

PULSE-LI: Photoacoustic and Ultrasound Lung Scanning for Evaluation of Lung Injury

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STUDY STATEMENT

This study will be conducted in accordance with the Health Canada-authorized protocol, the Medical Devices Regulations, Part 3, ISO 14155 principles for clinical investigation of medical devices, applicable Health Canada requirements, the TCPS 2, Unity Health Toronto policies, and REB approval. No protocol deviation or change will be implemented without required REB approval and, where applicable, Health Canada authorization, except where necessary to eliminate an immediate hazard to a participant.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to both the regulatory authorities and the REB for review and approval. Approval from both the regulatory authorities and the REB of both the protocol and the consent form(s) must be obtained before any participant is enrolled. Any amendment to the protocol will be approved by the REB and regulatory authorities before the changes are implemented at the study.

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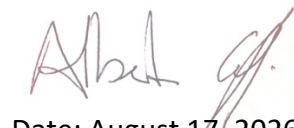


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ABSTRACT

Background: Pulmonary edema and pulmonary fibrosis represent distinct pathological processes with overlapping clinical and radiological presentations, yet they require fundamentally different management strategies. Current imaging modalities such as chest radiography (CXR) and computed tomography (CT) have limitations in bedside applicability, radiation exposure, and functional assessment. Conventional lung ultrasound (LUS), while portable and radiation-free, remains qualitative and operator-dependent, lacking the specificity to distinguish edema from fibrosis.

Objective: The PULSE-LI (Photoacoustic and Ultrasound Lung Scanning for Evaluation of Lung Injury) study aims to develop and validate a novel, non-invasive imaging platform that combines Quantitative Ultrasound (QUS) and Photoacoustic Imaging (PAI) to differentiate pulmonary edema from fibrosis at the bedside.

Methods: This is a prospective, two-phase study at St. Michael's Hospital. In Phase 1, we will optimize QUS/PAI imaging protocols in 20 healthy volunteers. In Phase 2, we will apply these protocols to 30 patients: 15 with acute decompensated heart failure (ADHF) and pulmonary edema, and 15 with idiopathic pulmonary fibrosis (UIP pattern on HRCT). QUS biomarkers will quantify lung structural alterations via analysis of raw radiofrequency data, including scatterer concentration, size, and spacing. PAI will provide functional insights into perfusion, oxygenation, and collagen/water content via multi-wavelength optical absorption profiling.

Outcomes: Primary outcome is the development and preliminary validation of structural and functional imaging biomarkers capable of accurately differentiating pulmonary edema from fibrosis. Secondary outcomes include reproducibility testing, assessment of patient-related imaging factors (e.g., skin tone), and correlation with clinical and physiological parameters.

Impact: PULSE-LI is an investigational, portable, radiation-free imaging approach designed to support development and preliminary validation of QUS- and PAI/MSOT-derived biomarkers for

lung injury. If validated in future studies, these biomarkers may support development of bedside tools for assessing pulmonary edema and fibrosis.

LAY ABSTRACT

Lung diseases such as fluid buildup in the lungs, called pulmonary edema, and lung scarring called fibrosis, can sometimes look similar on standard imaging tests, even though they have different causes and treatments. The PULSE-LI study will test whether two research imaging methods, quantitative ultrasound (QUS) and photoacoustic imaging (PAI), can identify imaging patterns that help distinguish pulmonary edema from pulmonary fibrosis . These imaging methods are non-invasive, do not use ionizing radiation, and may provide information about lung structure and tissue properties at the bedside. In this study, researchers will first perform imaging in healthy volunteers and then in participants with heart failure-related pulmonary edema and participants with pulmonary fibrosis. The study images and measurements will be used for research only. They will not be used to diagnose participants, monitor their illness, or guide treatment decisions. Participants will continue to receive their usual medical care based on standard clinical information and tests. The goal is to develop and preliminary validate research imaging “biomarkers” that may help characterize different types of lung injury. In the future, if these imaging biomarkers are validated in additional studies, they may support the development of bedside tools for assessing lung disease more quickly and safely.

1. BACKGROUND

1.1 Introduction

Respiratory diseases represent a major cause of morbidity and mortality in Ontario and globally, with chronic lung conditions ranking among the top causes of hospitalization and death(1). Among the wide spectrum of lung disorders, two key pathophysiological hallmarks - pulmonary edema and fibrosis - are especially prominent. These processes lie at the core of conditions such as cardiogenic pulmonary edema (CPE)(2) and interstitial lung diseases (ILDs)(3). Pulmonary edema, often secondary to acute decompensated heart failure (ADHF), affects over 1 million Canadians and contributes significantly to emergency department visits and admissions(4). Pulmonary fibrosis, including idiopathic pulmonary fibrosis (IPF), is a progressive interstitial lung disease with poor prognosis and rising prevalence in the aging population(5). Pathophysiological fluid accumulation or fibrotic remodeling of the lung parenchyma disrupts gas exchange and leads to progressive respiratory compromise. Although edema and fibrosis have distinct pathophysiology and require different therapeutic approaches(6), they often present with overlapping clinical symptoms and imaging features(7,8). Early and accurate bedside differentiation is essential, as treatment strategies - such as diuresis and cardiac support in CPE versus antifibrotic and immunomodulation therapy in ILDs - are condition-specific(7,9). Compounding this challenge is the need to monitor the progression or resolution of edema and fibrosis over time, a task that is critically important for evaluating response to therapy, adjusting clinical management, and predicting outcomes(10,11).

Yet, non-invasive bedside tools capable of both differentiating and quantifying these dynamic processes remain limited, often leading to diagnostic uncertainty, delayed care, or inappropriate treatment. Conventional imaging modalities such as chest radiography (CXR) and computed tomography (CT), while commonly utilized(12), are suboptimal for bedside use and longitudinal monitoring(13). CXR lacks the ability to map spatial heterogeneity of disease due to its 2D projection of a 3D volume and suffers from high inter-observer variability - even among trained clinicians(14,15). CT, while more accurate, requires significant infrastructure, exposes patients to

ionizing radiation (a single scan equivalent to approximately 70 CXRs), and is not feasible for frequent use or for unstable critically ill patients(16,17). Furthermore, CT imaging requires patient transport, which can be logistically and clinically risky in those with severe respiratory or hemodynamic instability. Importantly, both CXR and CT mainly offer static anatomical snapshots and lack the ability to capture real-time, functional, or physiological changes, such as shifts in tissue oxygenation or perfusion(18). Moreover, some conditions, like pulmonary edema, cannot be reliably tracked with CT at all and are currently monitored through invasive measures or non-specific biomarkers(19). These limitations highlight a pressing need for radiation-free, portable, and dynamic imaging techniques at the bedside. Lung ultrasound (LUS) is an attractive alternative due to its portability, safety, and real-time capabilities. However, conventional LUS is based on artifact analysis (e.g., B-lines) and remains largely qualitative(20) and operator-dependent(21). While it can detect fluid or tissue changes in the lung, it lacks the specificity needed to distinguish between pulmonary edema and fibrosis, and it does not provide quantitative or functional information.

1.2 Innovative Solution: PULSE-LI

To address these gaps, this project proposes the development and preliminary validation of PULSE-LI (**P**hotoacoustic and **U**ltrasound **L**ung **S**canning for **E**valuation of **L**ung **I**njury). PULSE-LI is a novel, non-invasive, radiation-free investigational imaging approach designed to evaluate whether QUS and PAI/MSOT biomarkers can differentiate and quantify imaging features associated with pulmonary edema and fibrosis at the bedside. It integrates two complementary and innovative technologies: **quantitative ultrasound (QUS)** and **photoacoustic imaging (PAI)**, designed to simultaneously assess the structural and functional state of the lung.

Quantitative Ultrasound (QUS): While ultrasonography is widely available and already established in acute care settings, traditional lung ultrasound (LUS) relies heavily on artifact-based, semi-quantitative assessments that are subjective(22) and operator-dependent(21). In diseases such as CPE and ILDs, fluid- and tissue-filled regions enable ultrasound signal

transmission, making LUS a potentially powerful tool for lung imaging(23–25). However, its full potential remains untapped due to reliance on subjective image interpretation and mostly qualitative scoring systems(21). QUS advances this by analyzing raw radiofrequency (RF) backscatter data, which contain rich, frequency-dependent information about tissue properties (26–28). Traditional B-mode LUS fails to exploit this content, making its acquisition very dependent on the machine utilized and the settings employed(29). Utilizing raw RF data instead allows for objective, reproducible, and quantitative assessments of lung pathology that go beyond qualitative interpretation.

Photoacoustic Imaging (PAI): PAI is an emerging hybrid modality that fuses optical and acoustic imaging(30). It works by illuminating tissues with low-energy laser light, which is absorbed by molecules such as hemoglobin, producing ultrasonic waves via thermoelastic expansion(31). These waves are then captured by conventional ultrasound detectors, which are coupled with laser fiber bundles, permitting hand-held scanning(32). Since tissue chromophores such as collagen, hemoglobin, and water absorb light at different optical wavelengths, PAI thus provides molecular-specific, wavelength-tunable contrast, enabling precise imaging of tissue physiology, such as oxygenation(33) and vascular structure(34) with high sensitivity and specificity at multiple biological scales, from single cells to entire organs(35,36). In the lung, this means that PAI can offer both spatial and functional information, including real-time assessment of red blood cell oxygenation and metabolic dynamics(37).

Together, QUS and PAI provide a powerful and complementary novel imaging framework. Their integration in PULSE-LI may support future development of bedside tools for differentiating pulmonary edema from fibrosis, tracking disease evolution, and quantitatively assessing treatment response. In the present study, these outputs will be used for research purposes only and will not guide clinical care.

1.3 Rationale and Scientific Basis

This project builds on prior work by our group and others demonstrating the utility of QUS and PAI in other organs and disease models. For example, QUS has been used to assess kidney fibrosis in transplant recipients (38), while PAI has shown promise in detecting perfusion and oxygenation abnormalities in vascular and inflammatory conditions(30,37,39). Our preliminary data suggest that fibrotic and edematous lungs exhibit distinct ultrasound and optical signatures that can be captured through advanced signal processing.

In pulmonary fibrosis, deposition of dense collagen alters tissue stiffness and reduces ultrasound backscatter at higher frequencies, producing a unique QUS signature. In pulmonary edema, excess fluid enhances scattering at specific frequencies while enabling stronger propagation of photoacoustic signals. PAI can further distinguish these tissues by analyzing optical absorption spectra of hemoglobin, water, and collagen, providing real-time metabolic and structural information.

