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PRESENTATION

Dr. Eva M. Sevick-Muraca:

Hi, my name is Eva Sevick, I'm the Kinder Distinguished Chair of Cardiovascular Research at the University of Texas Health Science Center, where I direct the Center for Molecular Imaging. Today I'm going to talk to you about imaging lymphatic function and dysfunction in human subjects. And I'll show you how we can actually use the imaging to monitor treatment and show how treatment affects lymphatic function and architecture.

My disclosures are as follows:

You'll be seeing human studies of Near Infrared Fluorescents that are conducted under IRB approvals, using an off-label administration of ICG or indocyanine green or IC cardio green. These human studies were funded by the Longaberger Foundation through the American Cancer Society, the National Institutes of Health, the Cancer Prevention and Research Institute of Texas, and Tactile Medical Systems.

I've also had financial interests in that I speak for Tactile Medical and lymphedema seminars; I'm a consultant to Sorrento Therapeutics on the lymphatics and there are royalty interests in the imaging technology that I'm going to be describing today.

This is unidirectional lymphatic vasculature and you're watching this video so you're interested in it. Let's make certain that we're all on the same page. The lymphatic system, the way that you enter into the lymphatic system is through the initial lymphatics that are located right underneath the epidermis. They also line all the organs, the internal organs of the body. In these initial lymphatics, the lymph epithelial cells are specialized. They don't have any basement membrane; they open and close and allow excess fluid, immune cells, and waste products, macromolecules, into the lymphatics. Once in the lymphatics, these entities become lymph and it's moved through the collecting vessels through the conducting vessels that consist of lymphangions. These are segments of lymph vessels that actually look like hearts or lymph hearts. They're bounded by valves and they have smooth muscle cells that pump in concert so that lymph is moved, oftentimes, against gravity through the thoracic duct. Along the way, that lymph transits through lymph nodes w3here immune surveillance occurs. Eventually, that lymph, after it transits against gravity up through the thoracic duct, empties into the supraclavicular vein, where all that material, the waste products, these immune cells, are returned to the blood system, so that those waste products are metabolized by the liver, the immune cells go out and fight infection, and the fluid is restored to the vascular system, so that we have fluid heliostatic.

There are conventional means of imaging the lymphatics. One that you may have heard of is lymphoscintigraphy. In that case, what one does is one injects right underneath the skin, intradermal injection, radiocol???, that's taken up by the initial lymphatics. And after several minutes of waiting around, one puts the patient underneath the gamma camera and counts the fluorescent radioactive decay. And this decay is then formed into an image called a lymphoscintigram and you see in this image, the injection sites at the webs of the feet or the webs of the toes of the feet or hands, and there's a grainy picture that marks

lymphatic vessels. In the United States we don't do an awful lot of lymphoscintigraphy. We also do lymphangiography. Lymphangiography consists of either cannulating a lymph vessel or under ultrasound guidance injecting into a lymph node. And what happens is that contrast media goes to the lymphatics and it captures this beautiful picture that you can get on MR or radiography or CT or X-ray. The problem with this is that this contrast agent, well actually the advantage of it is, this contrast agent, in the instance of a leak, can plug that leak. The problem is that it's not necessarily a conventional imaging modality for monitoring lymphatics. These are processes and procedures that are performed by radiologists and interventional radiologists.

We've decided to work for developing point-of-care imaging that's based upon near-infrared light. It's light, it's nonradioactive and so it's really simple to use. We're using Indocyanine Green, that's used in studies for hepatic liver function and what we do is we inject a trace amount right underneath the skin. That dye actually goes right into the lymphatics and that dye tends to have, actually has a fluorescent property. What we do is we illuminate the tissues with dim near-infrared light, that light penetrates into tissues, it excites the ICG that's located in the lymphatic vessels, and that causes fluorescence. That fluorescence propagates to the tissue surface and we have a camera system that we've developed so that we can collect that fluorescence and we can record it really quickly so we can get rapid images of the fluorescence, this indocyanine green that's in the lymphatic vessels. Now the secret to our technology, the secret sauce, is that we've actually the adapted the night goggle technology from the first Gulf War. If you might remember, Iraq invaded Kuwait in the evening hours and all those maneuvers were recorded using these night goggle technology goggles that the soldiers wore. What we did is after the first Gulf War, we contacted the army vendors and they sent us these. And we took them apart and adapted them on to these camera systems so that we can very sensitively image through several centimeters of tissue.

