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Assessment & Management of Chronic Lymphatic Filariasis Morbidity among Endemic Populations

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Doctor of Philosophy

Epidemiology

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Assessment & Management of Chronic Lymphatic Filariasis Morbidity among Endemic Populations

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2014

Abstract

Assessment & Management of Chronic Lymphatic Filariasis Morbidity among Endemic Populations
By Katherine E. Mues

Lymphatic filariasis (LF) is a neglected tropical disease characterized by lymphedema. The first clinical manifestation is usually an episode of adenolymphangitis (ADL). These recurrent episodes are characterized by swelling, lymphatic inflammation, high fever, and general malaise. The objectives of this dissertation were to 1) determine how a lymphedema management program and compliance to the program affects the rate of ADL episodes, 2) to estimate the effect of a regimen of anti-fungal cream use on ADL episodes, and 3) to estimate the proportion of ADL episodes attributed to specific pathogens measured through antibody levels.

Data for studies one and two are from a cohort of lymphedema patients enrolled in a morbidity management program in Odisha State, India. Data for study three are from a subset of a group of lymphedema patients enrolled in a morbidity management program in Léogâne, Haiti. Correlated Poisson and logistic models were used to estimate the efficacy of the program on the frequency of ADL episodes over time. Marginal structural Poisson models were used to estimate the effect of a regimen of anti-fungal cream on the frequency of ADL episodes. Absolute and relative changes of antibody levels for different antigens were calculated for study three.

Patients enrolled in the lymphedema management program in India experienced a 35% lower rate of ADL episodes at 24 months compared to baseline. Compliance to soap was associated with a decrease in the rate of ADL episodes in all disease groups except among those with entry lesions and early lymphedema. Study two suggests that an increase in the number of times one uses anti-fungal cream was associated with a slight decrease in the frequency of ADL episodes at 12, 18, and 24 months. Among the cohort of 41 lymphedema patients in Haiti, the Strep A antigen had the highest prevalence of antibody response.

Findings suggest that community-based lymphedema programs are effective in decreasing the frequency of ADL episodes and use of anti-fungal cream to treat entry lesions may decrease the frequency of ADL episodes. Study three provides evidence for infection with *Streptococcus A* as a potential contributing factor to ADL episodes.

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Chapter 1: Overview

1.1 Lymphatic Filariasis

Lymphatic filariasis (LF) is a neglected tropical disease (NTD) characterized by symptoms of swelling, lymphedema, and ultimately elephantiasis of the limbs. The disease affects over 120 million people throughout the world with over 1.3 billion at risk [1]. LF is caused by 3 different strains of parasitic nematode worms (roundworms), *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* [2], which are spread by several species of mosquitoes. These thread-like worms reside in the lymphatic vessels of humans, causing severe and permanent lymphatic damage. This damage results in disability, stigma, and loss of economic productivity. Currently, there is no cure for lymphatic filariasis. The focus is instead on prevention and morbidity management.

1.2 Life Cycle and Transmission

Humans become infected with worm larvae when bitten by an infected mosquito. Within several hours, the larvae molt and develop a new cuticle, introducing new antigens to the human host. The 4^{th} stage larvae reside in the lymphatic vessels where they develop into adults over 6-9 months. The adult worms live in the lymphatic system of the lower and upper limbs as well as the male genitalia (*W. bancrofti*). Female and male adult worms mate to produce sheathed microfilariae over the course of 5-10 years [2]. These microfilariae then migrate to the lymph and blood channels where they are then taken up by a mosquito vector during a blood meal (typically at night). Once ingested by a mosquito, the microfilariae shed their sheaths, enter the mosquito's gut, and then migrate to the thoracic muscles over a 24 hour period. It is here where the

microfilariae molt and develop into stage 1 and stage 3 larvae (10-14 days). The stage 3 larvae then travel to the mosquito's head and proboscis. During the mosquito's blood meal, the larvae then enter human skin to start the cycle over again. Mosquitoes of the *Culex*, *Anopheles*, *Mansonia*, and *Coquillettidia* genera are vectors for *Wuchereria bancrofti* [3], while the vector for *Brugia malayi* is typically of the *Aedes* or *Mansonia* generum [4]. Humans do not typically become infected from one single mosquito bite (as with malaria infection). To become infected, they must be exposed to infected mosquito vectors for an extended period of time and have endured many bites.

1.3 Clinical Effects of LF – Acute Attacks

Most individuals who are infected with the parasite are asymptomatic or have subclinical manifestations. Clinical symptoms of infection do not appear until many years after infection. The first clinical manifestation is usually an acute attack [2]. These acute attacks are characterized by swelling, lymphatic inflammation, high fever, general malaise, chills and edema. Acute attacks are a recurrent clinical aspect of lymphatic filariasis lasting 3-15 days each and often occur several times each year [5]. Patients who have undergone an acute attack most likely have had subclinical infection for several years prior.

There are several different definitions and categorizations of acute attacks in the literature, many of which overlap. Acute dermatolymphangioadenitis (ADL) is defined as a plaque-like area of relatively diffuse cutaneous inflammation with or without ascending lymphangitis or satellite adenitis [6]. ADL episodes typically last for several days and subside spontaneously following bed-rest [7]. Prostration, high fevers, and chills are common systemic manifestations of ADL. ADL episodes are often

accompanied or followed by distal edema of the effected leg [6]. Scientific evidence has shown that ADL episodes lead to the progression of chronic lymphedema [8-11]. Acute filarial lymphangitis (AFL) involves the presence of a circumscribed inflammatory nodule or cord in the arms, legs, or breast centered on adult worms in a lymphatic tissue [6]. These attacks spread in an ascending fashion and are rarely accompanied by systemic symptoms. It is hypothesized that AFL is of filarial origin while ADL is of bacterial origin [6]. ADL (Acute adenolymphangitis) characterized by sudden onset of high fever, painful, lymph node and lymphatic inflammation and dermatolymphangioadenitis characterized by high fever, chills, myalgias, and headache as well as edematous inflammatory plaques are also commonly seen in the literature [2]. There is still much debate as to the etiology of these acute manifestations.

1.4 Clinical effects of LF – Lymphedema and Elephantiasis

Lymphedema of the legs, arms, and breasts and hydrocele (among men) is the most visible symptom of lymphatic filariasis. Swelling of tissue and localized fluid retention is caused by lymphatic damage. Lymphedema is a chronic condition that can progressively worsen if not managed properly. Limbs affected by lymphedema are often at higher risk for infection. Swelling of the lower extremities is most common [2]. *Bancroftian* filariasis often results in swelling of the entire limb, unilateral or bilateral, while only the area below the knee is involved in *Brugian* filariasis [2]. Several clinical staging systems have been developed to classify the progression of lymphedema in humans. The Dreyer system involves seven stages characterized by reversibility of swelling overnight, presence of shallow and deep skin folds, presence of mossy lesions and knobs, and inability to go about daily tasks independently, ultimately leading to a

diagnosis of elephantiasis [12]. These clinical effects of LF often result in psychosocial problems [13], sexual problems [14], community stigma [15], and a loss of economic productivity [16].

Hydrocele is another frequent clinical effect of LF. It occurs among men when the scrotum swells. It is the result of the accumulation of fluid in the tunica vaginalis [7]. These vary in size from 5cm to over 30 cm [2]. These are seen only in areas endemic for *Bancroftian* filariasis and is often among the most common clinical manifestations (40-50% among infected men), although it is often underreported and underestimated. The fluid drained from the hydrocele is usually translucent; parasites are typically not found in the fluid. Hydrocele can develop into lymphedema of the scrotum when it is accompanied by thickening of the scrotal or penile skin. In extreme cases, the genitals may be grossly deformed.

1.5 Epidemiology

Lymphatic filariasis is endemic in 81 countries throughout the world, putting an estimated 1.3 billion people at risk [2]. *Wuchereria bancrofti* is prevalent throughout Sub-Saharan Africa with some endemic foci as far north as the Nile Delta. In South East Asia, LF is prevalent in India, Indonesia, the Philippines, and Papua New Guinea. In the Americas, it is found in Haiti and the Dominican Republic, as well as parts of Brazil and Guyana. The majority of infections (90%) are due to *Wuchereria bancrofti*. There are no animal reservoirs of this strain. *Brugia malayi* is endemic only in Asia. It is prevalent in China, India, Malaysia and some of the Pacific Islands, like Indonesia and the Philippines. *B. malayi* is also found among primate and feline reservoirs [2]. *Brugia*

timori only exists on the islands of Timor, Alor, Flores, Sumba, Roti, and Savu, all islands of Indonesia.

Within endemic areas, lymphatic filariasis has a focal distribution. Because the prevalence is highly dependent on the intensity of transmission, locational foci have developed within communities with high levels of heterogeneity [5]. This also results from ecological differences in mosquito breeding habits and short mosquito flight distances. In addition to variability based on geography, there is also marked variability in the proportion of persons in an endemic area who have patent infection [2].

There is an age related association with infection levels. Infection intensity is measured by the levels of microfilariae in the blood. These levels tend to increase with age until the 3rd or 4th decade[2] at which levels seem to stabilize with slight decreases. Symptomatic manifestations of the disease appear to increase gradually throughout the life span. They are rarely seen in young children. The likelihood of developing clinical disease varies by region, with about 1/3 of the 120 million infected worldwide having clinically overt disease. Again these variations are probably a result of ecological differences in endemic areas.

1.6 Lymphatic Filariasis Elimination Strategy

The World Health Organization (WHO) launched the Global Programme to Eliminate Lymphatic Filariasis (GPELF) in 1997 with a target elimination data of 2020. The goals of the program are two-fold [17]:

i. To interrupt transmission

ii. To alleviate the suffering and control morbidity among infected persons

To interrupt transmission, the entire at risk population must be treated with microfilariae-killing drugs for a period of time in order to ensure that blood microfilariae levels are below those needed to sustain transmission [18]. These endemic communities must be mapped and yearly mass treatment campaigns (also known as mass drug administration – MDA) must be completed. The WHO recommends that one of the following drug regimens be administered once annually for 5 years covering at least 65% of the at-risk population [17].

i. 6 mg/kg of body weight diethylcarbamazine citrate (DEC) + 400 mg albendazole
ii. 150 μg/kg of body weight ivermectin + 400 mg albendazole (in areas that are also endemic for onchocerciasis)

The use of table salt or cooking salt fortified with DEC for a period of one year is also an alternative regimen for endemic areas. GlaxoSmithKline and Merck & Co. Inc. have pledged to donate the drugs for as long as it takes to eliminate the disease.

To control morbidity and alleviate suffering, the program focuses on basic limb hygiene and the prevention of secondary bacterial and fungal infections of affected limbs. The goal of this is to stop further progression of lymphedema. Additionally, programs focus on education and understanding to create hope among affected individuals [18].

1.7 Diagnosis

The standard diagnosis procedure for lymphatic filariasis is detection of microfilariae (Mf) in a blood smear through microscopic examination [19]. Antigen detection with an immunoassay for circulating filarial antigen (CFA) is also useful. This can be done using a rapid immunochromatographic card test (ICT) or through an Og4C3

test using an ELISA. Serological assays for antifilarial antibodies in the blood are an alternate diagnostic technique. Earlier tests detected antigen on IgG antibodies consistently have poor specificity [20] while newer assays for IgG4 antibodies such as Bm14 (sensitive to both *W. bancrofti* and *B. malayi*) and BmR1 (*Brugia* infection only) provide excellent specificity. Identification of adult worms in the lymph system can be completed through biopsies or x-rays. Molecular diagnosis can be completed through PCR testing for *W. bancrofti* and *B. malayi*. Symptoms of lymphedema, elephantiasis, and hydrocele can be clinically examined for diagnosis of chronic disease. However, these patients will typically have negative Mf and antibody tests.

Diagnostic procedures for lymphatic filariasis are used at all points in the elimination strategy: 1) to map the prevalence of infection, 2) to measure the impact of MDA, 3) to detect recrudescent infection during MDA endpoint decision making, 4) for post-MDA surveillance [20].

1.8 Chemotherapeutic agents

Diethylcarbamazine (DEC) and ivermectin are the two mainstays of treatment for lymphatic filariasis [5]. DEC has both macro- and microfilaricidal properties in that it kills both adult worms and microfilariae. It is the treatment of choice for individuals(6 mg/kg daily for 12 days) [2]. Ivermectin only kills microfilariae; it has no macrofilaricidal effects. These drugs are most often used in combination with albendazole in control settings. Albendazole is primarily microfilaricidal, but has demonstrated macrofilaricidal effects [2]. Several studies have also shown the beneficial effects of the antibiotic doxycycline [21, 22]that targets the endosymbiont *Wolbachia*.

Control programs in Africa use a combination of albendazole/ivermectin, while albendazole/DEC is used elsewhere. Side effects of DEC include fever, chills, joint pain, headaches, nausea, and vomiting [2]. The severity of side effects is directly related to the number of microfilariae in the bloodstream. Albendazole has relatively few side effects.

1.9 Pathogenesis

The pathogenesis and spectrum of disease in LF has developed in recent years. As a result of ultrasound studies, it is now understood that all patients have some underlying pathology [23], although not all will present with clinical pathology. The heterogeneity of pathology among patients is not well understood, although it is probably related to the immunology in each patient. Among asymptomatic microfilaria-positive individuals, hidden lymphatic damage has been found. As the microfilaremia levels decrease over time, patients transition to become acutely symptomatic experiencing acute adenolymphangitis (ADL) and acute filarial attacks (AFL). The interval between detective microfilaremia and symptoms is the clinical incubation period [24] which may vary between 2 and 10 years in an endemic community. Following the acute phase, many patients begin to show signs of chronic lymphedema, which may lead to advanced stages of elephantiasis.

Tropical pulmonary eosinophilia is a rare outcome that involves paraoxysmal cough and wheezing, weight loss, low-grade fever, adenopathy, and pronounced blood eosinophilia (>3000 eosinophils (White blood cells)/μl) [2]. Tropical pulmonary eosinophilia is a result of intense immune response to circulating microfilaria. Persons with this outcome will have low mf levels, but will be antigen and antibody positive. In

most cases, there are restrictive pulmonary function abnormalities. If not treated with DEC, the disease may progress to chronic restrictive lung disease with interstitial fibrosis.

1.10 Immunology

The immunology of and host response to lymphatic filariasis is yet to be fully understood, and there is much heterogeneity of immunology among patients. Recent developments have provided more information on its relation to the clinical pathology and infection status. One of the most characteristic immunologic profiles is the down-regulation of the immune response among chronic filariasis patients [25], yet responses to other antigens remain normal. On the cellular side of the immune response, studies have shown that amicrofilaremic individuals usually have strong T helper cell type 1 (Th-1) anti-parasitic responses while microfilaremic individuals have strong Th-2 responses [26, 27]. Lymphedema patients (microfilaremic negative) are often found to have low interleukin (IL)-10 levels and elevated IFN-γ levels.

In a study of immunologic response and its association with lymphedema severity in Tanzania, it was found that IFN-γ levels were significantly elevated among asymptomatic uninfected individuals, as well as uninfected individuals with chronic lymphedema and elephantiasis [28] compared to infected, but asymptomatic LF patients. IL-4 levels were also elevated, but not to the same extent. The cellular immune response was also explored in relation to circular filarial antigen (CFA) and microfilaremia (mf) status. IFN-γ and IL-4 levels were elevated among CFA (-) and mf (-) patients, while they were lower among CFA (+) and mf (+) individuals. IL-10 was elevated among mf (-) and CFA (+) individuals. Furthermore, IL-10 was lowered when pathology was present among patients, suggesting it may play a protective role for the parasite.

Concerning the humoral immune response, it has generally been found that lymphedema patients (who are typically CFA (-) and mf (-)) have elevated levels of IgG1, IgG2, IgG3, and IgE compared to asymptomatic patients, while patients with active infection have higher IgG4 levels [29, 30]. Also, the ratio of IgG4/IgE is high among individuals with active infection, while it is lower among CFA (-) and mf (-) persons [29, 31]. The development of pathology and elimination of circulating parasites is associated with higher IGE/IgG4 ratios and expansion of other IgG subclasses [29]. This ratio has been important in understanding parasite survival and immunity. The IgG4 isotype is thought to be protective for the filarial parasite by stopping the release of human histamine. IgE is believed to be involved in the killing of the parasite through triggering of mast cell degranulation [32]. The skewing of the humoral immune response to filarial parasites toward IgE and IgG4 is interesting as IgE and IgG4 are now known to be promoted by the cellular IL-4, which reflects selective activation of the Th2 subsets [29]. The inverse correlation of T cell responsiveness with anti-filarial IgG4 isotype implies that active parasite infection leads to the T cell unresponsiveness shown for microfilaremics [33].

1.11 Lymphedema management programs

For the management of chronic lymphedema, the World Health Organization recommends simple measures like washing the effected limb with soap and water regularly, treating fungal and bacterial infections with antifungal and antibiotic creams, and elevating and exercising the limb. Additional techniques involve appropriate footwear, application of ointments, and clipping toe nails regularly. A study of a clinic-based trial of a foot care regimen plus several different combinations of drug treatment in

India, showed that the foot care regimen alone (with placebo drugs given) decreased the number of ADL episodes during the treatment year and the year following treatment [34]. This pattern of decrease for two years did not occur among the other regimens. All patients were instructed to continue the foot care regimen unsupervised, and upon long-term follow-up, it was found that the program reduced the frequency of ADL episodes by 72.5% [35]. Another clinical trial found reduction of limb edema and frequency of ADL attacks among regimens of 1) oral penicillin, 2) DEC, and 3) topical antiseptic ointment [36]. All 3 regimens involved foot care, which may owe itself to the observed reductions.

These clinical studies are important to understand the efficacy of hygiene and foot care programs themselves, but it is also important to demonstrate that these programs work in a field setting. A clinic-based lymphedema management program in Léogâne, Haiti demonstrated significant decreases in the incidence of ADL episodes and reduction of leg volume over a 4 year period [37]. The study also illustrated improved compliance to recommended practices over time. A study of a home-based lymphedema management program implemented by the national health system of Burkina Faso also demonstrated significant reductions in monthly ADL episodes [38].

1.12 Gaps in current knowledge

- Very little information on the efficacy and effectiveness of community-based lymphedema management programs
- Little information on the efficacy of anti-fungal cream to decrease the number of ADL episodes
- Still uncertainty in the literature on the etiology of ADL

1.13 Proposed projects

The dissertation will consist of three studies addressing three aspects of the clinical effects of lymphatic filariasis.

Study 1 Objective: To determine how a community-based lymphedema management program and compliance to the program in Khurda District, Odisha State, India affects the rate of ADL episodes and affects lymphedema stage and progression.

- **Aim 1:** Determine how the presence of a lymphedema management program affects the rate of ADL episodes through a pre- post-intervention comparison.
- **Aim 2:** Determine predictors of compliance to a community-based lymphedema management program.
- **Aim 3:** Assess the effect of compliance and factors that predict compliance to the program on the rate of ADL episodes over time among enrolled patients.
- **Aim 4:** Describe the progression of lymphedema among patients enrolled in the lymphedema management program in Khurda District, Odisha State, India over a 2 year follow-up period.

Study 2 Objective: To estimate the effect of anti-fungal cream use on episodes of acute adenolymphangitis (ADL) according to treatment regimen.

- **Aim 1:** To estimate this effect using marginal structural models for time varying confounding and compare to traditionally adjusted models.

Study 3 Objective: To quantify antibody levels to pathogens that potentially contribute to ADL episodes during and after an episode among lymphedema patients in Léogâne,

Haiti. We also sought to estimate the proportion of ADL episodes hypothesized to be attributed to specific pathogens in the development of ADL episodes.

- **Aim 1:** Quantify antibody levels during and after an ADL episode and determine the absolute and relative change for each person.
- **Aim 2:** Calculate the prevalence of antibody levels that showed a four-fold (300%) change from the ADL to convalescent period for each pathogen of interest.
- **Aim 3:** Calculate the prevalence of antibody response at different levels of antibody change.
- **Aim 4:** Examine the relative pattern of pathogen contributions under different levels of antibody change.

Chapter 2: Literature Review

2.1 Acute Attacks

There are two distinct forms of acute attacks associated with LF. Acute filarial lymphangitis (AFL) appears as a cord-like structure associated with retrograde lymphangitis in the limbs [7], involving the presence of a circumscribed inflammatory nodule or cord in the arms, legs, or breast centered on adult worms in a lymphatic tissue [6]. These attacks spread in an ascending fashion and are rarely accompanied by systemic symptoms. It is hypothesized that AFL is of filarial origin [6].

Episodes of adenolymphangitis (ADL) or acute dermatolymphangioadenitis are characterized by systemic symptoms such as fever, chills, myalgia, and headache [7]. Inflammatory plaques of the edematous area are frequent. ADL episodes are often accompanied or followed by distal edema of the effected leg [6]. Scientific evidence has shown that ADL episodes lead to the progression of chronic lymphedema [8-11]. For the purposes of this dissertation, we will be focusing on episodes of ADL. Henceforth, mention of an acute episode refers to an episode of ADL unless otherwise noted.

2.1.1 Incidence, Duration, and Risk Factors of ADL in Endemic Populations

The incidence of ADL varies somewhat by region, yet consistent risk factors include presence of chronic filariasis [39-44] and increasing age [39-45]. Other less consistent risk factors are gender [39, 42, 43, 45] (contrasting results of male vs. female), lymphedema grade [9, 46], as well as the rainy season [11, 43]. In two studies, microfilaremia was found to be a statistically significant effect modifier [11, 41]. In one study it modified the relationship between ADL and chronic filariasis [11], while in the

other it modified the relationship between ADL and age [41]. The mean duration of ADL episodes does not appear to exceed 7 days, while most studies averaged around 4-6 days. Hydrocele pathology was positively associated with duration of episode in two studies [39, 40]. The mean number of episodes (typically over a 1 year period) was relatively low, between 1 and 2. Risk factors for number of episodes include female gender [39, 40], chronic disease [39], season [40], and lymphedema severity [47].

2.1.2 Seasonal influences on ADL

There has been evidence for seasonal variation in the incidence of ADL. A randomized control trial exploring the efficacy of ivermectin and DEC combined with a limb-care regimen in Alleppey, India showed a significant increase in the number of ADL episodes [48] during the rainy season. The mean number of ADL episodes per month during the rainy season was 24.4 compared to 13.2 in the dry months ($P<0.0001$). These results are most likely internally valid because patients were closely followed for two years after treatment assignment, and most ADL episodes were directly observed in a clinic setting. An epidemiologic study on the natural occurrence of ADL in northern Ghana [43] also found a seasonal variation. The number of ADL cases decreased during the dry season and increased during the rainy season. In Rao's study of the natural occurrence of ADL among persons in a *B. malayi* endemic community [11], acute attacks were more frequent during the rainy months of June-September/November compared to the dry months of October and December.

The seasonal variation in ADL frequency can be explained by several factors. First, the increased number of episodes during the rainy season points to the presumed etiology of ADL: through fungal and secondary bacterial infections. The constant

moisture exposure to the foot/limb during the rainy season predisposes individuals to fungal infections, which then promote the secondary bacterial infection [48]. Also, the mosquito vector population may vary with season, becoming denser during the rains. It is possible that the increase in infective bites during the rainy season contributes to the increased frequency of ADL during that period [43]. Despite these findings however, the seasonal variation of ADL episodes is not consistent among all studies of ADL, with some showing little [42] or no seasonal variation [37].

2.1.3 Effect of MDA on ADL Incidence

Some studies have reported that the incidence of ADL may decrease following mass drug administration (MDA). A study in Flores, India evaluating the efficacy of DEC treatment over a 12 year period [49] found that the rate of ADL decreased from 46% in 1977 to 11% in 1988. However, 10 consistent days of DEC treatment was given en masse in 1977 and 1980, while it was selectively given in 1978, 1979, 1981, and 1982. Selective treatment was given in order of the following criteria: 1) all new residents 2) those who were mf (+) before initial treatment 3) those with a history of adenolymphangitis over previous 1-year period. Furthermore, those who presented with acute symptoms of adenolymphangitis were immediately treated with a single course of DEC. So results of this study may not be generalizable to MDA programs where all individuals in a community are treated with DEC annually. A 5-year follow-up of acute filarial morbidity (AFM) [44] found an overall decrease in AFM incidence after 4 rounds of annual MDA in Papua New Guinea. The incidence decreased from 0.39 per person-year in the pre-treatment year to 0.19 per person-year following a 4^{th} annual round of MDA. The lowest incidence occurred following the 2^{nd} annual round of MDA, 0.15 per

person-year. This detailed weekly follow-up of AFM is one of the most rigorous calculations of AFM incidence in an LF endemic community.

2.1.4 Effect of LF transmission on ADL

Several studies have commented on the effects of LF transmission levels on the incidence of ADL. The 5-year follow-up study in Papua New Guinea showed higher incidence of ADL among individuals from high-transmission communities [44]. After stratifying study communities by pre-MDA transmission potential, the authors found that the ADL incidence during pre-treatment year was significantly higher in high transmission communities.

2.2 The etiology of acute attacks in lymphatic filariasis

Despite extensive research, the etiologic cause of acute attacks in lymphatic filariasis is yet to be agreed upon in the scientific community. Several theories exist and have supporting scientific evidence. Part of the issue in defining the etiology of acute attacks is defining the type of acute attack itself. For this dissertation project, we will be exploring the etiology of adenolymphangitis (ADL) as opposed to acute filarial lymphangitis (AFL). Both will be defined and discussed in more detail below.

Another important distinction to make is between lymphangitis and lymphadenitis. These terms were often used in earlier literature to describe specific symptoms of an acute attack. Lymphangitis is the inflammation of the lymph channels that occurs as a result of infection at a site distal to the channel. Signs and symptoms include a deep reddening of the skin, warmth, lymphadenitis, and a raised border around

the affected area. The person may also have chills and a high fever along with moderate pain and swelling. Lymphadenitis is the inflammation of the lymph nodes.

2.2.1 Two clinical syndromes of ADL

In 1999, Dreyer distinguished between two specific types of acute attacks following the results of a 7-year follow-up of 600 patients referred to a filariasis clinic in Recife, Brazil [6]. Dreyer identified two clinically distinct syndromes: acute dermatolymphangioadenitis (ADL) and acute filarial lymphangitis (AFL). ADL is characterized by a plaque-like area of inflammation with or without ascending lymphangitis. It is accompanied by systemic symptoms of fever, malaise, and chills. A distal skin lesion that served as the point of entry for bacteria can be found in essentially all cases. The ADL episode is usually followed or accompanied by edema of the affected limb. The ADL episode itself will eventually digress, but recurrent attacks occur, leading to chronic lymphedema and elephantiasis. AFL attacks involve a circumscribed inflammatory nodule or cord in the affected limb. The cord or nodule is centered around the adult worms in the lymphatic system. The lymphangitis spreads in a descending fashion. There are rarely bacterial entry lesions in attacks of AFL, and systemic manifestations are mild or mostly absent.

In addition to their clinical distinctions, Dreyer established evidence for different etiologies of ADL and AFL attacks. Degenerating adult worms encased in granulomas were present in all biopsies of patients undergoing an AFL attack, adding to the evidence that AFL episodes are of a filarial origin. Other studies have found that treatment of an AFL-like attack with DEC did not improve the clinical condition, but may have aggravated it when adult worms are present at the site of the lesion [50, 51]. It is well

established that DEC has macro as well as microfilaricidal effects. Furthermore, DEC treatment may actually trigger a clinical syndrome resembling AFL, which occurred in 3 patients in Dreyer's study [6]. To support the bacterial etiology of ADL attacks, Dreyer found that all biopsies done in Recife yielded positive cultures of *Staphylococcus aureus, Corynebacterium*., *Bacillus subtilis*, *S. epidermis*, and group A *Streptococcus haemolyticus*. Furthermore, none of the ADL biopsies contained adult worms.

2.2.2 Bacterial cause of ADL

The etiology of ADL has been studied for most of the 20th century, yet is still not entirely understood. Observational research from the 1920's and 1930's in endemic areas argued for the bacterial cause of acute ADL episodes. In British Guiana streptococci was cultured from swollen lymph glands in 90% of cases [52]. Further studies of filariasis in British Guiana from 1926 to 1928 demonstrated a clear association between filarial lymphangitis and β haemolytic streptococcus [53]. Similar results were demonstrated in Fiji [54] and in Samoa [55]. A 1929 study of filarial lymphangitis in Calcutta, India [56] found that among the patients presenting to the Carmichael Hospital for Tropical Diseases for filarial lymphangitis or chyluria, the source of infection was of some internal or external septic focus, and was invariably caused by a streptococci or staphylococci bacterial strain. Contradictory to these studies, McKinley demonstrated a uniform absence of positive bacterial cultures taken under aseptic conditions among a series of cases of acute lymphangitis in Puerto Rico from 1929 to 1931[57]. Instead, he hypothesizes that there may be three types of acute lymphangitis of varying origin: 1) lymphangitis of bacterial origin, 2) lymphangitis of filarial origin 3) filarial lymphangitis with secondary bacterial infection.

More recently, Esterre demonstrated the important role of streptococcal infection among persons with ADL in French Polynesia[58]. A total of 22 patients with filarial ADL, 10 with chronic pathology, 10 with classic erysipelas (*Streptococcus* infection of the upper dermis and superficial lymphatics), and 20 endemic healthy controls were examined for clinical manifestations as well as laboratory investigations. Anti-streptolysin O (ASO) levels (indicative of recent streptococcal infection) were highest among the chronic pathology group and lowest among those with erysipelas (no significant differences). Anti-DNAse B levels (indicative of chronic streptococcal infection) were higher among those with ADL ($p=0.019$) and among those with erysipelas ($p=0.002$) when compared to controls. These results strengthen the view that bacteria have a significant role, even if not exclusive, in the development of ADL episodes among LF patients. They confirm the necessity to quickly initiate antibiotics among LF patients undergoing an ADL episode.

A case-control study evaluating whether tissues and edema fluids of lymphedematous extremities contained bacteria among LF patients in Tamil Vadu Province, India, found a significantly higher prevalence of bacterial isolates in lymphedema cases compared to healthy controls in surgical wound swabs, blood, and lymph fluids [59]. Furthermore, bacterial isolates and strains of the same phenotype and sensitivity to antibiotics from blood and tissue fluid, lymph, and lymph nodes were identified in the same patients with acute disease and also on the toe web surface, tissue fluid, lymph, and lymph nodes of the same extremity. These consistent findings (especially in the blood cultures) strengthen the hypothesis that bacterial penetration of the dermis to the lymphatic system and subsequent dissemination into the blood stream

may be the cause of ADL episodes. This study clearly demonstrates that microorganisms colonize the lymphedematous tissues of filarial patients and provides evidence for the bacterial cause of ADL episodes.

A study of filariasis in patients in Kerala, South India, demonstrated that the majority of ADL patients had a clearly marked point of entry (the web spaces and soles of the feet) through which bacterial or other microorganisms could gain entry [46]. Furthermore, 28 out of 65 patients had elevated ASO titers and elevated titers were observed in 40% of patients in whom a port of bacterial entry was detected.

Another study by Shenoy & Suma et al, examined streptococcal infection as a precipitating factor of ADL episodes in *Brugian* filariasis [60]. Working with a study population of 30 patients with ADL, 30 with chronic filarial edema, and 60 age and sex matched healthy adults, the authors found that ASO (anti-streptolysin O) titers were persistently elevated in 90% of patients in the ADL group compared with 20% in the chronic lymphedema group, and 0% in healthy adults over the 1 year period. Furthermore, in all 27 patients of the ADL group who had elevated ASO titers, a portal of entry for secondary bacterial infection was identified.

In a *W. bancrofti* endemic areas of Santo Domingo, Dominican Republic, Vincent et al demonstrated elevated anti-streptolysin O (ASO) levels among patients with recurrent episodes of adenolymphangitis compared to neighboring controls without episodes of adenolymphangitis [61]. The study took blood samples from both case and control patients at two different time points: during acute manifestations and 30 days following an acute episode. Comparing the paired ASO observations using a paired *t*-test, the authors found a significant mean log titer rise of 0.17 in convalescence (P=0.01).

Furthermore, the *t*-test for independent groups showed elevated ASO levels among adenolymphangitis patients during the acute period compared to the controls (t=2.9, P=0.0006) with a considerable increase in difference during convalescence (t=4.8, P=0.0004). Mean anti-DNAase B (ADAB) log titers rose between the acute and convalescent phases among cases, but did not reach statistical significance. Among the cases, almost half were unresponsive to ASO and ADAB tests. This suggests that these cases of cellulitis could be caused by another streptococci group (B, C, and G), *Staphylococcus aureus, Haemophilus influenzae* type B, or other pathogens.