This project will refine and validate these imaging biomarkers through the systematic study of three cohorts: healthy controls, patients with ADHF (representing pulmonary edema), and patients with a radiologically confirmed diagnosis of usual interstitial pneumonia (UIP) - representing fibrosis.

1.4 Current Knowledge Gaps

Despite the promise of ultrasound and optical imaging, there is no current platform capable of bedside, quantitative assessment of lung injury that can distinguish edema from fibrosis.

Key gaps include:

- Lack of validated QUS and PAI biomarkers for lung injury.
- Limited standardization of lung imaging protocols for functional imaging.
- Absence of bedside tools to guide therapy or monitor disease progression in real time.

Our project directly addresses these gaps through a translational, patient-oriented research approach.

2. HYPOTHESIS AND AIMS

2.1 Hypothesis

We hypothesize that the integration of Quantitative Ultrasound (QUS) and Photoacoustic Imaging (PAI) will enable non-invasive, real-time, and bedside differentiation between pulmonary edema and pulmonary fibrosis by capturing both structural and functional characteristics of injured lung tissue. QUS provides objective structural biomarkers by analyzing raw ultrasound radiofrequency (RF) data, while PAI detects functional molecular signatures (e.g., oxygenation, perfusion, collagen) using light-absorbing tissue characteristics. Together, they represent a powerful diagnostic strategy for characterizing lung pathology without radiation or invasive procedures.

2.2 Rationale

Current LUS techniques cannot reliably differentiate pulmonary edema from fibrosis(40), do not provide functional and metabolic information on oxygenation and perfusion, thus making them poorly suited for tracking changes in lung tissue over time. By leveraging vendor- and operator-independent QUS algorithms that analyze raw, unprocessed RF backscatter signals, we aim to develop structural biomarkers capable of detecting spatially heterogeneous and microscopic morphological changes in the lung parenchyma(41–44). The addition of PAI will enhance diagnostic and monitoring capabilities by providing functional and metabolic insights, including oxygenation and perfusion of the injured lung tissue, while estimating the fibrotic/edematous content arising from the fibrotic remodeling and fluid accumulation. Laser pulses used in PAI can traverse both air-filled and fluid-filled alveoli and selectively interact with specific chromophores in the injured regions(45–47). The PA effect, which generates ultrasound signals in response to localized light absorption (e.g., by hemoglobin), will only propagate through fluid-filled or fibrotic areas, which act as acoustic conduits. This creates endogenous contrast that inherently targets pathologic lung regions, dramatically increasing specificity. Thus, QUS and PAI together will enable sensitive, spatially resolved, and physiologically meaningful interrogation of injured lungs, offering a powerful bedside imaging strategy. We will develop QUS/PAI imaging biomarkers across three cohorts: healthy individuals; acute decompensated heart failure (ADHF) patients,

representing pulmonary edema; and pulmonary fibrosis patients, defined by a definite UIP pattern on HRCT scans. The technical development of the QUS/PAI biomarkers is described below with its application to the cohorts in 1c.

2.3 Aim #1: Develop structural QUS biomarkers capable of differentiating pulmonary edema from fibrosis

We will develop **three structural metrics** to track tissue changes that indicate lung damage due to edema and fibrosis, building on Dr. Hysi's experience with QUS(45–47) in the context of kidney transplants, most recently published in *Nature Medicine*(38). **1) Concentration biomarkers:** advanced scattering models will provide parametric maps of edematous/fibrotic distribution within the lung(48–50). **2) Size biomarkers:** the presence of collagenous regions is expected to shift the dominant energy of the power spectra towards lower frequencies, while edematous regions will induce a high frequency shift(51,52). **3) Spacing biomarkers:** we will use time-domain analysis of RF signals to quantify the spatial arrangement of scatterers, generating maps that reflect fluid and collagen deposition patterns in lung tissue. These frequency-based structural biomarkers will render lung ultrasound scans quantitative, reproducible, and capable of differentiating edematous and fibrotic lung tissue(29,53).

2.4 Aim #2: Develop functional PAI biomarkers capable of differentiating pulmonary edema from fibrosis

Building on pioneering preliminary PAI data from our group in animal(36,54–59) and human models(39,45,60) of disease, we will characterize functional alterations in lung physiology - specifically in oxygenation, perfusion, collagen and water content - using multi-wavelength PAI(56). Three **physiological biomarkers** will be developed for the injured lung from edema and fibrosis, relying on the unique optical absorption of chromophores of interest: **1) concentration/perfusion (HbT)**(61), **2) oxygen saturation (sO₂)**(62), and **3) water/collagen content**(63). This will be accomplished through novel implementations of spectral unmixing of the multiwavelength PA RF signal, an area of expertise of Dr. Hysi(57,64,65). This algorithm will

be used to also interrogate other relevant chromophores that are metabolically active in the injured lung such as **cytochrome c oxidase, lipids, glucose**(66). Currently, such information is only available through extensive and expensive histological staining protocols(67), which are not accessible in our patient cohort. This cohort of PAI biomarkers will enable spatially resolved mapping of physiological dynamics providing unprecedented insights into regional lung function at the bedside.

These aims will generate the first comprehensive anatomical and physiological biomarkers suite for differentiating and characterizing pulmonary edema and fibrosis using QUS and PAI. By combining structural and functional information in a non-invasive, real-time, and bedside-compatible platform, we will lead the way for further studies on diagnosis and longitudinal monitoring of lung injury, with direct clinical relevance for managing acute and chronic respiratory diseases.

3. Project Design, Methodology and Analysis

3.1 Overview

This is a prospective, observational study designed to develop and validate novel imaging biomarkers derived from quantitative ultrasound (QUS) and photoacoustic imaging (PAI) to differentiate between pulmonary edema and pulmonary fibrosis. The study will be conducted at St. Michael's Hospital, a tertiary academic medical centre in Ontario, and will consist of two sequential phases:

- **Phase 1:** Imaging protocol optimization and biomarker development in healthy volunteers (n = 20).
- **Phase 2:** Biomarker validation in patients with acute decompensated heart failure (ADHF, n = 15) and idiopathic pulmonary fibrosis (UIP pattern on CT, n = 15).

3.2 Study Objectives

- **Primary Objective:** Develop and preliminary validate QUS- and PAI/MSOT-derived investigational imaging biomarkers capable of differentiating pulmonary edema from pulmonary fibrosis at the bedside.
- **Secondary Objectives:**
 - Assess biomarker reproducibility
 - Evaluate the impact of participant characteristics (e.g., skin tone, sex) on imaging performance.
 - Explore associations between investigational imaging biomarkers and physiologic, laboratory, and standard imaging parameters.

3.3 QUS/PAI equipment

Ultrasound System

A commercially available ultrasound system with raw RF signal acquisition capability will be utilized for QUS. This is a pocket ultrasound probe that operates wirelessly through a smartphone application, enabling bedside imaging(68). Manufactured by Clarius Mobile Health (Richmond,

British Columbia, Canada), the L15 probe (a 192-element linear array transducer with 7.5 MHz center frequency) provides access to real-time B-mode images and the ability to extract the raw beamformed RF signals for each channel. This permits the capability of reconstructing the B-mode images offline, and developing the structural QUS algorithms described in Aim #1.

Photoacoustic System

The photoacoustic imaging component of PULSE-LI will use one investigational **TUPIL MSOT Acuity Echo (Investigational Device)** system(69). The original commercial name of the device is **MSOT Acuity Echo – Type 2 – 660–1300 nm**. The device was originally designed and manufactured by **iThera Medical GmbH**, Germany, which is no longer operational.

The TUPIL MSOT Acuity Echo is a multispectral optoacoustic tomography system that combines pulsed near-infrared laser illumination with ultrasound detection to acquire optoacoustic image data. Co-registered ultrasound imaging is used for anatomic reference. The system consists of a mobile acquisition unit, laser source, data-acquisition hardware, system computer/software, user interface, foot pedal, and handheld ultrasound/photoacoustic probe. The probe is applied externally to intact skin using ultrasound gel, similar to a conventional ultrasound examination, and will be positioned over protocol-defined thoracic/lung imaging regions to acquire investigational optoacoustic and ultrasound data.

The device unit used in this study is a single identified system, **model/catalogue number SYS-MSOTACUITYechoCE2, serial number 2-21-06**. The device is considered a **Class II investigational medical device** for the purposes of the Health Canada Investigational Testing Authorization. For the limited purposes of this ITA, **Dr. Eno Hysi** will act as both **manufacturer of record** and **importer of record** for this specific single device unit. This designation is limited to this device unit, the PULSE-LI study, and the Health Canada-authorized investigational testing protocol. It does not constitute commercial manufacturing, commercial sale, commercial distribution, or assumption of responsibility for the original iThera product line or for any other MSOT systems. The device will be used only by trained study personnel and only under the Health Canada-authorized PULSE-LI protocol. Operators will follow the study imaging protocol, local device-use procedures, laser-safety precautions, infection-prevention procedures, and stop-use criteria.

Local device controls will be maintained for the duration of the study, including investigational labelling, operator training records, device-use logs, service and functional-check documentation, complaint/deficiency handling, incident reporting, and stop-use procedures. MSOT outputs generated during PULSE-LI are investigational and will be used for research purposes only. They will not be used for routine clinical diagnosis, triage, prognosis, monitoring, treatment decisions, or patient-management decisions. Standard clinical care will continue to be determined by the treating clinical team using standard-of-care clinical information. No hardware or software modifications will be made for the PULSE-LI study unless documented assessment is completed and Health Canada notification or ITA amendment is submitted where required.

3.4 PHASE 1: Healthy volunteers – Protocol optimization and algorithm development

We will enroll 20 healthy adult volunteers (10 male/10 female) to optimize image acquisition protocols and develop baseline QUS and PAI algorithms. Although we will not be able to estimate the fibrotic or edematous content in healthy lungs, this phase will be crucial for establishing standard operating procedures and technical parameters necessary for reliable, reproducible, and high-quality data acquisition.

3.4.1 Inclusion Criteria

- Healthy subjects
- Age $\geq$ 18 years

3.4.2 Exclusion criteria

- Unable or unwilling to provide informed consent.
- Active smoker, defined as current cigarette smoking or smoking within the past 30 days
- History of lung or cardiac diseases
- Tattoos or exogenous pigments within the field of view
- Pregnancy
- Photosensitizing medication within 72 hours (e.g., sulfonamides, ampicillin, tetracycline)
- Current phototherapy

- History of photosensitive disease, such as porphyria or lupus erythematosus, treatment for a photosensitive disease, or active clinically relevant photosensitivity
- Open skin lesions/sensitivity in scan field
- Inability/unwillingness to wear laser safety eyewear
- Recent use of gas ultrasound contrast agent
- Inability to tolerate positioning/probe pressure.