And what you see here is this movie of lymphatic function in the lower legs of a person. And what you see is those lymphangions pumping that ICG-laden lymph from the feet all the way up into the groin area. So, these are the things that you can see using this military technology adapted to medical imaging.

So why is this so sensitive? Well it's because of that military technology. Usually in imaging we really want to have a high contrast, that is, you want to see the lymph vessel and discriminate it from the background, so that means you have to have a high contrast. And you want to have a high signal-to-noise ratio, you don't want to have a noisy image. So, the best imaging technology is basically up with high-signal noise and high contrast. And unfortunately, without that intensifier, without that military technology, your signal-to-noise and your contrast is actually quite low; actually, too low for you to be able to see and distinguish things for medical imaging. So, we use this technology and we develop our camera systems using phantoms that we developed with NIST so that we can certify the performance of our systems. And that seems to be the reason why we have this differential performance.

This is how you do it, you inject, this is a patient that has an injection of a tenth of a cc of indocyanine green, about 2 to 25 micrograms of this dye is a trace amount. And you see that the dye is readily taken up by the lymphatics. The camera is so sensitive that in order to actually see the lymph vessels, we cover that injection site with a band-aid and later on through this presentation, you'll see there's little black squares, that's like black electrical tape, 'cause that's the only thing that we could find to stop the light from oversaturating the camera. Now the interesting thing is that you have to do an intradermal injection. So, you have to do a Mantoux injection and have that wheal that's formed. If you go a little bit too deep, okay, you're not going to get access into the lymphatics and so you won't see these images.

So again, we place the dye right into these initial lymphatics, and you can do this anywhere in your body. It's taken up by the collecting and conducting vessels and pumped, oftentimes against gravity through the lymph

node, goes into the subclavian vein. There that trace amount of indocyanine green is basically clear through the blood system, so you really only see the vessels in the lymphatic vasculature.

This is an image that is, hopefully you can see the motion in the arm. These images are sped up about three time and there you'll note these black pieces of tape that cover the injection site so that you can see these beautiful pictures of lymphatic function. Well it turns out that pulsing and that movement of lymph is due to those lymphangions, which are essential to the transport of lymph. It's essential for ???, that's the movement of this fluid through your body.

And this is a video of a lymphedema subject with an injection in the heel, and you see the deep lymphatic vessels and the more superficial lymphatic vessels that are pumping. We can actually follow these lymphangions from the intensity as a function of time and you see that the intensity goes up as the ICG enters into the lymphangion, then that lymphangion squeezes it and you see that the intensity goes back down and you see this repetitious process. In the adjacent lymphangion upstream, you see when that lower lymphangion empties, the upper lymphangion fills up. These are characteristic wave forms that differ for everybody. It's not these wave forms and this contractile activity is not necessarily related to breathing or to heart rate. When a person is perfectly still, you'll still have this, so it's not muscle contraction. We do know that these lymphatic rates are dependent upon position and they're incredibly sensitive to the proinflammatory state of your regional tissues.

We see that this ICG will move through all the draining lymph node beds, whether in the groin, in the elbow, in the axilla, in the thigh. And everybody has lymph nodes in different locations, so this is a way to actually probe where your lymph nodes are. And again, we can do this noninvasively, so you don't have to open a person up.

These lymph nodes are taken out for staging, and as you know, that was oftentimes results is lymphedema. And in the normal arm you have straight lymphatic vessels that conduct lymph to the axilla, the axillary lymph nodes, but in the earliest stages of lymphedema, what you see is that we have these torturous vessels and we have this very fine lacy lymphatic vasculature that's close to the surface.

