



Lymphatic Education
& Research Network

GAPS IN THE FIELD OF LYMPHATIC RESEARCH



2024 Update for Distribution to
NIH, NHLBI, NIAMS, PRMRP/CDMRP,
ARPA-H, and CDC/CDEA

2024 Review prepared by the Lymphatic Education & Research Network (LE&RN) in collaboration with the Lymphatic Research Community for distribution to:

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Lymphatic Education
& Research Network

Since 1998, the mission of the **Lymphatic Education & Research Network (LE&RN)** has been to fight lymphatic diseases through education, research, and advocacy. We seek to accelerate the prevention, treatments, and cures for lymphedema, lipedema, lymphatic anomalies, and the continuum of lymphatic diseases. The lymphatic research field is at a turning point. For the first time in history, four federal public health agencies are focused on lymphatic biology and disease. These agencies and their leaders can take an unprecedented leap toward revolutionizing global health through their individual and collaborative actions.

The LE&RN community looks forward to playing its role in facilitating the communication between researchers and federal agencies to this end.

—William Repicci, President & CEO



We thank the global lymphatic research community who collaborated to create this document. We also thank the National Institutes of Health, who hosted the 2022 research workshop, Yet to Be Charted: Lymphatic System in Health and Disease, and all of the presenters who identified knowledge gaps and opportunities in lymphatic disease research that are included in this document.



Lymphatic research has experienced a near explosion of activity in recent years, as awareness of the importance of lymphatic mechanisms to the continuum of human biology and disease has grown. This ‘lymphatic continuum’ now easily encompasses cardiovascular disease, respiratory inflammation, obesity, autoimmune disease, and chronic transplant rejection, among many other pathologic conditions. An exponential growth in technological tools for research has made possible a similar growth in our comprehension of the molecular regulatory processes that govern the normal development and function of the lymphatic system.



—Stanley Rockson, M.D., LE&RN Co-Founder,
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CLINICAL GAPS

1. Early Diagnosis

One of the most frustrating aspects of caring for patients living with lymphatic disease is the relative lack of highly sensitive and specific non-invasive tests and blood biomarkers. Without these essential diagnostic tools, a delay in diagnosis is inevitable and often results in increased morbidity and mortality.

Imaging:

- An urgent need in novel non-invasive imaging techniques and devices.
- A need for further investigation of proposed techniques:
 - Fat-weighted whole-body MRI (e.g., Lipedema–fat to water ratio is greater than obesity)
 - Water-weighted MRI of soft tissue
 - Standardized sodium 23NA-MRI
 - MR Lymphangiogram techniques
 - Non-tracer MRL sensitive to edema and vascular morphology
 - Procedure for comparative analysis of ICG imaging of lymphatic vessels
 - Other—PET? Functional MRI?

There is a clinical need for development of *non-invasive imaging of lymphatic physiology* to aid diagnosis and understanding of all lymphatic diseases, including lymphedema and lipedema (e.g., non-invasive MRL, anatomical and sodium MRI, spectrometry MRI).

Biomarkers:

- Development of non-invasive assessment for genotyping of somatic mutations that are safer than biopsy (i.e., circulating free DNA).
- Novel biomarkers utilizing bodily fluids (urine, cyst fluid, pleural effusions, edematous fluid, saliva, and blood).

Omics:

Transcriptomics, proteomics and metabolomics, and microbiome studies:

- Define transcriptional machinery of lymphangiogenic pathways.
- Characterizing the role of the lymphatic vasculature in immune activation (outside lymphoid organs).
- Developing therapeutic lymphangiogenesis concepts.
- In solid organ transplants, would competent lymphatics enhance graft health?
- In breast cancer patients, can preemptive and targeted therapy safely accelerate lymphatic repair?
- Single Cell RNA techniques and atlas mapping
- How does the microbiome affect lymphatic health?

Standardized Definitions of Disease:

- Need objective documentation of appropriate standards for the diagnosis of lymphedema, lipedema, and other chronic edematous conditions.
- Need the study of lymphatic medicine included as a discipline taught in medical school and included as an integral part of the cardiovascular system.
- Need to better characterize the sex-specific patterns of obesity and regional adipose definition in order to understand when the pathology of lipedema is present.
- Develop and measure core outcome measures for lymphedema/lymphatic disease therapists to optimize clinical care and bring consistency to lymphedema/lymphatic disease research outcome goals.
- Awareness campaign to help providers better understand their patients with lymphatic disease.

