**Study Name:** Pilot Study: Prospective comparison of ICG microangiography and conventional angiography in severe frostbite

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1. Summary

Severe frostbite injury is a significant cause of morbidity in northern climates. Here in Minnesota, we have some of the highest numbers of severe frostbite injuries in North America [1]. As a result, at Regions Hospital we have the best opportunity to study this disease process and improve outcomes for frostbite patients. The diagnostic methods for severe frostbite injury vary from institution to institution and there is no standard practice. Commonly utilized methods include conventional angiography, Technetium 99 triple phase bone scans, SPECT studies, Indocyanine Green microangiography, and doppler studies [1-4].

Regions Hospital is one of the busiest institutions utilizing conventional angiography. Conventional angiography can fully delineate the vascular occlusion (cut off) found in severe frostbite patients in highly granular detail. However, this method of diagnosis is very labor intensive and not without risk (pseudoaneurysms, contrast induced nephropathy, complications associated with lying flat for up to 72 hours, etc[2,5-6])**.** By contrast, Indocyanine Green (ICG) microangiography is a relatively low risk imaging modality that can be done at the bedside. This method shows the microvascular soft tissue perfusion and demonstrates where the tissue is ischemic in severe frostbite injury. ICG imaging involves delivering a small amount of ICG dye (5 mg) via peripheral IV and using a specialized camera that captures the dye fluorescing in infra-red light.[7] This method of imaging is FDA approved and has been shown to be highly accurate at predicting final amputation level in severe frostbite injury [8].

The proposed pilot study aims to directly compare conventional angiography imaging to ICG microangiography in adult patients with severe frostbite. Severe frostbite is defined as 4$^{th}$ degree: frostbite resulting in vascular occlusion and tissue ischemia. Both imaging modalities have been used for the diagnosis and monitoring of severe frostbite injury but there has never been a study directly comparing these two imaging modalities[2,7-8]. We will be obtaining consent from adult patients with severe frostbite injury who are undergoing intra-arterial thrombolysis. During their treatment, an angiography will be performed according to the Regions Hospital standard of care protocol, followed immediately by an ICG microangiography image. These two diagnostic images will be directly compared by a radiologist and a burn surgeon to determine the image concordance or discordance. We will also monitor patients for potential side effects or adverse reactions to the ICG imaging, but as stated above, the ICG microangiography is low-risk and we do not anticipate any adverse reactions related to this procedure.

Pending the results of this study, we will pursue a full-scale version of this study, which will track patients in a longitudinal manner to determine the outcome of their frostbite treatments. The proposed pilot study is designed to assess feasibility, the degree of concordance or discordance between the two imaging modalities, and side effects of imaging.

2. Study Aims

Primary Outcome: Angiography and ICG microangiography imaging concordance/discordance.

Research question: Does the vascular cut off on conventional angiography found in severe frostbite patients correlate with the tissue ischemia demonstrated on ICG microangiography in severe frostbite?

Hypothesis: Conventional angiography can fully delineate the vascular occlusion (cut off) found in severe frostbite patients in highly granular detail. ICG microangiography shows the microvascular soft tissue perfusion and demonstrates where the tissue is ischemic in severe frostbite injury. We hypothesize that these two imaging modalities will be concordant at demonstrating the ischemic tissue present in severe frostbite.

Secondary Outcome: Safety.

Research question: Is indocyanine green safe when delivered in close association with contrast dye (used in conventional angiography)?

Hypothesis: Contrast dye used in conventional angiography is associated with nephrotoxicity and other risks. Indocyanine green dye is generally considered to be a highly safe product with minimal risks associated with administration. It has been used for decades with minimal side effects. It is hypothesized that ICG dye will not result in any increase in risk to the patient when delivered in temporal proximity to conventional dye.

3. Background, Rationale, Significance

Frostbite injury has been a significant cause of morbidity and mortality for as long as humans have experienced cold winters. When frostbite occurs, there are two primary mechanisms through which tissue damage occurs, though the main mechanism we will focus on here is systemic cooling. Frostbite is a multiphase process that starts with systemic cooling. As the body cools, blood is shunted from the extremities to the core to maintain heat in the vital organs. During this shunting, there is extensive vasospasm in the extremities and the tissue becomes ischemic. Upon further cooling, tissue ice crystals form within the cells and interstitial spaces causing desiccation and cell destruction. This occurs in the soft tissues and also affects the endothelium of the micro- and macrovasculature. Clots form in the microvasculature, worsening the ischemia. All ischemic tissues release pro-inflammatory factors, which increase inflammation and worsen clotting. [9-14]

Upon rewarming of the frozen tissues, the reperfusion can worsen the inflammatory state as these factors released by the ischemic tissue are now able to circulate. Rapid rewarming is used as a tool to mitigate this reperfusion injury, but it does not eliminate the damage [1,15]. After rapid rewarming, the tissue is assessed for the extent of damage to determine next treatment steps. Diagnosis varies by institution, but the main goal of all diagnostic modalities is to determine if there is a vascular cutoff causing tissue ischemia.