3.4.3 Study procedure

In the first phase of PULSE-LI study, we will enroll healthy volunteers to permit the development of the most feasible imaging sequences. Following the International Guidelines for Lung Ultrasound (70), we will perform a complete scan of the thorax, dividing the chest bilaterally in 6 regions of interest: anterior, lateral and posterior from the xiphoid-sternal line to the midclavicular, mid-axillary and posterior-axillary lines; each field will be divided in superior and inferior. We will acquire longitudinal and transversal scans for each of the six regions of interest. Longitudinal scans (i.e., aligned with cranio-caudal axis) allow clear identification of the pleural line; however, this approach provides visualization of shorter segments of pleura and therefore fewer lung artefacts. Transversal scans (i.e., aligned with intercostal space), while more technically challenging to obtain, allows the visualization of wider segments of the pleura(20).

Immediately after completing the recording of QUS images, precise location of the transducer will be marked using a dermatographic pen to allow precise correlation between QUS and PAI acquisitions. Given that PAI technology also utilizes an ultrasonic transducer, imaging will be acquired in the same manner as the LUS. There are no current guidelines for the PAI of the lungs, and this proposal presents a unique opportunity to develop them. Since the effect of tidal breathing on QUS and PAI signal stability is unknown, we will perform pilot testing in the healthy volunteers to define optimal imaging conditions, including: 1) breath-hold vs. tidal breathing protocols; 2) timing within the respiratory cycle; 3) probe positioning and insonation angles. To ensure data consistency, two sonographers will perform all assessments and undergo extensive training; we will also perform inter- and intra-operator reliability testing during Phase 1.

These imaging sessions performed on the healthy volunteer cohort will allow us to develop the most feasible imaging sequences, which will then be used with the patients enrolled in Phase 2.

Enrolled healthy volunteers will undergo one study procedure acquisition session (lung QUS and PAI). We are expecting each session to last no more than 80 minutes, with scanning time no longer than 30 minutes for the QUS and 30 minutes for the PAI (with approximately 2 minutes/region, with duration of optoacoustic scan of 10 sec plus time needed to determine adequate positioning).

PULSE-LI imaging will be performed for research purposes only. Investigational MSOT/QUS outputs will not be interpreted in real time for clinical care, entered into the medical record as diagnostic results, or used to guide treatment decisions. Standard clinical care, including any clinically indicated imaging, laboratory testing, monitoring, or treatment, will remain at the discretion of the treating clinical team.

3.4.4 Demographic data

For each participant, we will collect the following demographic information:

- Age
- Sex
- Weight
- Height
- Monk Skin Tone Scale(71)¹

¹ Skin pigmentation poses a challenge for PAI due to melanin's high absorption of light, particularly between 680–930 nm - the same range used to detect hemoglobin, water, and collagen. In darker-skinned patients, melanin reduces light penetration, weakens PA signals, and distorts spectral accuracy, particularly near the pleura. This concern gained attention during COVID-19, when pulse oximeters—also light-based—overestimated oxygenation in darker skin. To address this, we will recruit a racially diverse patient cohort, stratify imaging data according to the Monk Skin Tone Scale (MST), and apply Dr. Hysi's fluence correction and novel beamforming algorithms to mitigate bias. Skin tone will also be included in statistical analysis to prioritize equity in both design and validation, helping to close longstanding gaps in diagnostic accuracy among racially diverse populations.

3.5 PHASE 2: Patient enrollment – Identification of imaging biomarkers for fibrosis and edema

In Phase 2, we will enroll two patient cohorts in collaboration with colleagues in radiology, cardiology, and respirology.

3.5.1 Inclusion Criteria

- Edema: Patients hospitalized with acute decompensated heart failure (ADHF) with moderate-severe pulmonary edema. Moderate-to-severe pulmonary edema will be determined by the treating cardiology team and/or study physician based on clinical assessment and available standard-of-care investigations, such as chest radiography, lung ultrasound if clinically performed, oxygen requirement, natriuretic peptide testing, or other clinically indicated data.
- Fibrosis: Patients with diagnosis of IPF with a definite usual interstitial pneumonia (UIP) pattern on high-resolution computed tomography (HRCT), undergoing outpatient follow-up in pulmonology clinics. Definite UIP pattern will be confirmed by review of HRCT by a thoracic radiologist member of the study team, in conjunction with available clinical documentation and treating respirologist confirmation of clinical appropriateness.

3.5.2 Exclusion Criteria

- Unable or unwilling to provide informed consent
- Inability to cooperate with the ultrasound examination (e.g. severe agitation, altered mental status)
- Invasive mechanical ventilation or non-invasive mechanical ventilation at the time of the study procedure
- Chronic obstructive pulmonary disease (COPD)
- Lung emphysema
- Active malignancy with lung involvement
- End-stage renal disease requiring renal replacement therapy
- Recent or active lung infection
- Acute respiratory distress syndrome (ARDS)
- Recent thoracic surgery or trauma

- Active tuberculosis
- Known history of granulomatous lung disease.
- Active smoking, defined as current cigarette smoking or smoking within the past 30 days
- Tattoos or exogenous pigments within the field of view
- Pregnancy
- Photosensitizing medication within 72 hours (e.g., sulfonamides, ampicillin, tetracycline)
- Current phototherapy
- History of photosensitive disease, such as porphyria or lupus erythematosus, treatment for a photosensitive disease, or active clinically relevant photosensitivity
- Open skin lesions/sensitivity in scan field
- Inability/unwillingness to wear laser safety eyewear
- Recent use of gas ultrasound contrast agent
- Inability to tolerate positioning/probe pressure.

This focused inclusion strategy is designed to maximize the specificity of QUS and PAI biomarker development, targeting three clearly defined tissue states: normal, edematous, and fibrotic lungs. Conditions that may produce overlapping or confounding pulmonary features will be excluded. Intubated patients will be excluded to avoid variability introduced by positive pressure ventilation.

3.5.3 Study procedure

Enrolled patients will undergo one study procedure acquisition session (lung QUS and PAI) following the protocol of acquisition identified in the first phase of the study. We are expecting each session to last no more than 30 minutes, with scanning time no longer than 5 minutes for the QUS and 5 minutes for the PAI. The study team may use a dermatographic skin-marking pen to mark probe position on the skin in order to improve alignment between QUS and PAI acquisitions and, where applicable, with CT imaging.

In participants who provide separate consent for the optional NT-proBNP blood test, we will aim to obtain one blood sample on the same day as the QUS/PAI study procedure. For patients

hospitalized with acute decompensated heart failure (ADHF), the study team will aim to add NT-proBNP testing to clinically indicated bloodwork already being collected as part of routine care on the cardiology ward in order to avoid additional phlebotomy. For patients with definite UIP followed in the Respiratory Clinic, routine clinical bloodwork is generally not planned. In this cohort, one study-specific blood sample will be collected by a qualified member of the research team at the beginning of the QUS/PAI session. We are not expecting that this will meaningfully prolong the study visit. Healthy volunteers will not undergo NT-proBNP blood sampling as part of this study.

3.5.4 Demographic and Clinical Data

- Inpatient/Outpatient
- Days since admission (if inpatient)
- Age
- Sex
- Weight
- Height
- Monk Skin Tone Scale(71)
- Deyo Charlson Comorbidity Index
- Modified Medical Research Council (mMRC) Dyspnea scale
- Most Recent Pulmonary Function Tests (e.g., forced vital capacity FVC; volume exhaled in the first second – FEV1; lung volumes; diffusing capacity); date of test
- Most Recent Six-minute walk test; date of test
- Most recent imaging: 1) chest HRCT (Warrick score(72) and/or data-driven textural analysis DTA fibrosis score(73)); 2) chest radiography (fibrosis; interstitial edema; alveolar edema); date of test(s)
- Most Recent Laboratory tests (including NT-proBNP; troponin; creatinine; liver function tests; hemoglobin, white cell count; red blood cell count); date of tests
- Study-day NT-proBNP result, if an optional blood sample is obtained as part of the study
- Most Recent Echocardiogram with ejection fraction EF; date of test
- New York Heart Association NYHA classification

- Oxygen delivery device; FiO₂
- Oxygen saturation (SpO₂), respiratory rate, blood pressure and heart rate
- Most recent arterial blood gas analysis; date of test

We anticipate that a subset of patients will undergo chest CT imaging. For these patients, QUS and PAI will be performed immediately prior to the CT scan. To ensure precise anatomical correlation between insonated regions and corresponding CT slices, metal skin markers will be placed during ultrasound acquisition.

3.5.5. Biological Measurements

In Phase 2 patient participants only, we will aim to collect approximately 30 NT-proBNP samples in total, including 15 patients with ADHF/pulmonary edema and 15 patients with definite UIP/pulmonary fibrosis. No NT-proBNP blood sampling will be performed in healthy volunteers. NT-proBNP testing will be optional and will require separate consent from participants. Participants may agree to the imaging components of the study and decline the optional blood test.

Only one blood sample will be obtained per participant for NT-proBNP testing. The sample will be collected in one Gold Top Serum Separator Tube (SST; 3.5 mL). The minimum testing volume required is 1 mL, and the tube must be filled to at least one-half of its vacuum capacity in accordance with laboratory requirements.

For ADHF inpatients, whenever feasible, the NT-proBNP sample will be collected together with routine clinically indicated bloodwork so that no additional venipuncture is required.

For UIP outpatients, the blood sample will be drawn specifically for the study by a qualified member of the research team at the beginning of the QUS/PAI session.

Samples will be processed by the St. Michael's Hospital Biochemistry Laboratory using identifiable information required for clinical laboratory processing, including the participant's name and medical record number. NT-proBNP results will be reported in EPIC and may be viewed by the treating clinical team.

No storage of biological material is planned after analysis. Any remaining sample will be discarded once testing is complete, in accordance with standard laboratory procedures. The laboratory will store the specimens in the fridge and after 7 days, they will be discarded into a medical grade biohazardous bin to be picked up by Environmental Services.

