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An observational cohort study of patients with lymphatic anomalies complicated by effusions

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BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

**AN OBSERVATIONAL COHORT STUDY
OF PATIENTS WITH LYMPHATIC ANOMALIES
COMPLICATED BY EFFUSIONS**

by

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DEDICATION

I would like to dedicate this work to my parents and my sister, Melissa.

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ABSTRACT

This observational study was conducted to characterize those patients with lymphatic anomalies complicated by effusions, as well as factors that are associated with the occurrence of effusions and poor outcome. Furthermore, this research aimed to better describe the natural histories of patients presenting with effusions resulting from lymphatic disorders, including morbidity and mortality rates. Of a cohort of 230 registry patients, 145 registered patients suffering from lymphatic disorders who experienced at least one effusion during follow-up were eligible for inclusion in this study. Information was collected primarily from structured patient interviews, medical records, and interdisciplinary conference reviews and clinic visits at the Vascular Anomalies Center at Boston Children's Hospital. Among other characteristics, the age at presentation, the age at pulmonary presentation, concurrent symptoms and disease features were assessed. Of patients with effusions, 37% present with their first effusions within the first year of their lives. Of the deceased cohort of patients who presented with their first effusion in the first year of their lives, 66.67% also died within the first year of their lives. Overall mortality for those patients suffering from effusions was found to be 17% (25/145). Of the deceased cohort with a known cause of death, 53% died due to respiratory or

pulmonary complications of effusions. The median number of years from effusion presentation until death was 2.04 years. These data collected implied effusions are a critical determinant of mortality in patients with disorders of the lymphatic system.

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LIST OF ABBREVIATIONS

BCH	Boston Children’s Hospital
CCLA.....	Central Conducting Lymphatic Anomaly
GLA.....	Generalized Lymphatic Anomaly
GSD.....	Gorham-Stout Disease
KLA.....	Kaposiform Lymphangiomatosis
LM.....	Lymphatic Malformation
REDCap	Research Electronic Database Capture
VAC.....	Vascular Anomalies Center
VEGF	Vascular Endothelial Growth Factor

INTRODUCTION

Abnormal development of the lymphatic system, sometimes due to inheritable traits and sometimes due to random mutations, can cause anomalies of the lymphatic system and consequentially, lymphatic dysfunction. Although patients with lymphatic anomalies span a spectrum of different disorders, all of these patients share overlapping disease features and undergo many of the same therapies and treatments. This study was undertaken to identify those patients with lymphatic anomalies, complicated by effusions or ascites, to better understand the natural history, including the morbidity and mortality rates, of patients with these features.

Embryology of the Lymphatic System

Vasculogenesis, the differentiation of blood vessels from mesodermic endothelial precursors, begins to occur at the end of week three of embryogenesis (Pansky, 1982). These newly formed vessels further develop through endothelial sprouting and splitting. About two weeks after the hematologic vessels have developed, the lymphatic vasculature begins to form. Lymphatic channels grow out to form lymph sacs in weeks six through nine and lymph vessels develop along the major veins. By approximately the third month of development, these lymph sacs have evolved into fetal lymph nodes. There are six lymph sacs present in the embryo that ultimately develop into the lymphatic

vessels (Gray, 1985). The jugular sacs are the first to develop and form between the subclavian vein and the primitive jugular. The posterior sacs form where the iliac vein meets the cardinal vein. The retroperitoneal sac develops at the base of the mesentery vein in proximity to the suprarenal glands. Finally, the cisterna chyli grow across from the third and fourth lumbar vertebrae. The mature lymphatic system is responsible for intestinal fat absorption, immune cell transportation, and the recollection of interstitial fluid for return to the cardiovascular system.

The lymphatic vessels form alongside, but secondarily to the blood vascular system (Oliver, 2004). Furthermore, lymphatic vessels are usually not present in structures which lack the blood vascular system, such as the epidermis, hair, nails, cartilage and cornea. The lymphatics are also not present in some vascularized organs such as the retina and the brain. While one theory holds that lymphatic vessels form as outpouches of venous endothelial cells, another debates that, similar to blood vessels, lymphatic vessels form from mesenchymal clefts, which subsequently join with the venous system.

Secondary Lymphatic Disorders

Lymphatic disorders can either present at birth or be acquired later in life, such as post-operationally or through infectious diseases. While this work will focus on primary disorders of the lymphatic system, both congenital and acquired, the most common

lymphatic disorders worldwide are secondary to surgery, trauma or parasites. Secondary lymphatic disorders are presented here briefly for context.

Radical mastectomies or other surgical procedures which sever lymphatic channels can cause secondary lymphedema (Kumar, 2012). Following a mastectomy in which the axillary lymph nodes are removed, the lymphatic drainage system is disturbed, causing excess interstitial fluid to collect and present as lymphedema. This lymphedema occurs most frequently in the chest and arms and remains a higher risk for women who have had radical mastectomies for the remainder of their lives.

In addition to post-op presentation, secondary lymphedema can result from infection by parasitic agents. In lymphatic filariasis, microscopic worms, which can be transmitted between humans via mosquito bites, inhabit and clog human lymphatic channels (Centers for Disease Control and Prevention, 2014). Obstructed lymphatic channels are subject to increased hydrostatic pressure, which leads to the expansion of vessels and loss of chylous lymphatic fluid from the lymphatic system. Once in the lymphatic vasculature, these thread-like worms mature into adults, reproduce, and continue to live for approximately five to seven years. Filariasis is currently a primary cause of persistent disability globally, affecting 120 million people in seventy-three different countries across Asia, Africa, the Caribbean, South America, and the Western Pacific.

Genetics of Lymphatic Disorders

Congenital presentation, on the other hand, is sometimes detected prenatally on ultrasounds, but often times is not apparent until birth or later. Lymphatic malformations (LM), or abnormal collections of vessels that contain lymphatic fluid, result from errors that occur during fetal vascular development. It is believed that patients with these congenital anomalies express vascular endothelial growth factor (VEGF) transcripts, which are not expressed in normal lymphatics. The precise mechanism has not yet been identified and it remains unknown if any particular behaviors, foods, or medications during pregnancy cause lymphatic malformations. Lymphatic malformations typically occur without any indication of familial inheritance (Mulliken, 2013). Although it is possible in rare cases that some families may demonstrate inheritable malformations, most are believed to be caused by sporadic genetic mutations.