What we think is happening is that in the onset of lymphedema, before any swelling actually occurs, we think that these lymphangions that we have are weakening because of this proinflammatory state. And these lymphangions, when they pump, they create a pressure that moves lymph forward, that's from distal to proximal and it creates a backpressure that sucks lymph from these initial lymphatic capillaries. When this pump is actually weak, what happens is we don't generate sufficient pressures to propel lymph forward or to suck lymph from these initial lymphatic capillaries. And so the initial signs that we see, when patients that develop lymphedema, they get this dermal backflow. It can be this beautiful lacy lymphatic vessel that actually occurs right underneath the tissue. So, an injection in the wrist will show these beautiful structures upwards, closer to the elbow.

So, what we see is we see that as disease or this condition progresses, we see a movement from straight lymphatic vessels that pump to this dermal backflow where these lymphangions are becoming deficient, so we get even more dermal backflow up to the point where we don't have the vessels transporting lymph any further. What we have is we have this ICG-labeled lymph that's all within the tissues and it's extravascular. And that's the tough time, that's very difficult for lymph to move extravascularly. That's why we have lymph vessels so that we can move that lymph effectively through the vessels.

The therapists in our, who we've collaborated with, have actually used the technology to help them direct manual lymphatic drainage. So what we'll do is we'll use Bluetooth technology and we'll pipe the images on

to a plasma screen in the clinic wall and they'll watch that plasma screen. And they'll perform their therapy by directing their fluid, the new fluid to functional lymph vessels.

And what we'll see is recruitment of new vessels, we'll see filling of vessels that were not previously functional.

And you'll see that the therapist actually uses the image to actually find these draining lymph nodes and lymph vessels and lymphangions and trying to coax them to actually pump and move fluid.

In cases where patients have advanced disease, where most of the IC green-laden lymph is located extravascularly, we've actually been able to show that the advanced pneumatic compression garments, such as the Tactile Flexitouch, actually can be used to move very effectively this extravascular lymph. This is an image before treatment, where you see the dye that's been injected in the hand has gone all the way up to the elbow but no further, and then after a session of the pneumatic compression therapy with the Flexitouch, you see that the limb has more of this IC Green laden extravascular, but moving from distal to proximal. We see that in all of our cases, where before we don't have an awful lot of coverage of that IC Green lymph and we see that after the treatment it's always ???. In this last case, you see that after an injection near the wrist, there's no dye, but after the treatment you see that dye is moved proximally to those draining lymph nodes. The idea of moving that fluid and getting it away from, moving it towards a functional lymph draining bed is the basis of these technologies and the basis of MLD Therapy.

So, what I've talked to you about is how we've used this technology for looking at secondary, primary lymphedema and the treatment thereof. And this is a costly disorder, but I want to point out that most of our chronic conditions actually involve defective lymphatics. And what I'd like to do is talk about chronic and wounds and peripheral vascular disease.

And in venous disorders, it's always thought that whenever there's venous hypertension, the edema that's been formed is really due to this hypertension and that effective treatment is to basically reduce that hypertension so that you reduce the edema formation. Well we don't really have edema formation if the lymphatics are functional. So if the lymphatics are not compliant and don't respond to that excess fluid, we have edema formation. But in addition, those same symptoms of swelling could be due not to venous hypertension, but also just due to the fact that the lymphatics themselves aren't working. So whenever one has edema in the lower extremities, they could have it because you have venous disorders, you have lymphatic insufficiency, or you have a combination of both.

