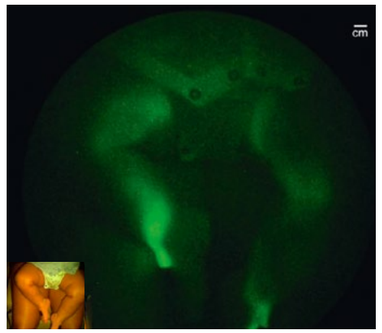
Imaging technique provides new diagnostic tool for lymphatic dysfunction in paediatric patients

It has long been established that the lymphatic vasculature plays an important role in cardiovascular health, but there have been few methods to diagnose lymphatic dysfunction. To aid real-time, point-of-care monitoring of the lymphatic system, **Dr Eva M Sevick** has devised a breakthrough investigational technique based on laser diode technology used by **Dr Matthew Greives** to diagnose vascular abnormalities in the paediatric population.

It is well understood that the lymphatic system is a key part of the body's vascular system. Made up of an extensive network of vessels, the lymphatic vasculature pumps excess tissue fluid (called "lymph") that carries waste products and foreign materials through a series of lymph nodes where they are exposed to white blood cells (lymphocytes). The pumps are miniaturised "lymph pumps" or lymphangions that propel lymph through its vasculature. The lymphocytes in the lymph node become "educated" by exposure to materials in "lymph" and then either secrete antibodies or even become activated to travel with lymph to ultimately drain to blood circulatory system in the veins in the neck and chest regions. Once antibodies or active lymphocytes are in the blood circulation, a whole body immune response is mounted. Over years of research, the lymphatic system has emerged as having a decisive role within a number of adult conditions, such as cancer-acquired lymphoedema, rheumatoid arthritis, peripheral vascular disease, cancer metastases, and various cancers like Hodgkin's lymphoma. In children, the lymphatics are increasingly recognised as having important roles in congenital lymphoedema and a myriad of vascular anomalies. Because the lymphatics can mediate a lifetime of health, it is essential to understand lymphatic disease, diagnose dysfunction, and where possible, deliver accurate treatments. Long-established lymphatic imaging techniques employ the use of contrast agents, and include computed tomography (CT), magnetic resonance (MRI) and lymphoscintigraphy. Although these methods have served physicians well, they lack the dynamic and fast imaging times required for eludicating the mechanics of lymph transport as well as for routine use in unsedated infants and children.



Fluorescent images of the lymphatics in the lower limbs of an infant with upper arm, congenital lymphedema. Reproduced with permission from Greives, *et al.*, Pediatrics, 2017

The non-invasive nature of the NIRF technique is a great improvement, especially for paediatric patients where excessive levels of stress need to be kept to a minimum

In a newly discovered breakthrough on the road to this aim, lead researcher Dr Eva M Sevick and her team at the Brown Foundation Institute of Molecular Medicine, The University of Texas have developed a novel, highly sensitive, non-invasive method of monitoring the human lymphatic system. Clinical Researcher, Dr Matthew Greives, has adapted the investigational technique to gain a better understanding of lymphatic involvement in infants, children, adolescents and even adults with vascular malformations or abnormalities in order to provide important, real-time diagnostic information.