Some lymphatic disorders, such as Milroy's disease and lymphedema-distichiasis, are known to be inheritable and have been linked to known genetic mutations (Stanford Hospital & Clinics, 2014). Milroy's disease, a genetic, congenital lymphedema, has been associated with the FLT4 gene, which is known to be involved with early lymphatic development. Lymphedema-distichiasis is characterized by a peri-pubertal presentation and lymphedema of the bilateral lower extremities confined to the areas below the knees. Affected patients also present with a second row of eyelashes. Mutations in the FOXC2 gene have been associated with this disorder. The group at the Vascular Anomalies Center at Boston Children's Hospital was able to identify a somatic mutation in PIK3CA

as the genetic association with CLOVES, a non-inheritable, complex disorder that also involves lymphatic dysfunction (Kurek, 2012). CLOVES patients exhibit different combinations of congenital lipomatous overgrowths, vascular malformations, epidermal nevi, scoliosis, and other skeletal dysfunction. While significant, this discovery did not provide much insight into morbidity and mortality rates or the natural history of CLOVES and other vascular malformations. However, identifying this mutation that is associated with not only CLOVES, but other vascular malformations, could guide the search for more efficient treatment and perhaps earlier detection in the future.

Complications of Lymphatic Anomalies

Although lymphatic malformations can develop anywhere on the body, they are most often found just beneath the skin in the neck and the axilla (Rockson, 2011). They result in edema, and in some cases, significant enlargement of soft tissues or bones. Swelling of the lymphatic malformation will also often result if the patient experiences infection. Dysfunction or disruption of small peripheral lymphatics results in the accumulation of lymphatic fluid, which dilates the lymphatic channels and causes lymphedema (Greene, 2014). This, in turn, produces changes in the skin, pain, scarring, and leakage. When cutaneous, serosal, or intestinal leaks occur, patients become susceptible to opportunistic infections. Leakage can account for IgG loss and can cause more serious repercussions, such as immunodeficiency and protein loss, which results in malnutrition and damage to the innate barrier against infection the skin provides. These

patients are not able to efficiently fight or clear infections due to their initial lymphatic anomalies.

Functional lymphatic systems use peristalsis and negative pressure gradients created by inspiration to drain lymph against the force of gravity (Rockson, 2011). While the presence of a lymphatic anomaly does not always cause an effusion, failure of the lymphatic system can cause the collection of fluids around vital organs, such as pleural effusions, pericardial effusions, or ascites. When dysfunction occurs distal to the cisterna chyli, fluid appears straw-colored, but when dysfunction occurs above the cisterna chyli, lipid converts this fluid to chyle. Tissue destruction, including bone loss, can result from reflux. Thrombocytopenia and bleeding also present as common risks due to resultant platelet binding. Additionally, lymphaticovenous anomalies often allow for bleeding into the lymphatic system.

Overview of Lymphatic Anomalies and Malformations

The spectrum of lymphatic malformations and anomalies diagnoses includes cystic lymphatic malformations (LM), generalized lymphatic anomalies (GLA), Gorham-Stout disease (GSD), central conducting lymphatic anomalies (CCLA), and kaposiform lymphangiomatosis (KLA). Regardless of the diagnoses made, the same group of therapies are applied across these disorders. Currently, there are no known preventative measures for these anomalies, but treatment options include sclerotherapy, surgical resection, embolization, thoracic duct procedures and medication.

Sclerotherapy closes cysts, induces clots, and causes shrinkage of a vascular malformation (Boston Children's Hospital, 2014). During the procedure, which normally takes approximately three to four hours, a sclerosant is injected directly into abnormal blood vessels by an interventional radiologist. Contrast material and special x-ray equipment are used to allow the doctor to clearly visualize the malformation that needs to be injected. Complication risks include damage to adjacent tissues, including nerve injury, numbness and a small risk of blood clot. Embolizations, also performed by interventional radiologists, block blood vessels in order to decrease flow through the vasculature to a lymphatic malformation. This is an image-guided, minimally invasive procedure intended to close up refluxing lymphatic channels and can be used to target the thoracic duct itself. Surgical resection could be a viable treatment option in situations where portions of lesions could be removed without risk to function or any vital organs. Most frequent complications include prolonged healing, infections, prolonged leaking, and re-expansion after excision. A host of medications, such as Sirolimus, Octreotide, Interferon, bisphosphonates, Thalidomide, Vincristine, and corticosteroids can also be used to control lymphatic disorders. Finally, dietary modifications and compression can be effective systemic therapies and help to control some symptoms, such as edema and effusions.

LMs are lesions of irregular or deformed lymphatic vasculature with fibrotic walls consisting of both smooth and skeletal muscle (Mulliken, 2013). The lesions are often found in the head or neck and can be localized or diffuse. Generalized lymphatic

anomaly describes a condition manifesting with both multifocal LM and skeletal involvement. This rare disorder presents with splenic cysts, soft tissue edema, lytic bone lesions, and, generally, chylous leaks. While many physicians and institutions may use the term lymphangiomatosis to classify GLA, the team at Boston Children's Hospital feels this categorization implies malignancy and prefer to refer to the condition as GLA. Central conducting lymphatic anomalies can affect the size and shape of central conduction channels with detrimental affects on channel function, resulting in lymphatic leaks or reflux.

Gorham-Stout disease, an exceptionally rare skeletal disease often referred to as "disappearing bone disease," is characterized by osteoclast-driven osteolysis, perhaps signaled by adjacent abnormal lymphatics (Lala, 2013). Soft tissue changes adjacent to Gorham-Stout bone lesions caused by abnormal lymphatic channels precede bone loss. While the condition's etiology remains unknown and there is no recognizable pattern of inheritance, it classically presents as localized osteolysis. A very common presentation of Gorham-Stout disease is the inability of a fracture to heal after trauma. Typically, the most affected areas of the body include the shoulder, the pelvic girdle, and the skull. In 2005, Duffy and colleagues reported that approximately 17% of patients suffering from Gorham-Stout Disease with involvement in the ribs, shoulder, or upper spine also experience expansion into the thorax. Without any treatment, the grave ramifications of resulting chylothoraces can raise the mortality rate in this patient population to about 64% (Duffy, 2005).

As set forth by Lala and colleagues, despite the fact that both GSD and GLA with extension into the bone can be classified as lymphatic disorders of the bone, some notable differences exist (Lala, 2013). Additionally, many radiologic similarities exist between GLA and GSD. However, while GSD causes cortical destruction of the affected bones, GLA does not. GLA is a disorder of multiple systems with dilated lymphatic channels. While it also typically affects bones, generalized lymphatic anomaly presents as a much more diffuse disorder relative to Gorham-Stout disease. Although the two share some common traits, GLA involves a more considerable number of bones along with greater incidence of visceral disease and cystic lymphatic malformations (Lala, 2013). Effusions are notably a leading factor of mortality across the spectrum of these diagnoses. Effusions can range from asymptomatic to causing symptoms such as shortness of breath, or dyspnea, chronic cough, oxygen dependence, and pain.

