvula completely visible
	2 = moderate	Moderate anterior or lateral tongue swelling, uvula only partially visible
	3 = severe	Severe diffuse swelling of tongue, soft palate and uvula not visible at all
	4 = very severe	Very severe diffuse tongue swelling that completely fills mouth orifice

M References

1. Banerji A, Blumenthal KG, Lai KH, Zhou L. Epidemiology of ACE Inhibitor Angioedema Utilizing a Large Electronic Health Record. The Journal of Allergy and Clinical Immunology: In Practice. 2017 Jun;5(3):744–9.

2. Messerli FH, Nussberger J. Vasopeptidase inhibition and angio-oedema. The Lancet. 2000 Aug;356(9230):608–9.

3. Baş M, Greve J, Stelter K, Havel M, Strassen U, Rotter N, et al. A Randomized Trial of Icatibant in ACE-Inhibitor–Induced Angioedema. N Engl J Med. 2015 Jan 29;372(5):418–25.

4. Sinert R, Levy P, Bernstein JA, Body R, Sivilotti MLA, Moellman J, et al. Randomized Trial of Icatibant for Angiotensin-Converting Enzyme Inhibitor-Induced Upper Airway Angioedema. The Journal of Allergy and Clinical Immunology: In Practice. 2017 Oct;5(5):1402–3.

5. Straka BT, Ramirez CE, Byrd JB, Stone E, Woodard-Grice A, Nian H, et al. Effect of bradykinin receptor antagonism on ACE inhibitor-associated angioedema. The Journal of Allergy and Clinical Immunology. 2017 Jul;140(1):242–2.

6. Hassen GW, Kalantari H, Parraga M, Chirurgi R, Meletiche C, Chan C, et al. Fresh Frozen Plasma for Progressive and Refractory Angiotensin-Converting Enzyme Inhibitor-induced Angioedema. The Journal of Emergency Medicine. 2013 Apr;44(4):764–72.

7. Bolton MR, Dooley-Hash SL. Angiotensin-converting enzyme inhibitor angioedema. Journal of Emergency Medicine. 2012 Oct;43(4):e261–2.

8. Karim MY, Masood A. Fresh-frozen plasma as a treatment for life-threatening ACE-inhibitor angioedema. The Journal of Allergy and Clinical Immunology. 2002 Feb;109(2):370–1.

9. Warrier MR, Copilevitz CA, Dykewicz MS, Slavin RG. Fresh frozen plasma in the treatment of resistant angiotensin-converting enzyme inhibitor angioedema. Annals of Allergy, Asthma & Immunology. 2004 May;92(5):573–5.

10. Chaaya G, Afridi F, Faiz A, Ashraf A, Ali M, Castiglioni A. When Nothing Else Works: Fresh Frozen Plasma in the Treatment of Progressive, Refractory Angiotensin-Converting Enzyme Inhibitor–Induced Angioedema. Cureus. 2017;:1–4.

11. Duan QL, Nikpoor B, Dube M-P, Molinaro G, Meijer IA, Dion P, et al. A variant in XPNPEP2 is associated with angioedema induced by angiotensin I-converting enzyme inhibitors. American Journal of Human Genetics. 2005 Oct;77(4):617–26.

12. Pare G, Kubo M, Byrd JB, McCarty CA, Woodard-Grice A, Teo KK, et al. Genetic variants associated with angiotensin-converting enzyme inhibitor-associated angioedema. Pharmacogenetics and Genomics. 2013 Sep;23(9):470–8.

13. Adebayo O, Wilkerson RG. Angiotensin-converting enzyme inhibitor-induced angioedema worsened with fresh frozen plasma. Am J Emerg Med. 2017 Jan;35(1):192.e1–192.e2.

14. Harjanne A. Automated kinetic determination of angiotensin-converting enzyme in serum. Clinical Chemistry. 1984 Jun;30(6):901–2.

15. Roberts JR, Lee JJ, Marthers DA. Angiotensin-converting enzyme (ACE) inhibitor angioedema: the silent epidemic. The American Journal of Cardiology. 2012;109(5):774–5.