3.6 Outcomes

This project is designed to develop and evaluate preliminary QUS- and PAI/MSOT-derived investigational imaging biomarkers for differentiating pulmonary edema from fibrosis at the bedside. Using QUS, we expect to generate structural biomarkers—scatterer concentration, size, and spacing—mapped across defined lung regions. PAI will produce functional biomarkers, including total hemoglobin (HbT), oxygen saturation (sO₂), and collagen/water content. Together, these will provide spatially resolved structural and physiological profiles of injured lung tissue.

A) STRUCTURAL QUS BIOMARKERS

We will develop **three structural metrics** to track tissue changes that indicate lung damage due to edema and fibrosis, building on Dr. Hysi's experience with QUS(45–47), most recently published in *Nature Medicine* in the context of kidney transplants(38).

(1) Concentration biomarkers: We will first characterize the frequency content of the US RF signals and remove system dependencies by using reference tissue-mimicking phantoms(48). Advanced scattering models such as the Fluid Filled Sphere or Gaussian(49) will provide parametric maps of edematous/fibrotic distribution within the lung once proper attenuation coefficient estimates have been implemented(50).

(2) Size biomarkers: Using matched filtering of the frequency spectrum of US signals via Gaussian-weighted Hermite functions(51), we will map the low and high frequencies dominating the RF signal. The presence of collagenous (fibrotic) regions is expected to shift the dominant energy of the power spectra towards lower frequencies, while edematous regions will induce a high frequency shift. We will then create a visual representation of the scatterer size within the heterogeneously injured lung, thus identifying the presence of edematous and fibrotic regions.

(3) Spacing biomarkers: We will analyze the spatial arrangement of scatterers in the injured and healthy lung regions by using statistical modeling of the US RF signal in the time domain(53). This will yield maps of metrics of fit parameters related to the spacing of the fluid and collagen depositions(29).

These frequency-based structural biomarkers will render LUS scans quantitative, reproducible, and capable of differentiating edematous and fibrotic lung tissue.

B) FUNCTIONAL PAI BIOMARKERS

Building on exciting preliminary PAI data from our group in animal(36,54–59) and human(39,45,60,65) models of disease, we will characterize functional alterations in lung physiology—specifically in oxygenation, perfusion, collagen and water content—using multiwavelength PAI. In the injured lung, the effective chromophore absorber will likely be the injured alveolar sacs and their network of blood vessels and surrounding collagen deposition. On average, 600 million alveoli in lungs are surrounded by a dense network of capillaries, circulating approximately 2.5 trillion RBC at a given time(74). Three **physiological biomarkers** will be developed for the injured lung from edema and fibrosis, relying on the unique optical absorption of chromophores of interest(30):

(1) concentration/perfusion (HbT)(61),

(2) oxygen saturation (sO₂)(62),

(3) water/collagen content(63).

This will be accomplished through novel implementations of spectral unmixing(64) of the multiwavelength PA RF signal(57,65), an area of expertise of Dr. Hysi. This algorithm will be used to also interrogate other relevant chromophores that are metabolically active in the injured lung such as **cytochrome c oxidase, lipids, glucose**(66). Currently, such information is only available through extensive and expensive histological staining protocols(67), which are not accessible in our patient cohort. This cohort of PAI biomarkers will enable spatially resolved mapping of physiological dynamics providing unprecedented insights into regional lung function at the bedside

3.7 Statistical Analysis

We will perform ANOVA or Kruskal-Wallis tests to compare biomarker distributions across healthy, ADHF and UIP groups. Receiver Operating Characteristic (ROC) curves will estimate preliminary discriminative performance for distinguishing ADHF/pulmonary edema from UIP/pulmonary fibrosis. Spearman correlations will quantify associations between imaging biomarkers and clinical metrics (e.g., PaO_2/FiO_2 ratio, *NT-proBNP*, *pulmonary function tests*). Intra- and inter-operator reproducibility will be evaluated using intraclass correlation coefficients. Exploratory multivariable and dimension-reduction analyses, such as *Principal Component Analysis* (PCA) and *Linear Discriminant Analysis* (LDA), may be used to explore biomarker combinations. Given the pilot sample size, these analyses will be interpreted as hypothesis-generating.

3.7.1 Sample Size

The primary performance metric will be the area under the receiver-operating curve (AUROC) of the combined QUS/PAI classifier to discriminate pulmonary edema from fibrosis. Based on the Hanley–McNeil method, assuming a two-sided α of 0.05, 80% power, and an expected AUROC of 0.80, we estimated that 30 participants (15 with pulmonary edema and 15 with fibrosis) would be sufficient. This sample size provides approximately 80% power to detect discrimination better than chance if the true AUROC is ≥ 0.75 , while also allowing an observed AUROC of 0.80 to be estimated with a 95% confidence interval of roughly 0.64–0.96.

4. Safety Procedures

4.1 Safety Reporting

4.1.1 Classification of Adverse Events

Safety events will be classified in accordance with ISO 14155 and the Health Canada ITA framework.

Adverse Events	Non-device-related	Device- or procedure-related	
Non-serious	Adverse event (AE)	Adverse device effect (ADE)	
Serious	Serious Adverse Event (SAE)	Serious Adverse Device Effect (SADE)	
		Anticipated	Unanticipated
		Anticipated Serious Adverse Device Effect (ASADE)	Unanticipated Serious Adverse Device Effect (USADE)

4.1.2 Definitions

- Adverse Event (AE): Any untoward medical occurrence, unintended disease or injury, or untoward clinical sign (including abnormal laboratory findings) in a participant, user or other person, whether or not related to the investigational medical device. This includes events related to the investigational device and events related to study procedures. Stable pre-existing conditions that do not worsen are not AEs.
- Adverse Device Effect (ADE): An adverse event related to the use of the investigational medical device. This includes events resulting from insufficient or inadequate instructions for use, deployment, installation or operation, and device malfunction.
- Device Deficiency: An inadequacy of the device with respect to identity, quality, durability, reliability, safety or performance, including malfunction, use error, and inadequate labeling. Near misses, use errors, and device deficiencies will be documented and assessed for corrective action and reportability, even if no participant injury occurs.
- Serious Adverse Event (SAE): AEs are classified as serious or non-serious. A Serious Adverse Event is any AE that led to 1) death; 2) serious deterioration in the health of the participant, including a life-threatening illness or injury, permanent impairment of a body structure or

function, in-patient or prolonged hospitalization, or medical or surgical intervention to prevent life-threatening illness/injury or permanent impairment; or 3) fetal distress, fetal death, or a congenital anomaly/birth defect. Planned hospitalization for a pre-existing condition or for a protocol-required procedure without serious deterioration in health is not an SAE. The term “life-threatening” in the definition of “serious” refers to an AE in which the participant was at risk of death at the time of the event. It does not refer to an AE that hypothetically might have caused death if it were more severe.

- **Serious Adverse Device Effect (SADE):** Any serious adverse event that is judged related to the investigational device.
 - **Anticipated Serious Adverse Device Effect (ASADE):** A SADE that is consistent with the known nature, incidence, severity, or outcome of the device as described in the current risk information.
 - **Unanticipated Serious Adverse Device Effect (USADE):** A SADE that by nature, incidence, severity, or outcome has not been previously identified for the study device.
- **Health Canada reportable incident (section 59):** A serious adverse incident that is related to a failure of the device, a deterioration in its effectiveness, or an inadequacy in its labelling or directions for use, and that has led to the death or serious deterioration in the state of health of a patient, user, or other person, or could do so if it recurred.

4.1.3 Severity/Intensity of Event

The intensity of each AE will be assessed by the Qualified Investigator (QI) or delegate as mild, moderate, or severe:

- **Mild:** transient or minimal symptoms; no or minimal treatment required; no meaningful interference with usual activities.
- **Moderate:** symptoms cause some interference with usual activities and may require treatment or additional observation.
- **Severe:** symptoms markedly interrupt usual activities, require significant therapeutic intervention, or are medically significant.

Severity describes intensity and does not by itself determine seriousness.

4.1.4 Relationship to Study

All Adverse Events (AEs) will have their relationship to the study assessed by a qualified physician who is part of the study team based on temporal relationship and their clinical judgment. For each reportable event, relationship will be assessed separately for: (1) the MSOT/photoacoustic device, (2) ultrasound/QUS acquisition, and (3) other study procedures such as positioning, coupling gel application, eye protection, or optional blood sampling. Relationship will be graded as unrelated, unlikely, possibly related, probably related, or definitely related.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention should be clinically plausible.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal.
- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the study intervention). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events).
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Unrelated** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology.

Where uncertainty exists, the event will be managed conservatively as at least possibly related until further information is available.

Events clearly attributable only to the participant's underlying disease or expected clinical course, with no reasonable possibility of a causal relationship to the device or study procedures, need not be classified as ADEs. However, worsening baseline conditions, events meeting SAE criteria, and any event for which device/procedure relationship cannot be excluded will be recorded and assessed.

4.1.5 Expectedness

Expectedness will be determined by the QI or delegate based on the current protocol, investigational plan, device risk analysis, investigator brochure/instructions for use, and other current risk information. An AE is unexpected if the nature, severity, specificity, or frequency of the event is not consistent with the current risk information for the device or study procedures.

4.1.6 Anticipated PULSE-LI safety profile

Category	Usually expected / should be collected if study-related	Non-serious changes that are clearly attributable to ADHF, UIP/ILD, or routine clinical care and have no reasonable possibility of relationship to the study device or study procedures
Imaging-contact / accessory effects	Transient coolness or discomfort from coupling gel Transient skin pressure marks or erythema from probe contact Brief discomfort from required eye protection or accessories Transient altered red-tinted vision or glare related to protective eyewear/filtering	Minor <u>baseline</u> skin findings, chronic visual symptoms, or symptoms clearly present before the study and unchanged by participation.

Category	Usually expected / should be collected if study-related	Non-serious changes that are clearly attributable to ADHF, UIP/ILD, or routine clinical care and have no reasonable possibility of relationship to the study device or study procedures
Procedure tolerance	Transient cough, dyspnea, fatigue, anxiety, pain, or positioning intolerance during the scan that resolves with rest, repositioning, or stopping the procedure Transient lightheadedness / vasovagal symptoms.	Routine dyspnea, cough, fatigue, edema, oxygen requirement fluctuation, or discomfort clearly attributable to ADHF, UIP/ILD, or standard inpatient/outpatient care.
NT-proBNP venipuncture (optional)	Brief venipuncture discomfort Minor bruising Minor bleeding Transient lightheadedness	Clinically expected lab abnormalities or disease-related findings unrelated to the blood draw procedure itself.
Serious but rare events	Clinically significant skin or eye injury Clinically significant respiratory / hemodynamic deterioration directly attributable to study procedures Fall or line/tube dislodgement during study positioning/transfer Serious device malfunction or deficiency that results in harm or could do so if repeated.	Hospitalization, arrhythmia, hypotension, hypoxemia, or other deterioration clearly explained by the underlying disease course and not temporally or clinically linked to study procedures.