Kaposiform lymphangiomatosis, which is frequently studied due to its poor prognosis, has been described by Croteau and colleagues as a clinicopathologically distinct lymphatic anomaly. Kaposiform lymphangiomatosis is classified as a vascular tumor, which grows from a preexistent malformation of a lymphatic channel (Croteau, 2014). KLA presents with localized regions of spindle cells and coagulopathy. These patients often suffer from hemorrhagic effusions and exhibit significant retroperitoneal and mediastinal involvement. To confirm a diagnosis of KLA, biopsy is necessary. Furthermore, “the intrathoracic component is most commonly implicated in morbidity and mortality; however, extrathoracic disease is frequent, indicating that KLA is not

restricted to pulmonary lymphatics. The mortality rate of KLA is high despite aggressive multimodal therapy,” (Croteau, 2014). These observations led to the hypothesis that pulmonary involvement of lymphatic anomalies may predict poor prognosis, despite multisystem involvement.

Interdisciplinary Care - The VAC model

Founded in 1976, the Vascular Anomalies Center (VAC) at Boston Children’s Hospital provides specialty expertise and leading-edge therapies for children affected with vascular anomalies (Fishman, 2014). In a lecture given at Boston Children’s Hospital, Dr. Steven Fishman, a co-director of VAC, described the initial stages of the center and how its database began as a rolodex of patients with no efficient system to effectively recall and compare patients, other than from memory. Today, the VAC team consists of twenty-five doctors spanning seventeen different specialties. This diverse team takes an interdisciplinary approach to treatment and care, meeting each Wednesday evening to review the charts and records of patients from around the world. During this conference, any available photographs, medical histories, imaging, and pathology slides are reviewed to give formal diagnoses, recommend treatment options, and provide answers to referring physicians and patients’ families. The team regularly communicates with other institutions to share their expertise and familiarity of these diagnoses. Patients are also seen with this interdisciplinary, patient-centered approach every Friday in VAC clinic. Although the database has grown significantly and other facilities across the

country have developed vascular anomaly centers of their own, current information continues to lack sufficient outcomes data to answer many questions relating to the longterm natural progression of these diseases.

The Lymphatic Anomalies Registry

The Lymphatic Anomalies Registry project is an observational study regarding complex lymphatic anomalies, which was established to improve current outcomes data (Boston Children's Hospital, 2014). Due to the lack of information available about the longterm effects of these malformations and effective therapies, this study aims to determine the best treatments and expected outcomes for patients suffering from these conditions. The primary goals of the Lymphatic Anomalies Registry, as laid out in IRB protocol, are: (1) to characterize the heterogeneity of lymphatic disorders, including demographics, presentation, and complications; (2) to identify factors that are associated with the occurrence of complications including effusions, coagulopathy, ecstatic draining veins, prior infections, visceral involvement, bone involvement, and development of cardiopulmonary symptoms; (3) to identify factors associated with poor outcome and use them to develop "staging" of lymphatic anomalies; (4) to describe the natural history of lymphatic anomalies, including morbidity and mortality; (5) to describe the therapies (medical and procedural), adverse events and responses to therapy in patients with lymphatic anomalies; and (6) to pilot quality of life, functional assessment and pain scoring tools in this patient population. Secondary objectives include: (1) estimating the

proportion of time that patients with lymphatic anomalies have affected offspring and (2) assessing for correlations of pregnancy complications or medications taken during pregnancy with the development of lymphatic anomalies. Additionally, this registry serves as a clinically-annotated dataset for correlative biomarker studies and clinical trials.

Information is obtained through structured patient interviews and reviews of medical records. After an initial interview, annual follow-ups are conducted to note any new developments. Interview questions cover diagnosis, prenatal history, pathology, disease features, genetics, fertility, medical therapies, procedural history, and functionality.

These complex lymphatic anomalies and the effusions they cause are associated with a range of diagnoses being studied in the Lymphatic Anomalies Registry project. However, across the spectrum, many patients with vascular anomalies suffer from effusions or ascites, most notably patients with Gorham-Stout disease and GLA. This investigation focusing on lymphatic anomalies patients suffering from effusions is an observational study using data from the Lymphatic Anomalies Registry, which sets out to determine natural histories for those patients suffering from effusions and to compare that data set to the results of KLA patients with similar pleural and pericardial effusions and ascites.

METHODS

IRB

The Lymphatic Anomalies Registry worked across two independent IRB protocols, which allowed for the cross-reference of a retrospective patient cohort. When the Lymphatic Anomalies Registry project began, the IRB permitted the review of retrospective patient data and inclusion of this retrospective cohort in the registry. However, when it was clear that a limitation of the retrospective reviews was loss of patient follow up and current outcomes data, approval to continue to follow patients prospectively was sought.

Objectives

Fundamentally, this cohort study focusing on lymphatic anomalies patients suffering from effusions intends to: (1) identify characteristics of those patients who suffer from effusions, including gender, age at presentation, and age at pulmonary presentation; (2) identify factors that are associated with the occurrence of effusions including concurrent symptoms and disease features; (3) to identify factors associated with poor outcome; (4) to describe the natural history of patients presenting with effusions resulting from lymphatic disorders, including morbidity and mortality; (5) to describe medical and procedural therapies and responses to these therapies in patients

with effusions; and (6) to gauge quality of life, functionality, and pain in this patient cohort.

Accrual and Data Collection

The Vascular Anomalies Center at Boston Children's Hospital consults on over 100 patients with complex lymphatic disorders each year, both in person in weekly interdisciplinary clinics and from afar in weekly interdisciplinary conference reviews. Eligible patients with vascular malformations, tumors or overgrowth syndromes with a significant lymphatic component were recruited to participate in this study. Patients who participated in the retrospective version of the study, who were believed to be highly interested candidates, were also contacted to participate again and be followed prospectively. We explained the goals of the research being conducted, who is conducting the research, how individuals were selected for this research, the risks of this research study, the benefits of this research, and the rights of the research participant while obtaining consent from patient participants.