And this is some of the cases that we've actually seen where whenever a patient presents with a swollen leg, you have to ask, is it a venous disease? Is it a lymphatic disease? Is it a combined venous lymphatic disease? This is the case of an adolescent girl, who in puberty ended up with a swollen leg. And she was treated with stinting, venous stinting, 'cause there was venous hypertension and there seemed to be venous compression. So once a stint was placed, it's expected that the venous hypertension would be resolved and the swelling would resolve. It did not and upon imaging what we see is her affected leg has dermal backflow. Clearly the lymphatics are involved. This is all compared to her normal leg, which has dilated lymphatic vessels, but clearly not the dermal backflow that we see in the contralateral leg. Interestingly, when we look at the ankle, we can actually see that dermal backflow that we see deep in that ankle; we see that functional lymphatic vessel pumping. So there's hope that we can basically make it, get those lymphatic angions working again so that we can create the sufficient pressures that move lymph distal to proximal and get rid of that dermal backflow. So this is a case where it was not just a venous disease. It could have been a purely lymphatic disease or a combination of a venous lymphatic disease, but yet, you still have to understand what the relative contribution from the lymphatics are to this edema.

This is a case, an interesting case, where a subject presented with Klippel-Trenaunay Syndrome, a venous disorder. And before the clinician was going to treat and oblate the veins, he wanted to see if there was a lymphatic abnormality. And what you see here is the lymphatic pumping that's occurring as this subject is actually standing. And you see in his normal leg, you get this wonderful pumping, lots of vessels, and compare that to the vessels that are in his affected leg. You see the port wine stain and you see those lymphatic vessels that are pumping, but they're not as many. Again, the swelling occurs because of the venous condition, but the lymphatics are insufficient to take up that excess fluid. So here again, even though we don't have dermal backflow, those lymphatics need a little bit of an assist.

In patients with venous insufficiency and venous wounds, the images are, you don't have to show any movies 'cause there's really not a lot of motion. We see that they have lymphatic vessels, but they seem to have this lymphatic pooling or dermal backflow at the sites where their vascular wounds are or where the vascular wounds will appear. So it suggests to us that these lymphatic disorders, or these venous disorders, inherently involve the lymphatics. Indeed, when you go to the classic textbooks' peripheral vascular disease, whether it's arterial disease or venous disease, these diseases are called the inflammation of the blood vessels. And so you would think that if there's inflammation and the lymphatics are supposed to remove these immune cells and the inflammation, if those aren't working, then your blood vessels become inflamed.

And that's what we see. This is a picture of a person with venous insufficiency, he's early stage, no vascular wound. An injection at the ankle and you see there's dermal backflow, not what you see in normal subjects, healthy subjects without venous disease.

Here's a subject that has mixed arterial disease, mixed arterial venous ulcer, pretty young ulcer. And what's interesting in this patient is that you don't see symmetrical lymphatics, which is what you normally would see. You see a lot of dermal backflow around the vascular wound. On the contralateral leg, which is healthy, you see this dermal backflow in the same regions, and perhaps there's going to be an ulcer that forms there, don't know. We know that in early ulcers, the immune system is really revved up and the lymphatics are supposed to take away that dying material, the immune cells take away the waste products. And what we see here is we see an awful lot of lymphatics, compared to the healthy contralateral leg. And we know that the lymphatics are really plastic 'cause they basically change, they're recruited in order to remove these immune cells and waste products from this area, which in this case is an ulcer. If we blow up this area of this lymphatic, these plastic lymphatics, they're working very hard. And I'm going to show this movie. You know that lymph is supposed to move distal to proximal and I want you to watch very carefully this movie. And remember, the lymph is supposed to move from the bottom of the screen to the top of the screen. When I move that, watch this. Did you see that? It moves downwards. It's almost like there's a lymphatic reflux. Like there's a venous reflux, there's a lymphatic reflux. And we think this is occurring because this inflamed site of this ulcer, basically spewing a lot of cytokines.