4.1.7 Reporting Procedures

All AEs and device deficiencies will be documented in the AE/SAE form, and adverse event log. SAEs that are ongoing at the end of study will be followed until resolution, stabilization, loss to follow-up, or until the QI determines that no further study-specific follow-up is required. The Qualified Investigator may also refer the patient for medical care and/or to be followed up with their primary physician or care team. Any baseline medical condition recorded before study entry that worsens after study procedures begin will be documented as an AE or SAE, as applicable. Changes in severity, seriousness, causality, or expectedness will be updated as new information becomes available.

The study team will notify the QI of any SAE, SADE, USADE, or potentially reportable device incident immediately and no later than 24 hours after first awareness.

- The QI will report any serious adverse incident that meets the Health Canada section 59 threshold to Health Canada and to the manufacturer/importer (Eno Hysi, PhD) within 72 hours of learning of the incident.
- The manufacturer/importer (Eno Hysi) is responsible for mandatory preliminary and final reports to the Minister. For incidents in Canada, the preliminary report must be submitted within 10 days of awareness if the incident led to death or serious deterioration in health, or within 30 days if it did not but could do so if it recurred.
- All SAEs, SADEs, USADEs, and other unanticipated problems will also be reported to the Research Ethics Board in accordance with institutional and REB requirements.
- Device deficiencies, whether or not associated with an AE, will be reported promptly to the manufacturer/importer (Eno Hysi) and evaluated for possible corrective action and reportability.
- The manufacturer/importer of record will maintain device complaint, deficiency, and incident records in the local ITA technical file.

4.1.8 Information to Participants

Participants will be informed in a timely manner of any new safety information that may affect their willingness to continue participation. If additional clinical evaluation or medical care is required, the participant will be referred to the appropriate care team.

4.1.9 Data and Safety Monitoring

No independent Data and Safety Monitoring Board (DSMB) will be established. PULSE-LI is a single-centre, non-invasive, observational, research-only imaging study with no investigational treatment assignment, no therapeutic intervention, and no use of investigational imaging outputs for clinical decision-making. The overall participant safety risk is therefore expected to be low, and an independent DSMB is not considered necessary.

Safety oversight will be provided by the Qualified Investigator, study Principal Investigators, manufacturer/importer of record, REB, and applicable Health Canada reporting pathways. All adverse events, serious adverse events, device deficiencies, protocol deviations, and stop-use decisions will be reviewed by the Qualified Investigator or delegate and assessed for seriousness, relatedness, expectedness where applicable, and reportability. Periodic study monitoring and

quality review will include review of consent documentation, eligibility documentation, device-use logs, adverse event/serious adverse event/device-deficiency forms, protocol deviation logs, data completeness, and compliance with study procedures. Findings will be documented, and corrective and preventive actions will be implemented as needed.

4.2 Potential Risks and Benefits

Adverse events will be monitored at any time by the qualified medical professional performing the scan; the US/PAI imaging will be immediately interrupted or aborted. The risk of irreversible injury is expected to be very low when the device is used according to the protocol and safety procedures; however, imaging will be stopped immediately if discomfort, skin change, device malfunction, or safety concern occurs. In this study population, adverse events may occur; however, we expect that most will be attributable to the participant's underlying disease and/or routine clinical care rather than to the study procedures.

LUNG ULTRASOUND

Lung ultrasound is routinely performed and is not associated with known complications. It does not involve radiation and the potential risks are limited to some discomfort from the probe and the moist sensation from the ultrasound gel.

PHOTOACOUSTIC IMAGING

The MSOT Acuity Echo device uses pulsed near-infrared laser light to induce optoacoustic signals for imaging. Based on the classification criteria for medical devices (Directive 93/42/EEC, Annex IX), the optoacoustic system of the MSOT Acuity Echo device corresponds to Class IIa (class 4 laser). According to Rule 10 of the Health Canada's MDR SOR/98-282, class II:

- Active diagnostic device
- Non-invasive
- Temporary use (<60 min)

The safety profile of the ultrasound and MSOT devices is well-characterised and extremely low. The Table below shows exemplary the reported events from a study performed at the University Medical Centre in Erlangen, Germany, evaluating children with Duchenne muscular dystrophy. The majority of reported (foreseeable) problems are related either to the use of

ultrasound gel for imaging or the need to wear eye protection. The use of filter glass also explains the reported red visibility. This phenomenon was reversible within seconds.

Table 2 Adverse events

	Muscular dystrophy Duchenne N=10	Healthy volunteer N=10
Reversible adverse events no.(%)	0 (0%)	0 (0%)
Pressure of safety goggles	2 (20%)	0 (0%)
Coolness of ultrasound gel	0 (0%)	4 (40%)
Red cast view	0 (0%)	1 (10%)
Serious adverse events- no.(%)	0 (0%)	0 (0%)

Although lasers are sometimes associated with thermal risks, the Acuity Echo system has been specifically engineered with multiple layers of safeguards to minimize the risk of skin or deep tissue heating or burns. These include both hardware limitations and software-enforced controls that maintain the laser output well below internationally recognized safety thresholds.

A. Laser output is strictly limited to remain below Maximum Permissible Exposure (MPE) The laser subsystem includes a proprietary power-control unit that enforces strict caps on the maximum energy delivered to the skin surface. The laser operates in the near-infrared spectrum, and pulse energies are carefully controlled to avoid reaching levels that could cause a rise in skin temperature. The device design and operation follow established standards such as IEC 60825-1 and ANSI Z136.1, which define the MPE limits for laser exposure. In clinical use, the energy applied per pulse is orders of magnitude below these limits, ensuring a wide margin of safety even in repeated or prolonged scans. Additionally, laser delivery is distributed over a relatively broad area via the probe's optical fibers, further reducing localized energy concentrations that could otherwise lead to heating. This spatial distribution is an intentional design feature to diffuse energy and avoid hotspots. Therefore, optoacoustic imaging does not result in any significant temperature increase in the tissue. The absorption of a laser pulse in the tissue results in a local transient temperature increase of a few millikelvin. Depending on the duration of the MSOT

imaging and the skin type of the participant, temperature increases occur typically in the range of less than one degree Kelvin/Celsius.

B. Built-in safety features prevent excess energy delivery. The system is equipped with a real-time energy monitoring system that continuously tracks laser performance during every scan. If any deviation from preset energy parameters is detected, the system is programmed to automatically halt laser emission and notify the operator. This interlock mechanism is a standard part of the safety protocol and is implemented both in hardware and firmware. These safeguards also include temperature sensors within the handheld probe and error-checking routines that are run before every scan to verify calibration, alignment, and energy control functionality. Together, these features ensure that even in the unlikely event of a hardware fault, the system will shut down automatically before any exposure exceeding safety thresholds can occur.

C. Extensive preclinical and clinical safety testing confirms no thermal injury. Across over 1,000 human scans performed using the CE-marked version of the Acuity Echo device, there were no reports of skin irritation, erythema, or thermal damage. All observed reactions were non-existent or unrelated to device operation. These real-world data demonstrate that the system is safe for clinical use, including on sensitive patient populations such as those with cardiopulmonary conditions. Furthermore, even when imaging was repeated in short succession or when applied to the same region multiple times, there were no measurable increases in skin surface temperature attributable to the laser. These findings confirm that, in practice, the energy levels used are not only theoretically safe but are empirically validated as such.

D. Compliance with international regulatory safety standards. The safety of the laser exposure used by the iThera Acuity Echo was a critical part of the device's CE Marking process. The laser subsystem underwent formal risk analysis and validation according to the European Medical Device Regulation (MDR) framework. The CE certification ensures that the device meets the stringent criteria laid out in international standards governing photonic exposure and electrical safety. This includes thorough testing of cumulative energy exposure and verification that all emitted radiation is within non-thermal, non-harmful limits. The successful attainment of CE certification underscores that the system's design incorporates effective risk controls and is suitable for human use under clinical conditions.

The near infrared light used in the MSOT can lead to retinal damage if the eye is irradiated. In order to prevent this, test participants and operators trained in using the MSOT device will wear appropriate laser safety glasses during the MSOT imaging.

NT-proBNP BLOOD SAMPLING (OPTIONAL) – Participants who agree to the optional blood test may experience brief discomfort from venipuncture, minor bruising, minor bleeding, light-headedness. For ADHF inpatients, we will aim to collect the sample together with routine bloodwork whenever feasible to minimize additional needle sticks. For UIP outpatients, one blood sample will be collected specifically for the study by qualified research staff. Participants may decline the optional NT-proBNP blood test and still participate in the imaging portion of the study.

Technician Training and Operator Expertise

In addition to the device's built-in technical safeguards, safe operation of the TUPIL MSOT Acuity Echo system depends on appropriate operator training, documented competency, and adherence to local device-use and safety procedures. Because iThera Medical GmbH is no longer operational, training for PULSE-LI operators will be conducted locally using available original iThera instructional materials, device manuals, safety documentation, prior user experience, and PULSE-LI-specific standard operating procedures. Training will be coordinated and documented under the oversight of Dr. Eno Hysi, who will act as manufacturer/importer of record for this single investigational device unit for the purposes of the Health Canada ITA, in collaboration with the qualified investigator and study team.

a. Device training will be standardized, documented, and competency-based

Before independently operating the device for study imaging, each operator must complete PULSE-LI-specific training and competency documentation. Training will include:

- **Laser safety procedures:** Operators will be trained in relevant laser-safety principles, device-specific laser precautions, use of protective eyewear, controlled-area

requirements, warning signage, safe activation/deactivation procedures, and response to suspected laser-safety hazards.