After consent was obtained, we asked participants to sign a medical record release form for permission to release outside medical records and for a member of our team to contact the doctor who primarily directs the care of the patient's condition. We collected available blood tests, imaging, medications, doses, and treatments. Subsequently, we completed structured interviews with either patients or patients' guardians, either by phone or during a clinic visit at the Vascular Anomalies Center in Boston. Interview

questions focus on overall diagnosis, past and present symptoms, disease features, medical therapies, procedures, topical therapies, pain, functionality, and prenatal and family histories. If any discrepancies exist between information gathered from medical records and patient interviews, the information found in official medical records is used over information from patient recall. We continue to follow these patients and conduct annual follow-up questionnaires regarding the ongoing management of patients' vascular anomalies, as well. These continue until patients withdraw from the study or are lost to follow-up.

Boston Children's Hospital is the coordinating center, and houses all data in a secure electronic database. There is always the risk of unintentional loss of privacy or breach of confidentiality during the process of requesting, storing, or transporting patient data. This risk was reduced by de-identifying all data and assigning each patient a code that identifies his or her unique responses to interview questions. This code does not contain any part of the patient's name, address, medical record number, or birth date. Only the research team at Boston Children's Hospital has access to identifiable patient information, which is kept in a separate internal REDCap database, available only to researchers at Boston Children's Hospital. All de-identified patient medical information is kept in the external REDCap database, which will eventually be expanded to include other clinical centers and research sites.

REDCap Data Capture

All acquired patient data is compiled and entered into software known as Research Electronic Data Capture (REDCap). REDCap is a clinical translational research tool intended for expedient collection and redistribution of electronic data (Harris, 2009). A study-specific database was created using questions selected by the research team. As this study becomes a multi-center registry, REDCap will allow researchers to access and input centralized data in compliance with standard HIPAA practices. REDCap is very valuable in terms of data export and lends itself to a well-organized, systematic study setup. REDCap presents a much simpler data storage method than Excel or other alternatives.

Data Analysis

The 145 registered patients comprising the cohort of patients with lymphatic disorders who experienced at least one effusion symptom during follow-up were eligible for data analysis. The median amount of time for patient follow-up was about nine years. Some patient characteristic information was gathered from the internal REDCap database, such as the number of patients interviewed and patient gender, and all other patient characteristics were gathered from the external database. The age at presentation was calculated as the age at first presentation of any disease feature or symptom. The age at pulmonary symptom presentation was calculated as the age at first pulmonary symptom presentation, including cough, shortness of breath, or effusion. Both

cardiopulmonary symptoms and effusion symptoms were considered for presentation of first pulmonary symptom. In either case, age at presentation could be less than zero because some patients presented signs of disease in utero. The follow-up time was calculated from first presentation until the date of last follow-up or the date of death. Overall survival curves were generated using the methods of Kaplan and Meier. Point estimates are presented +/- standard errors according to the methods of Peto. For Figure 1, in each of the three curves, the patient cohort analyzed remains the same, but the starting time differs for each curve.

RESULTS

The 230 total patients entered in the entire Lymphatic Anomalies Registry represent forty-two different states within the United States and twenty-three distinct countries. GLA was diagnosed with the highest frequency and at about 38% (87/230). Approximately 23% (54/230) of all patients reported a significant family history. Significant family history was defined as a family member with a vascular anomaly or a diagnosis of a bleeding or clotting disorder. However, it should be noted this statistic is based on self-reported patient information and the diagnoses of patients' relatives were not confirmed with medical records or by a clinician of the Vascular Anomalies Center at BCH. Of all 230 patients, 10% first presented within the first year of life, an additional 27% first presented within the first decade of life, and an additional 38% first presented within the first twenty years of life.

As can be seen in Table 1, attributes for just those patients suffering from effusions were analyzed, including characteristics such as gender, diagnosis, descriptive diagnosis, age at initial presentation, age at pulmonary presentation, and overall mortality. These statistics helped determine the age and gender of patients, as well as the distribution of patients across different diagnoses. The descriptive diagnoses serves to identify what features affected each patient and the distribution of disease involvement.

With 51% of patients with effusions being female, gender does not seem to be an increased risk factor for effusions. All patients received a descriptive diagnosis of

effusions, as that was the primary qualifying disease feature for inclusion in this study. Aside from effusions, the most common disease features were microcystic or macrocystic lymphatic malformations. Bone involvement and visceral involvement (defined as liver, kidney, or spleen involvement) were also disease features which presented with a notable frequency at 50% and 46%, respectively. Consistent with the statistics for the entire Lymphatic Anomalies Registry, GLA represented the diagnosis with the highest frequency among the cohort of patients with effusions. KLA patients have not yet been entered into the registry and therefore, represent 0% of this cohort. The median length of follow-up for effusions patients is approximately 8.6 years. This length of follow-up for current patients is potentially underestimated due to the rate of early mortality in this patient cohort.

Table 1. Patient Characteristics of Patients with Effusions. (n=145)

*p25=25th percentile; p75=75th percentile

Characteristics	n (%)
Patients with completed interview	47(32)
Female	74 (51)
Descriptive diagnosis	
Effusion	145 (100)
Bone involvement	72 (50)
Visceral involvement (liver, kidney, spleen)	67 (46)
Soft tissue mass	19 (13)
Overgrowth/Asymmetry	17 (12)
Capillary malformation/Stain	15 (10)
Venous malformation	3 (2)
Arteriovenous malformation	4 (3)
Lymphatic malformation	105(72)
Lymphedema	27 (19)
Lymphatic vesicles	16 (11)
Other	5 (3)
VAC Diagnosis	
Generalized lymphatic anomaly	68 (47)
Gorham–Stout	18 (12)
CLOVES	7(5)
Central conducting lymphatic anomaly	16 (11)
Kaposiform lymphangiomatosis	0
Lymphatic malformation	12 (8)
Other	24 (17)
Age at presentation; median (p25, p75*) in years	0.5(0, 9.0)
Age at pulmonary presentation; median (p25, p75*) in years	4.9(0.1, 12.1)
Overall mortality	25 (17)
Length of follow-up; median (range) in years	8.6(0.1,43.1)

Table 2 shows the number of patients with each presenting feature as a percentage of the total number of patients with effusions. Presenting features include pain, musculoskeletal dysfunction (including scoliosis, fractures, leg length discrepancies, osteopenia, etc.), overgrowth and asymmetries, visible anomalies, skin

lesions, edema, shortness of breath, effusions, bleeding, thrombosis, infections, chylous leaks, and abnormal labs. Presenting features manifesting most frequently were edema/swelling, pain, and shortness of breath.