2.2.3 Filarial cause of ADL

The other primary view on the cause of ADL episodes attributes them to the host's inflammatory/immune response to adult worm activity in the lymph system or microfilariae in the blood. A study of 63 established cases of *acute filarial fever* in Puri District, Odisha State, India found a significant rise in the microfilaria count during the acute episode compared to 1 month before and 1 month after the episode [62] suggesting fresh circulation of mf during the fever. It also found a significant decrease in the mean OD levels of filarial-specific IgG and IgG4 during the episode, a significant increase in the mean level of circulating immune complex (CIC = antibody + antigen) during the attack, suggesting that the excess antigen released into circulation during the episode had formed immune complexes after bonding to antibodies. Lastly, the study demonstrated no significant difference between the percentages of patients with a positive anti-streptolysin O (ASO) test (an indicator of recent streptococcal infection) before, during, or after the episode. In conclusion, this study suggests that the immune-mediated response to adult filarial worms may have induced the ADL episodes among these

patients. This may have been triggered by a new parturition of microfilariae released by the mother prior to the episode.

2.2.4 Fungal origins of ADL episodes

In addition to the bacterial and filarial causes of ADL episodes, the role of fungal infection has also been explored. Fungal infections can cause entry lesions, often found in between the toes, in between deep skin folds, and other places of warmth [51]. These entry lesions then serve as the point of entry for bacteria ultimately leading to an acute attack.

A study of randomly selected women from Léogâne, Haiti [63] included three distinct patient groups: 1) women with lymphedema; 2) microfilaria-negative and antigen-negative women with no lymphedema; 3) asymptomatic microfilaria-positive and antigen-positive women. The authors compared cellular and humoral reactivity to a panel of bacterial, fungal, and filarial antigens among the three groups of women. DTH skin testing was performed for seven antigens. The lymphedema group had an increased reactivity to the bacterium *Proteus.* Women who had experienced 3 or more acute attacks in the last 18 months were more responsive *Trichophyton* (a fungal pathogen) antigen ($P = 0.0006$) than those with fewer attacks. There was no association between number of acute attacks and the other skin test antigens. The authors also found that women with lymphedema had significantly increased responses to IgG for strepsolysin-O (SLO), indicating that this group experiences an increased number of *Streptococcus* infection, but there was no relationship found between the frequency of and time interval since the last acute attack and reactivity to SLO. These findings indicate that either SLO

is not the best measure of *Streptococcal* infection or that it is not the only cause of ADL. Other bacterial pathogens and fungi may be contributing causes to ADL episodes.

Additionally, a study in Sao Paulo, Brazil [64] obtained swab specimens from 21 lymphedema patients. Of these, 13 contained lesions suggesting infection with the fungus *Tinea pedis*. Furthermore, fungal infection was the lone agent in 7 out of the 13 patients. This was accompanied by a high prevalence of the bacterium *Corynebacterium minutissimum.* These patients were not known to be experiencing an ADL episode when the swab specimens were obtained. A case-control study in Georgetown, Guyana exploring the relationship between inter-digital lesions and number of ADL attacks [47] found evidence of fungal infections in a low proportion of skin and nail abnormalities among the patients, but regardless of etiology, the presence of inter-digital lesions was the most significant risk factor for ADL episodes.

2.2.5 Wolbachia Endosymbiont

Wuchereria and *Brugian* species of filarial nematodes carry an obligate bacterial endosymbiont, *Wolbachia* [2]. These intracellular bacteria were first recognized through ultrastructure studies of filarial worms and microfilariae in the 1970's [2]. Several studies on the molecular mechanisms of lymphangiogenesis in humans have shown that vascular endothelial growth factors C (VEGF-C) and D (VEGF-D) control lymphangiogenesis [65-68]. Furthermore, the expression of VEGF-C has been shown to be up-regulated by the proinflammatory cytokines, interleukin (IL)-1B and tumor necrosis factor (TNF). It has been hypothesized that in filarial species with the *Wolbachia* endosymbiont, the *Wolbachia* are major stimulators for proinflammatory cytokines like TNF and may induce production of VEGF-C in LF patients [21].

Treatment of filarial patients with Doxycycline has been shown to deplete the levels of *Wolbachia* in both Mf and adult worms of *W. bancrofti* patients [69, 70].

With these two lines of evidence, Debrah et al hypothesized that treatment of LF by doxycycline to target *Wolbachia* may ameliorate filarial pathology by down-regulating proinflammatory cytokines, TNF and IL-1B, and VEGF-C/VEGFR-3 [21]. The authors explored this hypothesis by treating lymphedema and microfilaremic patients in LF-endemic Ghana with 200 mg/d of doxycycline for 6 weeks and following for 12 and 24 months respectively. Doxycycline was found to have a strong macrofilaricidal effect among these patients. Furthermore, it was found to reduce plasma levels of VEGF-C/sVEGFR-3 which were associated with the amelioration of dilated supratesticular lymphatic vessels and improvement of lymphedema among patients.

Building on the previous study, Mand conducted a randomized control trial to address if the benefits of a 6-week course of doxycycline could be extended to lymphedema patients without active filarial infection (CFA negative) [22]. They found that a 6 week course of doxycycline provided mild to moderate improvements in lymphedema (all patients had lymphedema) over the 24 month follow-up period. The trial also found a significant difference in the number of ADL episodes reported among patients receiving doxycycline compared to placebo (P=.015) and among a third arm who received amoxicillin compared to placebo (P=.006). The authors conclude that individuals with stage 1-3 filarial lymphedema should take a 6-week course (200 mg/d) of doxycycline every other year and possibly annually. From their results, they conclude that the doxycycline targets the *Wolbachia* endosymbiont, which in turn may ameliorate

the host's inflammatory response to the filarial parasite, leading to improvements in in both lymphedema and the frequency of ADL episodes.

2.2.6 Summary of Etiology of ADL

Although the literature on the etiology of ADL episodes is vast, the results, study methods, and outcome definitions are varied. To understand the etiology of ADL episodes, an ideal study would randomly expose lymphedema patients to a variety of bacterial and fungal pathogens. These patients would then be followed up for ADL episodes. Those pathogens whose exposure resulted in an episode would then be prime candidates for being the causative agent in ADL morbidity. If more than one pathogen exposure resulted in ADL episodes among the patients, then perhaps a multiple agents are involved in the etiology. This type of study is unethical and therefore not feasible to conduct.

The studies done to date exploring the etiology of ADL have used a variety of techniques: comparing lymphedema patients with ADL to non-ADL lymphedema controls, comparing lymphedema patients with ADL to healthy controls without lymphedema, comparing two time points (an ADL and convalescent time point) among both lymphedema patients with ADL and lymphedema patients without ADL. They have also explored a variety of pathogens using a variety of laboratory indicator tests. Although many studies have found higher levels of both *Streptococcus* antibody and *Streptococcus* bacterial cultures among patients experiencing an ADL, most of the earlier work was descriptive; no analytical hypothesis tests were performed. Furthermore, some studies have specifically looked at the outcome of ADL defined by redness, swelling, and

systemic symptoms, while others have used such vague outcomes as acute filarial disease or acute filarial fever. These differences make studies difficult to compare.

Two other study design issues arise in trying to identify the cause of ADL episodes among lymphedema patients. First, understanding the temporal order of pathogen exposure and the occurrence of ADL cannot be underestimated when attempting to determine the etiology of ADL. Most of the literature on the etiology of ADL consists of cross-sectional studies with data from only one time point. Although one can show higher antibody levels to a certain pathogen among lymphedema patients with ADL compared to non-ADL controls, temporality cannot be established without multiple time points. If a study collects serologic samples during the ADL episode (acute period) and during a convalescent period following the episode, one can assess the change in antibody response and potentially implicate a specific pathogen or set of pathogens as the cause of the ADL episode. Another issue that has not been addressed in the previous literature is the issue of possible correlations between different pathogens or between different indicators of the same pathogens/same type of pathogens. Because the immunology and pathophysiology of ADL episodes are not well understood, it will be important to consider multiple pathogens in ascertaining the cause of ADL episodes. Therefore, it is essential to consider the correlations between these pathogens when doing analyses.

2.3 Epidemiology of chronic lymphedema

Lymphedema of the legs, arms, breasts and scrotum (also called hydrocele) is the most visible symptom of lymphatic filariasis. Lymphedema, a chronic condition that can progressively worsen if not managed properly, occurs when lymphatic damage results in

the swelling of tissue and localized fluid retention. Swelling of the lower extremities is most common [2]. *Bancroftian* filariasis often results in swelling of the entire limb, unilateral or bilateral, while only the area below the knee is involved in *Brugian* filariasis [2].

Lymphedema is typically measured through leg-volume and standard staging systems. The Dreyer system classifies lymphedema into seven distinct stages [12], with stage seven being the most severe:

- **Stage 1:** Swelling is reversible (goes away) overnight when the patient lies flat in a bed, not because of any specific treatment. These persons rarely have acute attacks, entry lesions, or bad odor.
- **Stage 2:** Swelling is not reversible (does not go away) overnight when the patient lies flat in a bed without lymphedema management. Patients may have acute attacks, lesions, and bad odor.
- **Stage 3:** Shallow skin folds are present in which the base of the fold can be seen when the patient moves the leg or foot so that the fold opens up. These patients may have occasional acute attacks as well as entry lesions and bad odor.
- **Stage 4:** Knobs are present on the limb. These knobs consist of bumps, lumps, or protrusions of the skin which predispose the limb to further trauma and to additional entry lesions. Additionally, protruding scars known as keloids may develop. The keloids are an abnormal healing process at the site of any earlier entry lesion. These patients experience acute attacks and will often have entry lesions and bad odor.

- **Stage 5:** Deep skin folds are present. These are folds whose base cannot be seen when the patient moves the leg or foot. The base of the fold can only be seen when the edges are separated by hand. Patients experience frequent acute attacks, will have entry lesions and bad odor.
- **Stage 6:** Mossy lesions are present on the surface of the skin. These are small elongated or round growths that cluster together. Mossy lesions contain fluid and are transparent or translucent. Since mossy lesions usually leak fluid, patients are at high risk of bacterial infection. Almost all patients have entry lesions between the toes with a bad odor and the toenails may be destroyed. Patients have acute attacks.
- **Stage 7:** The patient is unable to adequately or independently perform routine daily activities such as walking, bathing, and cooking. These patients have large legs, frequent acute attacks, extremely bad odor, and always have entry lesions.

The WHO method involves only four stages [71]:

- **Stage 0 (latent):** The lymphatic vessels have sustained some damage that is not apparent. Transport capacity is still sufficient for the amount of lymph being removed. Lymphedema is not present.
- **Stage 1 (spontaneously reversible):** Tissue is still at the non-pitting stage: when pressed by the fingertips, the tissue bounces back without any indentation. Usually upon waking in the morning, the limb is normal size.
- **Stage 2 (spontaneously irreversible):** The tissue now has a spongy consistency and is considered pitting: when pressed by the fingertips, the affected area indents

and holds the indentation. Fibrosis makes the beginning of the hardening of the limbs and increasing size.

- **Stage 3 (lymphostatic elephantiasis):** The swelling is irreversible and the limb or affected area is very large. The tissue is hard and unresponsive.

In addition to swelling of the limbs, men can develop hydrocele, swelling of the scrotum. It is the result of the accumulation of fluid in the tunica vaginalis [7]. These vary in size from 5cm to over 30 cm [2]. These are seen only in areas endemic for *Bancroftian* filariasis and is often among the most common clinical manifestations (40-50% among infected men), although it is often underreported and underestimated. Hydrocele can develop into lymphedema of the scrotum when it is accompanied by thickening of the scrotal or penile skin. In extreme cases, the genitals may be grossly deformed.

2.3.1 Gender distribution among persons with lymphedema

There has been some evidence that lymphedema of the leg is more common among women. Addiss's cohort of lymphedema patients in Léogâne, Haiti was 82.9% female [37]. An earlier study recruiting patients from the same clinic in Léogâne found of the 84 patients with limb lymphedema/elephantiasis, 74 (88%) were women [72]. Similar observations were in Pakistan [73] and in Ghana [74]. These observations may be related to each study's recruitment technique or may be a true association with lymphedema.

2.3.2 Repeated ADL episodes on chronic lymphedema

Most studies of the association between ADL episodes and chronic lymphedema have found a positive correlation. Both of Shenoy's randomized control trials of anti-filarial drugs found that those with higher grades of edema had more episodes of ADL [34, 48], while Suma's follow-up of these patients several years later also illustrated this relationship [35]. In a report of the progression of clinical disease among LF patients in a *Bancrofti* endemic area of south India, Pani and colleagues found the mean number of ADL attacks to increase with lymphedema grade [10]. In Addiss's Haitian cohort, the incidence of ADL increased with lymphedema stage [75]. Other studies, however, have found no significant relationship between ADL frequency and lymphedema [42, 43, 76]. The inconsistency of findings is due in part to the use of different staging systems [77] as well as different study designs.

2.3.3 Effect of MDA on lymphedema

There has been evidence for a protective association between intake of anti-filarial drugs and the occurrence of lymphedema in endemic populations. A randomized trial comparing the efficacy of DEC + ivermectin with that of DEC alone in Papua New Guinea [78] found a significant decrease in the frequency of lymphedema after 4 annual MDA's among both treatment groups. Findings from the study showed that the proportion of male subjects with advanced hydrocele was 15% before the trial began and 5% one year after the fourth treatment ($P<0.001$). The frequency of lymphedema of the legs was 5% pre-treatment and decreased to 4% one year following the 4^{th} round of MDA ($P=0.04$). Although this is a small change, it was statistically significant. For a chronic illness like lymphedema, it would be unexpected to see a large percent change in the

prevalence of any lymphedema over the course of 5 years. In a separate analysis of only individuals who had pre-existing lymphedema at the beginning of the trial, 87% of men who had advanced hydrocele and 69% of those with lymphedema of the legs at the beginning of the trial no longer had clinical signs of the disease by the end. The authors of the trial speculate that mass treatment with anti-filarial drugs decreases exposure to infective 3rd stage larvae, and this in turn diminishes the intensity of inflammatory responses to newly inoculated *W. bancrofti* or to the Wolbachia endosymbiont.

Several other studies have found a similar relationship between anti-filarial drugs and lymphedema. An early study of two rounds of DEC treatment in Indonesia found that nearly all of the patients with lymphedema post-treatment had complete regression of swelling within three years [79]. Those with elephantiasis regressed more slowly, taking two to four years for most. In Tanzania, Meyrowitsch showed that 1 annual and 2 semi-annual DEC treatments resulted in a decrease in the frequency of hydroceles [80]. One year after the start of treatment, 57.9% of males with hydrocele who took 1 annual dose of DEC showed improvement either through reduction in size or complete disappearance. Among men who took 2 semi-annual doses of DEC, 68.2% showed improvement. No such effect was observed on leg elephantiasis.

2.4 Lymphedema management programs

Although there is no cure for chronic lymphedema, simple hygiene and management techniques offer relief and can prevent further disability for lymphedema patients. As part of the Global Programme to Eliminate Lymphatic Filariasis (GPELF), the World Health Organization recommends simple measures like washing the effected limb with soap and water regularly, treating infections with antifungal and antibiotic

creams, and elevating and exercising the limb. Additional techniques involve wearing appropriate footwear, application of ointments, and clipping toe nails regularly. Lymphedema management programs have been implemented in many LF endemic areas throughout the world, yet their implementation strategy has differed. Also, the strength of the scientific evidence for the benefits of lymphedema management programs on acute attacks, chronic clinical outcomes, and quality of life varies.

2.4.1 Impact of lymphedema programs on ADL

To summarize the work done to date, the majority of studies have found that lymphedema management programs decrease the frequency of ADL episodes in LF endemic populations, offering substantive evidence that lymphedema management programs have a beneficial effect on the occurrence of acute ADL attacks. Despite this evidence, the studies vary in their methods of data collection, intervals and length of follow-up and statistical methods. There is a dearth of articles that calculate an actual rate of ADL and explore the effects of lymphedema programs using correlated GEE models for longitudinal data [37, 75]. Most of the articles reported decreases in the mean or total number of ADL episodes reported over time [34-36, 48, 81, 82]. Others reported a decrease in the percentage of patients who reported 1 or more ADL episodes [38, 83] then tested using a chi-square test. Furthermore, most studies did not adjust for potential confounding or test for interaction. Finally, the majority do not attempt to find a comparison group. Although it may be considered unethical to deny patients access to a lymphedema management program, few studies have mentioned this limitation in their research. Without a comparison group, it is difficult to scientifically prove that lymphedema programs are truly causing a decrease in the incidence of ADL episodes.

2.4.2 Impact of lymphedema programs on lymphedema atage

Several of the lymphedema management program studies also evaluated the effects of the programs on lymphedema stage. The Haiti cohort study found, of the 175 patients enrolled, 137 (78.3%) experienced a reduction in leg volume during the study. In normal legs and those with stage one lymphedema, the leg volume increased by 14mL and 5 mL respectively [37]. But for those with stage 2, 3, and 4 lymphedema, leg volume decreased significantly (P=0.01). Also, leg volume reduction was significantly associated with increasing lymphedema stage, lower frequency of ADL during the study period, and use of compressive bandages after controlling for study phase. In Shenoy's 1999 trial, there was little to no improvement in lymphedema grade throughout the course of the study [34].

Kerketta's work in Odisha, India showed that about 50% of all patients experienced oedema improvement after day 90 of treatment [36]. In all three regimens, about 20% of those whose oedema improved experienced reduction of 75-100%. Although the proportion of persons who experienced a reduction was smaller in regimen II, this was not statistically significant. Exploring differences in circumference between affected and normal limbs at different time points, there was no remarkable difference in efficacy between the 3 treatment regimens. ANOVA testing revealed that the reduction of oedema is high and significant among patients with grade-I lymphedema, followed by grade-II with the most remarkable reductions at along points 2 and 3 of the leg (distal part of the lower limb and proximal part of the lower limb) in grade-II and grade-III patients. In Wijesinghe's study in Sri Lanka, 11 patients transitioned from grade-II to grade-I lymphedema over the course of the program (P=0.012) [83].

2.4.3 Impact of lymphedema programs on quality of life

Although there is evidence that filarial lymphedema has negative effects on patient quality of life [84-86], few studies have directly explored the effects of lymphedema management programs on patient quality of life. McPherson explored the effects of a lymphedema hygiene and skin care regimen on quality of life among residents of Wismar, Guyana [87]. Fifteen patients were identified to be suffering from lymphedema. Patients completed knowledge, attitudes, and practices (KAP) survey and the Dermatology Life Quality Index (DLQI) questionnaire. Three lymphedema management interventions were then put in place in the community. Of the 15 lymphedema patients identified, 11 were present at the 1-year follow-up. Initial DLQI scores ranged from 2-18 out of 30 points (mean=10.9), and these initial scores showed only a weak association with lymphedema stage. At follow-up, the range of DLQI was 0-11 (mean=4.1), showing marked improvement ($P<0.0001$). Using the existing health infrastructure, this intervention resulted in significant benefits among lymphedema patients, while requiring very little follow-up. These results must be interpreted cautiously because of the very small sample size, lack of a control group, and no attempt to control for potential confounders or interactions between variables. It was however, the first study of lymphedema morbidity to use the DLQI, a quality of life assessment specific to dermatological clinical effects.

Colleagues at the Centers for Disease Control and Prevention explored the impact of a lymphedema management program in Khurda District, Odisha State, India over a 2 year follow-up period [88]. Using the World Health Organization's Disability Assessment Schedule II (WHO DAS II), they found composite disability decreased

significantly between the baseline and the twenty-four month assessment, from an average score of 66.2 at baseline to 60.4 at 24 months ($p<0.0001$), representing a perceived improvement in the quality of life. After stratifying by lymphedema category, patients with the most advanced lymphedema (Stages 4-7) saw the largest reductions in overall disability scores 24 months into the program.

Akogun's study of a home-based lymphedema management program in Burkina Faso found improvement of strained familial and social relationships in one of their lymphedema management groups [81], although this was not the main outcome of the study.

2.4.4 Compliance with lymphedema management programs and MDA campaigns:

Measuring compliance to lymphedema management programs is difficult and has not been done consistently across studies. In Addiss's study of the effectiveness of a basic lymphedema management program in Léogâne, Haiti, program compliance was measured through patient self-report, while ad-hoc home visits were made to assess patient capacity and to encourage compliance [37]. In a multivariable Poisson model exploring factors associated with ADL incidence, the authors found no significant relationship with program compliance.

A limb-care management program in Sri Lanka collected data on compliance through a questionnaire at baseline and post-intervention time points [83]. Among those suffering from at least one ADL episode, the percentage of patients following the recommended home care procedures increased for limb elevation and limb cooling following the intervention. Between the pre- and post-intervention periods, there was a significant increase in the percentage of patients who washed the affected limb using

soap, elevated, exercised, as well as in those who made efforts to reduce trauma to the limb. There was also a decrease in the percentage of those using an abrasive material to clean the skin.

As opposed to compliance with lymphedema management programs, there have been several studies exploring compliance with MDA programs and factors associated with MDA compliance. A study in Haiti found age, sex, and education to be associated with MDA compliance as well as people's perceived role in the community, their ability to take the pills, and overall knowledge of LF and LF elimination strategies [89]. In Khurda District, Odisha State India, Cantey evaluated MDA compliance in areas with and without an LF education program [90]. A multivariable analysis revealed three modifiable predictors of MDA adherence when controlling for age, gender, and education: 1) knowing about MDA in advance, 2) knowing the mosquitoes transmit LF, and 3) knowing that the MDA distributed was for LF. There was also a significant interaction between knowing about the MDA in advance and knowledge of mosquito transmission of LF.

Based on the barriers and predictors found in the latter MDA compliance evaluation, a refined educational campaign was implemented in Khurda District along with a lymphedema management program. Cantey and colleagues evaluated the efficacy of the refined educational campaign and lymphedema management program in increasing compliance with MDA [91]. In communities who had received the first educational campaign and the refined campaign, compliance increased from 59.5% after the first campaign to 90.2% following the refined campaign. Furthermore, communities that received the refined campaign only had a compliance rate of 75.0%, compared to 52.2%

at baseline. Even after controlling for other predictors of MDA compliance, knowledge of one of the three predictors discovered in the earlier evaluation independently predicted compliance. Also, the community-based lymphedema management program independently enhanced MDA adherence. These results support the need for programs covering both pillars of the elimination strategy, prevention of LF transmission through MDA and control of chronic lymphedema morbidity through management programs, simultaneously.

2.5 LF in India

India is endemic for both *Wuchereria bancrofti* and *Brugia malayi*, with an estimated 40 million persons infected. It is estimated that there are about 7 million people with chronic lymphedema in India [92]. Michael's 1997 reassessment of the global prevalence of LF determined there were 29.46 million microfilaremia cases, 6.58 million lymphedema cases, and 12.88 million hydrocele cases of *Bancroftian* descent in India [93], making up 42.88% of the global burden. Also, India carries 20.01% of the *Brugia malayi* global burden. Estimates from 1995 determined that nine states in India (Andhra, Pradesh, Bihar, Gujarat, Kerala, Maharastra, Odisha, Tamil, Nadu, Utter Pradesh and West Bengal) contributed to 95% of the total LF burden in India [92]. Sabesan's mapping study of prevalence data from 1951-1995 [94] illustrated four distinct regions of high endemicity: 1) eastern and south western coasts, 2) northern track along the Gangetic plain, 3) among the islands of Adaman and Nicobar, and 4) a belt in the central Deccan region in the South. It also noted that the prevalence of LF is very or moderately low in districts in the western half of the country.

2.5.1 LF in Odisha State, India

Odisha State, located along the eastern coast of India along the Bay of Bengal, has a population of 37 million distributed across 30 districts. It is the poorest state in India and is endemic for both *W. bancrofti* and *B. malayi*.

Sabesan's mapping study of lymphatic filariasis in India estimated the prevalence of LF (percent of mf (+) plus percent with filarial disease) in Odisha state to be 8.18% (1995 estimate)[94]. Twenty seven million people were determined to be at risk for LF with 2.39 million mf (+) and 1.51 million individuals with symptomatic LF. Furthermore, 6.31% of the total population of Odisha was determined to be at risk for LF. It also identified the districts along the eastern coast of Odisha as high priority regions for control because of their hyperendemicity (>10% prevalence for combined microfilaremia and clinical disease).

A study of Odisha's more western districts from 2007-2009 revealed higher than expected prevalence in this area considered to be less endemic [95]. Using ICT card testing on 1,563 individuals in the districts of Bargarh, Balangir, Kandhamal, Kendujhar, and Sambalpur were tested and interviewed on basic household information. Their results identify five districts of Odisha that represent a filariasis-endemic region not addressed by current MDA programs or surveillance. ICT card testing, which is more sensitive and a more direct marker of LF infection than traditionally measured mf (+) rates, may be the reason for this finding. The LF prevalence levels found in this study are much above the WHO threshold for MDA (1%), suggesting that a population-based study of LF prevalence should be completed and MDA should be considered for these western districts not currently targeted.

2.5.2 LF in Khurda District, Odisha State, India

A cross-sectional study of 12 villages in Khurda District, Odisha was carried out for a period of 3 months beginning in May 1999 [45]. During a door-to-door survey, each available subject was examined for both acute and chronic signs of LF and asked to recall information on the occurrence of ADL over the previous 3 months. Overall, the study examined a total of 5, 357 individuals. The prevalence of lymphedema (termed elephantiasis) was 3.43% (2.46% among males and 4.51% among females). Men recorded a prevalence of 4.10% for hydrocele. Furthermore, 6.67% of individuals (357) were affected with acute ADL during the previous 3 months. The prevalence of total disease due to filariasis was 12.53% (14.79% among males, 10.04% among females). The study also found that the prevalence of chronic filariasis is age dependent among both sexes. ADL prevalence was also age dependent: women 60+ suffered less attacks possibly as a result of their decreasing participation in agricultural work.

2.6 LF in Haiti

One of only four countries endemic for LF in the Americas, Haiti bears the largest burden of disease in the western hemisphere. It is endemic for *Wuchereria bancrofti*. The first reference of LF in Haiti was made in the mid 1700's, when it was referred to as pied-bottle [96]. Cases of elephantiasis of the leg were reported in Haiti in 1786 through the end of the 19th century [97, 98] [99]. Moving into the 20th century, Wilson used blood smears to detect *W. bancrofti* filarial infection in 1928 among Haitian workers in Cuba [100]. In the 1960's and 1970's, investigators explored the nationwide distribution of LF in Haiti, defining a triangular area in the north shaped by Cap-Haitien, Port de

Paix, and Gonaives [96]. Raccurt's work in 1988 revealed patent infection in 140 of 421 individuals (33%) in Léogâne, Haiti, located about 30 km west of Port au Prince[101].

From 2000-2001, Beau de Rochars conducted the first nationwide survey of school children in Haiti [102]. Using an adaptation of lot quality assurance sampling (LQAS), 5 schools within each of the 133 communes in Haiti were sampled. The goal of this study was not to determine the precise prevalence of LF in each commune, but to determine whether each commune met the WHO threshold for implementation of MDA (1%). Following consent, 100 μl of finger stick blood was collected from each child and tested for circulating filarial antigen (CFA) using an immunochromatographic test (ICT) card. The authors tested a total of 22,365 children in 440 schools across Haiti. A total of 517 (5.0%) boys and 384 (3.2%) girls tested positive for CFA. Overall, 117 (87.9%) communes had one or more children who were positive for CFA. An antigen prevalence of >10% was found mostly in communes located in the northern plains and in the coastal plains north, west, and east of Port Au Prince.

The results of Rochar's 2001 survey were used as the first step of the National Programme for Elimination of Lymphatic Filariasis (NPELF) in Haiti [103]. Hyperendemic communities (>10% antigenemia in children) were made a priority for initial MDA's of DEC and ALB. From 2000-2005, treatment was scaled up to reach all hyperendemic communes in Haiti. After a funding interruption in 2006, annual MDA's were resumed in 2007. National coverage for MDA was projected to occur by 2011 [104].

Chapter 3: Impact of a Community-Based Lymphedema Management Program on Episodes of Adenolymphangitis (ADL) and Lymphedema Progression - Odisha State, India

[Formatted for PLOS Neglected Tropical Diseases]

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Short Title:

Impact of Lymphedema Management Program on LF Morbidity

Abstract

Background: Lymphedema management programs have been shown to decrease episodes of adenolymphangitis (ADL), but the impact on lymphedema progression and of program compliance have not been thoroughly explored. Our objectives were to determine the rate of ADL episodes and lymphedema progression over time for patients enrolled in a community-based lymphedema management program. We explored the association between program compliance and ADL episodes as well as lymphedema progression.

Methodology/Principal Findings: A lymphedema management program was implemented in Odisha State, India from 2007-2010 by the non-governmental organization, Church's Auxiliary for Social Action, in consultation with the Centers for Disease Control and Prevention. A cohort of patients was followed over 24 months. The crude 30-day rate of ADL episodes decreased from 0.35 episodes per person-month at baseline to 0.23 at 24 months. The rate ratio comparing 24 months to baseline, taking into account the correlated nature of the data, was 0.57 (95% CI: 0.44, 0.74). Over the study period, the percentage of patients who progressed to more severe lymphedema decreased (P-value = 0.0004), while those whose lymphedema regressed increased over time (P-value<0.0001). Overall compliance to lymphedema management appeared to have little to no association with the frequency of ADL episodes among those without entry lesions (RR=0.87 (0.69, 1.10) (Table 6). Among those with entry lesions, compliance was associated with an increase in the rate of ADL episodes (RR=1.44 (1.11, 1.86)). Compliance to soap was associated with a decrease in the rate of ADL episodes in all disease groups except among those with entry lesions and early lymphedema.

Conclusions/Significance: Our results indicate that a community-based lymphedema management program is beneficial for lymphedema patients for both ADL episodes and lymphedema. It is one of the first to demonstrate an association between program compliance and rate of ADL episodes.

Author Summary

Lymphatic filariasis (LF) is characterized by clinical manifestations of limb swelling, lymphedema, and elephantiasis. LF is the world's second-leading cause of disability, with up to 15 million people with limb lymphedema or elephantiasis. The Global Programme to Eliminate LF aims to eliminate the disease through two pillars: (1) interruption of transmission and (2) treatment of clinical disease among those already affected. The Church's Auxiliary for Social Action (CASA), an Indian NGO, began a lymphedema management program in Khurda District, Odisha State, India in 2007. We evaluated the impact of the program on two clinical LF outcomes: acute episodes of adenolymphangitis (ADL) and chronic lymphedema progression. We found the monthly rate of ADL episodes decreased significantly after enrollment for two years in the program. Additionally, program compliance was found to be associated with ADL rate among some clinically distinct groups. Compliance to soap was associated with a decreased rate of ADL episodes among those without inter-digital entry lesions. The percentage of patients who progressed to more severe lymphedema decreased significantly over time, while those whose lymphedema regressed increased significantly. Our results indicate that a community-based lymphedema management program is beneficial for lymphedema patients for both ADL episodes and lymphedema. It is one of the first to demonstrate an association between program compliance and ADL episodes.

Introduction

Lymphatic filariasis (LF), caused by parasitic nematode worms, is characterized by clinical manifestations of swelling, lymphedema, and elephantiasis of the limbs. These thread-like worms reside in the lymphatic vessels of humans, resulting in severe lymphatic damage and dysfunction. Persons with lymphatic dysfunction caused by LF may suffer from adenolymphangitis (ADL) episodes characterized by swelling, inflammation of the limbs, fever, malaise and chills [2]. The frequency of ADL episodes has been shown to increase with more advanced lymphedema and studies have concluded that ADL episodes are a risk factor for lymphedema progression [9, 10, 34, 48, 75].

LF affects over 120 million people throughout the world with 1.3 billion at risk [105]. India accounts for over 40% of the global LF burden [93], with an estimated 40 million persons infected [2] and 7 million with chronic lymphedema. India has a national goal of LF elimination by 2015. The goals of the program are to 1) interrupt transmission and 2) control morbidity among infected persons [106]. To interrupt transmission, the entire at-risk population must be treated with microfilariae-killing drugs through yearly mass drug administration (MDAs). To control morbidity, lymphedema management programs focus on basic limb hygiene to prevent ADL episodes and stop further progression of lymphedema. The World Health Organization (WHO) has recently recommended that all LF endemic countries provide access to lymphedema management services [107].