16. Stone CJ, Brown NJ. Angiotensin-converting Enzyme Inhibitor and Other Drug-associated Angioedema. Immunology and Allergy Clinics of North America. 2017 Aug;37(3):483–95.

17. Jaspard E, Wei L, Alhenc-Gelas F. Differences in the properties and enzymatic specificities of the two active sites of angiotensin I-converting enzyme (kininase II). Studies with bradykinin and other natural peptides. Journal of Biological Chemistry. 1993 May;268(13):9496–503.

18. Ceconi C, Francolini G, Olivares A, Comini L, Bachetti T, Ferrari R. Angiotensin-converting enzyme (ACE) inhibitors have different selectivity for bradykinin binding sites of human somatic ACE. European Journal of Pharmacology. 2007 Dec;577(1-3):1–6.

19. Nussberger J, Cugno M, Cicardi M. Bradykinin-mediated angioedema. N Engl J Med. 2002 Aug;347(8):621–2.

20. Hubers SA, Kohm K, Wei S, Yu C, Nian H, Grabert R, et al. Endogenous bradykinin and B1-B5 during angiotensin-converting enzyme inhibitor-associated angioedema. The Journal of Allergy and Clinical Immunology. 2018 Nov;142(5):1636–1639.e5.

21. Molinaro G, Cugno M, Perez M, Lepage Y, Gervais N, Agostoni A, et al. Angiotensin-converting enzyme inhibitor-associated angioedema is characterized by a slower degradation of des-arginine(9)-bradykinin. The Journal of Pharmacology and Experimental Therapeutics. 2002 Oct;303(1):232–7.

22. Cyr M, Lepage Y, Blais CJ, Gervais N, Cugno M, Rouleau JL, et al. Bradykinin and des-Arg(9)-bradykinin metabolic pathways and kinetics of activation of human plasma. American Journal of Physiology - Heart and Circulatory Physiology. 2001 Jul;281(1):H275–83.

23. Emanueli C, Grady EF, Madeddu P, Figini M, Bunnett NW, Parisi D, et al. Acute ACE inhibition causes plasma extravasation in mice that is mediated by bradykinin and substance P. Hypertension. 1998 Jun;31(6):1299–304.

24. Ferreira PK, Campos MM, Calixto JB. The role of sensorial neuropeptides in the edematogenic responses mediated by B(1) agonist des-Arg(9)-BK in rats pre-treated with LPS. Regulatory Peptides. 2000 May;89(1-3):29–35.

25. O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F. The role of substance P in inflammatory disease. Journal of Cellular Physiology. 2004 Nov;201(2):167–80.

26. Saeb A, Hagglund KH, Cigolle CT. Research Article Using Fresh Frozen Plasma for Acute Airway Angioedema to Prevent Intubation in the Emergency Department: A Retrospective Cohort Study. Emergency Medicine International. Hindawi Publishing Corporation; 2016;:1–6.

27. Anish N, Gabremedhin AM. An encounter with enalapril-induced resistant, life-threatening angioedema at rural health center in Botswana. Indian Journal of Critical Care Medicine. India: Medknow Publications & Media Pvt Ltd; 2016 Oct 1;20(10):613–6.

28. Struthers AD, Anderson G, MacFadyen RJ, Fraser C, MacDonald TM. Nonadherence with ACE inhibitors is common and can be detected in clinical practice by routine serum ACE activity. Congestive Heart Failure. 2001 Jan;7(1):43–6.

29. Bonner N, Panter C, Kimura A, Sinert R, Moellman J, Bernstein JA. Development and validation of the angiotensin-converting enzyme inhibitor (ACEI) induced angioedema investigator rating scale and proposed discharge criteria. BMC Health Services Research. 2017 May;17(1):366.

30. Pandey S, Vyas GN. Adverse effects of plasma transfusion. Transfusion. 2012 May;52 Suppl 1:65S–79S.