Table 2. Presenting Features of Patients with Effusions. (n=145)

Presenting signs/symptoms	n (%)
Asymptomatic	20 (14)
Pain	27 (19)
Musculoskeletal dysfunction	4 (3)
Overgrowth/Asymmetry	12 (8)
Visible anomaly	21 (14)
Skin lesion	11 (8)
Edema/swelling	30 (21)
Shortness of breath	26 (18)
Effusion	56 (39)
Fracture	7 (5)
Bleeding/hemorrhage	0
Thrombosis	0
Abnormal laboratory study	1 (1)
Infection	9 (6)
Chylous leak	0
Other	35 (24)

Additionally, the concurrent symptoms in patients with effusions at the time of pulmonary presentation, including dysmorphology, are summarized in Table 3.

Dysmorphologies are presented relative to the total number of patients with hand or foot abnormalities, not the total 145 patients with effusions.

At the time the first pulmonary symptom presented, 88 patients (61%) had cardiopulmonary symptoms and 128 patients (88%) had effusions. Patients with

effusions and cardiopulmonary symptoms act as positive controls and some patients present with both effusions and cardiopulmonary symptoms, including cough and shortness of breath. Infections related to vascular anomalies (37%), pain (30%), and edema (26%) are the most notable concurrent symptoms at the first presentation of pulmonary symptoms. These data are taken from a combination of both medical records and patient interviews.

Table 3. Concurrent Symptoms in Patients with Effusions at the First Presentation of Pulmonary Symptoms. (n=145)

Concurrent symptoms at the first time of pulmonary presentation	n (%)
Bleeding	14 (10)
Coagulopathy	9 (6)
Edema	37 (26)
Infection-related to vascular anomaly	53 (37)
Lipomatous overgrowth	6 (4)
Malignancy	1 (1)
Functional limitations	6 (4)
Musculoskeletal involvement	30 (21)
Neurologic complication	8 (6)
Nutrition	16 (11)
Pain	43 (30)
Thromboembolism	6 (4)
Other	88 (61)
Dysmorphology	
Facial asymmetry	4 (3)
Macrocephaly	4 (3)
Epidermal nevus	2 (1)
Hand abnormality	6 (4)
Syndactyly	0
Polydactyly	0
Macrodactyly	3 (50)
Other	5 (83)
Foot abnormality	7 (5)
Syndactyly	1 (14)
Polydactyly	1 (14)
Macrodactyly	4 (57)
Delta-shaped foot	0
Sandal toe gap	1 (14)
Other	5 (71)

In Table 4, the age at first presentation of pulmonary symptoms in patients with effusions was recapped. Within the first decade, 69% present with their first pulmonary symptoms. Within the first twenty years of life, this statistic rises to 92%.

Table 4. Age at First Presentation of Pulmonary Symptoms in Patients with Effusions. (n=145)

Age at presentation	Number of patients	%
Before birth	13	9
0-1 years	40	28
2-10 years	46	32
11-20 years	34	23
21+ years	12	8
Total	145	100

Table 5 outlines the efficacy of medical therapies including antiplatelets, beta blockers, bisphosphonates, corticosteroids, Interferon, Octreotide, Sildenafil, Sirolimus, Thalidomide, and Vincristine. Percentages presented in this table sum to greater than 100% because many of these patients received more than one medical therapy. The number of patients who responded are presented as percentages of the number of patients treated with each medical therapy. Most patients in this cohort who received medical therapies received either bisphosphonates, steroids, Interferon, Octreotide, or Sirolimus.

Table 5. Efficacy of Medical Therapies in Patients with Effusions.

	Number of Patients with Effusions Treated	Number of Patients with Effusions who Responded n (%)
Antiplatelet	3	1 (33)
Beta blocker	6	6 (17)
Bisphosphonate	24	8 (33)
Steroid	30	7 (23)
Interferon	31	11 (35)
Octreotide	36	7 (19)
Sildenafil	1	0
Sirolimus	20	6 (30)
Thalidomide	9	1 (11)
Vincristine	5	1 (20)
Other	47	16 (34)

Similarly, Table 6 compares the efficacy of decortication, embolization, laser therapy, pleurodesis, surgical resection, sclerotherapy, and thoracic duct reconstruction in patients with effusions. The median (range) number of procedures applied per effusion patient and the number of effusion patients who responded to that procedure are also represented. The number of patients who responded are presented as percentages of the number of patients treated with each procedural therapy. Most patients of this cohort undergo surgical resections or pleurodeses. However, patients appear to respond most frequently to surgical resections, pleurodeses, and sclerotherapies. These procedures also lead to multiple procedures, implying these responses are only short-term.

Table 6. Efficacy of Procedural Therapies in Patients with Effusions.

*p25=25th percentile; p75=75th percentile

	Number of Patients with Effusions Treated	Median (p25,p75*) of Number of Procedures per Patients with Effusions	Number of Patients with Effusions who Responded n (%)
Decortication	11	1 (1, 1)	0
Embolization	14	1 (1, 1)	3 (21)
Laser (CO2/PDL)	4	1 (1, 1.5)	2 (50)
Pleurodesis	41	1 (1, 2)	24 (59)
Resection	47	1 (1, 2)	29 (62)
Sclerotherapy	25	1 (1, 2)	15 (60)
Thoracic duct reconstruction	23	1 (1, 1)	11 (48)
Other	103	1 (1, 3)	36 (35)

In Table 7, subject outcome was measured using mortality, recurrent effusions, development of new lesions, worsening pulmonary function tests (PFTs), thromboembolic complications, infections requiring antibiotics, persistent neurologic deficits, malignancies, and worsened quality of life (as reported by the parents of patients with effusions or patients with effusions themselves). It is shown that a high number of patients experience not just one effusion, but recurrent effusions. More than half of these patients also experience the development of new lesions and infections requiring treatment with antibiotics.

Table 7. Outcomes Summary at Any Time Through Most Recent Contact. (n=145)

Outcomes	Number of Patients with Effusions who Experienced this Outcome at least Once n (%)
Mortality	25 (17)
Recurrent effusion	136 (94)
Development of new lesions	79 (54)
Worsening PFTs	9 (6)
Thromboembolic complication	20 (14)
Infection requiring antibiotics	95 (66)
Persistent neurologic deficit	26 (18)
Malignancy	2 (1)
Worsened quality of life (patient report)	21 (15)

Finally, in Table 8, the cohort of deceased patients was outlined, including their diagnoses, ages at effusion presentation, ages at death, and the cause of death (if available). GLA patients represented the highest percentage of deceased patients and CCLA patients represent the least percentage of the deceased cohort. Slightly greater than one-third, or 36%, of deceased patients presented with their first signs of effusions within the first year of life. Of this subset of patients that presented with effusions during their first year of life, 66.67% (24% of the entire deceased cohort) also died within the first year of life. Of the total deceased cohort, 65.22% died within five years of effusion presentation and 48% of the total cohort within the first ten years of life. The median number of years from effusion presentation until death was 2.04 years. Of the patients with a known cause of death, 53% died due to respiratory or pulmonary complications of effusions.