At Regions Hospital, the main diagnostic method that has been used for over 20 years is conventional angiography. This clearly demonstrates the microvasculature of the tissues and allows the intra-arterial catheter directed sheaths to be placed to start thrombolytics, heparin, and vasodilators to treat the tissue ischemia [2]. Other diagnostic modalities used elsewhere include Technetium 99 triple phase bone

scans, SPECT imaging, and indocyanine green (ICG) microangiography [16]. All of these imaging modalities demonstrate the perfusion of the affected tissue but do not allow for directed delivery of thrombolytics. In institutions that use these imaging techniques, thrombolytics are typically delivered in an intravenous fashion rather than intra-arterial.

ICG microangiography is a technology that has had rapidly expanding applications in recent decades. These have included retinal imaging, determining perfusion of colorectal anastomoses, biliary imaging, and assessing the blood supply of tissue flaps [17-19]. It has recently been used in diagnosis of frostbite with good correlation with severe final amputation levels. It is easy to use, non-radiating, can be performed at the bedside, and cheaper than other imaging modalities [7-8].

Given that every hospital has different diagnostic and treatment algorithms, these different imaging modalities have rarely been compared in the same patient. The current best metric is comparing the imaging modality with the amount of tissue that is amputated as demonstrated in prior studies cited here. This research application provides a unique opportunity to compare two imaging modalities head-to-head in the same patient and determine their concordance or discordance.

4. Study Design

This pilot study is an unblinded prospective interventional imaging study designed to determine whether ICG microangiography is as accurate as conventional angiography in determining the ischemic tissue present in severe frostbite.

We will capture images of 10 patients who are undergoing conventional angiography and thrombolysis for severe frostbite. All patients will be adults (>18 years old) and able to provide consent for participation in the study. Once the patient has been started on thrombolysis, they will be evaluated for whether they meet the study's inclusion/exclusion criteria, and then approached by a CCRC Clinical Research Coordinator about possible involvement in this research project. If they consent to the study, then the ICG microangiography imaging will be performed immediately following their next conventional angiography session. When a patient is undergoing catheter directed thrombolysis, they have conventional angiography sessions to assess progression every 24 hours for up to 72 hours. In this pilot study, we would administer the ICG microangiography following one of these sessions, after consent is obtained. This imaging would be performed in the IR suite immediately following the conventional angiography session.

This study is quasi-experimental. The inclusion of a second (ICG) imaging technique is not a therapeutic modality and has limited risks to the study participants. ICG dye is very well tolerated and only contraindicated in iodine allergy. There is no radiation associated with this imaging study. Participants will serve as their own controls as they will be receiving both types of imaging.

*Population*

Patients will be identified by the burn surgeons and advance practice practitioners. They will evaluate the patients to determine whether they meet the inclusion/exclusion criteria of the study.

a. Inclusion criteria
    1. Adult (>18 years old) patients
    2. Diagnosed with severe frostbite by conventional angiography
    3. Undergoing thrombolysis with catheter directed lytics
    4. Clinically sober at the time of consent
    5. Cognitively able to provide consent as determined by clinician's best judgement
    6. Normal kidney function (GFR >60)

b. Exclusion criteria
    1. Pregnant. Pregnancy will be determined by standard of care pregnancy test performed on all female frostbite patients who are receiving lytics.
    2. Iodine allergy.

c. Sample size
    1. Ten ICG images will be performed on 10 unique patients who are already receiving conventional angiography as standard of care.
    2. Feasibility: Based on historical data from Regions Hospital between 2006-present, there are typically 20-70 patients per winter who undergo conventional angiography and catheter directed lytics who would be candidates to participate in this study. We anticipate approaching 20 patients to reach our target sample size of 10.