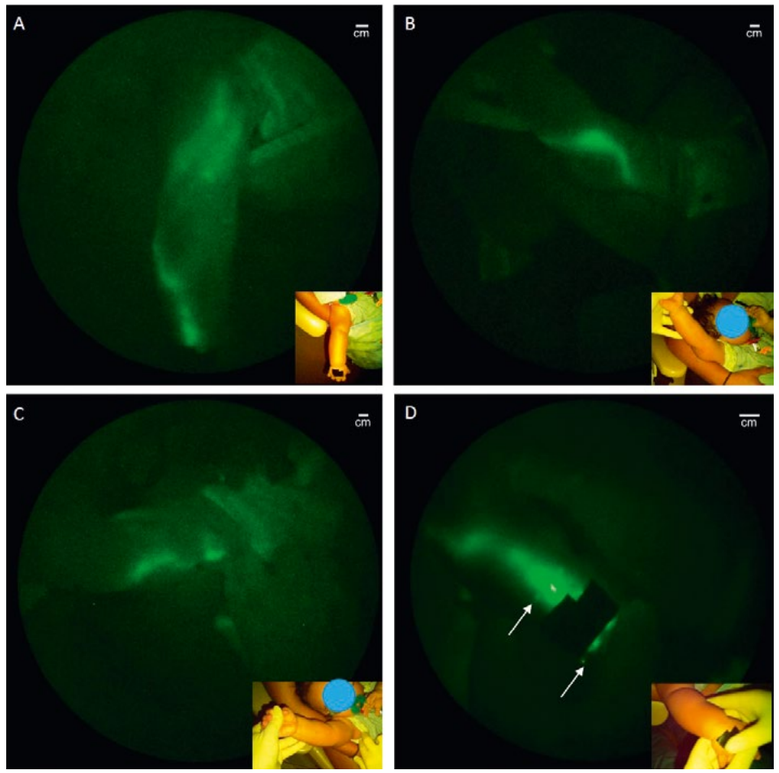
HOW IT WORKS

Extended from military equipment, the Near Infrared Fluorescence (NIRF) imaging method devised and developed by Dr Sevick and her team involves the use of the US Food and Drug Agency (FDA) approved dye, indocyanine green (ICG). This dye is injected intradermally for rapid uptake into the lymphatic plexus and drainage into the lymphatic vasculature. Trace doses of approximately 2- 25 micrograms of ICG in 0.1 cc or less are used in as little as two injection points. These low levels of contrast agent remain concentrated within the lymphatic region so they are easy to monitor, but are quickly cleared from the body once drained from the lymphatics into the blood circulation. In addition to the long-standing safety record of ICG at much higher doses, the trace dose and rapid clearance ensures that any potential adverse reactions are kept to a minimum. To conduct imaging, a dim laser diode emitting near-infrared light is shone on the tissue surface. The light penetrates several centimetres beneath the tissue surface and excites the ICG located in the lymphatic

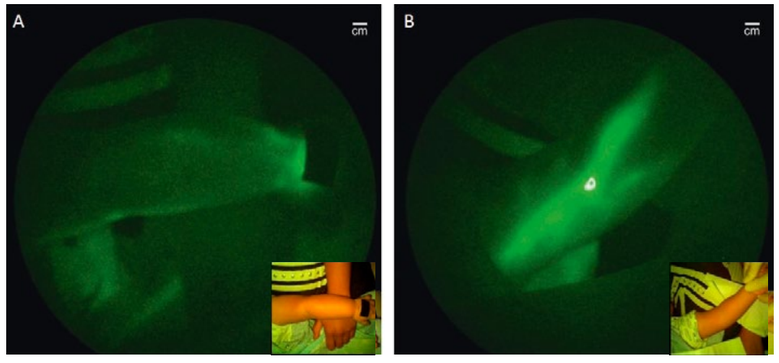
vasculature. The fluorescence that is emitted from the tissue surface is then collected using the night-vision technology coupled to a detection system that registers images within ten to 100's of milliseconds. The images can be compiled into movies showing the active "pumping" through the lymphatic vasculature in real-time. As Dr Sevick highlights: "It's amazing how just two injection sights, essentially 10 to 15 micrograms of ICG, will fill up the entire lymphatics, and once the contrast agent flows back into the blood vasculature via the subclavian vein, the trace dose of ICG is diluted and cleared to such an extent it's essentially gone. So essentially, we visualise only the lymphatics, and because the detection is so sensitive, we can actually see a real-time movie of lymph flow."

Historically, with established imaging modalities, such as lymphoscintigraphy or CT or MR lymphangiography, contrast agents are required for static imaging. In addition, lymphangiography techniques require lymphatic vessel cannulation or ultrasound guided injection into lymph nodes to introduce large amounts of contrast agents into the lymphatics. These techniques require the sedation of infants and children for both contrast administration and the comparatively long image acquisition times. The non-invasive and non-radioactive nature of trace contrast administration and rapid imaging provided by the NIRF technique is a great improvement, especially for paediatric patients where sedation can add complication to the diagnostic procedure. Recently, Drs. Greives and Sevick demonstrated the utility of the technology on a 21 month old subject diagnosed with congenital lymphoedema (Greives, *et al.,* Pediatrics, 2017).

Paediatric lymphoedema is thought to be caused by developmental malformations which, if left untreated, results in interstitial protein accumulation, tissue fibrosis and disfigurement, and reduced immune response. Early treatment is especially paramount for children who are at risk of a lifetime of sequelae arising from lymphatic impairment. Yet management of paediatric lymphoedema is typically based upon adult treatment, which lacks evidence for effective use in paediatric populations. The main impediment to understanding, diagnosing, and treating paediatric lymphoedema is the lack of an imaging modality that is safe, inexpensive, and effective. In their recently published work, the Greives and Sevick team used near-infrared fluorescence imaging to show intact, apparently normal lymphatic



Fluorescent images of lymphatics in the (A) lateral and (B) medial right arm that presents with lymphoedema and (C) left axilla (D). Possible, abnormal dermal backflow near right wrist. Reproduced with permission from Greives, *et al.*, Pediatrics, 2017.



Fluorescent images of the (A) lateral and (B) medial left unaffected arm with what appears to be a cubital lymph node. Reproduced with permission from Greives, *et al.*, Pediatrics, 2017.