Table 8. Deceased Cohort Summary Table. (n=25)

VAC Diagnosis	Age at effusion	Age at Death	Cause of Death	Retrospective Interview with family
GLA	3 months, 11 days	4 months, 29 days	Respiratory failure with pleural effusions and end organ failure	Y
GLA (Mediastinal LM with spleen, liver, lung pleura involvement)	15 years, 8 months	19 years, 1 month	Cardiopulmonary failure	Y
GLA	13 years, 7 months	18 years, 7 months	"Progressively weak lungs", respiratory failure	Y
GLA	17 years	21 years, 10 months	Sepsis, pleural effusions and pulmonary HTN	Y
Gorham Stout	4 years, 8 months	18 years, 7 months	Unknown- at time of death required ICU level care with chylous pleural effusions, presumed respiratory failure	N
Gorham Stout	9 years, 10 months	9 years	Died suddenly from bilateral chylothoraces	N
Gorham Stout	12 years	20 years, 6 months	unknown	N
Gorham Stout	3 years, 10 months	5 years, 7 months,	Sepsis with seizure and stroke	N
Gorham Stout	8 years, 5 months	16 years, 6 months	"general immune response and bleeding out" per mother. Requested extubation	Y
GLA	7 years	16 years, 3 months	Respiratory failure with pleural and pericardial effusions	N
GLA	2 months	Unknown (MD reported pt deceased but date unknown)	unknown	N

GLA	30 years	50 years	unknown	N
GLA	0 years (prenatal, 16 weeks gestation)	7 months	Recurrent effusions refractory to pleurodesis. Bradycardia and cardiac arrest at death.	N
GLA	2 years, 6 months	4 years, 5 months	unknown	N
GLA	8 years, 4 months	10 years, 4 months	Septic shock	N
GLA	0 years	6 months	unknown	N
GLA	1 year, 8 months	2 years, 9 months	Sepsis	N
GLA	8 years, 11 months	unknown	unknown	N
GLA	14 years, 5 months	35 years, 7 months	s/p bilateral lung transplant, respiratory failure.	Y
LM (Lymphatic malformation and possible genetic anomaly such as Noonan's)	0 years	4 months	Unknown, admitted to NICU until death	N
LM	0 years	24 years, 5 months	pneumonia and left ventricular dysfunction, respiratory failure	N
CCLA	6 years, 3 months (ascites)	8 years, 11 months	Lymphangiosarcoma	N
CCLA (also Noonan's)	3 months	5 months	Sepsis	N
LM	3 months	1 year, 11 months	unknown	N
Bilateral pleural effusions and ascites	1 month, 28 days	8 months	Renal failure and dehydration	Y

Figure 1 displays a Kaplan-Meier curve of overall survival generated for the effusions patient cohort. The curve starts from three different time points, including the time of first presentation (from the first date of any presenting symptom or disease feature to last follow-up or death), the time of first pulmonary symptoms (from the date of first reported pulmonary symptoms, including shortness of breath, cough, effusions, etc., to last follow-up or death), and the time of VAC diagnosis (from the date of diagnosis of lymphatic anomaly to follow-up or death). According to the curve, patients have the sharpest decline in overall survival after VAC diagnosis. Patients presenting initially with pulmonary symptoms statistically level out at about a 40% overall survival rate approximately five years sooner than those patients who first present with general disease features. It is important to note that overall survival represented by these curves captures not only vascular anomaly related, but all other mortality, as well as loss of follow-up. Additionally, overall survival is shown by years elapsed from each specific time point and not patient age at each specific time point.

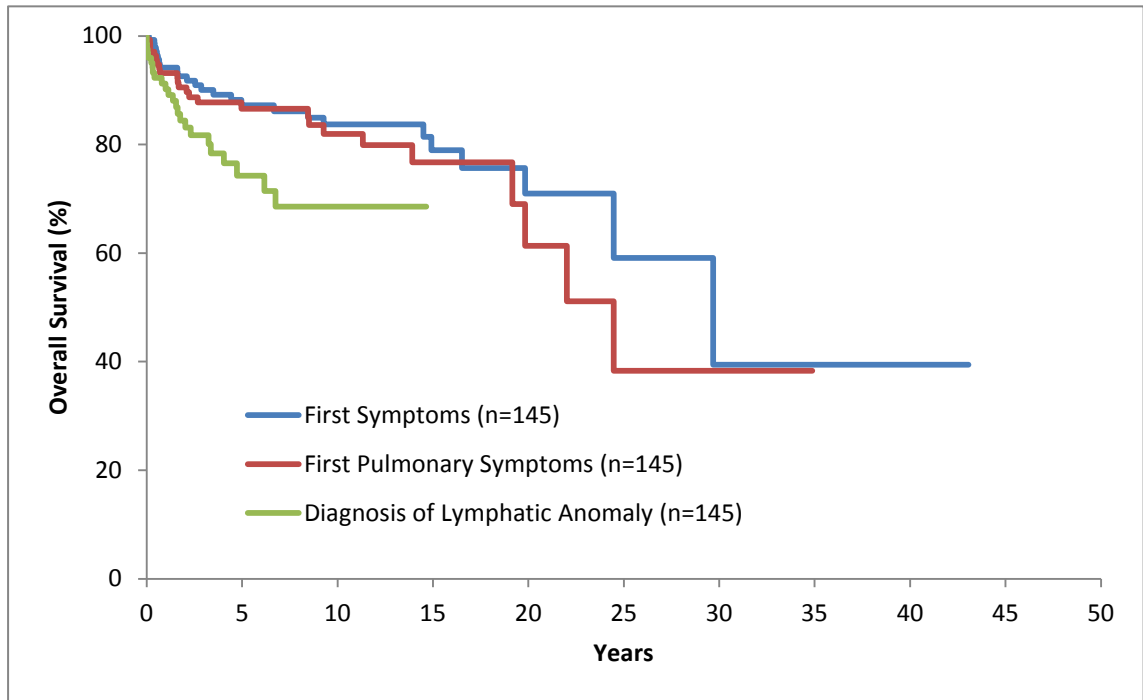


Figure 1. Kaplan-Meier Curve: Overall Survival of Patients with Effusions by Time Points

Figure 2 displays a Kaplan-Meier curve of the 133 patients of the 145 in the effusions cohort who received some form of treatment. Eleven patients who did not have any medical therapy or procedure and one patient who had a postmortem diagnosis were excluded. Curves demonstrate overall survival by treatment types, including procedure only, medical therapy only, and both procedural and medical therapies.