2. Lymph Collection and Analysis

- Development of clinically relevant approaches/methods/technologies for lymph sampling and comprehensive multi-omics analysis of lymph fluid *in vitro*.
- Integrate diagnosis and targeting of lymphatic cell populations.
- Surveillance of cancer cells in lymph flow to provide earlier insights into metastases and improve cancer.

3. Lymphatic Specific Pharmacotherapies and Targeted Therapies

- We lack medications that improve lymphatic function without causing significant off-target toxic effects.
- We lack drug mechanism studies.
- How do we evaluate drug resistance (pathway investigation)?
- Inhibition of lymphatic signaling in tumor metastasis.
- Novel methods of drug delivery (viral vectors, CAR T-cell therapy).
- Is there a role for pharmacology in lymphedema prevention?
- Is there a role for pharmacology in lipedema prevention or treatment?
- Does leukotriene biology offer opportunities for related lymphatic diseases like vascular malformation, lymphangiectasia, lipedema, and others?
- Causes and interventions for complications of lymphatic disease and lymphedema (e.g., lymphatic pain, disfigurement, fibrosis, and coagulopathy).

4. Therapeutic Devices, Surgical or Interventional Radiology Treatments of Lymphedema or other Lymphatic Diseases

- Improve lymphatic microsurgery by using robotics, virtual reality, augmented reality, and/or artificial intelligence to enhance training and increase the number of lymphatic micro-surgeons.
- How can the strategy of surgical prevention of lymphedema in cancer operations or treatment of other lymphatic diseases be further refined with regard to technique?
- Longer-term outcomes need to be evaluated for lymph node transfer and lymphaticovenous anastomosis for lymphedema.
- Further research to determine which patients vascularized lymph node transfer and/or lymphaticovenous anastomosis is effective to aid in patient selection.
- Large-scale prospective clinical trials are needed to determine optimal treatment algorithms. (Some do well, but those with an inflammatory component to lymphedema do not do as well.)
- Mechanisms of immunomodulation following vascularized lymph node transplant need to be elucidated to aid development of pharmacological therapies.
- Need for large scale prospective trials analyzing outcomes of lymphedema surgery with a goal of defining optimal surgical algorithms and analyzing these outcomes long-term. Studies like this are needed for insurance coverage.
- Understanding post-surgical lymphedema prevention and long-term surveillance for current breast cancer surgical reconstruction procedures.
- How to improve and tailor Phase 2 Complete Decongestive Therapy self-management activities for the person living with lymphedema.
- Examine the role of complete decongestive therapy in rehabilitation pre- and post-surgical treatment.
- Examine the adherence and outcomes of people living with lymphedema to use of pneumatic compression devices across the lifespan.
- Novel compression device development and comparison studies with current approved devices.
- Interventions to manage lymphedema in advanced cancer and innovation in palliative care.
- Need for further development of novel techniques in magnetic resonance lymphangiography.
- Need for novel interventional radiology preventative and therapeutic approaches to lymphatic disease.
- Need for interventional radiology drug delivery options.

5. Epidemiologic and Longitudinal Data

- Need for epidemiologic studies to realistically determine the incidence and prevalence of lymphatic diseases in the US (primary and secondary lymphedema, lipedema, vascular malformations and other rare lymphatic disorders).
- We lack natural history studies for lymphatic diseases and require them for FDA clinical trials.
- More comprehensive registries are needed with better collaboration across the lymphatic community to share and analyze the data.

6. Access to Care and Global Health

- Rural and global health disparities in people living with lymphedema.
- Impact of climate change on chronic edema/lymphedema.
- Remote education/telehealth interventions to support those living remotely and rural people living with lymphedema.

7. Standards for Outcomes Measures

- Development of validated patient reported outcomes in lymphatic disease.
- Development of validated clinical outcomes (i.e., radiologic measures).

8. Patient Advocates Emphasized These Gaps

Data collected from the NIH 2022 *Yet to Be Charted: Lymphatics in Health and Disease* Research Workshop

- Improved awareness and specialized training for medical staff to recognize, diagnose, and treat lymphatic diseases.
- Improved imaging of lymphatic diseases.
- Non-invasive diagnostics and emphasis placed on establishing lymphatic medicine as a discipline.
- Need for more Centers of Excellence and need for more specialists.
- Need for primary care givers to be more educated in lymphatic diseases.
- Need for creative state-of-the-art surgical approaches.
- Need for methods of “transition of care” from pediatric to adult vascular anomaly patients and specialty centers for pediatric and adult vascular anomaly patients.
- Need a method to include psychosocial counseling for patients with lymphatic diseases that have been historically neglected.
- Identify the barriers to the routine referral of at-risk people for lymphatic disease and/or lymphedema assessment, preventative exercises, self-management education, and treatment as needed across the lifespan.