Lymphedema management programs have been shown to decrease the frequency of ADL episodes and lymphedema severity both in clinical-trial [35, 36] and field settings [37, 38]. Patients enrolled in a clinic-based lymphedema management program

in Léogâne, Haiti demonstrated significant decreases in the incidence of ADL episodes and reduction of leg volume over a 4-year period [37]. A study of a home-based lymphedema management program in Burkina Faso also demonstrated significant reductions in the percent of patients experiencing at least one ADL episode over a month's time [38]. The studies completed to date vary in their methods of data collection, follow-up intervals, and study length. There are no studies on home-based lymphedema management programs that calculate an ADL rate and explore the effects of lymphedema programs using longitudinal models. Most studies did not adjust for potential confounding or test for interaction. Finally, there are few studies exploring the association between compliance to lymphedema management techniques and clinical outcomes of LF.

Following implementation of a community-based lymphedema management program in India, the aim of this study was to examine the rate of ADL episodes and progression of lymphedema over a two year period. We also aimed to determine predictors of compliance to the program and assess the effect of compliance on the rate of ADL episodes and lymphedema progression.

Methods

Ethics Statement

This project was submitted for human subjects review to the Center for Global Health at the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA. It was approved by CDC and determined to be program evaluation. Permission for the survey was obtained from the Odisha State Department of Health and Family Welfare. Participants were asked to give their written informed consent prior to participation. For those unable to write, consent was documented by recording the person's fingerprint or marking the signature line with an 'X' and by countersignature of survey personnel. For participants under 18 years of age, verbal consent of a parent or guardian was also obtained. Consent procedures were approved by CDC and the Odisha State Department of Health and Family Welfare.

Study Area

The lymphedema management program was implemented in Khurda district, Odisha State, India by the non-governmental organization, Church's Auxiliary for Social Action (CASA). Khurda district has a population of nearly 2.2 million and is highly endemic for LF caused by *Wuchereria bancrofti*. Based on 2001 estimates, there were 3.5 million – 6.3 million infected persons in Odisha state and 22,500-23,500 infected persons in Khurda district [45, 108-110].

Lymphedema Management Program

All patients enrolled in the program were trained in basic lymphedema management by physician-trained volunteers, including daily washing of limbs with soap

and water, daily exercise and elevation of the affected limb, and daily use of footwear outside the home. Patients were trained in the importance of early treatment and prevention of secondary bacterial and fungal infections with topical and oral antimicrobial agents. If inter-digital fungal infections were present, patients were instructed to use an antifungal cream on a daily basis. Patients were supplied with soap and antifungal cream for the first 6 months of the program; thereafter they were instructed to purchase these supplies at local stores and pharmacies.

Study Design

To evaluate CASA's community-based lymphedema management program, a cohort of individuals enrolled in the program was recruited. Of 189 villages in Bologarh block of Khurda district, 30 villages were eligible for inclusion in the study as they had not yet been enrolled in the lymphedema management program and were not located in the immediate vicinity of an already enrolled village. In the 30 selected villages, 533 persons with lymphedema were identified in June 2009. Of these patients, 456 persons were approached to be in the lymphedema program, as some patients had migrated out of their village during the 5-6 month period since the initial household census.

Enrollment eligibility included persons more than 14 years of age who reported lower leg swelling for at least 3 months. Of the 456 persons approached, 375 (82%) met the inclusion criteria and agreed to participate. Five patients were subsequently excluded from the analysis due to lack of lymphedema on examination (n=2), failure to meet age criteria (n=1), or mislabeling of survey forms (n=2) for a total sample size of 370 (81%).

There were no differences in the distribution of age, sex, and lymphedema stage among those who did not enroll compared to those who did enroll in the study (data not shown).

Patients were interviewed in Oriya (the local language) by trained interviewers at baseline (prior to enrollment) and at, 1, 2, 3, 6, 12, 18 and 24 months after enrollment in the program beginning in July 2009. We did not include a true control population in this study as it may be considered unethical to deny a person suffering from lymphedema access to a lymphedema management program as is recommended by the WHO for all LF endemic countries [107]. To evaluate the effectiveness of the program over time, we consider baseline, before patients were enrolled in the program, as the comparison group. Through a written questionnaire, interviewers collected information on patient demographics, frequency of compliance to lymphedema management techniques, ADL history and treatment, access to supplies, MDA history, and perceived disability using the World Health Organization Disability Assessment Schedule II (WHO-DAS II). Interviewers also completed a clinical assessment on each person in order to determine lymphedema stage.

Data were independently dual-entered into an Epi Info 7 (Stone Mountain, 2008) database. Data cleaning and analysis were performed in SAS 9.3 (Cary, North Carolina, USA). A sample size of 375 was calculated to detect a 5% decrease in the frequency of ADL episodes, with a 15% dropout rate, from baseline to 24 months post-enrollment.

Definitions

30-Day Rate of ADL Episodes

An ADL episode was defined by patient self-report of two or more of the following symptoms: redness, pain, or swelling of the leg or foot, with or without the presence of fever or chills, [40, 48]. Patients were asked how many times they had an ADL episode in the previous 30 days. The ADL rate per subject was calculated as the number of ADL episodes reported by each subject divided by 30. We assumed that each ADL episode reported initially occurred during the 30-day period and that each person remained at risk for ADL episodes during the entire 30-day retrospective period.

Lymphedema Progression

Both the interviewer and a supervisor performed independent lymphedema staging and photographs were taken of the affected limb(s). The 7-stage classification system [12] was used to stage lymphedema. Any discrepancies between interviewer and supervisor staging were resolved by independent evaluation of limb photographs by two physicians with extensive LF experience (P. Budge and L. Fox). Patients whose lymphedema stage increased from one time point to another were determined to have progressed, while those whose lymphedema stage decreased from one time point to another were determined to have regressed. Lymphedema stages 1-3 were categorized as early; stages 4-7 were categorized as advanced.

Compliance to Lymphedema Management Techniques

Compliance with specific lymphedema management techniques was measured by self-report. Patients were asked how frequently they performed each of five management techniques: washing affected leg with soap and water, treating inter-digital entry lesions with antifungal cream, elevating the limb, exercising the limb, and wearing footwear

outside. CASA's program did not provide oral antimicrobials for ADL episodes; therefore, compliance with oral antibiotics was not measured. A person was considered to be compliant to a lymphedema management technique if he/she reported performing that technique at least once per day.

We created an overall weighted compliance score for soap, cream, elevation, exercise, and wearing footwear outside. If patients reported performing a technique daily or more than once per day, they received a score of 2. If they reported performing a technique once per week or more than once per week, they received a score of 1, and if they reported performing a technique less frequently than once per week, they received a score of 0. Since cream was only indicated for patients with inter-digital entry lesions present, those without entry lesions had a potential maximum score of 8. To make the score of these patients comparable to those with entry lesions, the scores of patients without entry lesions were multiplied by a factor of 1.25. The scores for each technique were then summed for a maximum of 10. The summary score was then divided into two groups: compliant = 7-10 and non-compliant = 0-6.

Statistical Analysis

To determine how the presence of the lymphedema program influenced the number of ADL episodes experienced per person per month, Poisson models for correlated data (up to 8 observations per subject) were built with SAS's PROC GLIMMIX using an auto-regressive (1) correlation structure with a random intercept. The model contained dummy variables for time, variables for presence of inter-digital entry lesions, lymphedema status at baseline (advanced vs. early), and number of times a patient had participated in a MDA as this has been shown to be associated with the

frequency of ADLs episodes [44]. We also included interaction terms involving time with presence of entry lesions and lymphedema status at baseline. After assessment of interaction, confounding and precision [111], an adjusted rate ratio was calculated comparing the 30-day rate of ADL episodes at each time point to baseline.

To evaluate the association between compliance to lymphedema management techniques and the 30-day rate of ADL episodes, we used mixed effects Poisson models for correlated data. We evaluated overall compliance through the compliance score as well as compliance to each technique, defined dichotomously and lagged one time point. Each model used an auto-regressive (1) correlation structure and included a random intercept. The models controlled for confounding by access to water, soap, antifungal cream, and a hospital, as well as number of times a patient had participated in a MDA. Models of compliance to individual lymphedema management techniques also controlled for compliance to all other techniques at the lagged time point. Interaction by baseline lymphedema stage (advanced vs. early) and presence of inter-digital entry lesions at the lagged time point was assessed testing product terms with compliance. If no significant interaction was found, main effects of baseline lymphedema status and/or presence of entry lesions were included in the model.

Exploring the association between compliance to lymphedema management techniques and lymphedema progression, we used a fixed effects logistic model for correlated data with an auto-regressive (1) correlation structure. We explored both overall compliance and compliance to each technique lagged one time point, adjusting for the number of times a patient had participated in a MDA. Models of compliance to individual lymphedema management techniques also controlled for compliance to all

other techniques unless otherwise noted. Interaction by baseline lymphedema stage (advanced vs. early) and presence of inter-digital entry lesions at the previous time point was assessed testing product terms with compliance. If no significant interaction was found, main effects of baseline lymphedema status and/or presence of entry lesions at the previous time point were also included in the model.

Confounding was evaluated using both *a priori* causal pathways and data-driven methods. Only combinations of confounders that fell within 10% of a gold standard model with all potential confounders were considered for the final model.

To explore predictors (all of which were considered exposure variables) of compliance to lymphedema management techniques, correlated mixed effects logistic models were used. All models used an auto-regressive (1) correlation structure with a random intercept. Potential predictors of compliance that were statistically significant at alpha=0 .05 in bivariate models were included in a multivariable model.

Results

Baseline Demographic Characteristics

Table 3.1 displays the baseline demographic characteristics of the 370 lymphedema patients enrolled at baseline. The mean age was 57 years and the majority (59%) of the cohort was female. At baseline, about 27% of patients had inter-digital entry lesions, 14% had advanced stage lymphedema (stages 4-6), and patients reported having lymphedema symptoms for an average of 25 years.

Fifty-four (14.6%) patients were lost to follow-up during the 24 month study period; these patients did not contribute data at the 24 month time point. Over the course of the study, reasons for non-participation at any particular assessment were: absence from the village at the time of the assessment (70%), refusal (7%), illness (6%), or death (17%). In total, the study encompassed over 222 person-years of observation time (baseline to time of last follow-up). Two hundred thirty five patients (63.5%) contributed data at all 8 time points, 76 (20.5%) contributed data at 7 time points, and 29 (7.8%) contributed data at 6 time points. Only about 8% of patients contributed data at 5 time points or less. Censoring (i.e. contributing data at less than 8 time points) was not significantly associated with the rate of ADL episodes (RR= 0.89 (0.52, 1.53) nor with lymphedema stage progress (OR = 0.83 (0.61, 1.14)). Therefore, we assume that censoring was independent of the outcomes of interest.

During the two year study period, MDAs occurred in Odisha in June 2010 and March 2011 (Odisha State Ministry of Health and Family Welfare).

30-Day Rate of ADL Episodes

Over the follow-up period, patients reported a total of 687 ADL episodes. The sum of ADL episodes for each person over the follow-up period ranged from 0-24, with a median of 1, a mean of 1.86, and an inter-quartile range of 1-3. Looking at the distribution of the sum of ADL episodes over the study period, 44% of patients experienced 0 episodes, 17% experienced 1 episode, 21% experienced 2-3 episodes, and 19% experienced 4 or more episodes. The total number of reported episodes reported ranged from 129 at baseline to 45 at 6 months and was 72 at 24 months (Table 3.2). The mean duration of ADL episodes reported was 3.5 days.

The rate of ADL episodes (Figure 3.1a) decreased from 0.35 episodes per person-month at baseline to 0.14 at 6 months and plateaued to 0.23 at 24 months, representing a 35% decrease. Those who had entry lesions had a higher rate of ADL compared to those who did not (data not shown) (RR=1.88 (95% CI: 1.54, 2.29)), and those with advanced lymphedema at baseline had a higher rate of ADL compared to those with early lymphedema at baseline (RR = 2.66 (95% CI: 1.89, 3.75)).

Figure 3.1b displays the 30-day rate of ADL episodes by the presence of inter-digital entry lesions. Those with lesions have a higher rate of ADL episodes at each time point and also appear to have a greater difference when comparing the 24-month rate to the baseline rate. Figure 3.1c displays the 30-day rate of ADL episodes by lymphedema status at baseline. Those with advanced lymphedema at baseline have a higher rate of ADL episodes at each time point. Table 3.2 displays a Poisson model exploring the effect of time on the individual ADL rate controlling for number of MDAs and taking into account the correlated nature of the data. The rate ratios (RR) compare the rate at each time point to baseline. All of the RRs were less than 1 and statistically significant. The

RR with greatest magnitude was seen at 6 months. Comparing 24 months to baseline, the rate of ADL episodes was 0.57 that of the rate at baseline. In the Poisson model, we found significant interaction by baseline lymphedema status (advanced vs. early) and the presence of inter-digital entry lesions at the current time point. The stratified rate ratios (RR) of the final Poisson model controlling for the number of times a patient participated in a MDA are displayed in Table 3.3. Those with advanced lymphedema and entry lesions experienced a 43% reduction in ADL episodes from baseline to 24 months (RR=0.57 (95% CI: 0.35, 0.93)). Among those with advanced lymphedema and no entry lesions, the 30-day rate of ADL episodes decreased by 62% (RR=0.38 (95% CI: 0.17, 0.82)). Among those with early lymphedema, those with entry lesions had a 21% reduction in ADL episodes (RR=0.79 (95% CI: 0.46, 0.95)), while those without entry lesions experienced a 48% reduction (RR=0.52 (95% CI: 0.35, 0.76)).

Lymphedema Progression

The overall distribution of lymphedema stage, as classified in Table 3.4, changed significantly from baseline to 24 months (p-value = 0.0027). The percentage of patients with stage 3 or 4 lymphedema decreased over the study period (p-value = 0.0006) while the percentage of patients with stage 1 or 2 lymphedema increased (p-value = 0.0064) over the study period (Table 3.4). The small change in the percentage of patients with stage 5 or 6 lymphedema was not statistically significant. The percentage of patients who progressed to more severe lymphedema since the previous time point decreased (Table 3.4) (p-value = 0.0004), while the percentage of those whose lymphedema regressed increased over time (p-value<0.0001). Patients with advanced lymphedema at baseline were less likely to progress to a more severe lymphedema stage (data not shown); OR =

0.15 (95% CI: 0.07, 0.36). Patients with entry lesions at each time point were also less likely to progress, OR = 0.55 (95% CI: 0.38, 0.79), yet more likely to have advanced lymphedema, OR = 1.17 (95% CI: 1.08, 1.26).

Compliance to Lymphedema Management Techniques

Compliance to all lymphedema management techniques measured through the compliance score and dichotomized into two categories increased from 3% at baseline to 74% at 6 months and plateaued to 63% at 24 months (p-value 24 months vs. baseline <0.0001) (Figure 3.2). Compliance to soap use increased rapidly and remained high throughout the study (92%) (data not shown). Among those with inter-digital entry lesions, compliance to antifungal cream increased from 8% at baseline to 55% at 24 months (p-value <0.0001). Compliance to elevation, exercise, and wearing footwear outside the home reached moderate levels at 24 months: 88%, 62%, and 51% respectively.

Predictors of Compliance

Table 3.5 displays the frequency of potential predictors of compliance during the course of the study at 6 month time intervals. Patients' perceived knowledge on how to care for their lymphedema reached 100% at 6 months and remained for the rest of the study period. The mean number of MDAS in which patients reported participating increased significantly over time. Difficulty accessing water, soap, and antifungal cream, decreased significantly over time as did the mean number of ADL episodes and perceived disability assessment score.

In bivariate models, disease severity was associated with compliance to several individual lymphedema management techniques (data not shown). Those with advanced lymphedema at baseline (OR = 5.96 (95% CI: 4.16, 8.54)) and those with inter-digital entry lesions (OR = 3.49 (95% CI: 2.82, 4.30)) were more likely to comply with antifungal cream. Those with advanced lymphedema were less likely to exercise (OR = 0.63 (95% CI: 0.45, 0.88)). Patients with advanced lymphedema at baseline (OR = 0.39 (95% CI: 0.23, 0.66)) and with entry lesions (OR = 0.70 (95% CI: 0.55, 0.90)) were less likely to comply with wearing footwear outside.

Increasing age, increasing number of ADL episodes, difficulty accessing water, soap, antifungal cream, antibiotics, and the hospital as well as increasing disability assessment score were negatively associated with overall compliance to lymphedema management techniques in bivariate models (data not shown). Having at least a primary school education was positively associated with compliance. In a multivariable logistic model (Figure 3.3) difficulty accessing soap and antifungal cream were negatively associated with compliance to lymphedema management techniques.

Compliance to Lymphedema Management Techniques and Rate of ADL Episodes

We assessed the association between overall compliance to lymphedema management techniques measured through the compliance score and the 30-day rate of ADL episodes. Significant interaction by presence of entry lesions at the previous time point was present. Overall compliance to lymphedema management appeared to have little to no association with the frequency of ADL episodes among those without entry lesions (RR=0.87 (0.69, 1.10) (Table 3.6). Among those with entry lesions, compliance was associated with an increase in the rate of ADL episodes (RR=1.44 (1.11, 1.86)). To

explore this association further, we then lagged the total compliance score by two time points (data not shown) in order to allow enough time for the potential impacts of lymphedema management to take place. Using this technique, compliance was associated with a decrease in the rate of ADL episodes among those with entry lesions (RR=0.77 (95% CI: 0.59, 0.99)) and was somewhat associated with a decrease in the rate among those without entry lesions (RR=0.83 (95% CI: 0.64, 1.06)), although this finding was not significant.

We examined compliance to specific techniques at a previous time point that target the prevention of ADL episodes. Exploring compliance to soap, we found significant interaction by the presence of inter-digital entry lesions at the previous time point and baseline lymphedema stage (advanced vs. early). Compliance to soap was associated with a decrease in the rate of ADL episodes in all disease groups except among those with entry lesions and early lymphedema (Table 3.6). Since antifungal cream was only indicated among those with inter-digital entry lesions, we evaluated the association between compliance to cream use and ADL episodes only among those with inter-digital entry lesions present at the previous time point. Compliance to antifungal cream use had little to no association with the rate of ADL (Table 3.6). When we lagged the antifungal cream compliance two time points (data not shown), the rate ratio was further from the null, but was not significantly associated with a lower rate of ADL episodes, RR = 0.88 (0.63, 1.22) (only among those with lesions). Compliance to wearing footwear outside had a little to no association with the rate of ADL episode (Table 3.6).

Compliance to Lymphedema Management Techniques and Lymphedema Progression

We also looked at the association between overall compliance to lymphedema management techniques and lymphedema progression from one time point to the next. We found that those who are compliant to all management techniques were less likely to progress to a more advanced stage (OR=0.84 (0.62, 1.12)) (Table 3.7), yet the OR was not statistically significant.

We examined compliance to specific techniques at a previous time point that target the prevention of lymphedema progression. Compliance to soap was negatively associated with lymphedema progression (RR=0.63 (0.41, 0.98)). Compliance to antifungal cream at the previous time point, only among those with inter-digital lesions present at the previous time point, was associated with a decrease in the odds of lymphedema progression, although the finding was not statistically significant (Table 3.7). Compliance to elevation and exercise of the limb and wearing footwear outside had little to no association with lymphedema progression.

Discussion

This study found that patients enrolled in a community-based lymphedema management program experienced a 35% lower rate of ADL episodes at 24 months compared to baseline. The rate of ADL was lowest six months after enrollment, yet a decrease was sustained over the two year period of the study. These findings are consistent with other studies which have also found a decrease in ADL episodes following enrollment in a lymphedema management program [34, 37, 38, 46, 48, 82]. Other programs have found a similar plateauing of the ADL rate 3-12 months after beginning lymphedema management [37, 38]. Although our results show a significant decrease in the rate of ADL episodes over the 2 years since the lymphedema management program was implemented, we recognize that this decrease may be partially influenced by patient receipt of anti-filarial drugs during the two MDAs that took place over the course of the study [44]. To control for this possibility, we included the number of MDAs in which each patient reported ever participating in our multivariable model exploring the effectiveness of the program over time. In doing this, we found that the rate of ADL episodes at 24 months was 0.57 that of the rate at baseline. Even while controlling for MDA in our analyses, we do note the possibility for the decrease in ADL episodes to be partially influenced by decreased LF transmission as seen by others [21]. Data obtained from the Odisha State Ministry of Health for the entire state illustrate a decrease in the microfilaremia from 0.69% in 2009 to 0.42% in 2011 (personal communication). These data, however, are for the entire state of Odisha, and may not represent transmission levels in the district of Khurda.

In our cohort, the rise in ADL episodes after the 6 month time point may be partially explained by the fact that following 6 months in the program, patients were expected to procure their own soap and anti-fungal cream from local stores and pharmacies. Accompanying the dip in ADL rate at 6 months and subsequent rise, reported compliance to program techniques peaked at 6 months with a subsequent decline and plateau through 24 months, providing further evidence for this possible explanation.

Patients with advanced lymphedema at baseline and inter-digital entry lesions at each time point had the highest rate of ADL episodes throughout most of the study period. Although several studies have found an increased ADL rate among those with increasing lymphedema stage [9, 37, 46, 47] and among those with inter-digital entry lesions [46, 47, 112], none have found an interaction between the disease groups. The decrease in ADL rate was most striking among patients with advanced lymphedema at baseline and no entry lesions, suggesting the program was most impactful among these patients for decreasing ADL episodes.

Our study demonstrates that patients enrolled in the lymphedema management program experienced a moderate decrease in lymphedema stage. Given the concerns that advanced lymphedema may be difficult to reverse, it is not surprising that the majority of lymphedema regression occurred among patients with stages 3 and 4 transitioning to stages 1 and 2. These results are similar to the leg volume decreases found in Haiti among patients with stages 2, 3, and 4 lymphedema [37]. The percentage of patients whose lymphedema progressed to a more severe stage from one time point to the next also decreased over the study period suggesting that a basic hygiene and management program is capable of slowing the advancement of chronic lymphedema. Decreased

ADL rates and slowing the advancement of chronic lymphedema have important implications for the social stigma, debilitation, and quality of life impacts seen with LF disease. We have previously reported that patients in the lymphedema management program in Khurda district experienced a significant reduction in disability in every domain of the WHO-DAS II disability assessment score [26].

When evaluating predictors of compliance to all lymphedema management techniques, we found that reported difficulty accessing soap and antifungal cream were negatively associated with compliance indicating that access to the resources needed to perform the lymphedema management techniques may improve compliance. The study in Léogâne, Haiti found that compliance was associated with female gender and age >40 years [37], two associations we did not find. Patients enrolled in the limb-care program in Sri Lanka identified inconvenience, inability to find footwear large enough to fit, and forgetting to exercise the affected limb as the main reasons for non-compliance to the limb-care program [83].

Among patients without inter-digital entry lesions, overall compliance to lymphedema management was slightly associated with a reduced ADL rate, but this finding was not significant. Compliance was significantly associated with an increased rate of ADL episodes among those with entry lesions, which is difficult to explain biologically and has not been noted in other studies [37] [83]. However, when lagged two time points, compliance to lymphedema management techniques was associated with a decreased rate of ADL episodes among both groups. This suggests that the preventative benefits of the lymphedema management techniques may not be seen until

months after initiation, especially among those with entry lesions who are already at increased risk for ADL episodes.

Looking at the lymphedema management techniques separately, compliance to washing the limb with soap decreased the rate of ADL episodes in most disease groups. Compliance to antifungal cream among those with inter-digital entry lesions present had no effect on the rate of ADL. This finding will need to be explored further in order to better understand the efficacy of antifungal cream use in the prevention of ADL episodes. The only other studies evaluating antifungal cream compliance and the rate of ADL episodes did not find an association [37, 75] .

Evaluating the effects of compliance on the chronic form of LF, lymphedema progression, we found that patients who reported overall compliance to lymphedema management had slightly lower odds of lymphedema progression. Although this finding was not statistically significant, this study was not initially powered to detect a change in lymphedema progression, rather a change in ADL episodes. Compliance to soap had a significant negative association with the progression of lymphedema. Although studies of lymphedema management programs have found reductions in leg volume after implementation of the program [36, 37], no other studies have found an association between self-reported compliance to a lymphedema management technique and chronic lymphedema progression. One study found a slight positive association between uptake of diethylcarbamazine and ivermectin [78] and decreasing lymphedema stage. Therefore, we controlled for the frequency of MDA uptake among this population and still found a significant effect of soap compliance.

This study had several limitations. Aside from the physical examination of lymphedema stage, all results were based on patient self-report. Results involving the 30-day rate of ADL episodes may be subject to recall bias. Patients may have inaccurately recounted the number of ADL episodes they experienced or may have had a difficult time identifying distinct episodes. It would be ideal to have prospective information regarding ADL episodes with detailed information on the length of the episode and if any other episodes occurred simultaneously. This would allow us to calculate a more accurate rate of ADL episodes and to consider important aspects such as wash out period. Another major limitation of this study is the lack of a true control group: a subset of comparable patients who did not receive the community-based lymphedema program. Because of this limitation, we note the possibility that the decrease in the rate of ADL episodes over the study period may have occurred regardless of the intervention. A control group for this study was not deemed to be feasible since it would involve withholding knowledge of lymphedema management techniques from patients with lymphedema.

In addition, this study may have been limited by a social desirability bias leading patients to overestimate compliance to the lymphedema management techniques in order to please the interviewer. Selection bias may also be present as those who agreed to participate in the study may have been more willing to work to improve their lymphedema compared to those who did not enroll in the study. When exploring the association between compliance to lymphedema management techniques and the rate of ADL episodes, we lagged the compliance variable by one time point. This was done for both the 1 month and 6 month intervals of data points. For example, for ADL rate at 3

months, we looked at compliance reported at the 2 month time point. For the ADL rate at 12 months, we looked at compliance reported at the 6 month time point. We realize that the time intervals are unequal and this may be affecting the estimate of effect. Lastly, this study was conducted amongst a cohort of lymphedema patients in one district of Odisha State, India which may limit the generalizability of the results.

This study solidifies previous findings showing a decreased ADL rate among patients enrolled in a community-based lymphedema management program and demonstrates that this decrease can be maintained over a 2-year time period. This study is one of the first to demonstrate an association between self-reported compliance to soap and the 30-day rate of ADL episodes. We lagged the compliance variable at least one time point, in order to establish temporality of our results. In both analyses, we were able to assess the associations among different disease severity groups, explore interaction, and attempt to control for confounding. We also identified several predictors of program compliance in this population, providing information for current and future programs on how to increase compliance to lymphedema management among their patients.

Preliminary economic analyses have shown the per-person start-up cost of CASA's lymphedema management program varies from $6.5-$9.00, while the maintenance cost per person was $3.50 over the 24 months of follow-up [113]. The majority of the cost (64%) went to providing direct care to patients (i.e. training, follow-up, and supplies). Although lymphedema management programs may be more costly than annual MDA campaigns, we have demonstrated their effectiveness in this setting. In addition to the clinical benefits of the program, we have also found that persons enrolled in the program experienced a decrease of 2.5 work days lost due to their lymphedema

compared to baseline [114]. These benefits were maintained after the program stopped providing supplies such as soap and anti-fungal cream at 6 months. We hope that once patients see the benefits of consistently using these supplies, they will do their best to provide for themselves. Lymphedema management programs may have somewhat higher start-up costs, but the demonstrated benefits and lower sustaining costs may outweigh them.

Our results indicate that a community-based lymphedema management program is beneficial for lymphedema patients for both acute and chronic morbidity and these benefits can be sustained over a two year time period. It also illustrates that compliance to lymphedema techniques can be improved if patients are provided the proper resources such as soap and antifungal cream. Although there were differences of program effectiveness by disease severity group, we still support the broad implementation of lymphedema management efforts with integrated MDA campaigns. This work demonstrates the benefit of broad community-based lymphedema management programs implemented by volunteers or community health workers, while recognizing the need for referral care for patients with complicated lymphedema and more severe disease. Furthermore, our results suggest the clinical benefits of frequent soap use among this population. One may suggest that community-based lymphedema management programs focus strongly on the use of and continued access to soap for lymphedema patients.

This work demonstrates the benefits of scaling up lymphedema management programs, which is important since the global LF elimination program has recommended that all LF endemic countries provide access to morbidity management services [107]. Future research exploring the association between compliance and ADL episodes as well

as lymphedema progression should be performed amongst community-based lymphedema management programs in other endemic settings. Furthermore, the efficacy of antifungal cream and differences by disease severity need to be elucidated. Access to morbidity management and disability prevention services is a priority for the Global Programme to Eliminate Lymphatic Filariasis and developing cost-effective ways to provide these services will be needed even after mass drug administration for lymphatic filariasis has ended.

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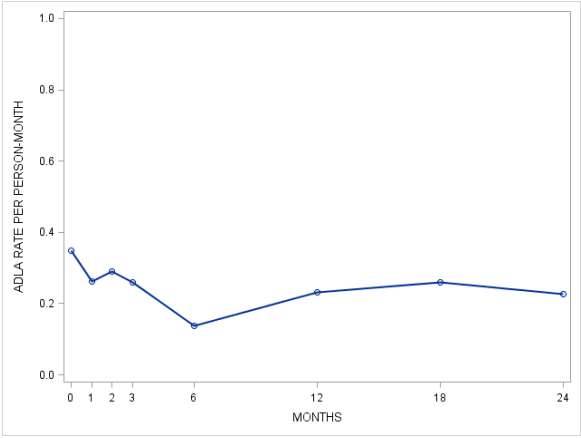
Table 3.1. Baseline Characteristics of Patients Enrolled in a Lymphedema Management Program, Khurda District, Odisha State, India. July, 2009. (N=370)

	N (mean)	% (SD)
Age	57	13.93
Female Gender	218	58.92
Education		
None at all	143	38.65
Completed at least primary school	226	61.08
Occupational Status		
Paid work	120	32.43
Non-paid work or unemployed	235	63.51
Caste		
General Caste	147	39.73
Other Backward Caste	175	47.30
Scheduled Caste	28	7.57
Scheduled Tribe	20	5.41
Presence of Chronic Health Condition other than Lymphedema	162	43.78
Comorbidities		
High blood pressure	62	16.76
Diabetes	12	3.24
Cancer	2	0.54
Heart problems	8	2.16
Stomach problems	66	17.84
Inter-digital Entry Lesions Present	100	27.03
Advanced Lymphedema Stage (4-6)	53	14.32
Years with lymphedema	25	16.04

A)



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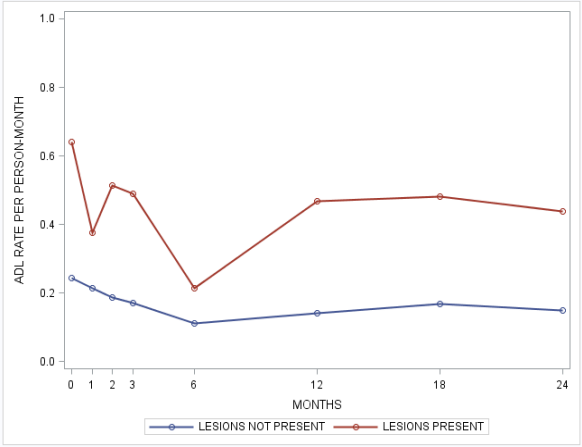

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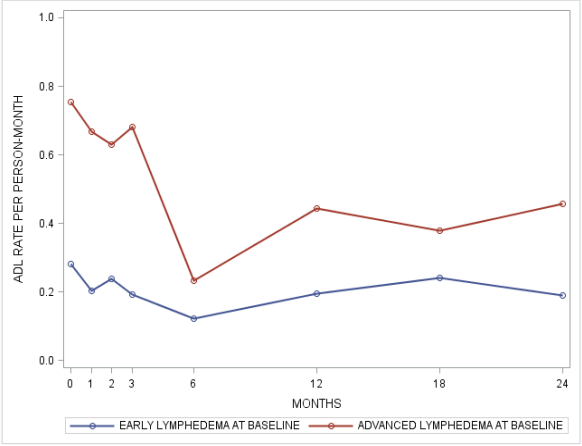


Figure 3.1. 30-day rate of ADL episodes, Khurda District, Odisha State, India. July 2009-July 2011. A. Overall 30-day rate of ADL episodes. B. 30-day rate of ADL episodes stratified by presence of inter-digital entry lesions. C. 30-day rate of ADL episodes stratified by baseline lymphedema status: early lymphedema (stages 1-3), advanced lymphedema (stages 4-7).

Table 3.2. 30-day rate of ADL over time among lymphedema management patients, Khurda District, Odisha State, India.