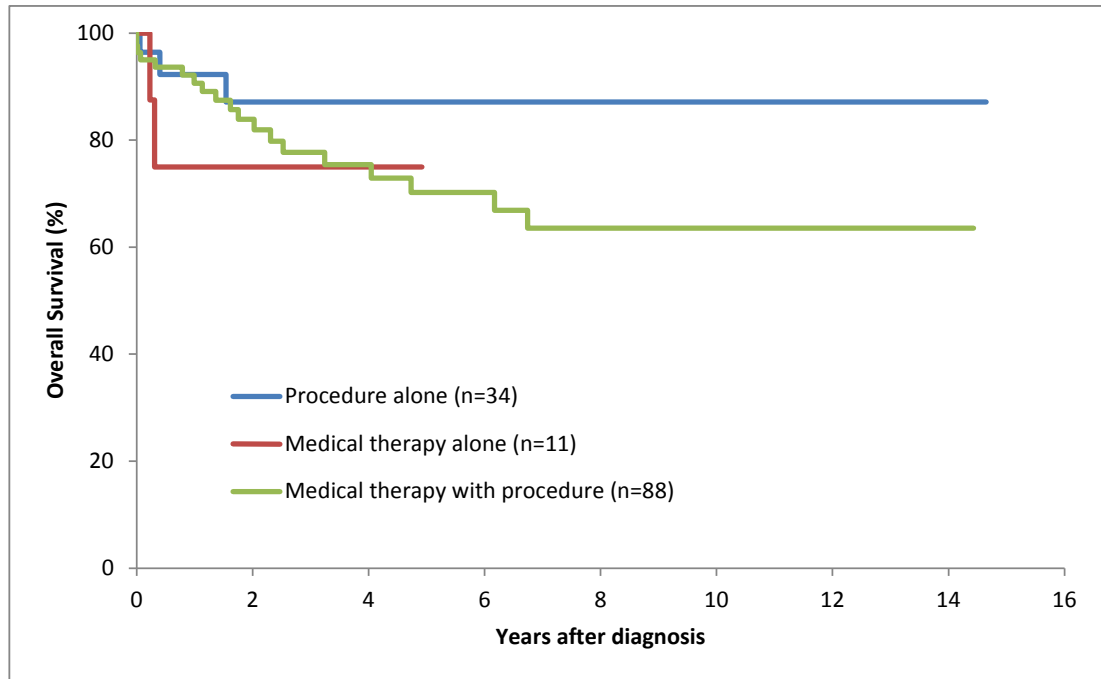


Figure 2. Kaplan-Meier Curve: Overall Survival of Treated Patients with Effusions by Treatment Types

Table 9 compares relative percents by diagnosis of patients in the registry, patients with effusions, deceased patients with effusions, and deceased patients by effusion and diagnosis. Other than the infrequency of effusions in CLOVES patients, this table demonstrates that although the VAC registry team prioritized the addition of effusions patients into the REDCap database for this study, the relative percentages of patients with effusions across each diagnoses are not widely discrepant with the percentages of patients in the registry across each diagnosis.

Table 9. Relative Percents by Diagnosis of the Entire Registry vs. Patients with Effusions.

	Patients in the Registry	Patients with Effusion	Deceased Patients with Effusions	Deceased by Effusion + Diagnosis
Total	230	145	25	
GLA	87/230 (37.83%)	68/145 (47%)	14/25 (56%)	14/68 (20.59%)
GSD	39/230 (16.96%)	18/145 (12%)	5/25 (20%)	5/18 (27.78%)
LM/other	48/230 (20.87%)	36/145 (25%)	4/25 (16%)	4/36 (11.11%)
CCLA	18/230 (7.83%)	16/145 (11%)	2/25 (8%)	2/16 (12.50%)
CLOVES	38/230 (16.52%)	7/145 (5%)	0/25 (0%)	0/7 (0.00%)

DISCUSSION

According to the information gathered, it was determined that the primary characteristics of patients suffering from effusions include a median age of six months at presentation of first symptoms and a median age of approximately four years and eleven months at presentation of first pulmonary symptoms. Whether the patients were males or females was not found to have an impact on patients' likelihoods to suffer from effusions.

Factors that were the most associated with the occurrence of effusions proved to be presenting symptoms of edema/swelling, pain, and shortness of breath. The percentage of patients suffering from edema and pain increased between the time of initial presentation of symptoms and initial presentation of pulmonary symptoms. Since these symptoms already represented the highest percentage of presenting features, it is implied that identifying these features could prove to be a valuable prognostic tool for clinicians treating patients at risk for developing effusions. Interestingly, although infection was only a presenting feature for 6% of the patient cohort, 37% of patients experience concurrent infections related to their vascular anomalies at the first presentation of pulmonary symptoms. Consistent with this finding at first pulmonary presentation, 66% of patients experienced infection requiring antibiotic treatment at least once. Furthermore, 47% of the deceased cohort of patients with a known cause of death died of sepsis or infection.

Overall mortality rate within this cohort of patients with effusions was found to be 17%. While referral bias could have potentially caused this statistic to be overestimated, it could also be underestimated due to loss of follow-up and difficulty in definitively confirming deceased status. Early presentation of effusion corresponds strongly with poor outcome. Of those patients who presented with effusions within their first years of life, 66.67% also died before their first birthdays. Overall, 48% of the deceased patients died before their tenth birthdays. Only 8% of patients presented with pulmonary symptoms after the age of twenty.

In terms of medical therapies, Interferon, bisphosphonates, antiplatelets, and Sirolimus appear to be the most effective, with the highest number of patients responding to these therapies. However, many incomplete medical records and frequent loss of patient follow-ups make it very challenging to draw any significant conclusions from this information. It was often difficult to definitively determine the efficacy of medical therapies from retrospective reviews of medical records. Additionally, the sample size for patients who received antiplatelet therapy is too small to draw any significant results from. Finally, with many patients receiving multiple treatments or medications, it is close to impossible to determine which therapy improved patient status could be attributed to. Similarly, it is difficult to definitively determine the efficacy of procedural therapies from retrospective review of medical records that are sometimes incomplete or lost to follow-up. If patients received medical therapies post-procedurally, it is hard to differentiate which intervention contributed to a positive patient response. However, of the available

data analyzed, pleurodesis, surgical resection, and sclerotherapy yielded the highest patient responses. Patient response proved hard to define because it often depends on patient baselines. When patient response is gathered from patient interviews, it could sometimes be a subjective measurement. Since this investigation was not a randomized, blind, controlled study, with each patient at the same starting baseline and receiving treatment from the same provider, it is hard to draw any conclusions from this data. For these same reasons, Figure 2 cannot be considered significant. Limitations due to referral bias, loss of patient follow-up, concurrent medical and procedural therapies, etc. are too great to justify any significance within the data.