The detection technique is similar in principle to night-vision goggles used by military personnel during night time operations, except it collects near-infrared light that is recorded and processed by a camera rather than captured by a human eye

Q&A

How long has current research into the technique been carried out?
The investigational technique has been deployed on the Texas Medical Center in Houston since 1994 and used safely in over 350 adults, children and infants.

Can you see NIRF imaging being used in other areas of medicine?
We use NIRF imaging for imaging the lymphatic system, but it has applications in image-guided surgery and other point-of-care procedures.

Has the technique revealed any surprises regarding the lymphatic system that were not currently known?
Yes. Because of the unprecedented sensitivity of near-infrared fluorescence imaging, we have been able to visualise for the first time the "lymph hearts" that exist throughout the human body, and that function to prevent tissue swelling and mediate immune surveillance. Using near-infrared fluorescence imaging, we've identified a number of chronic conditions where the lymphatic pump is dysfunctional and have started to use the technology to identify new therapeutic strategies to restore lymphatic function. Conditions such as rheumatoid arthritis, neuroinflammation, and peripheral vascular disease appear to have a lymphatic component that may be responsible for the onset or the worsening of these conditions.

It is pointed out that the technique will be highly valuable for paediatric patients. What is the minimum age of patient that it can be used on?
The most exciting thing about the technology is that image acquisition is fast, on the order of tens to 100's of milliseconds. This means infants and children do not need to be sedated for the procedure. In the past we have imaged infants as young as five weeks in the PICU (Pediatric Cardiology, 2014) and are imaging young children who have congenital lymphoedema (Pediatrics, 2017)

When do you expect the technique to be rolled out for general medical use?
The technology is currently used as an investigational tool in clinical research. With commercialisation, it should be ready for the market in 12-18 months.

anatomy in an infant with lymphoedema in the right arm, but with decreased lymphatic pumping function relative to the contralateral and lower extremities. Their work showed that the lack of pumping function rather than anatomical malformation was the source of irreversible arm swelling and that physiotherapies that stimulated lymphatic function could be effective therapies.

ADDITIONAL USES AND BENEFITS

The research has also helped to reveal how immune status is inextricably linked to the body's lymphatic system, revealing how the lymph flow is vital to health. Over the years, the team has used imaging to identify specific lymphatic phenotype dysfunctions to genetic mutations. This is invaluable information if tailored, specialised treatment plans are going to be delivered for rare genetic disorders such as Parkes Weber syndrome (Burrows, *et al.*, PNAS, 2013) or more common chronic conditions, such as inherited forms of lymphoedema (Agollah, *et al.*, Plos One, 2014; Gonzalez-Garay, Vascular Cell, 2016). The team has also pointed out the importance of near-infrared imaging in the treatment of paediatric patients where early treatment is critical for survival (Tan, *et al.*, Pediatric Cardiol. 2014). Because the near-infrared imaging technique can also be delivered without sedation and in a bedside environment or on the lap of a parent or caregiver, it is especially suited to applications in the paediatric population.

FUTURE AVAILABILITY

Owing to the record of ICG safety, its trace dose in this application, and the lack of radiation or radioactivity, the technique of near-infrared fluorescence lymphatic imaging has significant potential for point-of-care application in community-based health care practices. Future expansion into the medical community is predicted, with the technique to be rolled out in the not too distant future.

Detail

RESEARCH OBJECTIVES

Dr Eva Sevick, PhD has pioneered the development of near-infrared fluorescence optical imaging and together with Dr Matthew Greives, MD, Director of the UTHealth Vascular Anomalies Clinic, is using the technology to understand the lymphatic contribution to vascular disorders in children.

FUNDING

National Institutes of Health (NIH), The Plastic Surgery Foundation

COLLABORATORS

John C. Rasmussen, Melissa B. Aldrich, Banghe Zhu, J Rodney Morrow

BIO

Dr Sevick is Kinder Distinguished Chair of Cardiovascular Research, University of Texas Health Science Center at the Texas Medical Center, where she and her team developed from the bench and translated to the clinic, near-infrared lymphatic imaging in infants, children, lymphoedema patients, and animal models of human lymphatic diseases.

Dr Matthew Greives is Director of the Vascular Anomalies Clinic at UTHealth where is deploys the investigational lymphatic imaging technique in paediatric populations.

CONTACT

Eva M. Sevick, PhD, Professor and Kinder Distinguished Chair Director, Center for Molecular Imaging. The Brown Foundation Institute of Molecular Medicine
1825 Pressler, SRB330 | Houston, TX 77030
E: Eva.Sevick@uth.tmc.edu
T: +1 713-500-3560
W: https://www.uth.edu/imm/profile.htm?id=1005459

Matthew Greives, MD
Vascular Anomalies Clinic
6410 Fannin Street, Suite 950
Houston, TX 77030
E: Matthew.R.Greives@uth.tmc.edu
T: 713-500-7275
W: http://childrens.memorialhermann.org/services/vascular-anomalies/