- **Device operation and pre-use checks:** Operators will be trained to perform required pre-use checks, confirm device identity and investigational labelling, verify that the device is in an acceptable state for use, recognize system warnings or abnormal device behaviour, and follow stop-use criteria. The device will not be used for participant imaging unless current device configuration, investigational labelling, software version, probe integrity, laser status, electrical safety status, and functional readiness have been documented. If service status or functional readiness is uncertain, the device will be removed from use until assessed and documented as suitable for investigational use.
- **Device handling and troubleshooting:** Operators will be trained in safe handling of the acquisition unit, handheld probe, foot pedal, cables, user interface, and accessories. Operators will not perform hardware, software, or service modifications. Any device malfunction, warning, error code, unexpected shutdown, cable/probe concern, or other deficiency will be documented and escalated according to the PULSE-LI device-deficiency and stop-use procedures.
- **Participant preparation and positioning:** Operators will be trained in participant preparation, probe positioning, use of ultrasound gel and covers where applicable, infection-prevention procedures, and techniques to maintain adequate probe contact while avoiding excessive pressure, wounds, lines, drains, dressings, or areas of skin vulnerability.
- **Protocol-specific imaging procedures:** Operators will be trained in the PULSE-LI imaging protocol, permitted imaging regions, contraindications, use limitations, research-only status of MSOT outputs, adverse event reporting, and procedures for stopping or deferring imaging if participant safety or clinical care may be affected.

b. Only trained and authorized personnel will operate the system

At Unity Health Toronto / St. Michael's Hospital, the TUPIL MSOT Acuity Echo will be operated only by study personnel with appropriate background in medical imaging, ultrasound, optical/photoacoustic imaging, biomedical imaging, or related research procedures, and who have completed the PULSE-LI device-specific training and competency documentation. During study start-up, operators will be supervised by senior imaging scientists and/or experienced study personnel. Imaging procedures will be performed under the oversight of the qualified investigator and in coordination with the clinical team when imaging participants receiving clinical care.

Training records and operator authorization logs will be maintained in the local study and device-control files. Operators will be re-trained if there is a relevant protocol amendment, device-use procedure change, safety issue, recurring user error, or prolonged interval without device use.

No hardware or software modifications, reconstruction changes, service changes, or device-configuration changes will be made for PULSE-LI unless a documented technical, risk, and regulatory assessment is completed by the manufacturer/importer of record (Eno Hysi) and study team. Where required, Health Canada and/or REB notification or amendment will be submitted before implementation.

In summary, the TUPIL MSOT Acuity Echo will be used only by trained and authorized study personnel under documented local procedures. Available original iThera materials will be used as legacy training resources, but operator authorization for PULSE-LI will be based on local competency documentation, laser/device safety procedures, protocol-specific training, and oversight by the manufacturer/importer of record (Eno Hysi, PhD) and qualified investigator (Alberto Goffi, MD). QUS and MSOT outputs will remain investigational and will not be used for routine clinical diagnosis or patient-management decisions. However, NT-proBNP testing will be processed through the clinical biochemistry laboratory and the result will be reported in EPIC, where it may be available to the treating clinical team. Any clinical interpretation or treatment decision based on NT-proBNP will remain entirely at the discretion of the treating team.

Participants are not expected to receive direct clinical benefit from the investigational QUS/PAI imaging. QUS and MSOT/PAI outputs are investigational and will not be used for clinical diagnosis, routine monitoring, prognosis, treatment decisions, or patient-management decisions. For **patient participants**, standard clinical care will continue to be directed by the treating clinical team using standard-of-care information. For **healthy volunteers**, study imaging is not intended to provide clinical screening or diagnosis. Any medically actionable incidental finding will be managed in accordance with the participant's documented preference in the consent form. Participants who choose to receive such findings will be informed by the study team and, where appropriate, advised to seek assessment from their treating clinician or primary care provider. If an immediate and serious threat to the participant's health is identified, the study team will act in accordance with applicable legal, ethical, and institutional obligations.

4.3 Medical Care Related to the Study

For patients with ADHF and UIP, the medical care of the participant in the study will be performed as per local standard of care, without any deviation from clinical protocols. The present study is observational with respect to the QUS and PAI imaging procedures.

Participants may also choose to provide separate consent for an optional NT-proBNP blood test. For ADHF inpatients, this test will be added to routine clinically indicated bloodwork whenever feasible. For UIP outpatients, one study-specific blood sample will be collected by qualified research staff. The NT-proBNP result will be processed by the SMH Biochemistry Laboratory and reported in EPIC. Any clinical action based on this result will remain at the discretion of the treating team.

5. Ethical Considerations

5.1 Research Ethics Board

Approval of the protocol by the Research and Ethics Boards (REB) of Unity Health Toronto will be obtained before any participant is enrolled. Any substantial amendment to the protocol will require approval by the REB and regulatory authorities before the changes are implemented to the study. No deviation from, or changes to the protocol will take place without prior agreement from Health Canada and documented approval from the Research Ethics Board (REB), except where necessary to eliminate (an) immediate hazard(s) to the trial participants.

5.2 Recruitment and consent process

5.2.1 Healthy Volunteers

To recruit healthy volunteers for this study, we will use a snowball sampling approach. Initially, members of the research team will invite individuals from their professional networks - such as staff and trainees affiliated with the Li Ka Shing Knowledge Institute and St. Michael's Hospital - who may meet the eligibility criteria. These initial participants will be asked, if they feel comfortable, to share information about the study with others in their networks who may also be eligible and interested in participating. To ensure ethical recruitment practices and minimize the risk of coercion or perceived power imbalance, principal investigators and senior collaborators will not be directly involved in the recruitment process. This is particularly important as some potential participants may be trainees or staff under their academic or clinical supervision. Initial outreach will occur via a recruitment email distributed by the study's research coordinators, using email addresses identified from the investigators' personal contacts. The email will include a summary of the study, eligibility criteria, expected time commitment, and contact information for the research coordinator. Recipients will be invited to forward the message to others who may be interested, enabling a snowball-style expansion of recruitment. All individuals who express interest—regardless of referral source—will undergo the same eligibility screening and informed consent process. This approach offers a practical, low-burden

strategy for identifying healthy participants, especially given the study's current lack of external funding.

Potential participants who express interest will be contacted by the research coordinator, who will provide a copy of the consent form and address any questions. Written informed consent will be obtained prior to any study procedures. Participation will be entirely voluntary, and individuals will be informed that they may withdraw from the study at any time without consequence.

Participation or non-participation will have no effect on employment, trainee evaluations, academic assessment, clinical privileges, or relationships with supervisors. Principal investigators and supervisors will not be informed of who declines participation. Recruitment, eligibility screening, and consent will be conducted by study personnel who do not have supervisory authority over the potential participant whenever feasible.

5.2.2 Patients Admitted with ADHF

- Patients with ADHF and moderate-to-severe pulmonary edema will be recruited from inpatient cardiology wards at St. Michael's Hospital. The inpatient cardiology team will serve as the primary contact for the identification of eligible patients. Each morning following handover rounds, a member of the research team (e.g., research coordinator or fellow) will contact the cardiology ward team to inquire whether any new patients with ADHF and moderate-to-severe pulmonary edema were admitted in the previous 24 hours and whether these patients may be appropriate to approach for study participation.
- If a potentially eligible participant is identified, the research team will liaise with the patient's circle of care to obtain permission to approach the patient. A member of the patient's circle of care will first ask whether the patient is willing to hear about a research study. Only if the patient agrees the research team will approach the patient to discuss participation. Patients will only be approached if they have explicitly agreed to be contacted about the study and are competent and able to provide independent informed consent.
- Members of the research team will conduct the consent discussion directly with the patient, explain the study procedures, and provide a copy of the informed consent form (ICF). Ample time will be allowed for questions and for consideration of participation. Written informed

consent will be obtained prior to any study procedures. Participation will be entirely voluntary, and individuals will be informed that they may withdraw at any time without affecting the quality of their clinical care.

- When consent is obtained, the ICF will be signed and dated by both the participant and the member of the study team conducting the consent process. The participant will receive a copy of the signed ICF.
- Participants approached for this cohort will also be informed about the optional NT-proBNP blood test. Separate consent will be obtained for this component. If the participant agrees, the study team will aim to add NT-proBNP testing to routine clinically indicated bloodwork collected on the same day, in order to avoid additional venipuncture.

5.2.3 Patients with Definite UIP

Patients with idiopathic pulmonary fibrosis (IPF) and a radiologically definite UIP pattern will be identified from the outpatient population followed at the St. Michael's Hospital Respiratory Clinic.

- To identify potentially eligible participants, a thoracic radiologist from the research team will conduct a structured search of the radiology department database to identify individuals who have undergone chest CT imaging within the past two years and whose radiology report includes a diagnosis of "definite" or "probable" UIP.
- This preliminary list will then be cross-referenced with the database of patients currently followed by the St. Michael's Respiratory Clinic.
- For those patients appearing in both datasets, the thoracic radiologist will perform a secondary review of the CT images to confirm the presence of a definite UIP pattern, in accordance with current diagnostic guidelines. Only those with a confirmed definite UIP pattern will be considered eligible for the study. This limited pre-screening will be conducted under REB-approved partial waiver/alteration of consent for screening only. The information accessed will be limited to the minimum necessary to determine potential eligibility. No patient will be contacted by the research team unless the consent-to-contact pathway described below has been followed. Screening lists will be stored on secure Unity Health

servers, access will be limited to authorized study personnel, and individuals who are ineligible or decline contact will be removed from the active screening list.

- Following this step, the study team will contact the treating respirologist for each potentially eligible patient to ensure clinical appropriateness and to confirm that there are no exclusion criteria or contraindications to study participation.
- If the treating respirologist is in agreement, the study team will determine whether the patient has provided prior consent to be contacted for research purposes by reviewing documentation in the hospital electronic medical record (EPIC).
 - If consent to contact is documented in EPIC, a member of the research team will reach out directly to the patient by phone to describe the study, answer questions, and offer to provide the informed consent form (ICF) in advance by email or mail.
 - If there is no prior consent to contact documented in EPIC, the patient will be contacted a few weeks before a clinic or CT appointment by a member of the respiratory clinic (e.g., treating respirologist or administrative personnel) whether they are willing to be contacted for participation in a research study. If they agree, the research team will then follow up by phone to describe the study, answer questions, and offer to provide the informed consent form (ICF) in advance by email.
- For patients who express interest, a member of the study team will meet with the patient in person on the day of their scheduled respiratory clinic visit or CT scan. During this visit, written informed consent will be obtained prior to any study procedures. Ample time will be allowed for questions and for consideration of participation. Participation will be entirely voluntary, and individuals will be informed that they may withdraw at any time without affecting the quality of their clinical care. When consent is obtained, the ICF will be signed and dated by both the participant and the member of the study team conducting the consent process. The participant will receive a copy of the signed ICF.
- Participants approached for this cohort will also be informed about the optional NT-proBNP blood test, and separate consent will be obtained for this component. If the participant agrees, one blood sample will be collected by a qualified member of the research team at the

beginning of the QUS/PAI visit and sent to the SMH Biochemistry Laboratory for NT-proBNP testing. The result will be reported in EPIC.