Time (months)	N	# episodes	ADL Rate per person-month	Rate Ratio (95% CI)*	Adjusted Rate Ratio (95% CI)**
0	370	129	0.35	1.00	1.00
1	351	92	0.26	0.68 (0.54, 0.85)	0.68 (0.54, 0.86)
2	349	101	0.29	0.74 (0.59, 0.94)	0.73 (0.58, 0.93)
3	339	88	0.26	0.64 (0.50, 0.81)	0.63 (0.49, 0.80)
6	324	45	0.14	0.34 (0.25, 0.46)	0.32 (0.24, 0.44)
12	321	74	0.23	0.58 (0.45, 0.75)	0.57 (0.44, 0.74)
18	332	86	0.26	0.66 (0.51, 0.84)	0.65 (0.51, 0.84)
24	316	72	0.23	0.57 (0.44, 0.74)	0.57 (0.43, 0.74)

*Rate ratios were calculated using mixed Poisson models accounting for random effect of subject, using an auto-regressive (1) correlation structure. The models used data at the individual level.

**Adjusted for patient report of number of MDAs in which they had participated.

Table 3.3. 30-day rate of ADL over time among lymphedema study participants by presence of inter-digital entry lesions at current time point and lymphedema status at baseline, Khurda District, Odisha State, India. July 2009-July 2011.

Time	N†	# episodes	Observed ADL Rate per person-month	Adjusted Rate Ratio (95% CI)*	N†	# episodes	Observed ADL Rate per person-month	Adjusted Rate Ratio (95% CI)*
	Advanced Lymphedema at Baseline: Entry Lesions Present				**Advanced Lymphedema at Baseline: No Entry Lesions**			
0	43	38	0.88	1.00	9	2	0.22	1.00
1	37	27	0.73	0.77 (0.50, 1.17)	8	3	0.38	1.12 (0.58, 2.16)
2	37	25	0.68	0.70 (0.45, 1.10)	8	4	0.50	0.54 (0.27, 1.06)
3	39	29	0.74	0.78 (0.51, 1.19)	8	3	0.38	0.77 (0.38, 1.55)
6	36	5	0.14	0.26 (0.14, 0.49)	11	6	0.55	0.27 (0.11, 0.67)
12	37	19	0.51	0.54 (0.33, 0.88)	8	1	0.13	0.31 (0.15, 0.66)
18	41	17	0.41	0.45 (0.27, 0.75)	4	0	0.00	0.22 (0.10, 0.49)
24	38	20	0.53	0.57 (0.35, 0.93)	7	1	0.14	0.38 (0.17, 0.82)
	Early Lymphedema at Baseline: Entry Lesions Present				**Early Lymphedema at Baseline: No Entry Lesions**			
0	57	26	0.46	1.00	258	63	0.24	1.00
1	75	15	0.20	0.49 (0.29, 0.81)	226	47	0.21	0.71 (0.51, 1.00)
2	74	32	0.43	0.86 (0.55, 1.36)	228	40	0.18	0.66 (0.46, 0.95)
3	55	17	0.31	0.56 (0.33, 0.94)	237	39	0.16	0.56 (0.39, 0.80)
6	43	12	0.28	0.35 (0.17, 0.69)	231	21	0.09	0.35 (0.23, 0.54)
12	55	24	0.44	0.83 (0.51, 1.35)	212	30	0.14	0.48 (0.32, 0.72)
18	44	24	0.55	1.16 (0.70, 1.92)	234	40	0.17	0.58 (0.41, 0.84)
24	49	18	0.37	0.79 (0.46, 0.95)	220	33	0.15	0.52 (0.35, 0.76))

†The sample size at each time point may not add up to 370 because of missing data and/or lost to follow-up.

*Adjusted results of Poisson regression for correlated data with significant interaction by entry lesions and lymphedema status. Controlling for number of MDAs in which patients reported participating.

Table 3.4. Lymphedema progression over the study period, Khurda District, Odisha State, India, July 2009-July 2011.

	Baseline N (%)	**1 Month N (%)**	**2 Month N (%)**	**3 Month N (%)**	**6 Month N (%)**	**12 Month N (%)**	**18 Month N (%)**	**24 Month N (%)**	**P-value***
Lymphedema Stage									
1-2	184 (48.73)	166 (47.29)	183 (52.44)	181 (53.39)	175 (54.01)	179 (55.76)	173 (52.11)	190 (60.13)	0.0064
3-4	139 (37.57)	146 (41.60)	126 (36.10)	115 (33.92)	105 (32.41)	97 (30.22)	115 (34.64)	80 (25.32)	0.0006
5-6	47 (12.70)	39 (11.11)	40 (11.46)	43 (12.68)	44 (13.58)	45 (14.02)	44 (13.25)	46 (14.56)	0.4795
Lymphedema Progression Since Previous Time Point									
Progression	NA	46 (13.11)	27 (7.74)	16 (4.72)	18 (5.56)	28 (8.72)	42 (12.65)	16 (5.06)	0.0004
No change	NA	278 (79.20)	284 (81.38)	301 (88.79)	283 (87.35)	264 (82.24)	266 (80.12)	240 (75.95)	0.3139
Regression	NA	27 (7.69)	38 (10.89)	22 (6.49)	23 (7.10)	24 (7.23)	24 (7.23)	60 (18.99)	<0.0001
Lymphedema Progression Since Baseline									
Progression	NA	46 (13.11)	44 (12.61)	42 (12.39)	36 (11.11)	43 (13.40)	55 (16.57)	29 (9.18)	0.1088
No change	NA	278 (79.20)	268 (76.79)	257 (75.81)	245 (75.62)	227 (70.72)	232 (69.88)	218 (68.99)	0.0026
Regression	NA	27 (7.69)	37 (10.60)	40 (11.80)	43 (13.27)	51 (15.89)	45 (13.55)	69 (21.84)	<0.0001

*Denotes the p-value for difference between baseline and 24 months

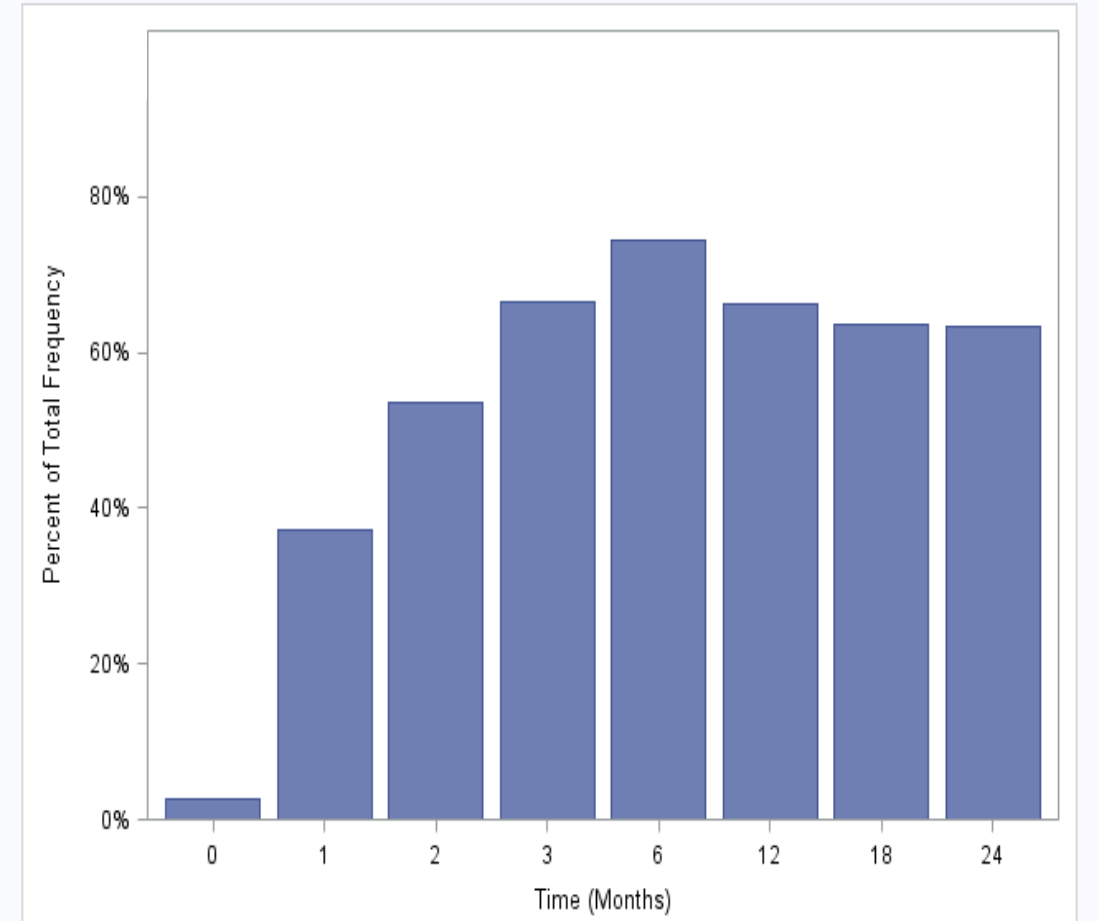


Figure 3.2. Frequency of program compliance over time, Khurda District, Odisha State, India. July 2009-July 2011. Program compliance was measured through a weighted compliance score to all techniques and dichotomized into two groups: compliant and non-compliant. Scores were weighted by the presence of inter-digital entry lesions.

Table 3.5. Potential predictors of compliance to lymphedema management techniques over time among patients enrolled in the lymphedema management program, Khurda District, Odisha State, India. July 2009-July 2011.

	Baseline (N=370)		**6 Months (N=324)**		**12 Months (N=321)**		**18 Months (N=332)**		**24 Months (N=316)**		**P-value***
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	
Knowledge on how to care for lymphedema	66	17.84	324	100.00	320	100.00	332	100.00	316	100.00	<0.0001
Difficulty accessing clean water	77	20.87	8	2.47	14	4.36	30	9.04	32	10.13	0.0001
Difficulty accessing soap	187	50.54	16	4.94	16	4.98	16	4.98	42	13.29	<0.0001
Difficulty accessing cream	185	50.00	20	6.17	18	5.61	50	15.06	41	12.97	<0.0001
Difficulty accessing oral medicine during ADL	205	55.41	110	33.95	119	37.07	162	48.80	187	59.18	0.2849
Difficulty accessing the hospital	226	61.08	138	42.59	159	49.53	174	52.41	188	59.49	0.5477
Own Mosquito Net	306	82.70	241	74.38	235	73.21	258	77.71	251	79.43	0.2743
Number of MDAs (Mean, SD)	2.2	1.5	2.2	1.4	2.2	1.4	2.1	1.5	3.2	1.7	<0.0001
Presence of inter-digital entry lesions	100	27.03	79	24.38	92	28.66	85	25.60	87	27.53	0.8936
ADL episodes in the past 30 days (Mean, SD)	0.42	0.92	0.14	0.45	0.23	0.71	0.26	0.72	0.23	0.69	0.0032
ADL episodes in the past 6 months (Mean, SD)	1.06	1.60	0.62	1.15	0.55	1.00	0.71	1.27	0.65	1.39	0.0007
Disability assessment score (Mean, SD)	66.18	22.76	58.21	16.36	60.08	18.00	63.85	19.68	60.41	19.04	0.0004

*Denotes the p-value for the difference between baseline and 24 months

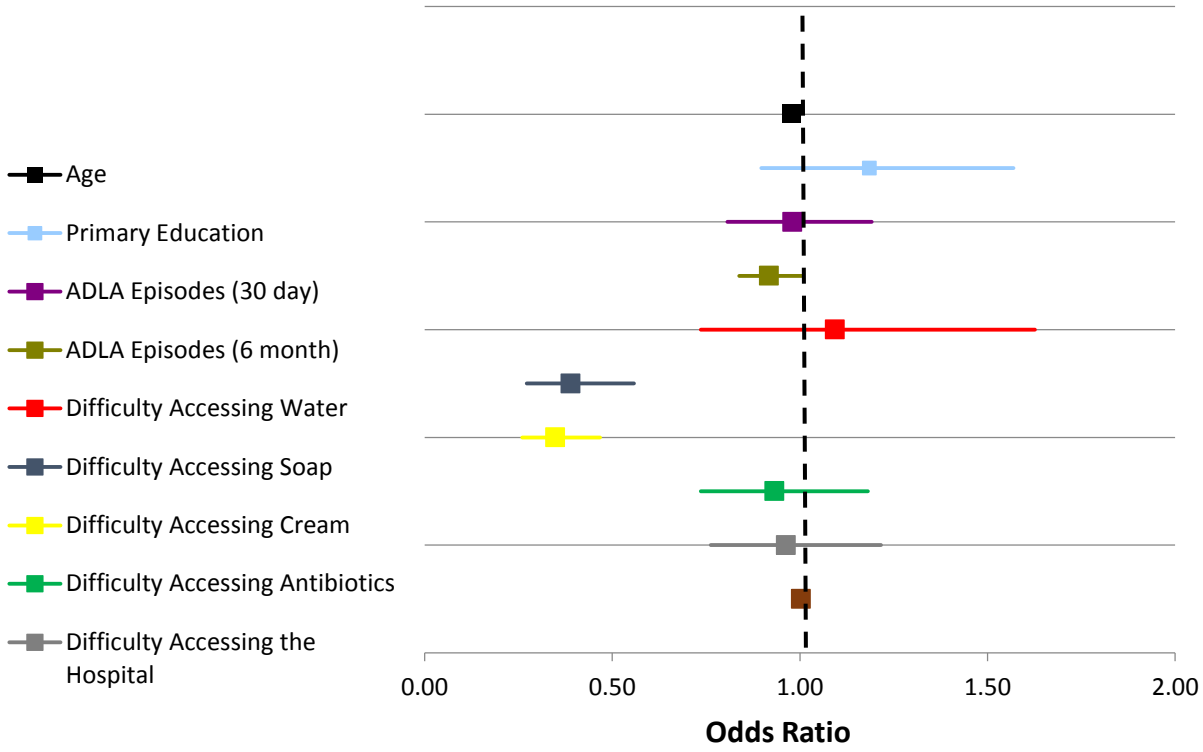


Figure 3.3. Predictors of compliance to lymphedema management techniques among patients enrolled in a lymphedema management program, Khurda district, Odisha state, India: results of a multivariable logistic model.

Table 3.6. Association between compliance to lymphedema management techniques at the previous time point and 30-day rate of ADL episodes, Khurda District, Odisha State, India. July 2009-July 2011.

Technique	Rate Ratio (95% CI)*
Overall compliance[γ]Þ	
Entry Lesions‡	1.44 (1.11, 1.86)
No Entry Lesions‡	0.87 (0.69, 1.10)
Soap*[δ]	
Entry Lesions‡, Advanced Lymphedema†	0.67 (0.43, 1.04)
Entry Lesions‡, Early Lymphedema†	1.45 (0.91, 2.29)
No Entry Lesions‡, Advanced Lymphedema†	0.26 (0.15, 0.45)
No Entry Lesions‡, Early Lymphedema†	0.57 (0.42, 0.77)
Antifungal cream[φ]	
Entry Lesions‡	1.09 (0.81, 1.46)
Footwear outside[#]	1.04 (0.85, 1.26)

* Adjusted results of Poisson regression for correlated data. Each rate ratio is comparing the rate of ADL episodes among those who are compliant to the rate among those who are not compliant within the specified clinical disease groups.

[γ]Dichotomized compliance score summarizing compliance to soap, cream, elevation, exercise, and footwear. Adjusted for baseline lymphedema status (advanced vs. early), access to water, access to soap, access to cream, access to the hospital, and number of time patient participated in MDA.

Þ Interaction with inter-digital entry lesions at previous time point was statistically significant.

‡Inter-digital entry lesions at previous time point

*Interaction with inter-digital entry lesions at previous time point and baseline lymphedema status statistically significant.

[δ] Adjusted for access to water, access to soap, access to cream, access to the hospital, number of time patient participated in MDA, and compliance to all other techniques.

†Lymphedema status at baseline

[φ] Only among those with inter-digital entry lesions at the previous time point. Adjusted for access to water, access to soap, access to cream, access to the hospital, number of time patient participated in MDA, lymphedema status at baseline (advanced vs. early), and compliance to all other techniques.

[#]Adjusted for baseline lymphedema status (advanced vs. early), inter-digital entry lesions at the previous time point, access to water, access to soap, access to cream, access to the hospital, number of time patient participated in MDA, and compliance to all other techniques.

Table 3.7. Association between compliance to lymphedema management techniques and lymphedema progression since previous time point. Khurda District, Odisha State, India. July 2009-July 2011.

Technique	OR (95% CI)*
Overall compliance[φ]	0.84 (0.62, 1.12)
Soap†	0.63 (0.41, 0.98)
Antifungal cream[γ]	
Entry Lesions‡	0.53 (0.21, 1.29)
Elevate†	1.11 (0.74, 1.67)
Exercise†	1.04 (0.74, 1.47)
Footwear outside*	0.93 (0.69, 1.26)

*Adjusted results of logistic regression for correlated data. Each rate ratio is comparing the rate of ADL episodes among those who are compliant to the rate among those who are not compliant within the specified clinical disease groups.

[φ] Dichotomized compliance score summarizing compliance to soap, cream, elevation, exercise, and footwear. Adjusted for baseline lymphedema status (advanced vs. early), inter-digital entry lesions at the previous time point, and number of time patient participated in MDA.

†Adjusted for baseline lymphedema status (advanced vs. early), inter-digital entry lesions at the previous time point, number of time patient participated in MDA, and compliance to all other techniques.

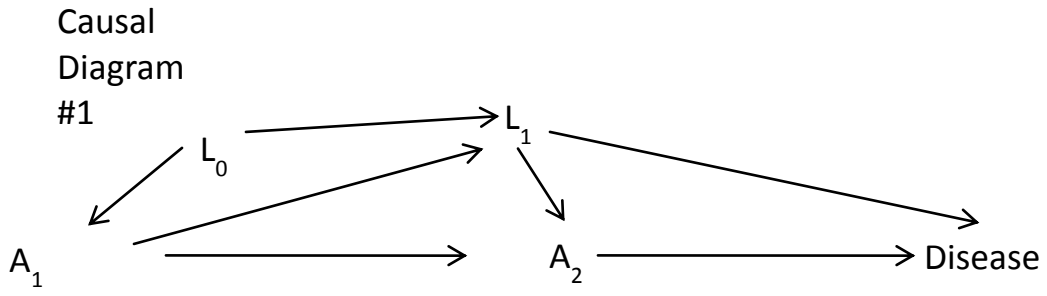
[γ] Only among those with inter-digital entry lesions at the previous time point. Adjusted for baseline lymphedema status (advanced vs. early), number of time patient participated in MDA, and compliance to soap, elevation, exercise, and wearing footwear.

‡Inter-digital entry lesions at previous time point

Chapter 4: Marginal Structural Models

Marginal structural models (MSMs) are a class of causal models for the estimation, from observational data, of the causal effect of a time-dependent exposure in the presence of time-dependent covariates that may be simultaneously confounders and intermediate variables[115] [116]. The parameters of MSMs can be estimated through inverse-probability-of-treatment weighted (IPTW) estimators. Robins has shown that traditional methods of adjustment for time-varying exposure using stratified analyses or parametric models may be biased [116] under the following two conditions: (1) there is a time-dependent covariate that is a risk factor for the outcome of interest and also predicts subsequent exposure, (2) past exposure history predicts subsequent level of the covariate.

The following causal diagram illustrates the issue raised when using traditional control methods in these situations:



We are interested in the total causal effect of A_1 and A_2 on disease. From causal diagram #1, we can see that L_1 confounds the relationship between A_2 and disease. However, if we control for L_1 we are simultaneously blocking the path from A_1 to disease, which we also want to measure. Through inverse probability of treatment weights (IPTW), the MSM creates a pseudo-population within each stratum of the

covariate so that there is no longer a confounding association from L_1.to A_2, while preserving the association between A_1 and L_1. Assuming no unmeasured confounding, the positivity assumption holds, and the MSM is correctly specified, the estimated parameter of interest will be a causal estimate of the relationship between the exposure regimen and the disease of interest.

In addition to the IPTWs, inverse probability of censoring weights (IPCWs) can be used in conjunction with the ITPWs to account for censoring due to loss to follow-up [116]. The IPCWs are analogous to the IPTWs, where censoring is treated as another time-varying treatment. Weighting by the inverse probability of censoring allows us to calculate the effect of interest, if contrary to the fact; all subjects had remained uncensored throughout the study period and allows us to control for potential selection bias due to *L*.

MSM Assumptions

The following assumptions must be made in order to interpret the estimate of effect as causal: 1) no unmeasured confounding exists, 2) the 'positivity' assumption [117]: all patients have a probability between 0 and 1 of receiving any treatment regimen conditional on their prior treatment history and baseline characteristics. Thus, there are no groups of patients who always receive a specific treatment or never receive treatment. 3) Our stated IPTW and IPCW models are correct.

The Positivity Assumption

The positivity assumption states: there is a positive probability of each treatment for each set of covariates (no perfect confounding) [118]:

$$f\left[\bar{a}(k-1), \bar{l}(k)\right] > 0 \rightarrow f\left[a(k)\middle|\bar{a}(k-1), \bar{l}(k)\right] > 0$$

Stabilized Inverse Probability of Treatment Weights

Let us define A_k as a dichotomous variable indicating treatment=1 or no treatment=0 on the k[th] day from the start of follow-up [118]. Let us define *Y* as a dichotomous outcome variable measured at the end of follow-up on day *K+1*. L_K represents the value on day *k* of the vector of all measured risk factors for the outcome. Let $\overline{A_k} = (A_0, A_1, A_2, \dots, A_k)$ be the observed exposure history through day *k* and let $\bar{A} = \overline{A_k}$. Let $\overline{L_k} = (L_0, L_1, L_2, \dots, L_k)$ be the observed covariate history through day *k* and let $\bar{L} = \overline{L_k}$. Let $Y_{\bar{a}}$ be the value of Y that would have been observed had all subjects received dose history $\bar{a} = (a_0, a_1, \dots, a_k)$ rather than their observed dose history $\bar{A}$. The following is the linear-logistic MSM that represents the causal effects of dose history on the outcome Y:

$$logit\ pr\ (Y_{\bar{a}} = 1) = \beta_0 + \beta_1 cum(\bar{a})$$

$where\ cum(\bar{a}) = \sum_{k=0}^{K} a_k$ is the cumulative dose through the end of follow-up associated with the dose history $\bar{a}$.

The MSM model below is for the observed data:

$$logit\ pr(Y = 1|\bar{A} = \bar{a}) = \beta'_0 + \beta'_1 cum(\bar{a})$$

If the observed data are unconfounded, the positivity assumption is met, and there is no loss to follow-up selection bias, then $\beta'_1 = \beta_1$ and the parameter estimates from the observed data using standard logistic regression will be unbiased. If the treatment is confounded by $\bar{L}$, then $\beta'_1 \neq \beta_1$ and our standard logistic regression parameter estimate

will be a biased estimate of the causal parameter. However, if there are no unmeasured confounders given data on the time-dependent covariates ($\bar{L}$) one can control for time-varying confounding by modifying the crude analysis by weighting each subject by the stabilized inverse probability of treatment weight SW. Observations where the time-varying factors are strong predictors of the current treatment are down-weighted in the analysis (because such observations are over-represented in the observed data).

$$SW = \prod_{k=0}^{t} \frac{f[A(k)|\bar{A}_{k-1}, V]}{f[A(k)|\bar{A}_{k-1}, \bar{L}(k)]}$$

In *SW* above, *A(k)* represents treatment at time *k*, $\bar{A}_{k-1}$ represents treatment history prior to time *k*, *V* represents a vector of time-independent covariates (baseline covariates), and $\bar{L}(k)$represents a vector of time-varying covariates through time *k*. The numerator is the probability a patient is on the observed treatment at time *k*, given the prior treatment history and baseline covariates. The denominator is the probability a patient is on the observed treatment at time *k*, give prior treatment history, baseline covariates, and time-varying covariates. Although not explicitly stated, *V* is included in the denominator since it is a subset of the variables in L_0.

The weighting creates a pseudo-population with the following important properties: treatment ($\bar{A}_k$) is not confounded by the time-varying covariates ($\bar{L}_{k-1}$) and the probability of the outcome given the treatment value in the pseudo-population is the same as in the true study population so that the causal estimate of effect is the same in both populations. Furthermore, the association between $\bar{A}_k$ and $\bar{L}_k$ is preserved. Because of these properties, we can calculate an unbiased estimate of the causal effect in the pseudo-population.

Estimating the Treatment Weights

Since the numerator and denominator of the stabilized weights are not known, we must estimate them from the observed data using a specified model. The following example of an estimated denominator says the probability of being treated on day *k* depends in a linear logistic fashion on the day *k*, the previous 2 days' treatment, the current and previous days' covariates, an interaction between yesterday's treatments and today's covariates, and the baseline covariates [119].

$$Denominator = \ logit\ pr\left(A_k = a_{ki} \middle| \bar{A}_{k-1} = \bar{a}_{(k-1))i}, \bar{L}_k = \bar{l}_{ki}\right) = \alpha_0 + \alpha_1 k + \alpha_2 a_{k-1} + \alpha_3 a_{k-2} + \alpha_4 l_k + \alpha_5 l_{k-1} + \alpha_6 a_{k-1} l_k + \alpha_7 V$$

One can fit the model above using standard logistic regression techniques. One can also estimate the numerator of sw_i by fitting the model above without the terms that include time-dependent covariates.

$$Numerator = \ logit\ pr\left(A_k = a_{ki} \middle| \bar{A}_{k-1} = \bar{a}_{(k-1))i}, V\right) = \alpha^*{}_0 + \alpha^*{}_1 k + \alpha^*{}_2 a_{k-1} + \alpha^*{}_3 a_{k-2} + \alpha^*{}_4 V$$

For each subject *i* we then have the estimated probabilities of the denominator of the weight at each time point, $\hat{p}_{0i}, \ldots, \hat{p}_{Ki}$. For each subject *i* we also have the estimated probabilities of the numerator of the weight at each time point, $\hat{p}^*{}_{1i}, \ldots, \hat{p}^*{}_{Ki}$. Then we estimate sw_i for each subject by taking the product of each numerator and denominator probabiilty:

$$SW_i = \frac{\prod_{k=0}^{K}\left(p^*{}_{ki}\right)^{a_{ki}}(1 - p^*{}_{ki})^{1-a_{ki}}}{\prod_{k=0}^{K}(\hat{p}_{ki})^{a_{ki}}(1 - \hat{p}_{ki})^{1-a_{ki}}}$$

The data analyst will need to write a small program to compute sw_i for each subject to obtain the predicted values output from the logistic regression models. Assuming no unmeasured confounders and that the models specified to estimate the denominator of the stabilized weights is correctly specified, the resulting estimate of the causal parameter β_1 will be unbiased. The stabilized weights would be put into a model in SAS's PROC GENMOD using the WEIGHT statement.

Non-Stabilized Inverse Probability of Treatment Weights

In contrast to the stabilized IPTWs, non-stabilized IPTWs have a one in the numerator:

$$W(K) = \prod_{k=0}^{K} \frac{1}{f[A(k)|\bar{A}_{k-1}, \bar{L}(k)]}$$

The non-stabilized weight is the basis of the marginal structural model theory, yet when the time-varying confounders are strongly associated with treatment, the values of w_i may vary greatly between subjects [116] which could result in very large weight values for some subjects. These subjects will thus contribute a large number of copies of themselves to the pseudo population. This variability can be avoided by calculating a stabilized weight, sw_i, where the probability of treatment x probability of remaining uncensored conditional on past treatment and censoring history replaces the one in the numerator. A subject that would normally have a very large or very small $w_{i,}$ will have a sw_i that is relatively closer to one, decreasing the overall variability of the weights. Robins has shown that the replacing the numerator of the stabilized weight with any other function of $(\bar{A}_k)$ will not influence the consistency of the estimator, but only the variance of the estimator will be influenced[120]. It is suggested that one use the stabilized

weights in MSMs, as the variance of the non-stabilized weights is often large with a non-normal sampling distribution [116].

Censoring Weights

A similar weight can also be estimated to adjust for selective patient drop out in the observational cohort.

$$SW_C(K) = \prod_{k=0}^{K} \frac{\Pr[C_{k+1} = 0 | C_k = 0, \bar{A}(k), V]}{\Pr[C_{k+1} = 0 | C_k = 0, \bar{A}(k), \bar{L}(k)]}$$

$C(k) = 0$ if a subject remains in the study beyond time *t*, 1 otherwise $C(k) = 1$. The probabilities of the numerator and denominator of the censoring weight can be estimated from the observed data in a similar way as the probabilities of the treatment weights were above.

The final weight for each patient observation is the product of the treatment weight and censoring weight.

$$Weight_{ik} = SW_{ik} * SW_{Cik}$$

The corresponding non-stabilized censoring weight:

$$W_C(K) = \prod_{k=0}^{K} \frac{1}{\Pr[C_{k+1} = 0 | C_k = 0, \bar{A}(k), \bar{L}(k)]}$$

Effect of Treatment Analysis

Following the estimation of the weights, a standard regression analysis is done incorporating the treatment and censoring weights for each subjects' observations. The following SAS Code is from a study of treatment for schizophrenia and the linear

outcome of change in Brief Psychiatric Rating Scale (BRPS) since baseline [119]. The model is a linear repeated measures model that incorporates the standardized treatment and censorship weights in the SAS WEIGHT statement. The REPEATED statement is used in order to produce robust standard errors.

```
PROC GEMOND DATA= WEIGHTS;
        WEIGHT STABWT;
        CLASS VIS PATSC GENDER ORIGIN2 INVSC TRT;
        MODEL CAVAR = TRT INVC BAVAR VIS AGEYRS GENDER ORIGINS2
        VIS*TRT/ DIST=NORMAL LINK=ID TYPE3;
        REPEATED SUBJECT = PATSC/ TYPE=IND;
RUN;
```

See Appendix 1 in chapter 6 for a detailed illustration of the weighting.

Chapter 5: The Effect of a Regimen of Anti-fungal Cream use on Episodes of Acute Adenolymphangitis (ADL) among Lymphedema Patients: an Application Using Marginal Structural Models.

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Abstract

Episodes of adenolymphangitis (ADL) are a recurrent clinical aspect of lymphatic filariasis lasting 3-15 days each and can occur several times each year. Previous studies have concluded that ADL episodes are a risk factor for the progression of chronic LF symptoms such as lymphedema and elephantiasis. Inter-digital entry lesions, thought to be caused by fungal infections, are often found on the web spaces between the toes of those suffering from lymphedema. Clinical research has shown that entry lesions contribute to the occurrence of ADL episodes. It has been suggested that the prevention of ADL episodes through recognition and treatment of interdigital entry lesions is a critical component of lymphedema management.

Our objective in this study was to estimate the observed effect of anti-fungal cream use on episodes of acute adenolymphangitis (ADL) according to treatment regimen among a cohort of lymphedema patients enrolled in a morbidity management program in Odisha State, India. We estimated this effect using marginal structural models for time varying confounding and compared these results to traditionally adjusted models.

Based on the marginal structural model using non-stabilized weights, we estimated that for every one unit increase in the number of times a patient used cream during the follow-up period, there was a 20% decrease in the rate of ADL episodes at 24 months (RR=0.80 (0.63, 1.03)). This result is compared to a 26% decrease estimated from an MSM using stabilized weights (RR=0.74 (0.54, 1.01)), a 17% decrease using a traditionally adjusted model (RR=0.83 (0.62, 1.11)), and an 8% increase using a crude model (RR=1.08 (0.88, 1.32)).

This is the first study to estimate the effect of a regimen of anti-fungal cream on the frequency of adenolymphangitis episodes among lymphedema patients enrolled in a morbidity management program. This study also highlights the importance of the consideration and proper handling of time-varying confounders that are also affected by previous exposure status in longitudinal observational studies.

Introduction

Patients with lymphatic filariasis (LF), a parasitic disease, often suffer from episodes of acute adenolymphangitis (ADL). These episodes are characterized by plaque-like areas of inflammation, swelling of the limbs, fever, malaise and chills [2], and are typically the first clinical sign of LF, occurring years after infection. ADL episodes are a recurrent clinical aspect of LF lasting 3-15 days each and can occur several times each year [34]. The frequency of ADL episodes has been shown to increase with more advanced lymphedema and studies have concluded that ADL episodes are a risk factor for the progression of chronic LF symptoms such as lymphedema and elephantiasis [9, 10, 34, 48, 75].