Lastly, patient outcomes, including quality of life, functionality, and pain in this cohort were dependent on patient self-reporting or the reporting of patients' parents. This data was often times reported from memory and could be skewed due to significant loss of patient follow-up. This information is often times not obtainable from medical records alone. Similarly, since only a small cohort of patients had PFTs done, and only a fraction of those patients had complete records containing multiple sets of PFTs, pulmonary function tests proved to be an incomplete way of measuring patient outcome.

The sharp contrast in mortality rate between GLA and CCLA proves interesting given the close relation and many overlapping characteristics between these two diagnoses. However, this might be attributed to the lower number of patients with a diagnosis of CCLA entered in the registry. On the other hand, lower presence of patients with CCLA could be due to general lower incidence of effusion in patients with this

diagnosis. The relative percents of deceased patients by diagnosis and effusions cited in Table 9 implies that it is not diagnosis that dictates mortality rates, but rather respiratory symptoms, and more specifically effusions, which correlate strongly with mortality.

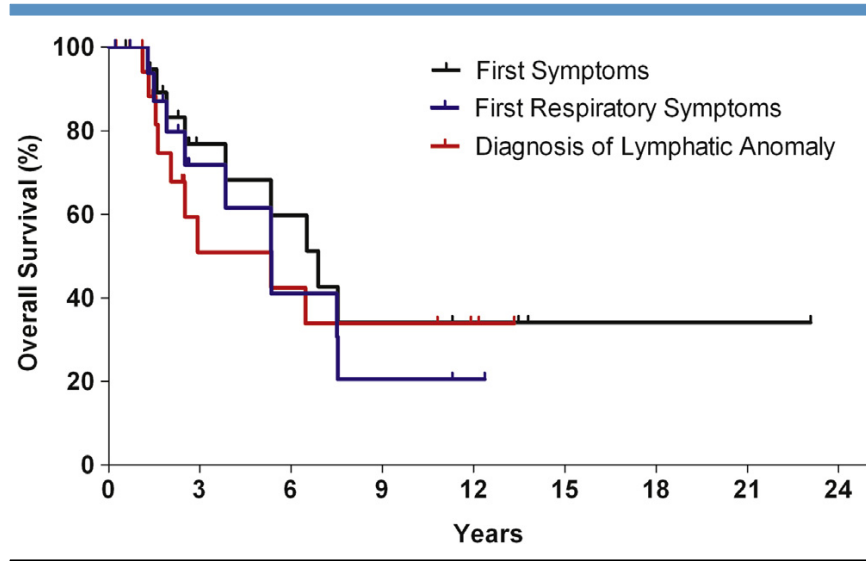


Figure 3. Kaplan-Meier Curve: Overall Survival of KLA Patients Compared from Three Time Points (Croteau, 2014).

As compared to Figure 2, Figure 3 demonstrates a similar sharp downward trend in overall survival from diagnosis of lymphatic anomaly. This could be attributable to referral bias or patients who are diagnosed by VAC later in their lives. Correspondingly, the overall survival rate of the cohort of patients with presentation of first respiratory symptoms drops faster than the overall survival rate of the cohort of patients with presentation of first general symptoms. Unlike Figure 2, the overall survival rate of patients presenting first with general disease symptoms never meets the survival rate of patients with first respiratory symptoms. This is due to the fact that the KLA patient

population does not comprise a cohort that represents 100% of patients with disease complicated by effusions. In Figure 2, the entire patient cohort ultimately presents with at least one effusion (and 94% with recurrent effusions) and therefore, the overall survival rate of patients who present first with general symptoms and patients who present first with pulmonary symptoms ultimately converge. This data suggests that effusions are the biggest determinant of mortality in patients with disorders of the lymphatic system. Furthermore, the initial trends of first symptoms and first respiratory symptoms for both Figure 1 and Figure 2 correlate very strongly. This is probably due to the fact that first symptoms could include patients whose first overall symptoms were pulmonary symptoms.

As previously stated, referral bias remains a primary limitation of this investigation. As a center with vastly comprehensive clinical expertise and unprecedented familiarity in the field of vascular anomalies, the group at Boston Children's Hospital often receives the sickest patients with the most extensive disease involvement. This considerably complex patient cohort may contribute to outcome bias indicative of lymphatic anomalies or malformations on the more severe end of the disorder spectrum.

Secondly, loss of patient follow-up also restricts the outcomes data of this research. Statistically, patients are classified into two categories, either "deceased" or "unknown". While "deceased" represents a very certain outcome with a definitive patient group, "unknown" is a more ambiguous patient categorization. With limited pertinent

medical information or no prospective results on some patients, rates of morbidity and mortality may be skewed. Even for subjects who maintain annual communication with the project, some medical records are incomplete and interviews largely rely on individuals' memories. These factors must be considered while interpreting results.

Going forward, we plan to expand patient cohorts included in the Lymphatic Anomalies Registry and to more deeply investigate therapies. We are beginning to incorporate imaging data into the registry, which would allow us to measure the extent of disease involvement more accurately and correlate this to patient outcomes. We are committed to minimizing patients lost to follow-up through this project through return clinic visits and short annual questionnaires. Based on the data presented, it seems measurements of patients' pain could prove useful for overall prognosis. Therefore, clinicians could benefit from improved methods of measuring and assessing the types and severity of pain experienced by these patients. Likewise, more attention should be paid to the incidence of infections in this cohort, as these patients are at a significant risk for developing and dying from infections. Since infection was not found to be strongly associated with first presentation of any disease symptoms in this cohort, perhaps more prophylactic antibiotic treatments could be considered to improve patient outcome before infections develop concurrent with pulmonary symptoms. We aim to use these natural histories and outcomes data to inform clinical trial design and current best practice.

To the best of our knowledge, this is the largest study investigating patients with complex lymphatic disorders complicated by effusions undertaken to date. This is

especially meaningful given the rarity of these disorders. The data has shown that effusions, especially effusions presenting earlier in life, are associated with poor outcome. Furthermore, patients suffering from effusions appear to be at increased risk of developing serious infections, which can lead to death. Clinicians should consider these findings, especially earlier administration of prophylactic antibiotics to patients who present with any symptoms or disease features of lymphatic disorders.

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