*Data collection process*

1. Process for identification of patients: All patients undergoing catheter directed thrombolysis for severe frostbite injury are managed primarily by the Burn Team. As such, the burn surgeons and burn advance practice practitioners will be able to identify patients who meet the inclusion criteria for this study.
2. Recruitment: The burn surgeon will identify patients and confirm that they meet the inclusion/exclusion criteria for the study. They will inform the Clinical Research Coordinator from the Critical Care Research Center (CCRC), who will approach the patient about the study and obtain informed consent.
3. Consent: Written consent will be obtained by the CCRC Clinical Research Coordinator who is the Lead Coordinator for this study (see attached ICF). Competency to provide consent will be confirmed by the coordinator obtaining consent by testing for comprehension after the consent conversation and before a signature is obtained.

*Data sources*

Data sources will include chart review, conventional angiography images, and ICG microangiography images. Images and data will be stored on an encrypted HealthPartners computer at Regions Hospital.

*Data acquisition*

- Data will be collected by Dr. Alexandra Lacey, burn surgeon.
- Patient contact by study personnel will include consenting the patient for this study (CCRC Clinical Research Coordinator) and performance of the ICG microangiography study (performed by Dr. Alexandra Lacey).
- Data points from the Electronic Medical Records will include:
    - MRNs
    - Last name, first name
    - Basic demographics: age, gender, race, ethnicity
    - Social: substance abuse, mental health diagnoses, housing status
    - Medical: comorbid conditions CAD/vascular disease, diabetes mellitus, renal insufficiency, smoking
- Data from the conventional angiography include:
    - Medications currently being infused
    - Hours of treatment
    - Areas of poor perfusion as measured by the Hennepin Frostbite Score [20] (see attached score sheet)
- Data from the ICG microangiography include:
    - Areas of poor perfusion as measured by the Hennepin Frostbite Score

**DATA DICTIONARY GOES HERE**

*Statistical Analysis Plan*

The primary dependent variable in this study is the Hennepin Frostbite Score that will be generated from each imaging technique. We will compute a correlation between the Hennepin Frostbite Score produced by the conventional angiography and the Hennepin Frostbite Score produced by the ICG microangiography. While we predict a high correlation between the scores generated by the two methods, a high correlation does not necessarily imply that there is good agreement between the two methods. Thus, we will also use a Bland-Altman (difference) plot to analyze the agreement between the two imaging techniques.

*Power analysis or statement of precision*

This will be a small pilot study that will not be adequately powered to determine significance.

5. Interventions and treatments

As previously stated, the main intervention in this study is the performance of an ICG microangiography study immediately following a conventional angiography image in patients who are undergoing catheter directed thrombolysis for severe frostbite injury. This will be a one-time diagnostic measure with no additional follow up or imaging required. This is not a therapeutic intervention, but a secondary imaging study with minimal risk.

6. Setting/Environment/Organizational feasibility

Study participants will be patients who are being treated primarily on the Burn Unit at Regions Hospital. Consent will be obtained while they are inpatient by a CCRC Clinical Research Coordinator. The ICG microangiography imaging will be performed in the Interventional Radiology (IR) suite immediately following the patient's conventional angiography imaging. This has been discussed with the IR leadership and they agree to performing this study in their space (see attached Letter of Support). Dr. Alexandra Lacey, burn surgeon, will be performing the ICG microangiography and staying in constant communication with the IR staff performing the conventional angiography imaging. Given the small number of images need for this pilot study, this method of communication and number of ICG providers will be adequate.

7. Risks and Benefits

ICG microangiography dye is very safe and well tolerated. It will be delivered via a peripheral IV, which the patient will already have in place per protocol for all patients with severe frostbite injury. The small 5mg dose used for this study has limited possible side effects. There is no radiation exposure for this imaging modality. The only known risk is allergic reaction to the ICG dye. Patients with a known iodine allergy will be excluded from this study due to possible reaction to the conventional angiography. All patients who participate in the study will be monitored following dye administration as standard of care for ICU level patients who have catheter directed lytics being delivered.

Psychological, social, legal, and economic risks are not foreseeable for this study. There is no direct benefit to the patient from participating in this study.

8. Strengths and Limitations

Strengths of the study: this is the first study looking at ICG microangiography and conventional angiography in the same patients. Both techniques have been studied individually but this has never been examined in a head-to-head manner in frostbite. Pending the results of this study, this may help to determine what the true 'gold standard' should be for frostbite imaging.

Limitations of the study: This will be a small pilot study that will not be adequately powered to determine significance. This is also a single institution study and is a single snapshot in time (not longitudinal). Concordance of imaging with long term outcomes will not be calculated as part of this preliminary study.