BASIC & TRANSLATIONAL SCIENCE GAPS

Basic research is often overlooked in terms of the impact and significance it can have in the development of novel therapies for diseases. Many of the advancements in the fields of cancer, cardiovascular disease, and neurology, for example, have been made due to the efforts of researchers who investigated the basic biology of those systems. Funding for this type of research for lymphatics is of the utmost importance to advance the knowledge and treatment of lymphatic diseases and lymphedema.

1. The Developmental Origin of the Lymphatic System

Including often neglected lymphatic muscle cells (where do lymphatic muscle cells originate in development?), contractile protein and ion channel expressions of lymphatics, and the regional differences.

2. Compendium of Lymphatic Biology and Physiology

Create a compendium of lymphatic biology and physiology to better understand the basic physiology of normal lymphatic tissue so that we can understand genetic and anatomic variations.

3. Quantification of Lymphatic Function

We lack an understanding of the pressures and flows in healthy and dysfunctional lymphatic networks.

- Mechanisms that maintain vessel homeostasis and role in optimizing function.
- Functional studies of human vessels, especially of healthy vessels.
- Studies to better understand the extent to which lymphatic contractile and valve dysfunction result from, or contribute to, other pathologies.
- Because it is technically difficult to study human lymphatic smooth muscle cells, this has resulted in relying on the study of lymphatic endothelial cells (LECs). We have a paucity of research on the diversity and function of lymphatic smooth muscle cells. This includes the developmental origin of lymphatic muscle cells, contractile protein and ion channel expressions of lymphatics, and the regional differences.
- Further evaluation required to understand the consequences of having or not having a normal functioning thoracic duct.
- Do primary lymphedema-causing genetic mutations affect lymphatic vessel contractility and permeability?

4. Evaluation of Lymphatic Anatomy

- How is the nervous system involved in lymphatic communication, and in which ways does nervous system innervation impact lymphatic capacity, flow, and function?
- Need to map the entire human lymphatic system in health and in disease.
- What are the main lymphatic anatomy variants of the upper extremity, lower extremities, and the body in general?
- How does the presence or absence of any anatomic variant potentially contribute to the incidence of lymphedema or other lymphatic diseases?

5. Understanding Cellular Behavior

- Explore role of lymphatic circulating tumor cells (CTCs) from various metastatic tumors to clarify their parallel, hematogenous and lymphogenous dissemination, and role of various subpopulations of lymphatic CTCs (e.g., cancer stem cells) in metastasis progression. Also, uncover the pathways and interactions between blood and lymphatic circulating cells, exosomes, and other lymph components.
- Use AI to develop models of cell behavior in a complex lymph microenvironment.

6. Need for Accurate and Widely Available Animal Models

A major gap and need in studying lymphatics is the availability of animal models that recapitulate the diverse biology and heterogeneity of human lymphatics (especially organ specific lymphatics in health and disease). For example: aspects of mouse lymphatic physiology do not apply to humans; i.e., visceral and thoracic collecting lymphatics do not pump, and mice are not exposed to chronic gravitational loads. No animal models exist for the human condition of lipedema.

7. Lymphatic Endothelial Cell (LEC) Specification

- Identify genes controlling the earliest steps of lymphatic endothelial cell (LEC) specification.
- Development of *in vitro* systems to derive LECs from human embryonic stem cells.
- Determine the plasticity of LEC phenotype and its regulation by metabolic factors.
- We need to identify relevant molecules and cells with which LECs communicate *in vivo*.
- How do mutant endothelial cells affect normal endothelial cells?
- How do complex lymphatic anomaly-causing genetic mutations affect different LEC populations and how do mutant LECs affect normal LECs?

8. The Role of the Lymphatic System in Different Organs of the Human Body in Health and in Disease

How and why do lymphatics of certain organs become dysfunctional, causing disease?