Inter-digital entry lesions, thought to be caused by fungal infections [121, 122], are often found on the web spaces between the toes of those suffering from lymphedema. Clinical research has shown that entry lesions contribute to the occurrence of ADL episodes [121]. A study of entry lesions among lymphedema patients in Recife, Brazil found that a higher number of lesions was associated with more advanced lymphedema stage and a higher frequency of ADL episodes [112]. The relationship between inter-digital entry lesions and the frequency of ADL episodes has also been seen in Guyana [47], but only a minority of lesions was found to have positive microscopy for fungal pathogens. Etiologically, it is hypothesized that entry lesions serve as a point of entry for bacteria, leading to ADL episodes [40, 121]. It is recommended that the prevention of ADL episodes through recognition and treatment of inter-digital entry lesions be a critical component of lymphedema management [112].

Little research has explored the effect of anti-fungal cream to treat inter-digital entry lesions on the frequency of ADL episodes. Several papers have illustrated the effectiveness of lymphedema management programs in decreasing the frequency of ADL over time [35-38], but few have evaluated the relationship between program compliance and ADL episodes. Those that have evaluated the association between overall compliance and ADL found no association [35, 75] or found a positive association among those with inter-digital entry lesions [16]. The only other study exploring the specific relationship between anti-fungal cream use and ADL episodes found a null association after restricting the analysis to only those who had inter-digital entry lesions and controlling for other covariates in a multivariable model [123].

Our objective was to estimate the observed effect of a treatment regimen of anti-fungal cream use on episodes of acute adenolymphangitis (ADL). We estimated this effect using marginal structural models for time varying confounding and compared these results to traditionally adjusted models.

Methods

A lymphedema management program was implemented in Khurda district, Odisha State, India by the non-governmental organization, Church's Auxiliary for Social Action (CASA). All patients enrolled in the program were trained in basic lymphedema management by physician-trained volunteers, including daily washing of limbs with soap and water, daily exercise and elevation of the affected limb, and daily use of footwear outside the home. Patients were trained in the importance of early treatment and prevention of secondary bacterial and fungal infections with topical and oral antimicrobial agents. If inter-digital fungal infections were present, patients were instructed to use an antifungal cream on a daily basis. Patients were supplied with soap and antifungal cream for the first 6 months of the program; thereafter they were instructed to purchase these supplies at local stores and pharmacies.

A cohort of 370 individuals was recruited and enrolled in CASA's community-based lymphedema management program. To be eligible, persons had to be at least 14 years of age and reported lower leg swelling for at least 3 months. See reference [123] for more details on the sampling and patient recruiting process. Patients were interviewed in Oriya (the local language) by trained interviewers at baseline (prior to enrollment) and at, 1, 2, 3, 6, 12, 18 and 24 months after enrollment in the program which occurred in July 2009. Through a written questionnaire, interviewers collected information on patient demographics, frequency of compliance to lymphedema management techniques, ADL history and treatment, access to supplies, MDA history, and perceived disability. Interviewers also completed a clinical assessment on each person in order to determine lymphedema stage using the 7-stage system [12] and to determine if inter-digital entry

lesions were present. Baseline lymphedema stage was categorized as advanced (stages 4-7) or early (stages 1-3). Data were independently dual-entered into an Epi Info 7 (Stone Mountain, 2008) database. Data cleaning and analysis were performed in SAS 9.3 (Cary, North Carolina, USA).

An ADL episode was defined by patient self-report of two or more of the following symptoms: redness, pain, or swelling of the leg or foot, with or without the presence of fever or chills, [40, 48]. Patients were asked how many times they had an ADL episode in the previous 30 days. The ADL rate per subject was calculated as the number of ADL episodes reported by each subject divided by 30 days. We assumed that each ADL episode reported initially occurred during the 30-day period and not before the beginning of that period.

Use of anti-fungal cream was measured through patient self-report at each time point. Patients were asked how frequently they used antifungal cream in the previous 30 days. A person was considered to be compliant to anti-fungal cream use if he/she reported using anti-fungal cream at least once per day. Cream use compliance at time point k (i.e., $cream_k$) was treated as a dichotomous variable: 1 if a patient was compliant, 0 if a patient was not compliant.

To measure the effect of a cream regimen, we used the sum of cream compliance over time [116] through *K*-1 (i.e. cum($\overline{cream}_{K-1}$)), where *K* indicates the time point at which ADL was evaluated and *k* indicates any time point previous to *K*: $cum(\overline{cream}_{K-1}) = \sum_{k=1}^{K-1} cream_k$. We did not include baseline cream use in the sum because patients had yet to be enrolled in the lymphedema management program, and

hence had not yet been instructed on anti-fungal cream use. The maximum sum of cream use possible varied by time of follow-up *K*. For example, when estimating the effect of cream through *K*-1 = 18 months on ADL episodes at *K* = *24* months, the maximum $cum(\overline{cream}_{K-1}) = 6$, representing cream use at 1, 2, 3, 6, 12, and 18 months. When estimating the effect of cream through *K*-1 = 6 months on ADL episodes at *K* = 12 months, the maximum $cum(\overline{cream}_{K-1}) = 4$, representing cream use at 1, 2, 3, and 6 months.

The presence or absence of inter-digital entry lesions was considered a potential confounder in this study as this variable is associated with anti-fungal cream use and is a risk factor for ADL episodes. Additionally, the presence or absence of inter-digital entry lesions is a condition that may come and go over a matter of days among LF patients, hence this variable is also time-varying in nature. Lymphedema status at baseline (advanced vs. early) and patient report of ever receiving drugs such as albendazole and diethylcarbamazine during mass drug administration (MDA) at time point *K* were considered as non-time-varying confounders.

Marginal Structural Models

Marginal structural models (MSMs) are a class of causal models for the estimation, from observational data, of the causal effect of a time-dependent exposure in the presence of time-dependent covariates that may be simultaneously confounders and intermediate variables [115, 116]. Robins has shown that traditional methods of adjustment for time-varying exposure using stratified analyses or parametric models may be biased [116] under the following two conditions: 1) there is a time-dependent covariate that is a risk factor for the outcome of interest and also predicts subsequent

exposure, 2) past exposure history predicts subsequent level of the covariate. The parameters of MSMs can be estimated through inverse-probability-of-treatment weighted (IPTW) estimators. The weighting creates a pseudo population in which the time-varying confounder is no longer associated with treatment, yet does not improperly control away the effect of the time-varying covariate as an intermediate in the causal pathway of interest. In addition to the IPTWs, inverse probability of censoring weights (IPCWs) can be used in conjunction with the ITPWs to account for censoring due to loss to follow-up, those subjects who drop out before the end of a study period [116]. The IPCWs are analogous to the IPTWs, where censoring is treated as another time-varying treatment. Weighting by the inverse probability of censoring allows us to calculate the effect of interest as if, contrary to what was observed, all subjects had remained uncensored throughout the study period

To estimate the cumulative effect of cream use measured at months 1 and 2 on the number of ADL episodes reported in the previous 30 days after K= 3 months of follow-up, let A_1 equal the observed sum of cream use at k=1 month of program enrollment and let L_1 represent the measured time-varying confounder, presence of inter-digital entry lesions, that may be associated with A_1 [116]. Let A_2 equal the observed cumulative cream use from 1 month to k=2 months of program enrollment and let L_2 represent the presence of inter-digital entry lesions that may be associated with A_2. Figure 5.1 shows the hypothesized causal diagram representing the causal relationship of interest through K=3 months of follow-up and the corresponding confounding relationships. The presence of inter-digital entry lesions (L_k) confounds the relationship between cream use at each time point (A_k) and the number of ADL episodes at the end of K=3 months of

follow-up (Y). If we control for lesions at k=2 months (L_2) in a traditional stratified model, we would also be blocking the causal pathway from cream at k=1 (A_1) to ADL through lesions at k=2 (L_2). Therefore, we would expect an estimate of the effect of cumulative cream use (from A_1 and A_2 to Y) on the number of ADL episodes at K=3 months obtained using traditional methods, in which inter-digital entry lesions is an independent time-varying variable in a regression model, to be a biased estimate of the true causal effect. In Figure 5.2, we expand the causal diagram of Figure 5.1 to represent the cumulative effect of cream use on the number of ADL episodes at K=24 months of follow-up (see Supplemental Appendix for additional causal diagrams).

Figure 5.3 incorporates censoring as another time-varying treatment in the causal diagram. Censoring at time k indicates that a subject was loss to follow-up at time point k. For example, if someone was censored at time k=3 months, that means we had data at baseline, 1 month, and 2 month, but we do not have data on them at 3 months. Even if they did provide data again at 12, 18, and 24 months, we still consider them censored at k=3 months and each proceeding time point. In Figure 5.3, we are assuming that the presence of interdigital entry lesions at time k is associated with censoring at time k. For example, someone who has lesions at k=3 months may be more likely to be censored at k=3 months (i.e. not provide data at k=3 months) than someone who does not have lesions. Thus, we are assuming there is some association between L_k and C_k. Of course, we cannot know this from the data because anyone who is censored at time k (C_k=1) will not have Lk information.

To account for a situation where inter-digital entry lesions at time k are associated with censoring at time k which then impacts use of cream at time k, we can treat

censoring as an additional time-varying exposure, as a part of the overall exposure history. To demonstrate these relationships in the causal diagram, we place the variable C_k, representing a subject's censoring status (C_k=1 if censored, C_k=0 if uncensored), before A_k, cream use at time k, and following L_k, lesions at time k (Figure 5.3). L_k confounds the relationship between C_k and Y, but also is an intermediate variable on the causal pathway from C_{k-1} to A_{k-1} to Y. Therefore, if you control for L_k in a stratified regression model, you are also controlling away some of the relationship between C_{k-1} to A_{k-1} to Y, which may result in a biased estimate of the effect of a treatment and censoring regimen on Y. We can create inverse probability of censoring weights (IPCWs) analogous to inverse probability of treatment weights (IPTWs) to control for impact of L_k on C_k. When we consider both the treatment and censoring weights in the analysis, we are considering treatment and censoring as a joint effect, where censoring history is a part of treatment history.

We used MSMs to deal with censoring and the proposed time-varying confounding of inter-digital entry lesions while estimating the effect of a cream use regimen on ADL episodes. We modeled the number of ADL episodes as a Poisson variable with a corresponding offset of 30 days at risk. Weighting each subject by their inverse probability of treatment, we controlled for the confounding effect of inter-digital entry lesions without controlling for it as an intermediate variable in the relationship between earlier and later anti-fungal cream use. Also, weighting each subject by their inverse probability of censoring, we adjusted for selection bias due to loss to follow-up [18]. All marginal structural models were fit to subjects who were uncensored up until the time point K of interest.

In the Poisson marginal structural model (1), we treated the cumulative sum of cream use as a continuous variable:

$$\ln E\,(Y_{iK}) = \beta_0 + \beta_1 cum\,(\overline{cream}_{K-1}) + \beta_2(Baseline\ Lymphedema) + \beta_3(MDA_K) + \ell_i \qquad (1)$$

where $cum\,(\overline{cream}_{K-1}) = \sum_{k=1}^{K-1} a_k$ is the sum of cream use through the end of follow-up *K-1*.

We estimated the cumulative effect of cream use on ADL through several follow-up time points *K*: 3, 6, 12, 18, and 24 months. We calculated inverse probability of treatment weights (IPTWs) and inverse probability of censoring weights (IPCWs) and fit a MSM for each follow-up time point *K* controlling for baseline lymphedema and MDA history at *K*. Because we considered the outcome at one time point *K*, each model included one observation per subject. For example, at follow-up point *K*=6 months, each subject had one line of data with the following variables: ADL at 6 months, sum of cream use from *k=1* month through *K*-1=3 months, MDA history at *K*=6 months, and baseline lymphedema status (advanced vs. early). Standard errors for the MSMs were calculated using robust estimates to appropriately adjust for the sampling weights.

Calculating the Weights

We used both stabilized and non-stabilized weights for the MSM. A non-stabilized weight, w_i= (IPT x IPC), is the inverse of one's probability of treatment (IPT=1/P(A_k)) multiplied by the inverse probability of remaining uncensored (IPC=1/P(C_k)), conditional on previous treatment, previous censoring history, and time-

varying confounders. When the time-varying confounders are strongly associated with treatment, the values of w_i may vary greatly between subjects [116], which could result in very large weight values for some subjects. These subjects will thus contribute a large number of copies of themselves to the pseudo population. This variability can be avoided by calculating a "stabilized weight", sw_i.

A subject that would normally have a very large or very small $w_{i,}$ will have a sw_i that is relatively closer to one, decreasing the overall variability of the weights. Robins has shown that replacing the numerator of the stabilized weight with any other function of (A_k) will not influence the consistency of the estimator, but only the variance of the estimator [120]. It is suggested that stabilized weights be used in MSMs, as the variance of the non-stabilized weights is often large with a non-normal sampling distribution [116].

Calculating a Stabilized Treatment Weight

All treatment weights are conditional upon the subject remaining uncensored through time point *k*. The stabilized treatment weight for the i^{th} subject at the k^{th} time point is calculated as follows, where *j* indicates the number of time points between *k* and the 1 month time point:

$$sw_{ik} = \frac{P\left(A_k = a_{ik} \middle| A_{k-1} = a_{i(k-1)}, C_k = 0, C_{k-1} = 0 \dots C_{k-j} = 0\right)}{P\left(A_k = a_{ik} \middle| A_{k-1} = a_{i(k-1)}, L_k = l_{ik}, L_{k-1} = l_{i(k-1)}, C_k = 0, C_{k-1} = 0 \dots C_{k-j} = 0\right)}$$

To estimate the numerator of sw_{ik}, we calculate the probability of cream use at time point *k* conditional on cream use at the previous time point *k*-1 and on the subject not being censored up through time point *k* using the following logistic model:

$$Numerator\ sw_{ik} = {p^*}_{ik}$$
$$= logit\ P\left(A_k = a_{ik} \middle| A_{k-1} = a_{i(k-1)}, C_k = 0, C_{k-1} = 0 \dots C_{k-j} = 0\right)$$
$$= {\delta^*}_0 + {\delta^*}_1 a_{i,(k-1)}$$

To estimate the denominator of sw_i, we calculate the probability of cream use at time point *k* conditional on cream use at the previous time point *k*-1, the presence of entry lesions at the current time point *k*, the presence of entry lesions at the previous time point *k*-1, and on the subject not being censored up until time point *k* using the following logistic model:

$$Denominator\ sw_{ik} = p_{ik}$$
$$= logit\ P\left(A_k = a_{ik} \middle| A_{k-1} = a_{i(k-1)}, L_k = l_{ik},\ L_{k-1} = l_{i(k-1)}, C_k = 0, C_{k-1} = 0 \dots C_{k-j} = 0\right)$$
$$= \delta_0 + \delta_1 a_{i,(k-1)} + \delta_2 l_{i,k} + \delta_3 l_{i,(k-1)}$$

After estimating the numerator (${p^*}_{i1}, \dots, {p^*}_{iK-1}$) and denominator ($p_{i1}, \dots, p_{iK-1}$) of the treatment weight at each time point *k* =1, 2, 3, 6, 12, and 18 months, we then calculate the product of each subject's weights at each time point to produce an overall stabilized treatment weight for each subject:

$$sw_i = \frac{\prod_{k=1}^{K-1}\left({p^*}_{ik}\right)^{a_{ik}}(1 - {p^*}_{ik})^{1-a_{ik}}}{\prod_{k=1}^{K-1}(p_{ik})^{a_{ik}}(1 - p_{ik})^{1-a_{ik}}}$$

Calculating a Stabilized Censoring Weight

All censoring weights are conditional upon the subject remaining uncensored through time point *k.* The stabilized censoring weight, sw_{Cik}, for the i^{th} subject at the k^{th} time point is calculated as follows, where *j* indicates the number of time points between *k* and the 1 month time point.

$$sw_{Cik} = \frac{P\left(C_k = c_{ik} \middle| A_{k-1} = a_{i(k-1)},\ C_{k-1} = 0 \ldots C_{k-j} = 0\right)}{P\left(C_k = c_{ik} \middle| A_{k-1} = a_{i(k-1)}, L_k = l_{ik},\ L_{k-1} = l_{i(k-1)},\ C_{k-1} = 0 \ldots C_{k-j} = 0\right)}$$

To estimate the numerator of sw_{Cik}, we calculate the probability of remaining uncensored at time point *k* conditional on cream use at the previous time point *k*-1 and on being uncensored prior to time point *k* using the following logistic model:

Numerator of sw_{Cik}:

$$p^*_{Cik} = \ logit\ P\left(C_k = c_{ik} \middle| A_{k-1} = a_{i(k-1)},\ C_{k-1} = 0 \ldots C_{k-j} = 0\right) = \delta^*_{C0} + \delta^*_{C1} a_{i,(k-1)}$$

To estimate the denominator of sw_{Cik}, we calculate the probability of remaining uncensored at time point *k* conditional on cream use at the previous time point *k*-1, the presence of entry lesions at the current time point *k*, the presence of entry lesions at the previous time point *k*-1, and on being uncensored prior to time point *k* using the following logistic model:

Denominator of sw_{Cik}:

$$p_{Cik} = \ logit\ P\left(C_k = c_{ik} \middle| A_{k-1} = a_{i(k-1)}, L_k = l_{ik},\ L_{k-1} = l_{i(k-1)},\ C_{k-1} = 0 \ldots C_{k-j} = 0\right) = \delta_c + \delta_{c1} a_{i,(k-1)} + \delta_{c2} l_{i,k} + \delta_{c3} l_{i,(k-1)}$$

After estimating the numerator ($p^*_{Ci1}, \ldots, p^*_{CiK-1}$) and denominator ($p_{Ci1} \ldots p_{CiK-1}$) of the censoring weights, we then calculate the product of each subject's weights at each time point to produce an overall stabilized censoring weight for each subject:

$$sw_{Ci} = \frac{\prod_{k=1}^{K-1}(p^*_{Cik})^{a_{ik}}(1-p^*_{Cik})^{1-a_{ik}}}{\prod_{k=1}^{K-1}(p_{Cik})^{a_{ik}}(1-p_{Cik})^{1-a_{ik}}}$$

Full Stabilized Weight

The stabilized treatment and censoring weights were multiplied together to create a full stabilized weight:

$$stabilized\ msm\ weight = sw_i \times sw_{Ci}$$

Calculating a Non-Stabilized Treatment Weight

The non-stabilized treatment weight for the i^{th} subject at the k^{th} time point is calculated as follows, where *j* indicates the number of time points between *k* and the 1 month time point:

$$w_{ik} = \frac{1}{\left(A_k = a_{ik} \middle| A_{k-1} = a_{i(k-1)}, L_k = l_{ik},\ L_{k-1} = l_{i(k-1)}, C_k = 0, C_{k-1} = 0 \ldots C_{k-j} = 0\right)}$$

To estimate the denominator of w_{ik}, we calculate the probability of cream use at time point *k* conditional on cream use at the previous time point *k*-1, the presence of entry lesions at the current time point *k*, the presence of entry lesions at the previous time point *k*-1, and on the subject not being censored up until time point *k* using the following logistic model:

$$Denominator\ w_{ik} = p_{ik}$$

$$= \ logit\ P\left(A_k = a_{ik} \middle| A_{k-1} = a_{i(k-1)}, L_k = l_{ik},\ L_{k-1} = l_{i(k-1)}, C_k = 0, C_{k-1} = 0 \ldots C_{k-j} = 0\right)$$

$$= \delta_0 + \delta_1 a_{i,(k-1)} + \delta_2 l_{i,k} + \delta_3 l_{i,(k-1)}$$

After estimating the denominator ($p_{i1}, \ldots, p_{iK-1}$) of the non-stabilized treatment weight at each time point, we then take its inverse and calculate the product of each subject's weights at each time point to produce an overall non-stabilized treatment weight for each subject:

$$w_i = \frac{1}{\prod_{k=1}^{K-1}(p_{ik})^{a_{ik}}(1-p_{ik})^{1-a_{ik}}}$$

Calculating a Non-Stabilized Censoring Weight

The non-stabilized censoring weight for the i^{th} subject at the k^{th} time point is calculated as follows, where *j* indicates the number of time points between *k* and the 1 month time point.

$$w_{cik} = \frac{1}{P\left(C_k = c_{ik} \middle| A_{k-1} = a_{i(k-1)}, L_k = l_{ik},\ L_{k-1} = l_{i(k-1)},\ C_{k-1} = 0 \ldots = C_{k-j} = 0\right)}$$

To estimate the denominator of w_{cik}, we calculate the probability of remaining uncensored (C_k =0) at time point *k* conditional on cream use at the previous time point *k*-1, the presence of entry lesions at the current time point *k*, the presence of entry lesions at the previous time point *k*-1, and on being uncensored prior to time point *k* using the following logistic model:

$$Denominator = p_{col} =$$

$$logit\ pr\left(C_k = c_{ik} \middle| A_{k-1} = a_{i(k-1)}, L_k = l_{ik},\ L_{k-1} = l_{i(k-1)},\ C_{k-1} = 0 \ldots C_{k-j} = 0\right) =$$

$$\delta_c + \delta_{c1} a_{i,(k-1)} + \delta_{c2} l_{i,k} + \delta_{c3} l_{i,(k-1)}$$

After estimating the denominator ($p_{ci1} \ldots p_{ciK-1}$) of the non-stabilized censoring weight, we then take its inverse and calculate the product of each subject's weights at each time point to produce an overall non-stabilized censoring weight for each subject:

$$w_{cik} = \frac{1}{\prod_{k=1}^{K-1} (p_{cik})^{a_{ik}} (1 - p_{cik})^{1-a_{ik}}}$$

The non-stabilized treatment and censored weights are multiplied together to create a full non-stabilized weight:

$$non - stabilized\ msm\ weight = w_i \times w_{ci}$$

Assuming our causal diagram is correct, the time-varying confounding only exists through time point *K-1*. Thus, for each follow-up time *K*, the weights were multiplied only through time point *K-1*. For example, for the model exploring the effects of a cream use regimen through 18 months on the number of ADL at 24 months, the weights for time points 1 month through 18 months were multiplied together. For a model exploring the effects of a cream use regimen through 12 months on the number of ADL episodes at 18 months, the weights for time points 1 month through 12 months were multiplied together.

Traditional Poisson Models

We also estimated the effect of cream regimen on the expected number of ADL episodes using traditional Poisson models that were not weighted. These models

controlled for the presence of inter-digital entry lesions by including the time-varying confounder in the model statement. To model the effect of cumulative cream use on the expected number of ADL episodes at the specified follow-up time point *K* while simultaneously controlling for lesions at each prior time point *k,* we built the following model (2) for each specified *K.* We estimated the cumulative effect of cream use on ADL through several follow-up points *K*: 3, 6, 12, 18 and 24 months. Thus, a model with one observation per subject was fit for each follow-up point *K*.

$$\ln E\,(Y_{iK}) = \beta_0 + \beta_1 cum\,(\overline{cream}_{K-1}) + \beta_2(Baseline\ Lymphedema) + \beta_3(MDA_K) + \sum_{k=1}^{K-1} \beta_k(Lesions_k) + \ell_i \quad (2)$$

where ℓ_i denotes the offset for this model computed as the log of 30 days at risk.

Assumptions

To estimate causal effects, the MSM approach is based on the following assumptions: 1) no unmeasured confounding exists, 2) the 'positivity' assumption [117]: all patients have a probability between 0 and 1 of receiving any treatment regimen conditional on their prior treatment history and baseline characteristics. Thus, there are no groups of patients who always receive a specific treatment or who never receive treatment. 3) Our hypothesized causal diagram, IPTW and IPCW models are correct.

Results

Three hundred seventy lymphedema patients were enrolled in the lymphedema management program at baseline. Each model was fit to subjects who remained uncensored at each time point *K*: 312 were uncensored at 3 months, 280 at 6 months, 261 at 12 months, 247 at 18 months, and 235 were uncensored at 24 months (Table 5.1). Throughout the study period, about 13% of patients had advanced lymphedema (stages 4-7) (Table 5.1). The mean number of reported ADL episodes per 30 days was 0.27 at 1 month, 0.14 at 6 months (Table 5.1) and was 0.24 at 24 months. The distribution of the presence of inter-digital entry lesions and MDA history is also listed in Table 5.1. Lesions appear to remain relatively stable over time, while the percent of participants who ever participated in an MDA rises from 84% at 1 month to 89% at 24 months. A previous paper concerning this cohort [123] provides more detail on demographic and LF disease characteristics. Table 5.2 shows the distribution of anti-fungal cream use regimens for each time point *k* among the uncensored lymphedema patients at time *K*. After *k*=18 months of follow-up, about a third (28%) of patients had not used anti-fungal cream, while about a fifth (18.3%) had used it at least one time. Another quarter (26.8%) of the cohort had used cream at least four times during follow-up.

Table 5.3 shows the estimated rate ratios (RR) obtained from the MSM using stabilized weights, MSM using non-stabilized weights, the traditional adjusted Poisson regression controlling for lesions at each previous time point without weighting, and the crude Poisson regression which does not control for the presence of inter-digital entry lesions or baseline covariates. All models estimate the effect of cumulative anti-fungal cream use through *K*-1, treated as a continuous variable, on the number of ADL episodes

reported in the previous 30 days at each specified follow-up period, *K*. Table 5.4 displays the corresponding parameter estimates from each model.

The stabilized MSM results suggest that an increase in the number of times one uses anti-fungal cream was associated with a slight decrease in the frequency of ADL episodes at 12, 18, and 24 months. However, this association was only significant for ADL episodes at 18 months, suggesting that for every one unit increase in the number of times one uses anti-fungal cream through 12 months, there was a 23% (RR=0.77 (0.62, 0.96)) decrease in the number of ADL episodes at 18 months. Using the parameter estimates from Table 5.4, based on the stabilized MSM, a patient who used anti-fungal cream at all six previous time points (months 1, 2, 3, 6, 12, and 18) had an average number of ADL episodes at 24 months that was 73% lower than the number of ADL episodes for a patient who did not use anti-fungal cream at any of the six previous time points (stabilized MSM = -0.22 x 6 = -1.32, RR = $e^{-1.32}$= 0.27).

The non-stabilized MSM results also suggest that an increase in the frequency of using anti-fungal cream was associated with a slight decrease in the frequency of ADL episodes at 6, 12, 18, and 24 months. Again, the RR was only significant at 18 months suggesting that for every one unit increase in the number of times one uses anti-fungal cream through 12 months, there was a 45% (RR=0.55 (0.39, 0.76)) decrease in the frequency of ADL episodes at 18 months. The results of the traditional regression, controlling for lesions at each time point, suggest a slightly negative association between the frequency of cream use over time and the frequency of ADL episodes at a subsequent time point. However, none of these were statistically significant. The crude model

results suggest a positive association between cream use and the frequency of ADL episodes, yet none were statistically significant.

Discussion

Using marginal structural Poisson models, our results suggest that an increased anti-fungal cream use over time was slightly associated with a decreased frequency of ADL episodes at a subsequent time point among a cohort of lymphedema patients enrolled in a morbidity management program, although this finding was only statistically significant at K=18 months of follow-up. The estimated effect was found after adjusting for both time-varying (inter-digital entry lesions) and non-time-varying (baseline lymphedema status, history of MDA) confounders.

Based on the marginal structural model using stabilized weights, we estimated that for every one unit increase in the number of times a patient used cream during the follow-up period, there was a 20% decrease in the rate of ADL episodes at 24 months, although this finding was not statistically significant. Compare this to an estimated 26% decrease using a non-stabilized MSM, a 17% decrease using a traditionally adjusted model, and an 8% increase using a crude model, none of which were statistically significant. As expected, the stabilized and non-stabilized MSM estimates were consistently further from the null than those of the traditionally adjusted regression. We suspect the traditional models may be controlling away some of the effect of anti-fungal cream by controlling for the intermediate lesion variable. The marginal structural models are able to control for the time-varying confounding by entry lesions, without controlling for it as an intermediate variable on the proposed causal pathway. Assuming no unmeasured confounding and correctly specified treatment, censoring, and marginal structural models, we interpret the estimated effects as causal and conclude there is a slightly protective effect of anti-fungal cream on the frequency of ADL episodes.

Treating the cumulative sum of cream use over time as a continuous variable, it appears that the protective effect strengthens as the follow-up period for ADL episodes is expanded to 18 months and then recedes at 24 months. The rate ratios for the non-stabilized MSMs intensify from 0.94 for ADL episodes at 3 months to 0.81 at 12 months, to 0.55 at 18 months and then attenuate to 0.78 at 24 months. There is a similar pattern for the stabilized MSMs, although the RRs at 3 and 6 months were somewhat above the null. The same pattern is seen in the traditional adjusted Poisson model, although the rate ratio at 3 months is in the positive direction. In both the stabilized and non-stabilized MSMs, the rate ratios are only statistically significant at 18 months, while none of the rate ratios for the traditionally adjusted and crude models are statistically significant. This may be due to the loss of statistical power to detect a significant estimated effect. Since the models used only uncensored individuals through time point *K*, they may be under powered to detect a significant change in the frequency of ADL episodes. Specifically, the models estimating the effect of a cream regimen on ADL episodes at 24 months only included 235 of the original 370 individuals. The original sample size was powered to detect a 5% decrease in ADL episodes over two years, and was not powered to detect a difference by anti-fungal cream use. Although we adjust for selection bias due to loss to follow-up using the inverse probability of censoring weights, the model may still be under powered to detect significant differences.

Within the context of lymphedema management programs, our study suggests that consistent use of antifungal cream when indicated may slightly decrease the frequency of ADL episodes among lymphedema patients. Although not statistically significant at all follow-up time points, this finding provides evidence for the effectiveness of anti-fungal

cream in decreasing ADL episodes, while also providing further etiologic evidence for entry lesions as an entry point for bacterial pathogens that in turn lead to ADL episodes [47, 63]. Current and future lymphedema management programs should continue to emphasize the importance of patient awareness of inter-digital entry lesions and the use of anti-fungal creams to treat them when they are present [112]. This is the first study to explore the effects of a regimen of anti-fungal cream on ADL episodes and the first to use marginal structural models to control for time-varying confounding by inter-digital entry lesions when estimating this effect. Although slight, the differences between the MSMS and traditional adjusted models indicate that consideration of time-varying confounding is important when attempting to estimate this causal effect. We also believe conceptually, the marginal structural model is the most appropriate way to estimate the effect within the context of the time-varying nature of inter-digital entry lesions.

Our results should be considered in the context of the following limitations. The frequency of anti-fungal cream use and ADL episodes was based on self-report. Patients may have inaccurately recounted the number of ADL episodes they experienced or may have had a difficult time identifying distinct episodes. Furthermore, during one's time enrolled in the lymphedema management program, patients may have exhibited a desire to please the interviewer, leading them to overestimate frequency of anti-fungal cream use.

The results of this study may also be subject to another form of measurement error. The presence of inter-digital entry lesions was observed through a physical examination by a volunteer and supervisor at each time point. Yet inter-digital entry lesions are a condition that may come and go over a matter of days, so that the

observation of lesions at a certain time point may not indicate whether a patient had lesions during the entire time interval between data collection points. Conversely, cream use and ADL episodes were measured through a retrospective self-report that represents an average frequency over the time interval. The frequency of cream use and ADL episodes at any given time point represent an average over the previous 30 days, while the presence of inter-digital entry lesions represents their actual occurrence at the given time point, resulting in a lack of clarity in the temporal sequence of cream and entry lesions. The difference in the way these variables were measured may have biased the estimate of effect for which we were unable to control in the analysis.

Although only 64% of patients enrolled in the lymphedema management program remained uncensored for the two year duration of data collection, we took into account the possibility of selection bias due to loss to follow-up by using inverse probability of censoring weights in the marginal structural models. These weights create a pseudo population of patients in which censoring is not associated with previous cream use or the presence of inter-digital entry lesions. Thus, we hope to properly control for any selection bias arising from censoring in estimating the effect of cream use on ADL episodes.

In a sensitivity analysis, a subject was said to be censored if he/she had two or more adjacent missing observations and therefore could not be imputed and/or did not have 24 month data. For those who had missing observations adjacent to non-missing observations, we imputed missing values for cream use and inter-digital entry lesions from their previous non-missing observation and considered these subjects as uncensored. The estimates of effect were then calculated using marginal structural,

traditional adjusted and crude Poisson models on the new "uncensored" population. The estimates for the MSMs and adjusted model were closer to the null and the estimate from the crude model was further from the null than in our main analysis, yet the pattern of differences between the estimates from the models remained. We also completed an analysis using only inverse probability of treatment weights (w_i= IPT) on truly uncensored subjects, which yielded almost similar estimates as the weights accounting for both time-varying confounding and censoring, w_i = (IPT x IPC).