9. Data Confidentiality and Privacy

All data will be stored on the private HealthPartners computer of Dr. Alexandra Lacey. This computer and its contents are stored in a locked office in the Burn Unit and are not accessible to any other members of the research or healthcare teams. Any data that are extracted for analysis and/or publication will be de-identified. After completion of this pilot and any follow up studies, identifiable data will be destroyed by the PI. There is no plan to share these data with other entities.

10. Timeline

The goal timeline for this limited project is to begin winter 2022 (January 2022 – March 2023) to make sure we have enough patients to allow us to collect all 10 images for this study. Data analysis will be completed over the spring and summer to allow for the creation any necessary follow up project for the following winter.

11. Dissemination of data

Findings of scientific significance will be submitted for presentation at the American Burn Association annual meeting. The typical deadline for abstract submission is early October so all analyses will be completed by that time. Findings will also be written up for publication in a peer-reviewed journal. If a follow up study is needed, that would be pursued prior to publication.

12. References


1. Lacey AM, et al. An Institutional Protocol for the Treatment of Severe Frostbite Injury – A 6 Year Retrospective Analysis. *J Burn Care Res*. 2021 Aug;42(4):817-820
2. Gonzaga T, et al. Use of Intra-arterial Thrombolytic Therapy for Acute Treatment of Frostbite in 62 Patients with Review of Thrombolytic Therapy in Frostbite. *J Burn Care Res.* 2016; 37(4):e323-34
3. Gao Y, et al. Research progress in the pathogenic mechanisms and imaging of severe frostbite. *Eur J Radiol*. 2021 Apr;137; 109605
4. Millet JD, et al. Frostbite: Spectrum of Imaging Findings and Guidelines for Management. *Radiographics*. 2016;36(7):2154-2169
5. Twomey JA, Peltier GL, Zera RT. An open-label study to evaluate the safety and efficacy of tissue plasminogen activator in treatment of severe frostbite. *J Trauma*. 2005 Dec;59(6):1350-4
6. Heard J, et al. Thrombolytic Use in Management of Frostbite Injuries: Eight Year Retrospective Review at a Single Institution. *J Burn Care Res.* 2020;41(3):722-726
7. Masters T, et al. Microangiography to Monitor Treatment Outcomes Following Severe Frostbite Injury to the Hands. *J Burn Care Res.* 2018;39(1):162-167
8. Lacey AM, et al. Microangiography: An alternative tool for assessing severe frostbite injury. *J Burn Care Res.* 2019;40(5):566-569
9. Weatherly-White RC, et al. Experimental studies in cold injury. II. The pathogenesis of frostbite. *J Surg Res*. 1964;4:17-22
10. Rohrer MJ, Natale AM. Effect of hypothermia on the coagulation cascade. *Crit Care Med*. 1992;20:1402-5
11. Zook N, et al. Microcirculatory studies of frostbite injury. *Ann Plast Surg*. 1998;40:246-53; discussion 254.

12. Bourne MH, Piepkorn MW, Clayton F, Leonard LG. Analysis of microvascular changes in frostbite injury. *J Surg Res.* 1986;40:26-35
13. Robsen MC, Heggers JP. Evaluation of hand frostbite blister fluid as a clue to pathogenesis. *J Hand Surg Am*. 1981;6:43-7
14. McCauley RL, et al. Frostbite injuries:a rational approach based on pathophysiology. *J Trauma.* 1983;23:143-7.
15. Rogers C, et al. The Effects of Rapid Rewarming on Tissue Salvage in Severe Frostbite Injury. *J Burn Care Res.* 2022;43(4):906-911
16. Millet JD, et al. Frostbite: spectrum of imaging findings and guidelines for management. *Radiographics*. 2016;36(7):2154-2169
17. Braun JD, et al. Early quantitative evaluation of indocyanine green angiography in patients with critical limb ischemia. *J Vasc Surg.* 2013;57:1213-8
18. Jafari Md, et al. The use of indocyanine green fluorescence to assess anastomotic perfusion during robotic assisted laparoscopic rectal surgery. *Surg Endosc*. 2013;27:3003-8
19. Zhi Z, et al. Optical microangiography of retina and choroid and measurement of total retinal blood flow in mice. *Biomed Opt Express.* 2012;3:2976-86
20. Nygaard RM, et al. The Hennepin Score: Quantification of Frostbite Management Efficacy. *J Burn Care Res.* 2016;37(4):e317-e322.