Examples of organ specific gaps in lymphatic knowledge:

- **Lymphatics of the eye** -> Understanding pathophysiology of glaucoma, retinal and optic nerve diseases, and corneal transplant rejection.
- **Lymphatics of the liver (a key organ of lymph production)** -> Understanding the molecular mechanisms of lymphangiogenesis, the role of the lymphatic system in different etiologies of liver disease, and the modulation of hepatic lymphatic system as strategy for treating liver disease and its complications.
- **Lymphatics of the heart** -> Understanding lymphatic signaling vs. lymphatic drainage function during cardiac repair following MI, does lymphatic signaling affect other cell types in the heart during development of cardiac disease, understanding the origins of injury-induced lymphatic vessels, need to identify the novel players regulating lymphatic sprouting during embryonic development.
- **Lymphatics of the GI tract** -> Understanding whether lymphatics of the GI are a conserved feature of epithelial cell niches (local microenvironment), does lymphatic intestinal stem cell communication respond dynamically to infection, inflammation, changes in nutrient availability. How do stem cells and crypt-based lymphatics regulate access of immune cells to the intestinal stem cell niche? How might stem cells regulate their lymphatic niche? How LECs sense and respond to tissue alterations, microbiome, and immune cues? What are the homeostatic LECs role in the intestine and its implication in disease?
- **Lymphatics of the kidneys** -> Understanding the evidence that promotion of lymphangiogenesis is beneficial in several kidney diseases. Studies demonstrate that there is great heterogeneity of LECs across microvascular beds, vascular cell type, developmental stages, health vs. disease states. But why is that?
 - We need to better understand the lymphatic substrates (wall problem, valve problem, or pumping problem) that results in lymphatic failure with organ dysfunction (when lymph becomes dysfunctional how does it affect the organ).
 - We need to better understand the contribution of lymphatic dysfunction to end organ dysfunction.
 - We need to develop better treatment options for patients with lymphatic failure due to organ dysfunction.

9. The Role of Lymphatics in Acute Injury, Chronic Disease, and Sex-Related Differences in Disease Outcomes

- Better understanding of the lymphatic system and lymphatic valves in obesity, lipedema, and lymphedema.
- How does aging impact lymphatic biology and function? Do cells change (i.e., button junctions increasing)?
- Special emphasis on the impact of sex on lymphatic function and dysfunction as lymphatic diseases impact women.
- How are lymphatics involved in metabolic syndrome and chronic inflammation?
- How are lymphatics involved in the development of osteoarthritis?
- How are lymphatics involved in proper wound healing and fighting infections?
- How are lymphatics involved in neurological disorders such as stroke, dementia, and traumatic brain injury?
- Lymphatic involvement in musculoskeletal injury, repair, and regeneration.
- Investigating lymphatic physiology in the context of heart failure, lung disease, kidney disease.
- Impact of the restoration of lymph flow on healing and change in disease outcomes.
- Study how inflammatory bowel disease affects lymphatic vasculature and how it is associated with immunity.

10. Bench to Bedside Technologies

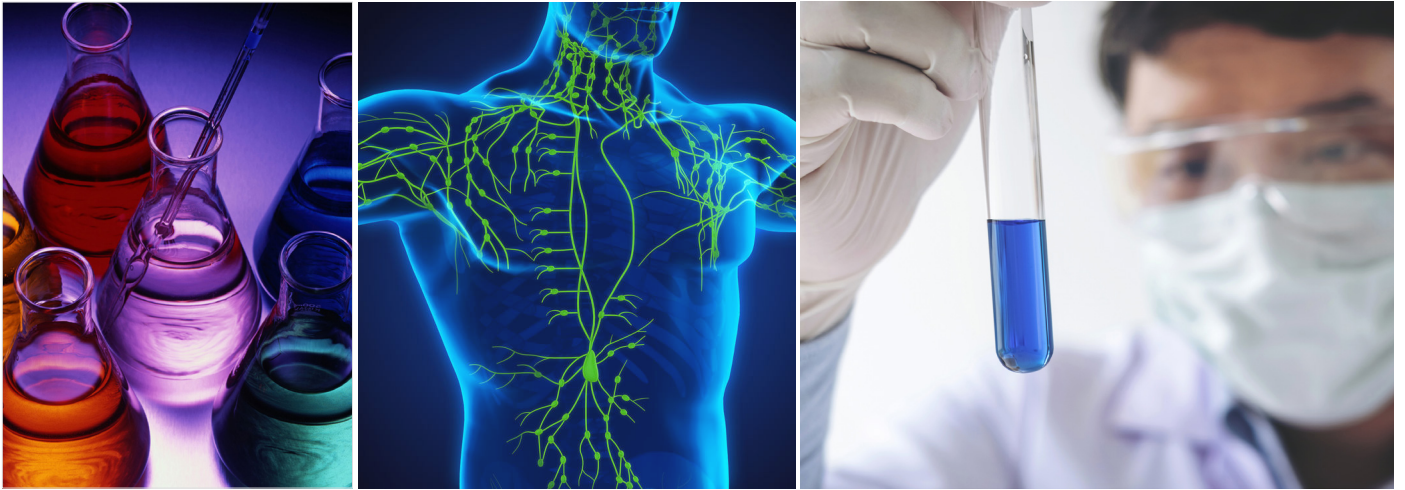
- Need to understand the gaps in pathological processes that have been identified such as inflammation and lymphangiogenesis.
- Need to identify causative mutations in novel genes underpinning primary lymphedema and complex lymphatic and vascular anomalies in humans.
- Need for development of cell therapy or biomaterials that can improve lymphatic regeneration.