This is the first study to illustrate the potential preventative effects of anti-fungal cream on episodes of adenolymphangitis among lymphedema patients enrolled in a morbidity management program. It also highlights the importance of the consideration and proper handling of time-varying confounders that are also affected by previous exposure status in longitudinal observational studies. Future research with greater power and more reliable measurement of inter-digital entry lesions, cream use, and ADL episodes is needed to further elucidate the potential benefits of consistent use of anti-fungal cream when inter-digital entry lesions are present.

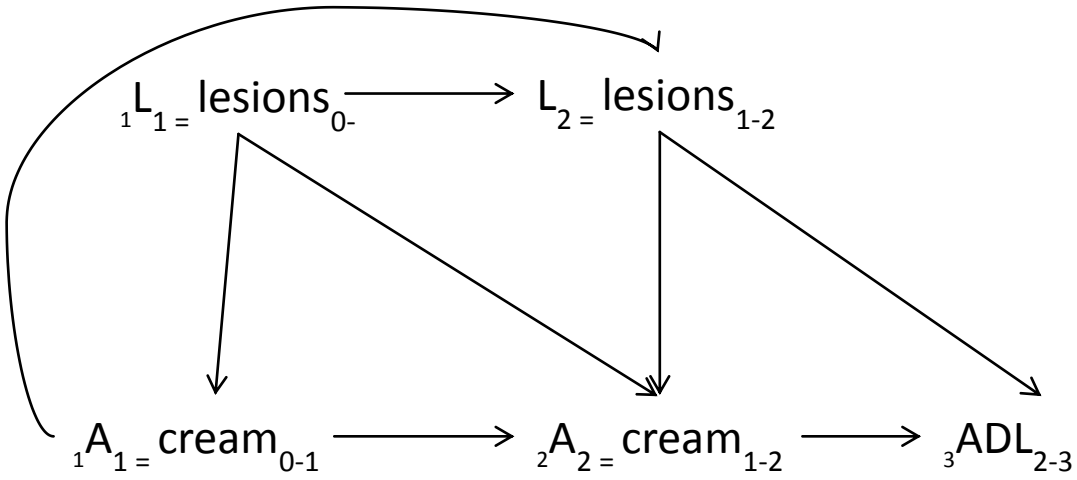


Figure 5.1. Causal diagram of the effect of cream use through 2 months on ADL episodes between 2 and 3 months illustrating the relationships between time-varying confounders L, cream treatment A, and the outcome of interest Y, ADL between 2 and 3 months.

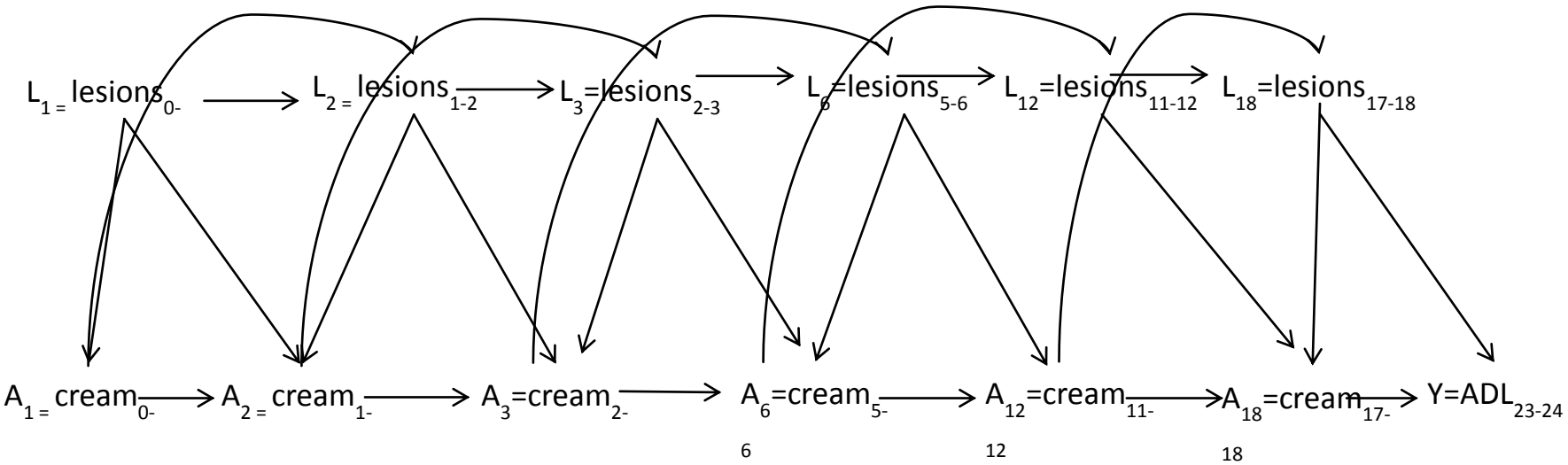


Figure 5.2. Causal diagram of the effect of cream use through 18 months on ADL episodes between 23 and 24 months illustrating the relationships between time-varying confounders L, cream treatment A, and the outcome of interest Y, ADL between 23 and 24 months.

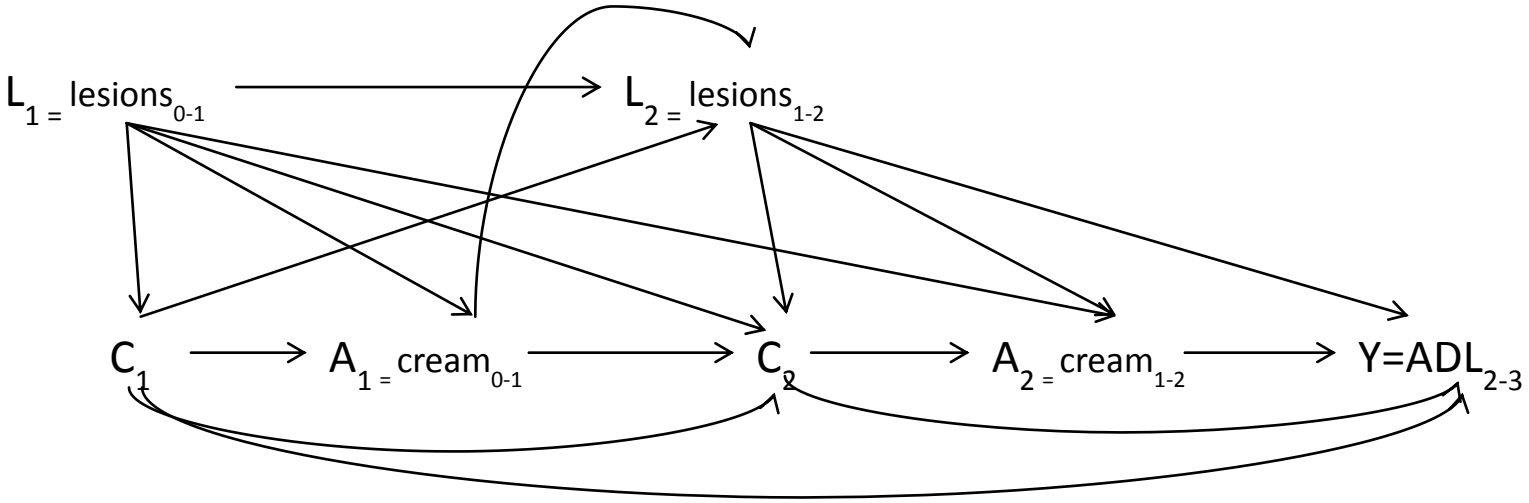


Figure 5.3. Causal diagram of the effect of cream use through 2 months on ADL episodes between 2 and 3 months illustrating the relationships between time-varying confounders L, cream treatment A, censoring C, and the outcome of interest Y, ADL between 2 and 3 months.

Table 5.1. Outcome information, treatment and confounding history among uncensored lymphedema patients enrolled in a lymphedema management program. Khurda District, Odisha State, India, 2009-2011. N (%)

# Uncensored Individuals (% of 370)	**1 month** N=351 (94.86)	**2 month** N=334 (90.27)	**3 month** N=312 (84.32)	**6 month** N=280 (87.57)	**12 month** N=261 (70.54)	**18 month** N=247 (66.76)	**24 month** N=235 (63.52)
Advanced Lymphedema Stage	44 (12.54)	39 (11.68)	38 (12.18)	36 (12.86)	35 (13.41)	32 (12.96)	33 (14.04)
Mean Number of ADL Episodes (max)	0.27 (4)	0.28 (4)	0.24 (3)	0.14 (5)	0.26 (8)	0.30 (7)	0.24 (8)
Presence of Inter-digital Entry Lesions	112 (31.91)	107 (32.04)	84 (26.92)	66 (23.57)	77 (29.50)	62 (25.10)	62 (26.38)
Ever Participated in MDA	294 (83.76)	283 (84.73)	266 (85.26)	239 (85.36)	223 (85.44)	209 (84.62)	210 (89.36)

Table 5.2. Distribution of cream use regimens among uncensored lymphedema patients. Khurda District, Odisha State, India. 2009-2011. N (%)

		Cream Use Sum						
Time point *k*	N[1]	6	5	4	3	2	1	0
18 months	235	21 (8.94)	23 (9.79)	19 (8.09)	30 (12.77)	33 (14.04)	43 (18.30)	66 (28.09)
12 months	247		26 (10.53)	25 (10.12)	34 (13.77)	30 (12.15)	53 (21.46)	79 (31.98)
6 months	261			29 (11.11)	41 (15.71)	35 (13.41)	61 (23.37)	95 (36.40)
3 months	280				37 (13.21)	52 (18.57)	65 (23.21)	126 (45.00)
2 months	312					45 (14.42)	72 (23.08)	195 (62.50)

[1]N represents the number of uncensored patients at follow-up time *K*, the subsequent time point to the corresponding time point *k* in the table. For example, N=235 represents the number of uncensored patients through *K*=24 months, from which we sum the number of times each subject used cream through *k*=18 months.

Table 5.3. Rate ratios (RR) of number of ADL episodes at follow-up time point *K* per one unit increase in the sum of cream use among a cohort of lymphedema patients enrolled in a lymphedema management program who remained uncensored until time point *K.* Khurda District, Odisha State, India, 2009-2011.

Time (*K*)	Marginal structural model with stabilized weights[a] RR (95% CI)	Marginal structural model with non-stabilized weights[b] RR (95% CI)	Traditional adjustment for time-varying covariates[c] RR (95% CI)	Crude model[d] RR (95% CI)
3 months	1.08 (0.70, 1.67)	0.94 (0.64, 1.37)	1.12 (0.77, 1.64)	1.76 (1.30, 2.38)
6 months	1.04 (0.60, 1.81)	0.83 (0.45, 1.51)	0.92 (0.47, 1.82)	1.23 (0.89, 1.71)
12 months	0.63 (0.36, 1.09)	0.81 (0.40, 1.60)	0.84 (0.54, 1.30)	1.27 (0.97, 1.67)
18 months	0.77 (0.62, 0.96)	0.55 (0.39, 0.76)	0.86 (0.59, 1.26)	1.07 (0.90, 1.26)
24 months	0.80 (0.63, 1.03)	0.74 (0.54, 1.01)	0.83 (0.62, 1.11)	1.08 (0.88, 1.32)

[a] This Poisson model used stabilized inverse probability of treatment and inverse probability of censoring weights. It is adjusted for the following covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.
[b] This Poisson model used non-stabilized inverse probability of treatment weights and inverse probability of censoring weights. It is adjusted for the following covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.
[c] This Poisson model was unweighted and adjusted for the time-varying covariate, presence of inter-digital entry lesions, by including them in the model as regressors. It also adjusted for the following covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.
[d] This Poisson model was unweighted and did not control for any time-varying or time-independent covariates.

Table 5.4. Parameter estimates from Poisson models estimating number of ADL episodes at follow-up time point *K* per one unit increase in cream use among a cohort of lymphedema patients enrolled in a lymphedema management program who remained uncensored until time point *K*. Khurda District, Odisha State, India, 2009-2011.

Time (*K*)	Marginal Structural Model with stabilized weights[a] $\hat{\beta}$ (95%CI)	Marginal Structural Model with non-stabilized weights[b] $\hat{\beta}$ (95% CI)	Traditional Adjustment for Time-Varying Covariates[c] $\hat{\beta}$ (95% CI)	Crude Model[d] $\hat{\beta}$ (95% CI)
3 months	0.08 (-0.36, 0.51)	-0.07 (-0.45, 0.32)	0.12 (-0.27, 0.50)	0.56 (0.26, 0.87)
6 months	0.03 (-0.52, 0.59)	-0.20 (-0.79, 0.40)	-0.08 (-0.76, 0.60)	0.21 (-0.12, 0.54)
12 months	-0.47 (-1.02, 0.09)	-0.22 (-0.91, 0.47)	-0.18 (-0.62, 0.26)	0.24 (-0.03, 0.51)
18 months	-0.26 (-0.48, -0.05)	-0.60 (0.39, 0.76)	-0.15 (-0.53, 0.23)	0.07 (-0.10, 0.23)
24 months	-0.22 (-0.47, 0.03)	-0.31 (-0.62, 0.06)	-0.18 (-0.47, 0.10)	0.07 (-0.13, 0.28)

[a] This Poisson model used stabilized inverse probability of treatment and inverse probability of censoring weights. It is adjusted for the following covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[b] This Poisson model used non-stabilized inverse probability of treatment weights and inverse probability of censoring weights. It is adjusted for the following covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[c] This Poisson model was unweighted and adjusted for the time-varying covariate, presence of inter-digital entry lesions, by including them in the model as regressors. It also adjusted for the following covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[d] This Poisson model was unweighted and did not control for any time-varying or time-independent covariates.

SAS CODE

Sample SAS Code for calculating numerator of stabilized IPTW: the probability of cream use at time point *k* conditional on cream use at time point *k-1*:

```
*INVERSE PROBABILITY OF TREATMENT WEIGHTS;
/*MODEL 1 STABILIZED NUMERATOR*/
proc genmod data=censoring descending;
     model cream = creamlag / link=logit dist=binomial;
     output out=model1 p=probcream_n;
run;
```

Sample SAS Code for calculating denominator of both the stabilized and non-stabilized IPTW: the probability of cream use at time point *k* conditional on cream use at time point *k-1*, lesions at time *k*, and lesions at *k-1*:

```
/*MODEL 2 DENOMINATOR*/
proc genmod data=censoring descending;
     model cream = creamlag lesionsfinallag lesionsfinal /link=logit dist=binomial;
     output out=model2 p=probcream_d;
run;
```

Sample SAS Code for calculating numerator of stabilized IPTC: the probability of censoring at time point *k* conditional on cream use at time point *k-1*:

```
*INVERSE PROBABILITY OF CENSORING WEIGHTS;
/*MODEL 3 STABILIZED NUMERATOR*/
proc genmod data=censoring ;  *PROBABILITY OF NOT BEING CENSORED;
     model censor = creamlag /link=logit dist=bin;
     output out=model3 p=probcens_n;
run;
```

Sample SAS Code for calculating denominator of both the stabilized and non-stabilized IPTC: the probability of censoring at time point *k* conditional on cream use at time point *k-1*, lesions at time *k*, and lesions at *k-1*:

```
/*MODEL 4 DENOMINATOR*/
proc genmod data=censoring ;  *PROBABILITY OF NOT BEING CENSORED;
     model censor = creamlag lesionsfinallag lesionsfinal/link=logit dist=bin;
     output out=model4 p=probcens_d;
run;
```

Sample SAS Code for calculating the stabilized and non-stabilized weights:

```
proc sort data=model1; by newid time; run;
proc sort data=model2; by newid time; run;
proc sort data=model3; by newid time; run;
proc sort data=model4; by newid time; run;

/*CALCULATING THE WEIGHTS*/
data main_w;
      merge model1 model2 model3 model4;
      by newID time;

/* variables ending with _0 refer to the numerator of the weights
  Variables ending with _w refer to the denominator of the weights */

/* reset the variables for a new patient */
      if first.newid then do;
            k1_0=1; k1_w=1; k2_0=1; k2_w=1;
          end;

      retain k1_0  k1_w  k2_0 k2_w;


/* Inverse probability of treatment weights */

     /* patients not using cream */

     if cream_dich2=0 then probcream_n=1-probcream_n;
     if cream_dich2=0 then probcream_d=1-probcream_d;

     /* patients who were censored */

     if censor2=1 then probcens_n=1-probcens_n;
     if censor2=1 then probcens_d=1-probcens_d;

     /* subjects who are not missing, but have a missing probability because missing
     information on one or more variables that predict treatment or censoring at that time
     point:  assuming these subjects are not contributing to the confounding; do not want
     the proceeding weights to be missing*/
     if probcream_n=.      and missing=0 then probcream_n=1;
     if probcream_d=.      and missing=0 then probcream_d=1;
     if probcens_n=.   and missing=0 then probcens_n=1;
     if probcens_d=.   and missing=0 then probcens_d=1;
```

```
      k1_0=k1_0*probcream_n;
      k1_w=k1_w*probcream_d;
      k2_0=k2_0*probcens_n;
      k2_w=k2_w*probcens_d;


   /* Stabilized and non stabilized weights */
       stabweight=(k1_0*k2_0)/(k1_w*k2_w);
       weight=1/(k1_w*k2_w);
run;
```

From here, we merged in the 24 month outcome data to the 1-18 month data and subset only subjects who remained uncensored throughout the study period.

```
*24 MONTH DATA ONLY AMONG THOSE WHO WERE NOT CENSORED;
data twentyfouruncen;
      set twentyfour;
      where censor=0;
run;
*235 obs;

*WEIGHT DATA ONLY AMONG THOSE WHO WERE NOT CENSORED;
data uncen;
      set main_w;
      where censor=0;
run;

/*MERGING 24 MONTH AND WEIGHT DATA*/
data one;
      set uncen twentyfouruncen ;
run;

proc sort data=one; by newid time; run;
proc print data=one; by newid; run;

/*CREATING PERMANTANT DATASET */
data mues.msmweight;
      set work.one;
run;
```

We then transposed the data so that each subject only has one row of data. We transposed the data for each variable we were interested in keeping in the data set, resulting in 6 transposed datasets. We also merged in the baseline stage and MDA variables from the 24 month time point.

```
/*TRANSPOSING THE DATA*/
proc transpose data=one out=two prefix=weight;
      var weight;
      id time;
      by newid;
run;

proc transpose data=one out=three prefix=stabweight;
      var stabweight;
      id time;
      by newid;
run;

proc transpose data=one out=four prefix=ADL;
      var acute30_num;
      id time;
      by newid;
run;

proc transpose data=one out=five prefix=cream;
      var cream_dich2;
      id time;
      by newid;
run;

proc transpose data=one out=six prefix=lesions;
      var lesionsfinal;
      id time;
      by newid;
run;

proc transpose data=one out=seven prefix=lesionslag;
      var lesionsfinallag;
      id time;
      by newid;
run;

/*MERGING TOGETHER THE TRANSPOSED DATA SETS*/
proc sort data=two; by newid; run;
proc sort data=three; by newid; run;
proc sort data=four; by newid; run;
proc sort data=five; by newid; run;
proc sort data=six; by newid;run;
proc sort data=seven; by newid; run;

data eight;
```

```
      merge two three four five six seven;
      by newid;
run;

*adding in baselinestage and mda variables;
data nine;
      set twentyfouruncen (keep=newid baselinestage mda_ever);
run;

data full;
      merge eight (drop=stabweight24 weight24) nine;
      by newid;

      logpt30=log(30);
run;

/* CREATING PERMANANT DATASET */
data mues.msmtxcensuncen;
set full;
run;
```

We then defined the cream treatment regimen:

```
/* CREATING SUM VARIABLE FOR ANALYSIS */
data creamsum;
      set mues.msmtxcensuncen;

      creamsum=sum (cream1, cream2, cream3, cream6, cream12, cream18);

run;
```

Sample MSM SAS Code (using stabilized IPT and IPC weights):

K = 24 months, continuous cream sum variable

```
proc genmod data=creamsum ;
      class newid;
      weight stabweight18;
      model ADL24 = creamsum  baselinestage mda_Ever/ dist=poisson link=log
offset=logpt30;
      repeated subject=newid/type=ind; /*TO GET ROBUST STANDARD ERRORS*/
      estimate 'cream sum' creamsum 1/exp;
run;
```

Sample SAS Code Traditional Adjusted Poisson Model:

K = 24 months, continuous cream sum variable

```
*TRADITIONAL ADJUSTED MODEL;
```

```
proc genmod data=creamsum;
      class newid;
      model ADL24 = creamsum baselinestage mda_ever lesions1 lesions2 lesions3
      lesions6 lesions12 lesions18/
      dist=poisson link=log offset=logpt30;
      repeated subject=newid/type=ind; /*TO GET ROBUST STANDARD ERRORS*/
      estimate 'cream sum' creamsum 1/exp;
run;
```

Sample SAS Code Crude Poisson Model:

K = 24 months, continuous cream sum variable

```
proc genmod data=creamsum;
      class newid;
      model ADL24 = creamsum /
      dist=poisson link=log offset=logpt30;
      repeated subject=newid/type=ind; /*TO GET ROBUST STANDARD ERRORS*/
      estimate 'cream sum' creamsum 1/exp;
run;
```

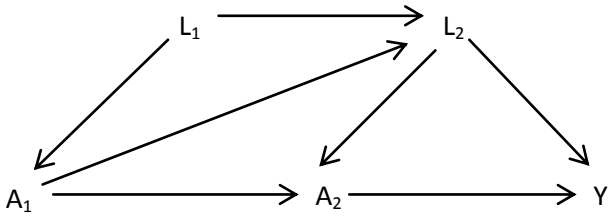
Chapter 6: Appendix: The Effect of a Regimen of Anti-fungal Cream use on Episodes of Acute Adenolymphangitis (ADL) among Lymphedema Patients: an Application Using Marginal Structural Models.

APPENDIX 1: Illustration of inverse probability of treatment weights in a marginal structural model

To illustrate the utility of inverse probability of treatment weights in the presence of a time-varying confounder that is also on the causal pathway, we provide a simple example. Suppose we have the following causal diagram (causal diagram 1) when evaluating the effect of a treatment regimen $\bar{A}$ on an outcome Y. This causal diagram represents the observed cohort data.

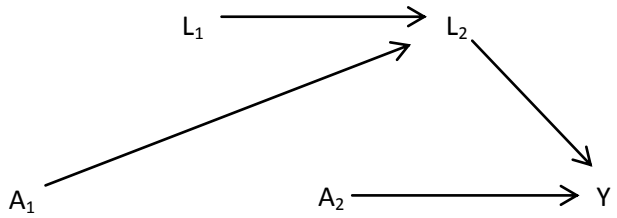
Let $\bar{A} = (A_1, A_2)$ denote the observed treatment history and $\bar{L} = (L_1, L_2)$ denote the measured time-varying confounders. We are interested in estimating the effect of treatment history $\bar{A}$ on the outcome Y. From the causal diagram, we can see that L_2 confounds the relationship between A_2 and Y yet L_2 is also on the causal path from A_1 to Y. If we control for L_2 in a standard regression model, we will also be controlling away some of the effect of A_1 on Y through L_2 .

Causal Diagram1:



We now consider a second causal diagram 2, which represents the relationships that would exist between $\bar{A}$, $\bar{L}$, and Y in a randomized trial where A_1 and A_2 are both randomized:

Causal Diagram 2



Because of treatments A_1 and A_2 are randomized in causal diagram 2, L_1 and L_2 are no longer associated with treatment. Furthermore, treatment A_1 is no longer associated with treatment A_2. Through randomization, we have removed from causal diagram 1 the confounding arrows from L_1 and L_2 leading into A_1 and A_2 as well as the arrow leading from A_1 to A_2.

Next, we will show how weighting by the inverse probability of treatment (IPTW) at time 2 allows one to control for confounding by L_2 without also controlling for L_2 as an intervening variable. Hence we allow the arrow between A_1 and L_2 to remain, while removing the arrows into A_2. We can remove the arrow from L_1 to A_1 by either weighting by an IPTW for A_1 or controlling for it as a regression in a model since L_1 is not an intervening variable as well. For the purposes of this example, we are going to ignore the association between L_1 and A_1.

Suppose we have a population of 311 individuals, of which 102 have L_2=1 and 209 have L_2=0. Those 66 individuals who have A_1=1 are more likely (46/66= 0.70) to have L_2=1 than the 245 individuals who have A_1=0 (56/245= 0.23) (i.e. A_1 is a determinant of L_2). Additionally, in this population, L_2 confounds the effect of A_2 on the outcome Y of interest because

a. the probability of being treated with A_2 when L_2=1 (53/102 = 0.52) is greater than the probability of being treated with A_2 when L_2=0 (30/209 = 0.14), so $OR_{A2,L2}$ = 6.45, and
b. the probability of the outcome Y is greater among those with L_2=1 (44/102 = 0.43) compared to those with L_2=0 (29/209 = 0.14),so $OR_{Y,L2}$ = 4.71.

The following 2x2 tables display the relationship between A_2 and D, stratified by levels of A_1 and L_2. Along with each table, we calculated the probability of observed A_2 status, the risk of disease, and the risk ratio for A_2 and disease.

Table 1

	A_1=1, L_2=1		
	A_2=1	A_2=0	
D+	15	2	17
D-	24	5	29
	39	7	46

Table 2

	A_1=1, L_2=0		
	A_2=1	A_2=0	
D+	1	2	3
D-	5	12	17
	6	14	20

$$Prob\ (A2 = 1)_{A1=1,L2=1} = \frac{39}{46} = 0.8478 \qquad Prob\ (A2 = 1)_{A1=1,L2=0} = \frac{6}{20} = 0.30$$

$$Prob\ (A2 = 0)_{A1=1,L2=1} = \frac{7}{46} = 0.1522 \qquad Prob\ (A2 = 0)_{A1=1,L2=0} = \frac{14}{20} = 0.70$$

$$Risk\ \ Disease_{A1=1,L2=1} = \frac{17}{46} = 0.3696 \qquad Risk\ \ Disease_{A1=1,L2=0} = \frac{3}{20} = 0.15$$

$$RR_{A2,D|A1=1,L2=1} = \frac{\frac{15}{39}}{\frac{2}{7}} = 1.3462 \qquad RR_{A2,D|A1=1,L2=0} = \frac{\frac{1}{6}}{\frac{2}{14}} = 1.1667$$

Table 3

	A1=0, L2=1		
	A2=1	A2=0	
D+	9	18	27
D-	5	24	29
	14	42	56

Table 4

	A1=0, L2=0		
	A2=1	A2=0	
D+	4	22	26
D-	20	143	163
	24	165	189

$$Prob\ (A2 = 1)_{A1=0,L2=1} = \frac{14}{56} = 0.25 \qquad Prob\ (A2 = 1)_{A1=0,L2=0} = \frac{24}{189} = 0.1270$$

$$Prob\ (A2 = 0)_{A1=0,L2=1} = \frac{42}{56} = 0.75 \qquad Prob\ (A2 = 0)_{A1=0,L2=0} = \frac{165}{189} = 0.8730$$

$$Risk\ \ Disease_{A1=0,L2=1} = \frac{27}{56} = 0.4821 \qquad Risk\ \ Disease_{A1=0,L2=0} = \frac{26}{189} = 0.1376$$

$$RR_{A2,D|A1=0,L2=1} = \frac{\frac{9}{14}}{\frac{18}{42}} = 1.50 \qquad RR_{A2,D|A1=0,L2=0} = \frac{\frac{4}{24}}{\frac{22}{165}} = 1.25$$

If we calculate the crude risk ratio for the relationship between A_2 and D ($i.e., RR_{A_2,D}$=1.8105), we see that it is in fact confounded, as it is higher than all stratified risk ratios (i.e., 1.35, 1.17, 1.50, and 1.25).

$$Crude\ RR_{A_2,D} = \frac{\frac{29}{83}}{\frac{44}{228}} = 1.8105$$

Suppose we are interested in the estimated effect of a treatment regimen $\bar{A} = (A_1, A_2)$ by comparing the presence of treatment at both time points (A_1=1, A_2=1) on D to the absence of treatment at both time points (A_1=0, A_2=0). The 2x2 table below shows the crude RR = 1.84 when comparing the risk of disease among those with (A_1=1, A_2=1) compared to those with (A_1=0, A_2=0).

Crude 2x2 Table

Table 5

	A_1=1,A_2=1	A_1=0,A_2=0	
D+	16	40	56
D-	29	167	196
	45	207	252

$$crude\ RR = \frac{\frac{16}{45}}{\frac{40}{207}} = 1.84$$

We could also be interested in the effect of treatment regimen $\bar{A} = (A_1, A_2)$ by comparing the presence of treatment at only one time point (A_1=1, A_2=0) or (A_1=0, A_2=1) to the absence of treatment at both time points (A_1=0, A_2=0). However, for this example we will only explore the effect of treatment at both time points (A_1=1, A_2=1) on D compared to the absence of treatment at both time points (A_1=0, A_2=0).

The following 2x2 tables display the distribution of disease among the two treatment regimens (A_1=1, A_2=1) and (A_1=0, A_2=0) stratified by L_2. We can see here that the stratified RR's (0.90, 1.25) differ from the crude RR (1.84), and that L_2 does in fact confound the effect of a treatment regimen on disease status.

Table 6

	L_2=1		
	A_1=1,A_2=1	A_1=0,A_2=0	
D+	15	18	33
D-	24	24	48
	39	42	81

Table 7

	L_2=0		
	A_1=1,A_2=1	A_1=0,A_2=0	
D+	1	22	23
D-	5	143	148
	6	165	171

$$RR_{A2\ A1,D|\ L2=1} = \frac{\frac{15}{39}}{\frac{18}{42}} = 0.90 \qquad RR_{A2\ A1,D|\ L2=0} = \frac{\frac{1}{6}}{\frac{22}{165}} = 1.25$$

We will now illustrate how weighting Tables 1-4, which display the association between A_2 and D stratified by A_1 and L_2, by inverse probability of treatment weights (IPTW) appropriately controls for the confounding by L_2 on the relationship between A_2 and D while not controlling for it as an intervening variable. We will weight each strata by the inverse of the probability of observed treatment A_2 to create an unconfounded pseudo population. Each weight is conditional upon the value of A_1 and L_2.

$Inverse\ Prob\ (A2 = 1)_{A1=1,L2=1} = \frac{46}{39} = 1.1795$ $\qquad$ $Inverse\ Prob\ (A2 = 1)_{A1=1,L2=0} = \frac{20}{6} = 3.3333$

$Inverse\ Prob\ (A2 = 0)_{A1=1,L2=1} = \frac{46}{7} = 6.5714$ $\qquad$ $Inverse\ Prob\ (A2 = 0)_{A1=1,L2=0} = \frac{20}{14} = 1.4286$

$Inverse\ Prob\ (A2 = 1)_{A1=0,L2=1} = \frac{56}{14} = 4.00$ $\qquad$ $Inverse\ Prob\ (A2 = 1)_{A1=0,L2=0} = \frac{189}{24} = 7.875$

$Inverse\ Prob\ (A2 = 0)_{A1=0,L2=1} = \frac{56}{42} = 1.3333$ $\qquad$ $Inverse\ Prob\ (A2 = 0)_{A1=0,L2=0} = \frac{189}{165} = 1.1455$

The weighting gives more weight to subjects who are most unrepresented in terms of their observed treatment (A_1=0, L_2=0, A_2=1) and (A_1=1, L_2=1, A_2=0), and lower weight to subjects who are more represented in terms of their observed treatment (A_1=0, L_2=0, A_2=0) and (A_1=0, L_2=1, A_2=0). In particular, the weight for (A_1=0, L_2=0, A_2=1) is 7.875 when $Prob\ (A2 = 1)_{A1=0,L2=0} = 0.1270$ from Table 4; the weight for (A_1=1, L_2=1, A_2=0) is 6.5714 when $Prob\ (A2 = 0)_{A1=1,L2=1} = 0.1522$ from Table 1; whereas the weight for (A_1=0, L_2=0, A_2=0) is 1.1455 when $Prob\ (A2 = 0)_{A1=0,L2=0} = 0.8730$ from Table 4; the weight for (A_1=0, L_2=1, A_2=0) is 1.3333 when $Prob\ (A2 = 0)_{A1=0,L2=1} = 0.7500$ from Table 3.