And our work with animals shows that whenever there's these proinflammatory cytokines such as ???, when those go up, in our mass models, what we find is that there's reflux. This is a mouse on the side. Mice don't have as many lymphatics as humans do. This is the inguinal lymph node. And they have a single lymphatic vessel that propels lymph from the inguinal lymph node to the axillary lymph node. It's supposed to go distal to proximal, right? So when we give this mouse, we administer these proinflammatory cytokines, what you see is some of the same behavior. We see lymph going backwards. Do you see that? So we think what's happening is that there's this inflamed state, the lymphatic vessels are dilating in response, the valves aren't working, the pump is weakened, and so we're not moving lymph effectively, and as a result, those immune cells and all those cytokines are building up and perhaps may be responsible for the formation of the ulcer in the first place.

We've used the Flexitouch therapy to actually try to move this toxic lymph away from the wound site, and these are some of the images of patients with active wounds, where we're actually moving that lymph away from the wound after the Flexitouch treatment. So the idea of moving the metabolic waste products, the dead cells and these cytokines out of the area, gives the wounds and those ulcers a chance to heal.

So what's interesting about this is that for the first time, I think, we're looking at longitudinal changes and that we can actually ameliorate this dermal backflow that we have used in the past to characterize the initial onset of lymphedema. And so perhaps now, we're able to manipulate the lymphatics so that we can relieve the symptoms associated with these very chronic conditions.

You know, before there was blood vascular imaging and geography, we didn't have a lot of treatments for the blood vasculature. Now we're imaging the lymphatics routinely. So, now you might imagine, now we might be able to come up with some cures for not only the chronic conditions that I've discussed, but several other chronic conditions. Thank you for your time and your interest in the lymphatics.

Dan Carlson:

On behalf of Tactile Medical Dr. Sevick, thank you so much for sharing your passion and your research. And attendees across the country, thank you for taking the time to tune in and join us. At this time, as we don't have questions on the phone, we'd like to go to some of the questions that have come in through the webinar portal Q & A. So first Dr. Sevick, can you please explain further why ICG isn't available as a clinical diagnostic tool in the U.S. today?

Dr. Eva Sevick:

Well ICG is approved for an IV administration for looking at liver function, vascular abnormalities and looking at skin profusion, not skin profusion but profusion in surgical procedures. It's not approved for the intradermal administration, so that's it's an off-label administration. So hopefully, we're going to be launching the lymphatic imaging system for ICG administration shortly, once we get the FDA clearance for market approval. So ICG is off-label right now; it's used off-label, but once it's approved for the market, then it should become available in the United States.

Dan Carlson:

Alright thank you, and another question from the webinar portal. You mentioned lymphangion pulse rates differing from one subject to the next. Has there been any research on correlation of lymphangion pumping rate to activity levels and exercise? And then the same question for BMI?

Dr. Eva Sevick:

Antidotally we've seen, we've done about 500 patients on the Texas Medical Center and in the surrounding Houston area. And every now and then we get an athlete that enrolls into our studies. And antidotally I can tell you that those athletes always have incredible lymphatic pumping. They have lots of lymph vessels and they pump. We believe that cardiovascular health is synonymous to lymphovascular health. So although we don't have the studies, the specific study to prove it, it's an observation that we've made. Once we get these devices in other people's hands, it would be interesting to see if others can make that association in terms of activity and lymphatic function. As far as BMI goes, we expected to see that as the BMI increases the lymphatic pumping would decrease. That was our expectation and I think a lot of people think that, would expect that, because obesity is often also associated with systemic inflammation. And we know that inflammation ??? the lymphatic pump. We did a retrospective study with some interns a year ago and two

years back and we asked them to look at all the images and see if they could find this correlation between BMI and lymphatic pump rate. And it was really interesting, that correlation did not exist. We did not find a correlation, we did not find expected correlation that with increasing BMI, the lymph pumping function decreases, which was a little bit surprising to us. And it may very well be that the lymphatic pumping function is more directly correlated to inflammatory states. And BMI and inflammation is probably a looser correlation; perhaps that's the reason why. More research needs to be done in this area to fully answer that question.