BASIC SCIENCE RESEARCH: GAPS AND NEEDS FOR RARE LYMPHATIC DISEASES

- How is the microenvironment altered in complex lymphatic anomaly (CLA) tissues and does it contribute to disease progression?
- What is the full complement of CLA-causing genetic mutations? Is it more than “one hit” that causes mutations?
- What makes the responsible somatic mutations for CLAs different from cancer causing mutations?
- What is the best method to genotype patients? Can we use cell-free DNA or circulating biomarkers to diagnose CLAs and monitor response to treatments?
- Are there other circulating diagnostic biomarkers for CLA?
- How do CLA-causing genetic mutations affect different LEC populations and do mutant LECs affect normal LECs?
- Do CLA-causing genetic mutations affect lymphatic vessel contractility and permeability?
- What other genes, factors, and pathways contribute to the pathogenesis of vascular and lymphatic anomalies?
- How do mutant endothelial cells affect normal endothelial cells?
- Why do lymphatics invade bone? Is it just by chance?
- What causes coagulopathy (trapping of platelets and clotting factors) in some patients presenting with complex lymphatic anomalies?
- What causes the LECs to become spindle shaped? Do these abnormal cells affect surrounding cells? How?
- Is crosstalk between LECs and osteoclasts important for the progression of CLAs?
- We need cell lines that will allow *in vitro* work and will allow *in vivo* xenografts or allografts.
- We need genetically engineered animal models.
- We need a repository of biospecimens (blood, body fluids, and tissue).
- How does the chikungunya virus infect LECs and cause lymphatic injury, and can we create therapies against it?

CLINICAL SCIENCE RESEARCH: GAPS AND NEEDS FOR RARE LYMPHATIC DISEASES

- There are big gaps in the collaborative collection of data and biospecimens for rare lymphatic and vascular anomalies.
- We need natural history studies.
- We need broader, more comprehensive, and collaborative registries.
- Gaps in early diagnosis and therefore with delayed diagnosis comes morbidity and mortality.
- We need more non-invasive diagnostic tests and biomarkers for lymphatic and vascular anomalies.
- Drug mechanism studies: how does sirolimus or any drug used currently for CLAs work (e.g., trametinib, apelesib). How do we assess pharmacokinetics response? How do we evaluate drug resistance (pathway investigation) with all of these drugs?
- Do we need dual therapy, working on both the PIK3 and RAS pathways?
- Better evaluation of response (radiologically) and standardized outcome measures.
- Development of validated patient reported outcomes and other clinical outcomes.
- Other assessments for genotype that are safer than biopsy (i.e., circulating free DNA).
- Need federal legislation for clinical trials and genomic testing.
- Need more pilot studies for novel therapies.
- Need orphan drug designations of medications.
- Need PK analysis for pediatric dosing.
- Need novel methods for drug delivery directly into affected tissue and cells to avoid the toxic side effect profile of existing and new drugs (with accompanying animal models to test these methods).
- Need to be able to target mutant endothelial cells and reduce toxic side effects of drugs used for treatment.
- Is trametinib a better treatment for RAS pathway driven CLAs than sirolimus? What other MEK-inhibitors may be better with less side effects?
- How do mutations in genes that cause CLAs affect initial lymphatics and collecting lymphatics?
- Are there therapies besides obvious targeted therapies that would be effective at treating CLAs?
- Can the lymphatic disease be monitored by imaging (e.g., FDG-PET, fMRI, or other novel techniques)?
- Why do mutations in PIK3CA cause different phenotypes?



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