- The ultrasound/photoacoustic imaging session will then be performed at the same visit to minimize patient inconvenience.

As this is a study targeting a defined clinical population (UIP), we are requesting permission to access relevant radiology and respirology data without prior individual patient consent for the purposes of feasibility pre-screening. It would be impractical to obtain consent from all individuals followed in the respirology clinic, as many will not meet imaging criteria for definite UIP. We anticipate reviewing approximately 200 patient charts during this pre-screening process. The identifiers used during this phase will be limited to: Patient name; Medical record number; Date and findings of relevant CT imaging; Clinical status (e.g., active patient of the respirology clinic) These identifiers will only be used for the purpose of screening and cross-referencing eligibility and will be kept confidential in accordance with institutional privacy policies. The final list of potentially eligible patients will be stored on secure, access-controlled servers at Unity Health. Patients who have been already contacted and who have been either consented or declined consent will be immediately deleted from the list. This list will be retained only until either all 15 participants required for the study have been recruited or all identified patients have been contacted. At that point, the list will be securely deleted in accordance with institutional privacy and data retention policies.

5.2.4 Time given to consider participation

Healthy Volunteers:

Healthy volunteers will be given up to 7 days to consider participation. They will receive the Letter of Information and Informed Consent Form (ICF) via email and will be invited to contact the study coordinator with any questions before providing written consent.

ADHF Patients:

This group represents the most time-sensitive population, as pulmonary edema can resolve rapidly with treatment. For this reason, consent will need to occur within a limited timeframe. Patients will be given up to 3 hours to consider participation and ask questions. The study team

will ensure that the consent process is respectful, unhurried, and conducted in a quiet environment conducive to decision-making.

UIP Patients:

Patients with UIP will be contacted in advance by telephone. During this call, a member of the study team will describe the study, answer questions, and offer to send the Letter of Information and ICF via email or mail. This initial contact will occur at least one week prior to the participant's scheduled clinic appointment or CT scan.

On the day of their appointment, a member of the study team will meet with the patient in person at a pre-arranged time to review the study again, answer any additional questions, and obtain consent if the individual wishes to proceed. While ample time will be allowed for this discussion, we recognize that participants may have limited availability due to clinic schedules or transportation arrangements. As such, the time allotted for consideration on the day of the visit will be guided by the patient's preferences.

5.2.5 Ongoing participant willingness and cooperation

At each time point, members of the study team will approach patients to explain the study and its procedures in clear, accessible language. Only patients who are competent and able to provide independent informed consent will be enrolled in this study. This approach ensures that participants are engaged in the process to the extent possible and given the opportunity to express questions, preferences, or concerns. As the study involves the performance of ultrasound and photoacoustic imaging at the bedside, patient cooperation (e.g., remaining still, tolerating the procedure) is essential. If a participant shows signs of distress, refusal, or is otherwise unable to meaningfully engage with the procedure, the imaging session will not be conducted. This approach respects participant autonomy and ensures that patients are actively involved in the decision-making process to the extent of their ability.

5.2.6 Study Withdrawal / Termination Criteria

Participation in the study will be terminated if there is noticeable warming or reddening of the skin during MSOT imaging or significant discomfort at any point. The MSOT imaging time per

anatomical region will be limited to 2 minutes (with less 10 seconds of optoacoustic scan), making warming or reddening highly unlikely.

Patients may request to withdraw from the study at any time. Upon withdrawal, no further data will be collected. If the declaration of consent is revoked, data collected up to this point can be considered. The participant has the right to demand that the data be destroyed, provided that legal provisions do not prevent such destruction. Participant can request that their individual data be removed from analysis. However, once data has been aggregated and analyzed it would no longer be possible to remove their individual data from the larger anonymous aggregate data set.

5.3 Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design, procedures, requirements, or processes defined in the Health Canada-authorized and REB-approved protocol, whether intentional or unintentional. Protocol deviations may occur at the participant level, study-procedure level, device-use level, documentation level, or study-site level.

Investigational testing will be conducted in accordance with the authorized protocol. The investigational device will not be used, and study procedures will not be conducted, outside the approved protocol except where necessary to eliminate an immediate hazard to a participant.

If a conscious decision to depart from the procedures specified in the protocol is anticipated, contemplated, or made, prior review by the Qualified Investigator or delegate is required. If the proposed departure represents a change to the protocol, study procedures, eligibility criteria, informed consent form, investigational device use, safety assessments, follow-up, sample size, analysis plan, or adverse event reporting procedures, the change will not be implemented until REB approval and, where applicable, Health Canada authorization through a revised Investigational Testing Authorization have been obtained.

Prior approval is not required when unforeseen circumstances beyond the control of the investigator or study team result in a deviation, such as inability to complete a study procedure, equipment malfunction, scheduling limitations, or inadvertent error. Such deviations will be documented and reviewed as soon as possible after discovery.

A deviation may be implemented without prior approval only when necessary to eliminate an immediate hazard to a participant. In such cases, the Qualified Investigator will document the deviation or change, the reason it was necessary, and any corrective actions taken. The deviation or change will be reported to the REB as soon as possible in accordance with institutional requirements. Where the change affects the Health Canada-authorized protocol, investigational device use, participant safety, or study conduct, the study team will assess whether notification to Health Canada and/or submission of a revised ITA is required.

All protocol deviations will be documented in a study deviation log, regardless of whether they meet criteria for reporting to the REB. For each deviation, the study team will document the date of occurrence, date of discovery, participant study ID if applicable, description of the deviation, reason or contributing factors, whether the deviation was planned or unplanned, impact on participant rights, safety or well-being, impact on data completeness, accuracy, reliability or interpretability, corrective and preventive actions, and whether reporting to the REB, Health Canada, manufacturer/importer, or other institutional authorities was required.

Protocol deviations that may significantly affect participant rights, safety, or well-being, or that may significantly affect the completeness, accuracy, reliability, or interpretability of study data, will be reported to the REB in accordance with institutional requirements. Examples include, but are not limited to, failure to obtain or properly document informed consent before study procedures, use of an incorrect consent form version, enrolment of a participant who does not meet eligibility criteria, use of the investigational device outside the authorized protocol, imaging of a participant or anatomical region excluded by the protocol, failure to follow required safety precautions, failure to adhere to required imaging procedures, loss or inappropriate disclosure of identifiable information, device use by personnel not authorized or trained for study procedures, or conduct of study procedures during a lapse in REB approval.

If a protocol deviation is associated with an incident involving the investigational device that meets Health Canada's mandatory reporting criteria, including an incident related to device failure, deterioration in effectiveness, or inadequacy in labelling or directions for use that led to death or serious deterioration in health, or could do so were it to recur, the Qualified Investigator will report the incident and the circumstances surrounding it to Health Canada and to the

manufacturer/importer within 72 hours of becoming aware of the incident. Manufacturer/importer reporting obligations will be completed in accordance with the Medical Devices Regulations.

6. Data Handling and Record Keeping

Everyone involved in the study is committed to respecting patient's privacy according to all local, provincial, and national standards. Personal health information (PHI) will be accessed only by authorized members of the study team. No other persons will have access to patient personal health information without consent, unless required by law. The research team will keep patient's personal health information private and confidential in accordance with all applicable privacy legislations, including the Personal Health Information Protection Act (PHIPA) of Ontario. To help ensure the privacy of subjects, we will not collect any data beyond the minimum necessary to complete the research objectives.

Study team members will only use Unity Health computers in research space with adequate privacy to access medical records.

6.1 Data Storage

All electronically collected data will be stored in secure, access-controlled directories on Unity Health servers, which are protected by institutional firewalls and comply with hospital data security protocols. All data will be handled in accordance with established standards for secure storage, including locked physical storage, password-protected backups, and encrypted digital systems. Robust safeguards will be implemented to protect against accidental or unlawful destruction, loss, alteration, unauthorized access, disclosure, or misuse of data. Upon request, investigators will provide relevant regulatory authorities with the necessary documentation to verify compliance with these data protection protocols. All procedures will adhere to applicable federal and provincial privacy regulations, including the Personal Information Protection and Electronic Documents Act (PIPEDA, 2000) and Ontario's Personal Health Information Protection Act (PHIPA, 2004).

No participant name, medical record number, date of birth, or direct identifier will be entered into the Clarius Cloud or MSOT device for research image acquisition. Ultrasound images will initially be stored in either password-protected ultrasound systems or in the Clarius Cloud

infrastructure. The Clarius software uses elliptical curve cryptography to keep data safe; even in the unlikely event that data are intercepted, they will be indecipherable. All data stored on Clarius Cloud are stored in data centers located in Canada. No patient information will be collected in the cloud infrastructure, with images de-identified at the time of study creation using the study code (see below). Moreover, on the Clarius Cloud, study ID and images are stored in separated logical servers; the image files do not store any patient information within their records. Clarius uses Amazon AWS standard encryption method (AES256) for storing cases, which is FIPS compliant (<https://aws.amazon.com/security/>). Clarius adopts the HITRUST CSF (Common Security Framework) as its security framework. The HITRUST CSF Assurance program is a common, standardized methodology to effectively and consistently measure compliance. The CSF integrates and harmonizes requirements from many authoritative sources such as ISO, NIST, PCI, HIPAA and others; it tailors the requirements to a healthcare organization based on specific organizational, system and regulatory risk factors (<https://hitrustalliance.net/>). Clarius' Privacy Policy can be found at: <https://www.clarius.com/privacy/>. QUS data will then be securely transferred from the ultrasound devices or the Clarius Cloud to the Unity Health network, where it will be stored on encrypted servers protected by institutional firewalls and access controls in compliance with data security standards.

Photoacoustic data will initially be stored on the password-protected hard drive of the TUPIL Multispectral Optoacoustic Tomography (MSOT) Acuity Echo (investigational device) system. The data will then be securely transferred to the Unity Health network, where it will be stored on encrypted servers protected by institutional firewalls and access controls in compliance with data security standards.

Research image data will be transferred from device storage to the approved Unity Health secure research storage location as soon as feasible. Residual study data on acquisition devices will be deleted after transfer and verification.

6.2 Data De-Identification

All study participants will be assigned a unique study identification (ID) code. The linkage between study IDs and personal identifiers (master linking log) will be maintained in a separate, secure file stored on Unity Health's encrypted servers, protected by institutional firewalls and restricted access protocols. All study records and datasets will reference participants only by their assigned study ID, ensuring that the data used for analysis remains de-identified.