Our new weighted stratified 2x2 tables represent a pseudo population without confounding by L_2:

Table 8

	A_1=1, L_2=1		
	A_2=1	A_2=0	
D+	17.7	13.1	31
D-	28.3	32.9	61
	46	46	92

Table 9

	A_1=1, L_2=0		
	A_2=1	A_2=0	
D+	3.3	2.9	6
D-	16.7	17.1	34
	20	20	40

$$Prob\ (A2 = 1)_{A1=1,L2=1} = \frac{46}{92} = 0.50$$

$$Prob\ (A2 = 0)_{A1=1,L2=1} = \frac{46}{92} = 0.50$$

$$Risk\ \ Disease_{A1=1,L2=1} = \frac{31}{92} = 0.3370$$

$$RR_{A2,D|A1=1,L2=1} = \frac{\frac{17.7}{46}}{\frac{13.1}{46}} = 1.35$$

$$Prob\ (A2 = 1)_{A1=1,L2=0} = \frac{20}{40} = 0.50$$

$$Prob\ (A2 = 0)_{A1=1,L2=0} = \frac{20}{40} = 0.50$$

$$Risk\ \ Disease_{A1=1,L2=0} = \frac{6}{40} = 0.15$$

$$RR_{A2,D|A1=1,L2=0} = \frac{\frac{3.3}{20}}{\frac{2.9}{20}} = 1.14$$

Table 10

	A_1=0, L_2=1		
	A_2=1	A_2=0	
D+	36	24	60
D-	20	32	52
	56	56	112

Table 11

	A_1=0, L_2=0		
	A_2=1	A_2=0	
D+	31.5	25.2	57
D-	157.5	163.8	321
	189	189	378

$$Prob\ (A2 = 1)_{A1=0,L2=1} = \frac{56}{112} = 0.50$$

$$Prob\ (A2 = 0)_{A1=0,L2=1} = \frac{56}{112} = 0.50$$

$$Risk\ \ Disease_{A1=0,L2=1} = \frac{60}{112} = 0.54$$

$$RR_{A2,D|A1=0,L2=1} = \frac{\frac{36}{56}}{\frac{24}{56}} = 1.5$$

$$Prob\ (A2 = 1)_{A1=0,L2=0} = \frac{189}{378} = 0.50$$

$$Prob\ (A2 = 0)_{A1=0,L2=0} = \frac{189}{378} = 0.50$$

$$Risk\ \ Disease_{A1=0,L2=0} = \frac{57}{378} = 0.1508$$

$$RR_{A2,D|A1=0,L2=0} = \frac{\frac{31.5}{189}}{\frac{25.2}{189}} = 1.25$$

The probability of A_2 is now equal (0.50) within all four strata (i.e. $OR_{L2,\ A2}$= 1)), as we have controlled for the confounding by L_2.

The weighted population still has an association between A_1 and L_2 however, illustrating that we did not control for L_2 as an intermediate variable between A_1 and D, i.e., the arrow between A_1 and L_2 remains.

Table 12

	A_1=1	A_1=0	
L_2=1	92	112	204
L_2=0	40	378	418
	132	490	622

$$Crude\ RR_{A1,L2} = \frac{\frac{92}{132}}{\frac{112}{490}} = 3.05$$

We will now calculate the RR using the weighted pseudo population. Again, we are estimating the effect of treatment regimen (A_1=1, A_2=1) on D compared to the effect of an absence of treatment at both time points (A_1=0, A_2=0) on D:

Pseudo Population: 2x2 Table

Table 13

	A_1=1,A_2=1	A_1=0,A_2=0	
D+	21	49.2	70.2
D-	45	195.8	240.8
	66	245.0	311

$$MSM\ RR = \frac{\frac{21}{66}}{\frac{49.2}{245}} = 1.58$$

Comparing the MSM RR (1.58) from Table 13 to the RRs stratified by L_2 (0.90, 1.25) from Tables 6 and 7 above, we can see that the MSM RR is closer to these stratified estimates than is the crude RR (1.84) from Table 5. As indicated previously, we have controlled for the confounding by L_2. The traditional approach, modeling the effect

of the treatment regimen on D while controlling for L_2 in the model, will be illustrated in the SAS example below. We show that the traditional adjusted RR is closer to the null than is the MSM RR, underestimating the effect of the treatment regimen on D.

We now show the SAS code and output for our example above.

A) The crude, un-weighted SAS code and output: The 'tx' variable was classified into four groups, indicating the four possible treatment regimens: 1=(A_1=1, A_2=1), 2=(A_1=1, A_2=0), 3=(A_1=0, A_2=1), 4=(A_1=0, A_2=0). For all analyses, we are comparing treatment group 1 (A_1=1, A_2=1) to treatment group 4 (A_1=0, A_2=0), as indicated in the code.

```
proc genmod data=appendix descending;
class tx (param=ref ref='4');
model d = tx / link=log dist=bin;
estimate 'tx' tx 1/exp;
where tx in (1, 4);
run;
```

Table 14 (compare with Table 5)

Analysis Of Maximum Likelihood Parameter Estimates

Parameter		DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Intercept		1	-1.6438	0.1420	-1.9222	-1.3655	133.98	<.0001
tx	1	1	0.6098	0.2459	0.1279	1.0916	6.15	0.0131
Scale		0	1.0000	0.0000	1.0000	1.0000		

fixed.

Contrast Estimate Results

Label	Mean Estimate	Mean Confidence Limits		L'Beta Estimate	Standard Error	Alpha	L'Beta Confidence Limits		Chi-Square	Pr > ChiSq
tx	1.8400	1.1364	2.9792	0.6098	0.2459	0.05	0.1279	1.0916	6.15	0.0131
Exp(tx)				1.8400	0.4524	0.05	1.1364	2.9792		

B) The SAS code to calculate the probability of the observed A_2 and corresponding table of probabilities by A_2, A_1, and L_2.

```
/*DENOMINATOR OF INVERSE PROBABILITY OF TREATMENT WEIGHT
*/
```

```
proc genmod data=appendix2 descending;
model A2 = A1 L2 A1L2 /link=log dist=binomial;
output out=probA2 p=probA2;
run;
```

Notice the model above includes an interaction term between A1 and L2 (A1L2). In order for the weights and MSM results to match those we calculate by hand, this model must be unconstrained, meaning it must have an interaction term between each of the covariates.

Table 15 (compare with Tables 1-4)

probA2	a1	l2	a2	a1l2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
0.126984127	0	0	1	0	24	7.72	24	7.72
0.152173913	1	1	0	1	7	2.25	31	9.97
0.25	0	1	1	0	14	4.50	45	14.47
0.3	1	0	1	0	6	1.93	51	16.40
0.7	1	0	0	0	14	4.50	65	20.90
0.75	0	1	0	0	42	13.50	107	34.41
0.847826087	1	1	1	1	39	12.54	146	46.95
0.873015873	0	0	0	0	165	53.05	311	100.00

C) The SAS code to calculate the inverse probability of treatment weights and corresponding table of the weights by A_2, A_1, and L_2.

```
/*CALCULATING THE WEIGHTS*/
data weight;
set probA2;
/*IF OBSERVED A2=0, THEN TAKE CONVERSE OF PREDICTED
PROBABILITY*/
if A2=0 then probA2=1-probA2; weight=1/(probA2);
run;
```

Table 16 (Compare with weights on page 4)

weight	a1	l2	a2	a1l2	Frequency	Percent	Cumulative Frequency	Cumulative Percent
1.1454545455	0	0	0	0	165	53.05	165	53.05
1.1794871795	1	1	1	1	39	12.54	204	65.59
1.3333333333	0	1	0	0	42	13.50	246	79.10
1.4285714286	1	0	0	0	14	4.50	260	83.60
3.3333333333	1	0	1	0	6	1.93	266	85.53
4	0	1	1	0	14	4.50	280	90.03
6.5714285714	1	1	0	1	7	2.25	287	92.28
7.8749999998	0	0	1	0	24	7.72	311	100.00

D) The weighted stratified 2x2 tables – from the pseudo population:

Table 16 (compare with Table 8)

$A_1=1, L_2=1$

Table of d by a2			
d	a2		
	A2=1	A2=0	Total
D+	17.6923	13.1429	30.8352
D-	28.3077	32.8571	61.1648
Total	46	46	92

Table 17 (compare with Table 9)

$A_1=1, L_2=0$

Table of d by a2			
d	a2		
	A2=1	A2=0	Total
D+	3.33333	2.85714	6.19048
D-	16.6667	17.1429	33.8095
Total	20	20	40

Table 18 (compare with Table 10)

$A_1=0, L_2=1$

Table of d by a2			
d	a2		
	A2=1	A2=0	Total
D+	36	24	60
D-	20	32	52
Total	56	56	112

Table 19 (compare with Table 11)

$A_1=0, L_2=0$

Table of d by a2			
d	a2		
	A2=1	A2=0	Total
D+	31.5	25.2	56.7
D-	157.5	163.8	321.3
Total	189	189	378

E) The SAS code for the weighted MSM and corresponding output. Since we are using sampling weights in the MSM, we must use the repeated statement in order to get robust standard errors.

```
/*WEIGHTED MSM*/
proc genmod data=weight descending;
class i tx (param=ref ref='4');
model d = tx / link=log dist=bin;
weight weight;
estimate 'tx' tx 1/exp;
where tx in (1, 4);
repeated subject=i /type=ind; /*TO GET ROBUST STANDARD
ERRORS*/
run;
```

Table 20 (Compare with MSM result without computer in Table 13)

Analysis Of GEE Parameter Estimates							
Empirical Standard Error Estimates							
Parameter		Estimate	Standard Error	95% Confidence Limits		Z	Pr > |Z|
Intercept		-1.6054	0.1411	-1.8818	-1.3289	-11.38	<.0001
tx	1	0.4615	0.2719	-0.0714	0.9943	1.70	0.0896

Contrast Estimate Results										
		Mean					L'Beta			
Label	Mean Estimate	Confidence Limits		L'Beta Estimate	Standard Error	Alpha	Confidence Limits		Chi-Square	Pr > ChiSq
tx	1.5864	0.9311	2.7028	0.4615	0.2719	0.05	-0.0714	0.9943	2.88	0.0896
Exp(tx)				1.5864	0.4313	0.05	0.9311	2.7028		

F) The SAS code and weighted model for relationship between A1 and L2 to show relationship still exists in the pseudo population

```
proc genmod data=weight descending;
model l2=a1 / link=log dist=bin;
weight weight;
estimate 'a1 on l2' a1 1/exp;
run;
```

Table 21 (Compare with Table 12)

Analysis Of Maximum Likelihood Parameter Estimates							
Parameter	DF	Estimate	Standard Error	Wald 95% Confidence Limits		Wald Chi-Square	Pr > ChiSq
Intercept	1	-1.4759	0.0830	-1.6386	-1.3132	316.26	<.0001
a1	1	1.1149	0.1009	0.9171	1.3127	122.08	<.0001
Scale	0	1.0000	0.0000	1.0000	1.0000		

held fixed.

Contrast Estimate Results										
Label	Mean Estimate	Mean Confidence Limits		L'Beta Estimate	Standard Error	Alpha	L'Beta Confidence Limits		Chi-Square	Pr > ChiSq
a1 on l2	3.0492	2.5021	3.7160	1.1149	0.1009	0.05	0.9171	1.3127	122.08	<.0001
Exp(a1 on l2)				3.0492	0.3077	0.05	2.5021	3.7160		

G) 2x2 table of Tx (A_1=1, A_2=1 vs. A_1=0, A_2=0) and D: Pseudo population:

Table 21 (Compare with Table 13)

Table of d by tx			
	tx		
d	A1=1,A2=1	A1=0,A2=0	Total
D+	21.0256	49.2	70.2256
D-	44.9744	195.8	240.774
Total	66	245	311

H) SAS Code and Output for Traditional Adjusted Model: Adjusting for L_2

```
/*TRADITIONAL MODEL*/
proc genmod data=weight descending;
class tx (param=ref ref='4');
model d = tx l2/ link=log dist=bin;
estimate 'tx' tx 1/exp;
where tx in (1,4);
run;
```

Analysis Of Maximum Likelihood Parameter Estimates							
Parameter		DF	Estimate	Standard Error	Wald 95% Confidence Limits	Wald Chi-Square	Pr > ChiSq
Intercept		1	-2.0034	0.1941	-2.3839 -1.6229	106.48	<.0001
tx	1	1	-0.0868	0.2618	-0.6000 0.4264	0.11	0.7402
l2		1	1.1466	0.2599	0.6372 1.6560	19.46	<.0001
Scale		0	1.0000	0.0000	1.0000 1.0000		

xed.

Contrast Estimate Results									
		Mean				L'Beta			
Label	Mean Estimate	Confidence Limits		L'Beta Estimate	Standard Error	Alpha	Confidence Limits		Chi-Square	Pr > ChiSq
tx	0.9168	0.5488	1.5317	-0.0868	0.2618	0.05	-0.6000	0.4264	0.11	0.7402
Exp(tx)				0.9168	0.2401	0.05	0.5488	1.5317		

I) Comparison of results:

To summarize these results, we can see that the traditional RR=0.9168 is closer to the null than the MSM RR=1.5864 when estimating the effect of a regimen of treatment on disease. This example illustrates how traditional adjustment for a time-varying covariate like $L_{2,}$ controls away some of the effect of A_1 on D because L_2 is an intervening variable in that path. The weighted MSM allows us to control for the confounding effect of L_2 on A_2 and D, without controlling for it as an intervening variable.

J) Data layout for the weighted MSM:

Obs	i	d	a1	l2	a2	tx	a1l2	probA2	weight
1	123	1	0	0	1	3	0	0.12698	7.87500
25	16	1	1	1	0	2	1	0.15217	6.57143
32	67	1	0	1	1	3	0	0.25000	4.00000
46	47	1	1	0	1	1	0	0.30000	3.33333
52	48	1	1	0	0	2	0	0.70000	1.42857
66	76	1	0	1	0	4	0	0.75000	1.33333
108	1	1	1	1	1	1	1	0.84783	1.17949
147	127	1	0	0	0	4	0	0.87302	1.14545

K) Data layout for traditional adjustment:

Obs	i	d	a1	l2	a2	tx
1	123	1	0	0	1	3
25	16	1	1	1	0	2
32	67	1	0	1	1	3
46	47	1	1	0	1	1
52	48	1	1	0	0	2
66	76	1	0	1	0	4
108	1	1	1	1	1	1
147	127	1	0	0	0	4

MSM Applied to a Sequence of Time-Varying Confounders over Time

In a marginal structural model with a sequence of the time-varying confounder over time, we compute an IPTW at each time point corresponding to the confounder. In our causal diagram above, the $IPTW_2$ would control for confounding by L_2. Expanding the time series, $IPTW_3 \ldots IPTW_k$ would control for confounding by $L_3 \ldots L_k$ without controlling for the relationship between $A_2 \ldots A_{k-1}$ and $L_3 \ldots L_k$. Typically, the IPTW at time *k* is calculated by a logistic regression model with the exposure at time *k* as the outcome and the previous exposure history and time-varying confounder at time *k* as the independent variables. The IPTWs at some time points may also be conditional on a lagged covariate, L_{k-1}, depending on the causal diagram assumptions.

The SAS Code to calculate the weights is displayed below. Note, this SAS code is implemented on a SAS dataset with multiple observations per subject where A_k = treatment at each time point, A_{k-1} = treatment at preceding time point, L_k = covariate at each time point, and L_{k-1} = covariate at preceding time point.

```
proc genmod data=appendix2 descending;
model Ak = Ak-1 Lk Lk-1 /link=logit dist=binomial;
output out=probAk p=probAk;
run;
```

This code will output the predicted probability of treatment =1. Since we are weighting by each subjects' observed treatment history, we must take the converse of the predicted probability for those subjects who were not treated (i.e., A_k=0). Each subject will have an IPTW at each time point. A running product of $IPTW_2$, $IPTW_3 \ldots IPTW_k$ is calculated to create an overall IPTW for each subject at the outcome time point. The following SAS

code illustrates how the retain statement is used to calculate a running product of the weights:

```
/*CALCULATING THE WEIGHTS*/
data msm;
set probAk;
by id time;

/* reset the variables for a new patient */
if first.id then do;
k1_0=1;
end;

retain k1_0  ;


/* patients not receiving treatment */
if Ak=0 then probAk=1-probAk;

k1_0=k1_0*probAk;

/* Inverse probability of treatment weights */
weight=1/(k1_0);

run;
```

After calculating the weights at each time point, the data must be transposed so that each subject has one row of data with $A_1 \ldots A_k$, $L_1 \ldots L_k$, Y_k, $IPTW_1 \ldots IPTW_k$, and any non-time-varying confounders. The marginal structural model is weighted by the $IPTW_k$ for each subject. The marginal structural model will have Y_k as the dependent variable, and the defined treatment history based on $A_{1,\ldots}A_k$, and other non-time-varying confounders ($X_1 \ldots X_k$) as independent variables.

$$logit\ P(Y = 1) = \beta_0 + \beta_1(tx\ history) + \beta_2(X_1) + \cdots + \beta_{k+2}(X_k)$$

The corresponding MSM SAS code will output the weighted MSM odds ratio.

```
proc genmod data=msm ;
   class id;
   weight weightk;
   model Y = txhistory X1...Xk/ dist=bin link=logit;
   repeated subject=id/type=ind; /*TO GET ROBUST STANDARD
ERRORS*/
   estimate 'tx hisory' txhistory 1/exp;
run;
```

Although the data is transposed to include only one row per subject, we use the repeated statement with an independent correlation structure in order to get robust standard errors, as the MSM weights are essentially sampling weights and this must be accounted for in the calculation of the standard errors.

APPENDIX 2: Causal diagrams

In addition to the causal diagrams displayed in Figures 5.1-5.3, below are the causal diagrams corresponding to the hypothesized causal relationships between cream regimen and ADL episodes at K=6, 12, and 18 months (Figures 6.1-6.3). Figures 6.4-6.8 display \ causal diagrams that incorporate censoring as another time-varying treatment to illustrate how censoring was accounted for in this study.

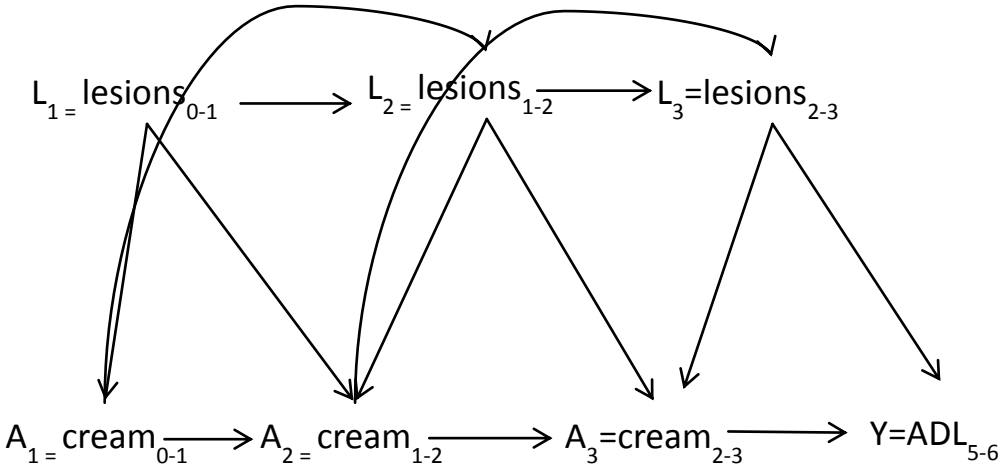


Figure 6.1. Causal diagram of the effect of cream use through 3 months on ADL episodes between 5 and 6 months illustrating the relationships between time-varying confounders$\bar{L}$, cream treatment $\bar{A}$, and the outcome of interest Y, ADL between 5 and 6 months.

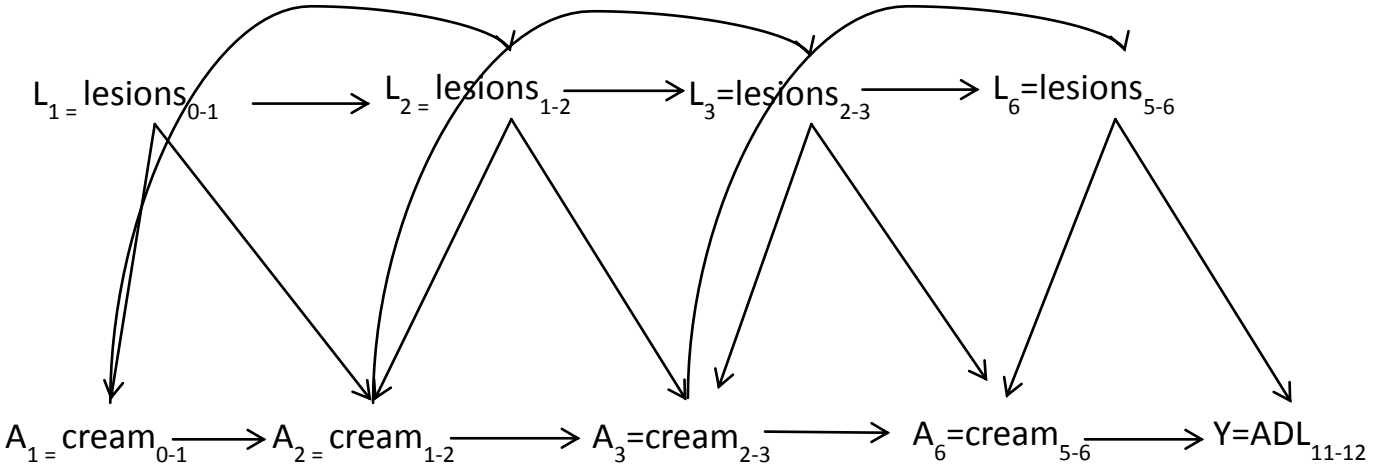


Figure 6.2. Causal diagram of the effect of cream use through 6 months on ADL episodes between 11 and months illustrating the relationships between time-varying confounders $\bar{L}$, cream treatment $\bar{A}$, and the outcome of interest Y, ADL between 11 and 12 months.

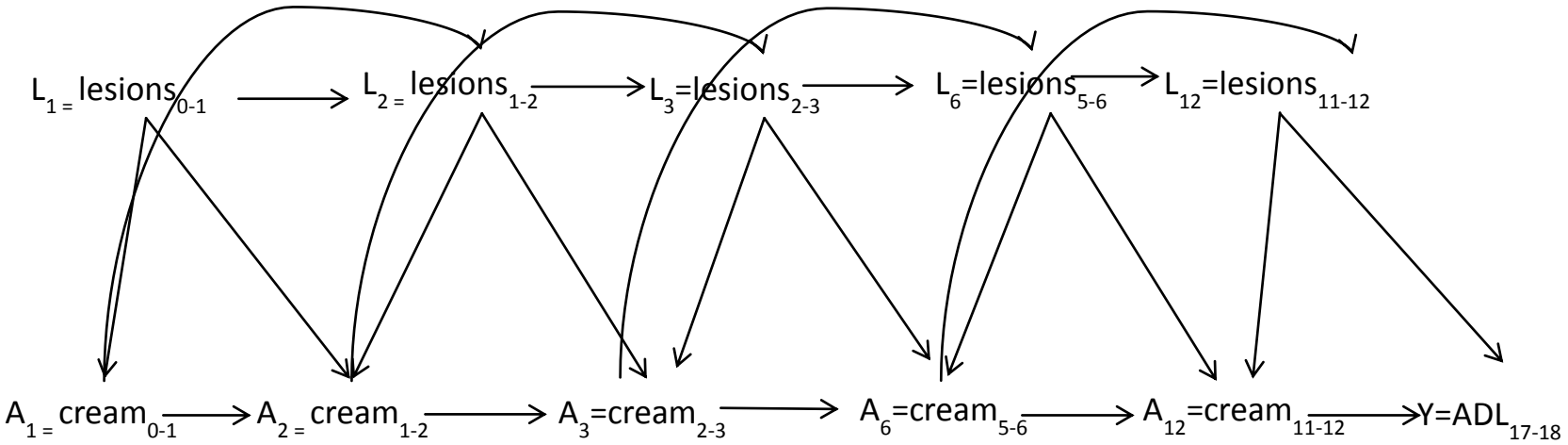


Figure 6.3. Causal diagram of the effect of cream use through 12 months on ADL episodes between 17 and 18 months illustrating the relationships between time-varying confounders $\bar{L}$, cream treatment $\bar{A}$, and the outcome of interest Y, ADL between 17 and 18 months.

The following five causal diagrams illustrate the same proposed causal relationships shown in the three previous causal diagrams, but with the addition of the variable *C*, representing a subject's censoring status at each time point. If a subject is censored at time point *k*, then C_k=1 for that time point and all subsequent time points. If a subject is not censored at time point *k* or any time preceding time point *k*, then C_k=0 for that time point. In order to adjust for censoring in the marginal structural models, we created an inverse probability of censoring weight analogous to the inverse probability of treatment weight. As discussed by Robins [116], to adjust for censoring is to estimate the causal effect of a treatment regimen, if all subjects had remained uncensored. To add the idea of censoring to the causal diagram, one adds at time *k* the variable C_k before A_k and following L_k. Under the assumption that no arrows from unmeasured causal risk factors *U* go directly into C_k or A_k, then the measured covariates L_k are sufficient to adjust for both confounding and selection bias due to censoring.

In each of the following causal diagrams, C_k has been placed before A_k and following L_k. Censoring is treated essentially as another time-varying treatment. Given the proposed causal diagram, if we weight by the inverse probability of censoring, conditional on cream use at *k*-1, lesions at *k*-1, and lesions at *k*, then the estimates will be adjusting for the selection bias due to censoring. In the MSMs, we multiplied the IPC weights by the IPT weights at each time point to create a full MSM weight. The following causal diagrams incorporate the censoring variable through *k*=3 months, *k*=6 months, *k*=12 months, *k*=18 months, and *k*=24 months.

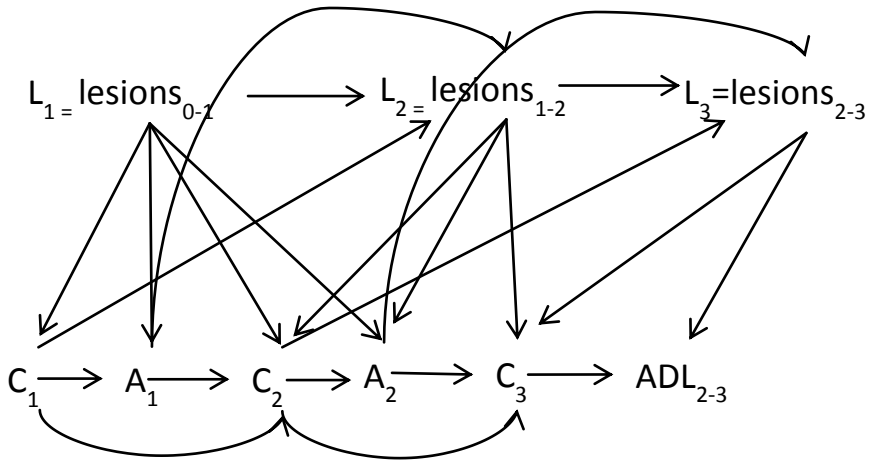


Figure 6.4. Causal diagram of the effect of cream use through 2 months on ADL episodes between 2 and 3 months illustrating the relationships between time-varying confounders $\bar{L}$, cream treatment $\bar{A}$, censoring $\bar{C}$, and the outcome of interest Y, ADL between 2 and 3 months.

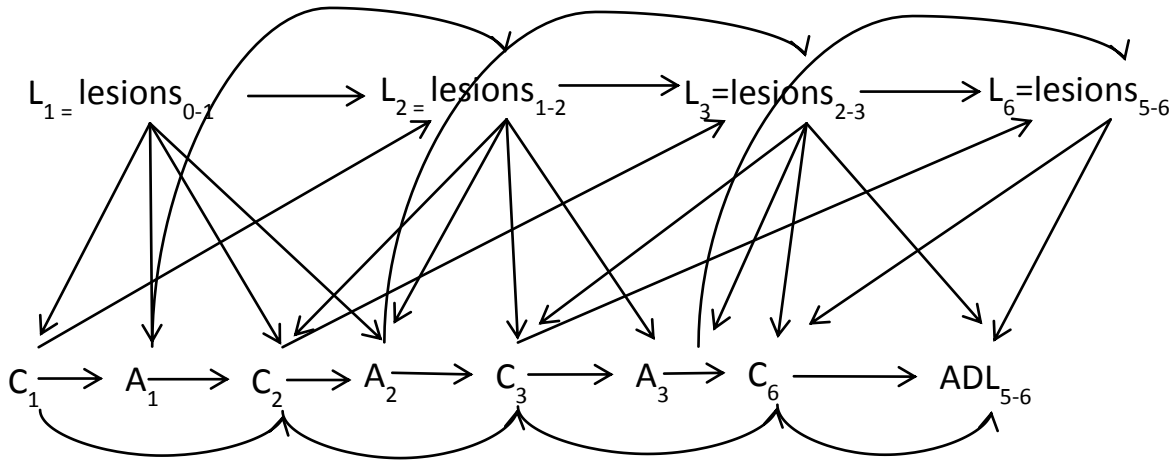


Figure 6.5. Causal diagram of the effect of cream use through 3 months on ADL episodes between 5 and 6 months illustrating the relationships between time-varying confounders $\bar{L}$, cream treatment $\bar{A}$, censoring $\bar{C}$, and the outcome of interest Y, ADL between 5 and 6 months.

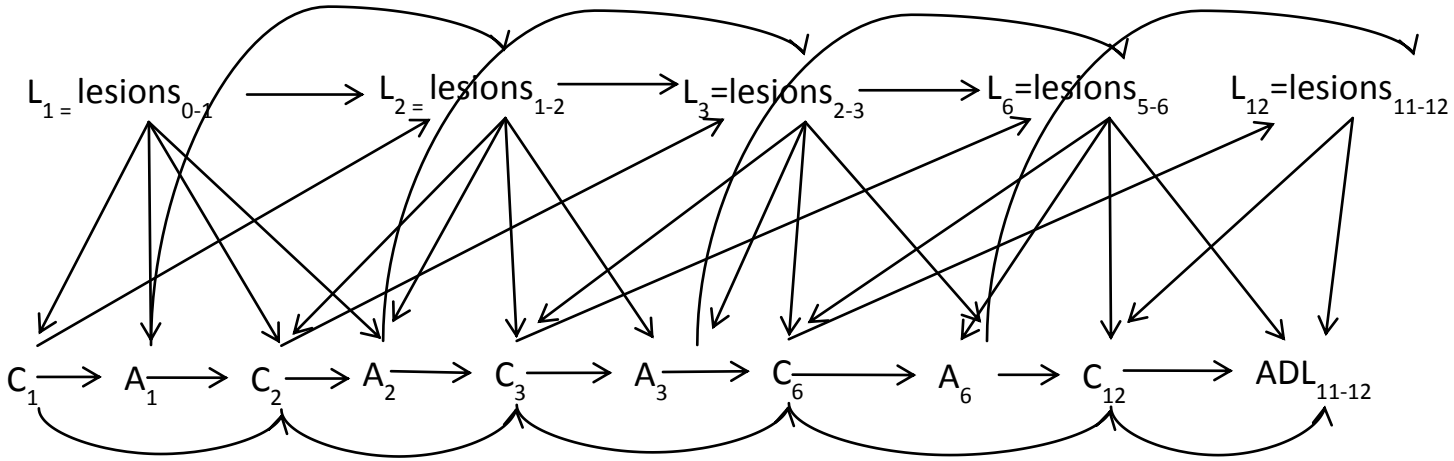


Figure 6.6. Causal diagram of the effect of cream use through 6 months on ADL episodes between 11 and 12 months illustrating the relationships between time-varying confounders $\bar{L}$, cream treatment $\bar{A}$, censoring $\bar{C}$, and the outcome of interest Y, ADL between 11 and 12 months.