Dan Carlson:

Alright thank you. Now going back to the webinar portal, we have a question that came in from Stephanie Amadi at Lymphatic Therapy. Thank you, Stephanie for identifying yourself on this. The question is, I was wondering if you are able to locate or see the damage blocked lymph node or the area with nerthly imaging?

Dr. Eva Sevick:

So remember that when we put an administration of the indocyanine green Intradermally, it's taken up and it's taken up by functional lymphatics, okay? So we only see functional lymphatics. That's kind of an interesting aspect, 'cause you have to have some function in order to draw that indocyanine green into the lymphatic vessels. Now when there is a damage upstream of the watershed, what we'll see is we'll see uptake near the injection site and it'll go so far and then we'll start to see that dermal backflow coming back. Or, we might see lymphatic rerouting, is another thing that we see. And so that's where you infer that there is a blockage at that point. So right now, the lymphatics really depend upon a contrast stage for medical imaging. And these lymphatic vessels won't take up that contrast agent unless they're functional. That was a really good question.

Dan Carlson:

Alright, thank you Stephanie. And so Dr. Sevick, another question from the webinar portal, have your imaging studies shown any difference in lymph flow rates or patterns between primary lymphedema and secondary lymphedema?

Dr. Eva Sevick:

At this point, we don't believe so. I think we, as you understand that, primary lymphedema is relatively rare, so it may very well be that as we secure more patients with primary lymphedema, some correlation might pop out. But at this point, our imaging just says look, you've got defective lymphatics. It doesn't really ascertain the cause. And I guess you get that, it comes from medical history to infer what the cause might be. However, we've had several study subjects over the years that have come in with lymphedema with no apparent causation and suggesting that there might be familial or inherited or primary lymphedema. And one of the things that we do often look to see in those cases is if there's a lymphatic abnormality not only in the affected leg, but perhaps in the unaffected leg or unaffected limbs. And usually when we see any abnormalities in the "unaffected limbs," that's kind of a clue that we have a primary, we suspect primary even more strongly. But there really isn't a way to ascertain what the cause is of that lymphatic dysfunction. So more research needs to be done in this area. We have used lymphatic imaging to actually phenotype and find genotypes and we found a couple new gene variants that are caused, that causes lymphedema. We've taken family members who are asymptomatic but carry a gene mutation or gene variant. And when we image them, they have abnormal lymphatics. And so with the imaging, we've been able to actually do, to identify new gene variants that are associated with lymphedema and some of those have been published an now are being validated by other genetics laboratories around the world.

Dan Carlson:

Thank you very much for that answer. It's really definitely an interesting and emerging subject as we learn more about genetics and some of the speculation that even primary is more common than we ever appreciated. Another question that came in through the online portal, Dr. Sevick, have you been able to see any distinguishing patterns in lipedema as compared to lymphedema in your imaging studies?

Dr. Eva Sevick:

Yes, as you may know, lymphedema appears in very late stages, lipedema. And in lymphedema we see that dermal backflow. But in early stages of lipedema, we actually see more dilated lymphatic vessels. And in the very early stages, it appears to us that we have a lot of lymphatic pumping. And that makes sense, because if you have an inflammation, then the lymphatics pump. And then they wear out and then they become dilated and pump less. So this is research that we're conducting with the Lipedema Foundation and we're still in the process of doing that. But we don't typically see dermal backflow. We see lymphatic straight vessels and we see pumping, we tend to see that they appear to be dilated. And again, in early stages we'll see more pumping and in late stages it appears that it slows, and then when lymphedema sets in, we'll see that dermal backflow. I hope that answered the question.

Dan Carlson:

Thank you. So at this time we do not have any additional questions through the online portal and there are no more questions on the phone from what we can see, so again Dr. Sevick, thank you so much on behalf of Tactile Medical for sharing your passion and your research and your expertise with us all and thank you to those sites across the country that took the time to join us today on the webinar. For those of you who do have questions that come up afterward in discussion, please submit those to your Tactile product specialist as we'd like to collect those and then be able to follow up through email with Dr. Sevick.