An exception to de-identification will apply to the optional NT-proBNP blood sample at the time of clinical laboratory processing. Because samples will be processed by the SMH Biochemistry Laboratory and the result will be reported in EPIC, identifiable information including participant name and medical record number will be used on the laboratory requisition and within the hospital clinical record. After result generation and extraction for research purposes, study datasets will reference participants by study ID.

6.3 Data Management

De-identified ultrasound images and clinical data collected during this study may be used by the study investigators for future ethically approved research. De-identified aggregate data and study results may be shared in publications, presentations, and scientific reports. De-identified individual-level data may be shared only where permitted by participant consent, REB approval, institutional policy, data-sharing agreements, and applicable privacy legislation.

To enable monitoring, evaluation, and/or audit by Health Canada and the REB, the Principal Investigators will securely retain study records for seven years after the end of the study. These records will include the master linking log and other information required to identify participants and link study records, case report forms, and relevant hospital records; original signed informed consent forms; copies of case report forms; source documents; and other required study documentation. Coded study data will be retained for the same seven-year period. Access to records containing personally identifying information will be restricted to authorized individuals.

At the end of the seven-year retention period, personally identifying information maintained specifically for the study, the master linking log, and coded study data will be securely and permanently destroyed in accordance with institutional policies and applicable privacy legislation. Information contained in participants' hospital medical records, including NT-proBNP results reported in EPIC, will remain subject to Unity Health Toronto's applicable clinical-record retention policies.

If the Investigators relocate, retire, or for any reason withdraw from the study, then the study records must be transferred to an acceptable designee, such as another Investigator at the site, or to another institution.

7. Timeline

The PULSE-LI project is designed to be completed within a 12-month period through a structured, two-phase prospective study with clearly defined milestones for recruitment, imaging, biomarker development, and data analysis. The feasibility of this timeline is supported by robust institutional infrastructure, a multidisciplinary team with relevant expertise, and detailed pre-planning of imaging protocols and cohort logistics.

PHASE 0 - Regulatory and technical readiness

- Application and approval by Research Ethics Board
- Application to Health Canada for medical device investigational testing authorization (ITA)
- PAI system training and Standard Operating Procedure (SOP) development
- Finalization of case report forms and data management plan.

Phase 1 (Months 1–3): Healthy Volunteer Enrollment and Protocol Optimization

Objective: Optimize imaging protocols and establish baseline QUS and PAI biomarkers using healthy volunteers (n = 20).

Key Activities:

- Recruit and image 10 male and 10 female healthy volunteers.
- Test and compare imaging sequences
- Perform pilot testing to refine scanning parameters: probe positioning, respiratory phase (tidal breathing vs. breath-hold), and insonation angles.
- Use acquired data to develop and validate QUS signal processing and PAI spectral unmixing algorithms.

Milestone: Completion of optimized QUS and PAI imaging protocols and finalization of the acquisition and signal processing pipeline for application in Phase 2.

Phase 2 (Months 4–9): Patient Enrollment and Imaging

Objective: Apply the optimized imaging protocol to patient cohorts with pulmonary edema (n = 15, ADHF) and fibrosis (n = 15, UIP) to develop disease-specific imaging biomarkers.

Key Activities:

- Recruit eligible patients with ADHF from inpatient units and UIP from outpatient respirology clinics or CT imaging appointments.
- Apply rigorous inclusion/exclusion criteria to ensure well-defined cohort phenotypes and minimize confounding.
- Coordinate closely with clinical teams (cardiology, radiology, and respirology) to identify, recruit, and schedule eligible patients.
- When CT imaging is available, use skin markers during ultrasound/PAI to enable accurate correlation between insonated and radiologically imaged regions.

Milestone: Completion of imaging and data acquisition for 30 patients, ensuring a complete dataset for downstream biomarker extraction and validation.

Phase 3 (Months 9–12): Data Analysis, Validation, and Dissemination

Objective: Extract, analyze, and validate QUS and PAI biomarkers; assess reproducibility; and prepare results for publication.

Key Activities:

- Perform statistical analyses comparing biomarker distributions across healthy, ADHF, and UIP groups
- Assess diagnostic reproducibility
- Incorporate skin tone data into algorithm validation to ensure robustness and equity in image quality and biomarker accuracy.

Milestone: Completion of biomarker validation and submission of at least one manuscript and/or conference abstract summarizing study findings.

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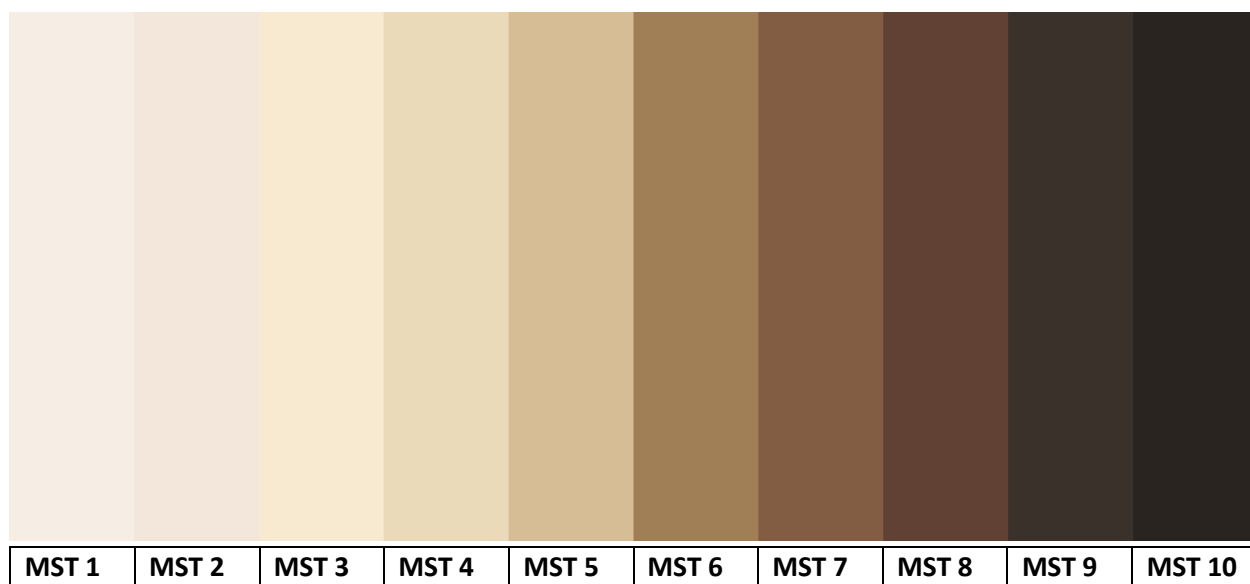
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9. Appendices

MST Swatches

MST Swatches are 10 single-colored rectangles, and each swatch provides the most representative color within its MST group. The swatches are created for researchers who need to use the exact color values of the MST scale for their studies and for reference when running evaluations



Deyo-Charlson Comorbidity Index

AIDS
Any malignancy
Cerebrovascular disease
Chronic pulmonary disease
Congestive heart failure
Dementia
Diabetes with complications
Diabetes without complications
Hemiplegia or paraplegia
Metastatic solid tumor
Mild liver disease
Moderate/severe liver disease

Myocardial infarction
Peptic ulcer disease
Peripheral vascular disease
Renal disease
Rheumatologic disease

Modified Medical Research Council (mMRC) dyspnea scale

Grade	Description of breathlessness
0	I only get breathless with strenuous exercise
1	I get short of breath when hurrying on level ground or walking up a slight hill
2	On level ground, I walk slower than people of the same age because of breathlessness, or have to stop for breath when walking at my own pace
3	I stop for breath after walking about 100 yards [91 meters] or after a few minutes on level ground
4	I am too breathless to leave the house or I am breathless when dressing

Adapted from: Fletcher CM, Elmes PC, Fairbairn AS, Wood CH. The significance of respiratory symptoms and the diagnosis of chronic bronchitis in a working population. Br Med J 1959; 2:257.

Warrick Score

Severity score		Extent score	
Abnormality	Grading	Bronchopulmonary segments for each of the abnormality, score by number of segments involved	Grading
Ground Glass Opacities	1	1 to 3 segments involved	1
Irregular Pleural Margins	2	4 to 9 segments involved	2
Septal or Subpleural Lines	3	>9 segments involved	3
Honeycombing	4	N.B. extent of disease measured for each of the abnormalities.	
Subpleural Cyst	5		
Maximal severity score	15	Maximal extent score	15

Step	What you do	Points awarded
1. Identify the five key parenchymal abnormalities	• Ground-glass opacities (GGO) • Irregular pleural margin/thickening • Septal or sub-pleural lines (reticulation) • Sub-pleural cysts / traction bronchiectasis • Honeycombing	Severity weight (fixed) GGO = 1 Pleural margin = 2 Septal lines = 3 Sub-pleural cysts = 4 Honeycombing = 5
2. Gauge how widespread each abnormality is (count involved bronchopulmonary segments; 18 in total)	• 1–3 segments → 1 point • 4–9 segments → 2 points • > 9 segments → 3 points	Extent points (0–3)
3. Add severity + extent for every abnormality	Range per lesion: 0 (absent) to 8 (5 + 3)	
4. Sum across all five lesions	<i>Maximum totals</i> Severity subtotal = 15 Extent subtotal = 15 Global Warrick score = 0–30	
5. Grade overall disease burden	1–8 = <i>Mild</i> 9–16 = <i>Moderate</i> ≥ 17 = <i>Severe</i>	

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New York Heart Association (NYHA) Functional Classification

Class	Functional capacity	Typical activity threshold that provokes symptoms*
I	No limitation of physical activity. Ordinary activity does not cause undue fatigue, palpitations, dyspnoea or angina.	Brisk walking, climbing several flights of stairs, recreational sports all tolerated.
II	Slight limitation. Comfortable at rest, but ordinary activity results in symptoms.	Walking ≥ 2 –3 city blocks, climbing one flight at normal pace may trigger breathlessness or fatigue.
III	Marked limitation. Comfortable at rest, but less-than-ordinary activity provokes symptoms.	Activities of daily living such as dressing, slow walking on level ground, or light housework lead to dyspnoea, fatigue, or angina.
IV	Unable to carry on any physical activity without discomfort. Symptoms occur at rest and are worsened by any activity.	Dyspnoea, fatigue, or angina even while seated or lying down; minimal exertion (e.g., toileting) is intolerable.