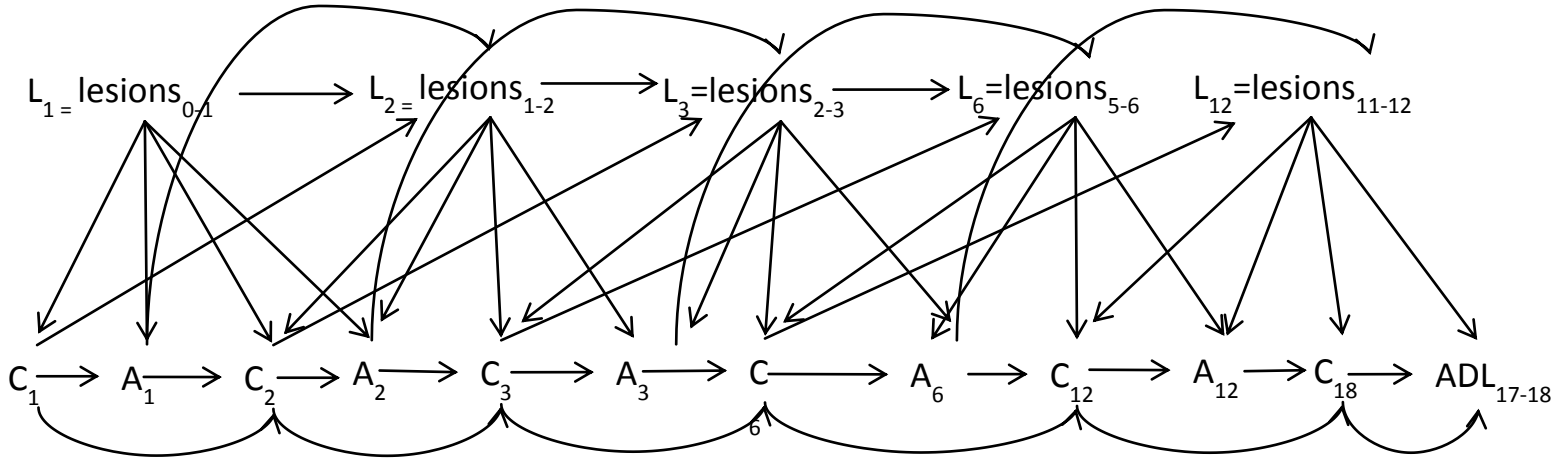


Figure 6.7. Causal diagram of the effect of cream use through 12 months on ADL episodes between 17 and 18 months illustrating the relationships between time-varying confounders $\bar{L}$, cream treatment $\bar{A}$, censoring $\bar{C}$, and the outcome of interest Y, ADL between 17 and 18 months.

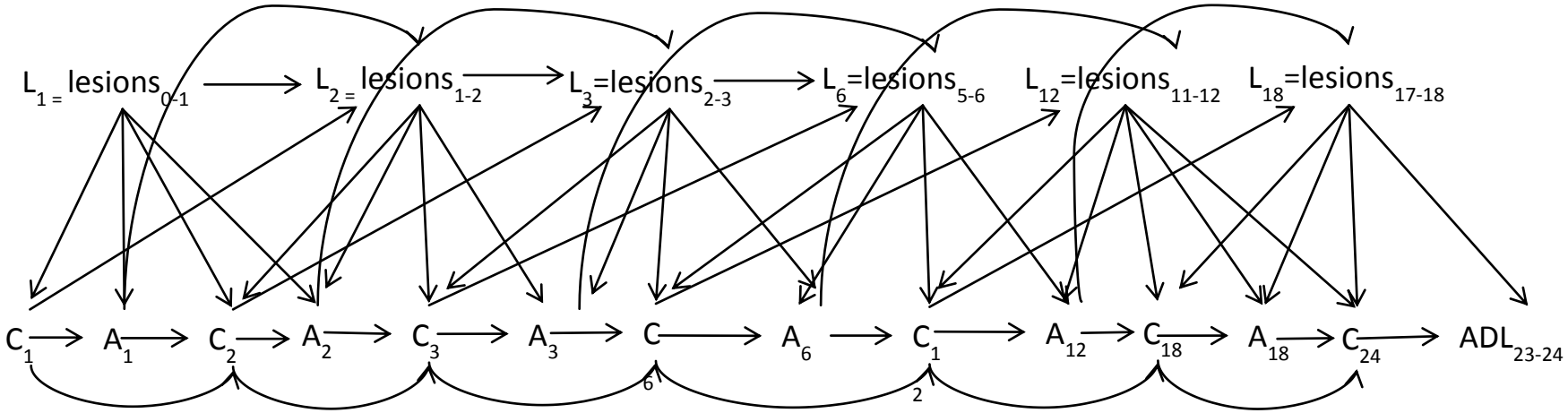


Figure 6.8. Causal diagram of the effect of cream use through 18 months on ADL episodes between 23 and 24 months illustrating the relationships

APPENDIX 3: Relationships between time-varying confounders, treatment history, and ADL

Tables 6.1-6.5 illustrate the associations between cream treatment history, the time-varying confounder lesions, and the outcome ADL episodes, as proposed in the causal diagrams. Odds ratios and 95% confidence intervals are presented adjusted for non-time-varying confounders, baseline lymphedema status (advanced vs. early) and history of MDA.

Table 6.1. Odds ratios and 95% confidence intervals estimating the association between lesions at time *k*-1 and cream use at time *k* among patients enrolled in a lymphedema management program, Khurda District, Odisha State, India, 2009-2011. Results from repeated measures logistic regression.

	OR	**95% CI**	
Time-varying covariates			
Presence of entry lesions at *k*-1	2.83	2.19	3.68
Non-time-varying covariates			
Baseline lymphedema stage (advanced vs. early)	4.01	2.78	5.77
History of MDA	1.29	0.86	1.96

Table 6.1 shows that there is a significant positive relationship between inter-digital entry lesions at time*k*-1 and cream use at time *k*. Those who have entry lesions present at *k*-1 have 2.83 times the odds of using anti-fungal cream at the subsequent time point *k*.

Table 6.2. Odds ratios and 95% confidence intervals estimating the association between cream use at time *k*-1 and the presence of entry lesions at time *k* among patients enrolled in a lymphedema management program, Khurda District, Odisha State, India, 2009-2011. Results from repeated measures logistic regression.

	OR	**95% CI**	
Time-varying covariates			
Cream use *k*-1	1.15	0.89	1.49
Non-time-varying covariates			
Baseline lymphedema stage (advanced vs. early)	19.20	10.21	36.08
History of MDA	0.98	0.63	1.54

Table 6.2 illustrates the association between cream use at *k*-1 and inter-digital entry lesions at time *k* is minimal. Although not statistically significant, the odds of entry lesions being present at time *k* among those who used cream at time *k*-1 are 0.15 times greater than those who did not use cream at time *k*-1. This illustrates that perhaps the relationship between cream use at time *k*-1 and lesions at time *k*, is not present in this cohort of lymphedema patients. However, we still maintain our hypotheses as presented in the causal diagrams.

Table 6.3. Rate ratios and 95% confidence intervals estimating the association between cream use at *k*-1 and the frequency of ADL episodes at time *k* and the presence of entry lesions at time *k*-1 and the frequency of ADL episodes at time *k* among patients enrolled in a lymphedema management program, Khurda District, Odisha State, India, 2009-2011. Crude odds ratios from repeated measures logistic regression.

	RR	**95% CI**	
Cream use *k*-1	1.38	1.11	1.72
Presence of entry lesions at *k*-1	2.06	1.55	2.73

Tables 6.3 illustrates that there is a significant positive association between cream use at *k*-1 and the frequency of ADL episodes at time *k* as determined by a crude Poisson regression model. The crude model indicates an adverse relationship between cream use and the frequency of ADL episodes. Table 6.3 also shows that there is a significant positive association between the presence of inter-digital entry lesions at time *k*-1 and the

frequency of ADL episodes at time *k*, indicating that entry lesions are a risk factor for ADL episodes in this cohort.

Table 6.4. Cream use at time *k* by the presence of entry lesions at time *k* over time among patients enrolled in a lymphedema management program, Khurda District, Odisha State, India, 2009-2011.

	Cream use N(%)						
	1 month	2 month	3 month	6 month	12 month	18 month	24 month
Lesions present	50 (44.6)	72 (64.9)	71 (75.5)	57 (72.2)	64 (69.6)	52 (61.2)	48 (55.2)
Lesions not present	18 (7.7)	33 (14.0)	76 (31.3)	69 (28.5)	54 (24.6)	59 (24.8)	61 (26.9)

Table 6.4 shows the distribution of cream use at time *k* by the presence of entry lesions at time *k*. The percent represent the percent of persons with lesions present who used cream and the percent of those without lesions present who used cream. The distribution indicates that over time persons with and without lesions are reporting cream use, although those with entry lesions at time *k* have a higher frequency. The corresponding odds ratio for this association was 3.77 (2.99, 4.76).

Table 6.5. Cream use at time *k* by the presence of entry lesions at time *k*-1 over time among patients enrolled in a lymphedema management program, Khurda District, Odisha State, India, 2009-2011.

	Cream use N(%)						
	1 month	2 month	3 month	6 month	12 month	18 month	24 month
Lesions present	43 (46.2)	69 (63.9)	74 (66.7)	65 (68.4)	52 (74.3)	54 (54.6)	53 (62.4)
Lesions not present	26 (10.2)	35 (14.9)	72 (32.1)	64 (28.0)	65 (26.3)	60 (26.6)	52 (23.3)

Table 6.5 shows the distribution of cream use at time *k* by the presence of entry lesions at time *k*-1. The percent represent the percent of persons with lesions present at time k-1 who used cream and the percent of those without lesions present who used cream. The distribution indicates that over time persons with and without lesions are reporting cream use, although those with lesions at time *k*-1 have a higher frequency. The corresponding odds ratio for the association was 4.01 (3.14, 5.11)

APPENDIX 4: Distribution of the weights

Tables 6.6 and 6.7 show the distribution of the non-stabilized and stabilized weights. These weights are representing the 235 uncensored subjects that were used in the marginal structural models. Since they are a running product of the weights up until time *k*, the non-stabilized weights increase from 1 to 18 months. The stabilized weights do not increase over time. The non-1 value for the numerators of the stabilized weights prevent this from happening, resulting in a much lower variance of the stabilized weights.

Table 6.6. Distribution of the non-stabilized weight by time among the 235 uncensored patients included in the marginal structural models.

	Mean	Median	IQR	Min	Max
1 month	2.00	1.34	1.34, 2.11	1.0	7.16
2 months	4.26	2.45	1.79, 5.00	1.0	28.11
3 months	11.75	4.13	2.40, 12.84	1.34	101.94
6 months	28.02	10.59	3.22, 28.53	1.79	314.88
12 months	75.12	23.02	4.31, 59.83	2.74	1451.49
18 months	197.48	45.79	8.68, 154.35	3.22	3980.97

Table 6.7. Distribution of the stabilized weight by time among the 235 uncensored patients included in the marginal structural models.

	Mean	Median	IQR	Min	Max
1 month	0.96	0.92	0.92, 1.03	0.20	1.95
2 months	0.92	0.86	0.69, 0.91	0.11	2.68
3 months	0.96	0.79	0.59, 1.21	0.17	4.39
6 months	0.98	0.73	0.65, 1.11	0.14	7.20
12 months	0.97	0.68	0.51, 1.03	0.08	7.87
18 months	1.04	0.63	0.38, 0.97	0.06	11.60

APPENDIX 5: Nominal cream sum results

The following table 6.8 displays the stabilized and non-stabilized MSM, traditional adjusted, and crude model results for the sum of cream use treated as a nominal variable. Each rate ratio compares the frequency of ADL among those with the specified cream sum value to those who have a cream sum of zero at each designated time point *K*.

The nominal model equation:

$$\ln E\ (Y_{iK}) = \beta_0 + \beta_1(cream_{K-1}) + \cdots + \beta_j\left(cream_{K-j}\right) + \beta_3(Baseline\ Lymphedema) + \beta_4(MDA_K) + \ell_i$$

where *j* indicates the number of time points between *K* and the 1 month time point.

Table 6.8. Rate ratios of number of ADL episodes at follow-up time point *K* of the effect of anti-fungal cream regimen treated as a nominal variable at follow-up time point *K* among a cohort of lymphedema patients enrolled in a lymphedema management program who remained uncensored until time point *K*. Khurda District, Odisha State, India, 2009-2011.

	Marginal Structural Model with stabilized weights[a] RR (95% CI) ¥	Marginal Structural Model with non-stabilized weights[b] RR (95% CI) ¥	Traditional Adjustment for Time-Varying Covariates[c] RR (95% CI) ¥	Crude Model[d] RR (95% CI) ¥
K= 3 Months				
Cream Sum = 2	1.26 (0.48, 3.28)	0.87 (0.39, 1.95)	1.26 (0.59, 2.71)	3.11 (1.71, 5.66)
Cream Sum = 1	0.93 (0.47, 1.84)	0.99 (0.52, 1.86)	1.08 (0.58, 2.00)	1.63 (0.91, 2.93)
Cream Sum = 0	REF	REF	REF	REF
K = 6 Months				
Cream Sum = 3	1.29 (0.26, 6.44)	1.01 (0.17, 5.97)	0.75 (0.09, 6.49)	1.99 (0.64, 6.13)
Cream Sum = 2	0.91 (0.26, 3.18)	0.45 (0.10, 1.98)	1.05 (0.22, 5.00)	1.62 (0.51, 5.16)
Cream Sum = 1	0.89 (0.20, 4.01)	1.09 (0.22, 5.54)	1.68 (0.47, 5.95)	1.94 (0.64, 5.91)
Cream Sum = 0	REF	REF	REF	REF
K = 12 Months				
Cream Sum = 4	0.38 (0.06, 2.24)	0.30 (0.04, 2.33)	0.52 (0.08, 3.29)	3.28 (1.05, 10.20)
Cream Sum = 3	0.28 (0.06, 1.40)	0.39 (0.07, 2.25)	0.62 (0.13, 2.99)	1.91 (0.59, 6.16)
Cream Sum = 2	0.03 (0.002, 0.35)	0.01 (0.001, 0.10)	0.07 (0.01, 0.62)	0.16 (0.02, 1.39)
Cream Sum = 1	0.61 (0.18, 2.10)	0.39 (0.09, 1.74)	0.82 (0.23, 2.89)	1.83 (0.60, 5.58)
Cream Sum = 0	REF	REF	REF	REF
K=18 Months				
Cream Sum = 5	1.05 (0.11, 10.22)	0.84 (0.05, 12.91)	0.93 (0.16, 5.38)	2.80 (1.16, 6.75)
Cream Sum = 4	0.25 (0.05, 1.27)	0.24 (0.05, 1.08)	0.35 (0.07, 1.67)	0.90 (0.25, 3.31)
Cream Sum = 3	0.53 (0.11, 2.57)	0.27 (0.06, 1.25)	0.37 (0.07, 1.85)	0.61 (0.16, 2.29)
Cream Sum = 2	0.73 (0.16, 3.40)	1.65 (0.32, 8.63)	1.06 (0.22, 5.09)	1.73 (0.60, 5.02)
Cream Sum = 1	4.29 (1.21, 15.21)	2.41 (0.67, 8.62)	2.26 (1.01, 5.07)	2.45 (1.00, 6.04)
Cream Sum = 0	REF	REF	REF	REF
K=24 Months				
Cream Sum = 6	0.05 (0.004, 0.73)	0.02 (0.001, 0.16)	0.19 (0.03, 1.05)	0.30 (0.06, 1.49)
Cream Sum = 5	0.38 (0.08, 1.77)	0.29 (0.05, 1.67)	0.54 (0.12, 2.47)	0.89 (0.21, 3.69)
Cream Sum = 4	0.41 (0.07, 2.26)	0.28 (0.07, 1.10)	0.38 (0.10, 1.51)	0.63 (0.15, 2.68)
Cream Sum = 3	1.00 (0.23, 4.36)	1.02 (0.24, 4.30)	0.44 (0.10, 1.86)	0.64 (0.17, 2.47)
Cream Sum = 2	0.74 (0.17, 3.22)	0.38 (0.07, 2.16)	1.00 (0.27, 3.73)	0.80 (0.22, 2.97)
Cream Sum = 1	0.99 (0.22, 4.46)	0.80 (0.18, 3.57)	0.51 (0.14, 1.94)	0.59 (0.14, 2.49)
Cream Sum = 0	REF	REF	REF	REF

[a] This Poisson model used stabilized inverse probability of treatment and inverse probability of censoring weights. It is adjusted for baseline covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[b] This Poisson model used non-stabilized inverse probability of treatment weights and inverse probability of censoring weights. It is adjusted for baseline covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[c] This Poisson model was unweighted and adjusted for the time-varying covariates, presence of inter-digital entry lesions, by including them in the model as regressors. It also adjusted for baseline covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[d] This Poisson model was unweighted and did not control for any time-varying or baseline covariates.

¥ Standard errors are robust estimates.

Evaluating the Ordinal Nature of Cream Sum

To evaluate whether it was appropriate to treat the sum of cream use as a continuous variable, as we did in the main text of this article, we used a likelihood ratio test. For K=24 months, we tested the following null hypothesis of the nominal stabilized MSM model. The subscript for each cream variable represent the sum of cream use over the 18 months preceding *K*=24 months. For example, $cream_1$ is the dummy variable indicating someone who used cream 1 time from 1 to 18 months, while $cream_6$ is the dummy variable indicating someone who used cream 6 times from 1 to 18 months.

$$\ln E\ (Y_{iK}) = \beta_0 + \beta_1(cream_1) + \beta_2(cream_2) + \beta_3(cream_3) + \beta_4(cream_4) + \beta_5(cream_5) + \beta_6(cream_6) + \beta_7(Baseline\ Lymphedema) + \beta_8(MDA_{24}) + \ell_i$$

$$H_0\colon \beta_6 = 2\beta_5,\ \beta_6 = 3\beta_4,\ \beta_6 = 4\beta_3,\ \beta_6 = 5\beta_2,\ \beta_6 = 6\beta_1$$

This null hypothesis illustrates the relationships that would exist between the nominal indicator variables if the sum of cream use had an ordinal nature. That for every increase in the sum of cream use, the estimated parameter estimate, beta, would increase in an ordinal fashion. The likelihood ratio test was distributed chi-square with 5 degrees of freedom. The calculated p-value = 0.9378 indicating that we cannot reject the null hypothesis. This supports our treatment of cream use as a continuous variable in the main analyses.

We also subjectively evaluated the nominal RR estimates to see if they appear ordinal (Table 6.8). Looking at the RR estimates in Table 6.5, it appears there is a somewhat ordinal pattern for the stabilized MSM estimates. We chose to treat the sum of cream use as continuous because of these results and also for data efficiency.

APPENDIX 6: Results of sensitivity analyses

Sensitivity Analysis 1: MSMS weighted by Inverse Probability of Treatment Weights (IPTW), only uncensored subjects.

Table 6.9. Rate ratios (RR) of number of ADL episodes (dependent variable) at follow-up time point *K* per one unit increase in cream use treated as a continuous variable among a cohort of lymphedema patients enrolled in a lymphedema management program in Khurda District, Odisha State, India, 2009-2011.

Cream Sum	**Marginal Structural Model with stabilized weights[a]** **RR (95% CI) ¥**	**Marginal Structural Model with non-stabilized weights[b]** **RR (95% CI) ¥**	**Traditional Adjustment for Time-Varying Covariates[c]** **RR (95% CI) ¥**	**Crude Model[d]** **RR (95% CI) ¥**
K=3 months	1.09 (0.70, 1.68)	0.94 (0.64, 1.38)	1.12 (0.77, 1.64)	1.76 (1.30, 2.38)
K=6 months	1.03 (0.59, 1.80)	0.82 (0.45, 1.49)	0.92 (0.47, 1.82)	1.23 (0.89, 1.71)
K=12 months	0.63 (0.36, 1.09)	0.79 (0.40, 1.58)	0.84 (0.54, 1.30)	1.27 (0.97, 1.67)
K= 18 months	0.77 (0.62, 0.96)	0.55 (0.39, 0.76)	0.86 (0.59, 1.26)	1.07 (0.90, 1.26)
K=24 months	0.81 (0.63, 1.03)	0.75 (0.55, 1.02)	0.83 (0.62, 1.11)	1.08 (0.88, 1.32)
Cumulative cream use up to *k-1**	0.81 (0.68, 0.96)	0.80 (0.65, 0.98)	0.96 (0.86, 1.06)	1.09 (0.99, 1.21)

[a] This Poisson model used stabilized inverse probability of treatment and inverse probability of censoring weights and adjusted for baseline covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[b] This Poisson model used non-stabilized inverse probability of treatment weights and adjusted for baseline covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[c] This Poisson model was unweighted and adjusted for the time-varying covariate, presence of inter-digital entry lesions, by including them in the model as regressors. It also adjusted for baseline covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[d] This Poisson model was unweighted and did not control for any time-varying or baseline covariates.

¥ Standard errors are robust estimates.

*This is a Poisson repeated measures model with cumulative cream use up to *k-1* as the exposure variable.

Sensitivity Analysis 2: MSMs weighted by both Inverse Probability of Treatment and Inverse Probabilit of Censoring Weights (IPTW and IPTC), with imputed values for those missing observations adjacent to non-missing observations

Table 6.10. Rate ratios (RR) of number of ADL episodes (dependent variable) at follow-up time point *K* per one unit increase in cream use treated as a continuous variable among a cohort of lymphedema patients enrolled in a lymphedema management program in Khurda District, Odisha State, India, 2009-2011.

Cream Sum	**Marginal Structural Model with stabilized weights[a] RR (95% CI) ¥**	**Marginal Structural Model with non-stabilized weights[b] RR (95% CI) ¥**	**Traditional Adjustment for Time-Varying Covariates[c] RR (95% CI) ¥**	**Crude Model[d] RR (95% CI) ¥**
K=24 months	0.95 (0.76, 1.18)	0.81 (0.61, 1.08)	0.96 (0.75, 1.22)	1.17 (0.98, 1.40)
Cumulative cream use up to *k-1**	0.86 (0.73, 1.02)	0.85 (0.70, 1.04)	1.01 (0.91, 1.12)	1.14 (1.04, 1.25)

[a] This Poisson model used stabilized inverse probability of treatment and inverse probability of censoring weights and adjusted for baseline covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[b] This Poisson model used non-stabilized inverse probability of treatment weights and adjusted for baseline covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[c] This Poisson model was unweighted and adjusted for the time-varying covariate, presence of inter-digital entry lesions, by including them in the model as regressors. It also adjusted for baseline covariates by including them as regressors: lymphedema status (advanced, stages 4-7 vs. early, stages 1-3) and history of MDA by time point *K*.

[d] This Poisson model was unweighted and did not control for any time-varying or baseline covariates.

¥ Standard errors are robust estimates.

*This is a Poisson repeated measures model with cumulative cream use up to *k-1* as the exposure variable.

Chapter 7: Changes in Antibody Levels during and following an Episode of Acute Adenolymphangitis (ADL) among Lymphedema Patients in Léogâne, Haiti

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Abstract:

Background: Episodes of acute adenolymphangitis (ADL), characterized by plaque-like areas of cutaneous inflammation, are often the first clinical sign of lymphatic filariasis (LF). They are often accompanied by swelling of the affected limb, inflammation, fever, and general malaise and lead to the progression of lymphedema. Although ADL episodes have been studied for a century or more, questions still remain as to their etiology. We quantified antibody levels to pathogens that potentially contribute to ADL episodes during and after an episode among lymphedema patients in Léogâne, Haiti. We estimated the proportion of ADL episodes hypothesized to be attributed to specific pathogens.

Methods: Through ELISA assays, we measured patient antibody levels to specific pathogens during and following an ADL episode among 41 lymphedema patients enrolled in a cohort study in Léogâne, Haiti. We calculated the absolute and relative changes in antibody levels between the ADL and convalescent time points. We calculated the prevalence of antibody response for each antigen of interest as the number of patients whose antibody levels increased from the ADL to convalescent time point at a specified cut-point divided by the number of patients undergoing an ADL episode. We explored relative patterns of antibody response at the following levels of percent change from ADL to convalescent time point: 5%, 10%, 25%, 50%, 100%, 200%, and 300%. We framed our hypotheses and results in the context of sufficient component cause models.

Results: There were no significant differences between serum antibody levels during the ADL episode and at the convalescent time point. There were only a few positive antibody changes at the 300% (four-fold) level for the filarial antigen BpG2, Strep A, and

Candida antigens. Looking at patterns of the prevalence of antibody response across levels, we found that among bacterial pathogens, the Strep A antigen had the highest prevalence of antibody response. Among filarial antigens, BpG2 had the highest prevalence, and among the fungal pathogens, *Candida* had the highest prevalence.

Conclusions: Although our results are limited by the lack of a control group and few antibody responses, they provide some evidence for infection with *Streptococcus A* as a potential contributing factor to ADL episodes. Our results add to the current evidence and illustrate the importance of determining the causal role of bacterial and fungal pathogens and immunological antifilarial response in ADL episodes. These findings are especially important within the context of a greater focus on LF morbidity control as the infection is being eliminated in endemic areas.

Introduction:

Lymphatic filariasis (LF) is a neglected tropical disease affecting 120 million people throughout the world with over 1.3 billion at risk [105]. The disease is caused by 3 different species of parasitic nematode worms, *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* [2], which are spread by several genera of mosquitoes. These thread-like worms reside in the lymphatic vessels of humans, causing severe and permanent lymphatic damage.

While often characterized by chronic lymphedema and ultimately elephantiasis of the limbs, the first clinical sign of lower limb LF disease is typically an episode of acute adenolymphangitis (ADL). An ADL episode is defined as a plaque-like area of relatively diffuse cutaneous inflammation with or without ascending lymphangitis or satellite adenitis [6]. ADL episodes are accompanied by swelling, lymphatic inflammation, high fever, general malaise and chills. The episodes are a recurrent clinical aspect of LF lasting 3-15 days each and may occur several times each year [5]. ADL episodes are often accompanied or followed by distal edema of the affected leg [6]. Clinical evidence has shown that ADL episodes can lead to the progression of chronic lymphedema [8-11].

ADL episodes are inflammatory reactions most likely trIgEred by bacterial, fungal, or filarial pathogens through points of entry on the infected individuals' affected limb. Despite extensive research, the etiology of ADL episodes is yet to be fully understood. Although there has been evidence for bacterial pathogens such as *Streptococcus A* and *Staphylococcus* as the causal agent in ADL episodes [46, 58-61], other studies have attributed them to the host's inflammatory/immune response to adult filarial worm activity in the lymph system or microfilariae in the blood [62, 124]. Other

studies suggest that filarial larvae likely contributed to observed ADL episodes [44] or have found associations between filarial intensity and ADL incidence [78]. Additionally, recent work has focused on the role of the *Wolbachia* endosymbiont in ADL episodes [22]. The role of fungal infections in predisposing to ADL episodes has also been explored [47, 63], which can cause entry lesions, often found between the toes, and within deep skin folds [51]. These entry lesions can then serve as the point of entry for bacteria leading to the ADL episodes.

The importance of understanding the etiology and immunology during an ADL episode cannot be overestimated as ADL episodes contribute to the progression of chronic LF. Our objective was to quantify antibody levels to pathogens that potentially contribute to ADL episodes during and after an episode among lymphedema patients in Léogâne, Haiti. We also sought to estimate the proportion of ADL episodes hypothesized to be attributed to specific pathogens in the development of ADL episodes.

Methods:

Study population

Between June 1995 and December 1997, lymphedema patients were enrolled in a study to test the feasibility and effectiveness of a basic lymphedema management program in a resource-poor setting [37]. Patients were recruited at the outpatient clinic of Ste. Croix Hospital in Léogâne, Haiti, which is endemic for *Wuchereria bancrofti*. Patients were included if they had lymphedema of the leg, agreed to return to the clinic for follow-up evaluations, provided written informed consent, and did not have an obvious cause of edema such as a tumor. Data were collected on demographic information, number of ADL episodes, and leg volume. Patients' lymphedema was classified into stages using the 7-stage system of Dreyer and colleagues [121]. Stages 4-7 were collapsed into a single fourth-stage category [37]. Out of 302 patients who first received lymphedema care at the clinic during the enrollment period, 230 eligible patients were enrolled. Of these, 175 returned to the clinic for at least 5 routine follow-up visits over a period of 6 months and were included in the original cohort. All patients continued through the end of the study until December 1998.

A subset of 41 patients of the original 175 was used for this analysis. In addition to the demographic, ADL, and leg volume information, serum samples were collected from these patients. During an ADL episode, patients were instructed to report to the clinic at which point a serum sample was taken. They were then instructed to return to the clinic after the ADL episode had ended for collection of a convalescent serum sample. Attempts were made by the investigator to obtain serum samples from all 175 patients enrolled in the original cohort, but not all patients were able to get to the hospital

during an ADL episode as they may have been disabled or did not consent to have their blood drawn. Eight of the 41 patients had serum samples taken at multiple ADL and convalescent time points (3 had serum drawn during and after 3 episodes, and 5 had serum drawn during and after 2 episodes) for a total of 52 paired samples and 104 observations.

Pathogen Specific Antigens

We measured the antibody level for a variety of filarial, bacterial, and fungal pathogens hypothesized to be associated with ADL episodes. To test for filarial antibodies we used *Brugia pahangi* antigens that were extracted from adult worms as previously described [63]. These assays measure antibody responses to filarial antigens following *Brugia* and *Wuchereria* infections. Isotype-specific responses were measured with isotype-specific monoclonal antibodies. An IgG1 response to Brugia (BpG1) is considered to be a measure of filarial exposure [125],whereas BpG4 measures filarial IgG4 antibody which is associated with active filarial infection [126] and is higher among microfilaremic patients (those with microfilariae circulating in the blood) [127, 128]. We also measured filarial IgG2 and IgG3 antibodies through the BpG2 and BpG3 antigen tests.

IgG antibodies to the fungal pathogens *Candida* and *Trichophyton* were measured using crude extracts from culture organisms. We measured IgG antibody to the bacterial pathogen *Streptococcus A* with several different antigens: the virulence factors Streptococcal Pyrogenic Exotoxin A (SPEA) and Streptococcal Pyrogenic Exotoxin B (SPEB), the hemolytic exotoxin Streptolysin O (SLO), and the carbohydrate antigen *Streptococcus* group A (StrepA). In addition to the streptococcal bacterial pathogens, we

measured IgG responses to *Pseudomonas* using an exotoxin antigen and *Staphylococcus* using *Staphylococcus* enterotoxin B (SEB).

Serologic Assays

The Og4C3 ELISA assay [129] to detect circulating *Wuchereria bancrofti* antigen was used to determine which lymphedema patients were currently infected with the parasite. Assays to detect antibody response to adult filarial antigen were performed using biotinylated monoclonated antibodies (Zymed Laboratories, San Francisco, CA) specific for IgG1, IgG2, IgG3, and IgG4. Assays for antibody response to *Streptococcus* group A (Lee Laboratories, Grayson, GA), Streptolysin O, *Pseudomonas* exotoxin (List Biological Laboratories), *Staphylococcus* enterotoxin B (Sigma Chemical Co., St. Louis, MO), *Candida*, and *Trichophyton* were done using biotinylated monoclonal antibodies for total IgG. All antibodies were standardized against a high titered serum sample included in each assay and arbitrarily assigned an antibody level of 10,000 units per ml. Antibody levels were then transformed into arbitrary units (AU). Plasma levels for all antigens were assayed in triplicate at a final dilution of 1:50.

Sufficient component cause models

In exploring potential filarial, bacterial, or fungal pathogens associated with ADL episodes, we conceptualized them using sufficient component cause models (SCCM) [130]. We assume that infection with these pathogens leads to an antibody response. These infections may or may not occur in combination with a number of measureable factors such as age and gender and unknown factors such as genetics and the surrounding environment ultimately leading to the ADL episode. In this framework, each risk factor

for the outcome of interest is defined as a component cause or predisposing factor of the outcome of interest. Each component cause contributes to one or more sufficient causes, which contain the minimal set of conditions that result in the outcome [131-133]. An outcome may have a single or multiple sufficient causes.

Figure 7.1 displays three potential SCCMs for ADL episodes. Model 1 states that the presence of a bacterial infection such as *Streptococcus A* along with unknown factors U_1 are sufficient to cause an episode of ADL. Model 2 states that at minimum, a reaction to filarial worm infection in combination with unknown factors U_2 must be present for ADL episode to occur. Model 3 states that at minimum, the presence of a fungal infection such as *Trichyophtyon* in combination with unknown factors U_3 are sufficient to cause an episode of ADL. Model 4 involves a biological interaction of infection with both a bacterial and fungal pathogen. The fungal pathogen causes a lesion on the skin of the affected limb, which then provides a pathway for the bacteria and the subsequent response to infection in the form of an ADL episode. The unknown factors stated in these SCCMs may comprise a multitude of environmental, genetic, and immunologic factors. The examples of sufficient component causes for ADL episodes displayed in Figure 7.1 are only a few hypotheses of many potential sufficient causes.

Specific Aims and Assumptions

The specific aims of this study were mainly descriptive and based on several assumptions. Our first aim was to calculate the prevalence of antibody levels that showed a four-fold (300%) change from the ADL to convalescent period for each pathogen of interest, i.e. they had an antibody response at the four-fold level. Under the

following assumptions, we then calculated a population attributable fraction (AF_{pop}) for each pathogen of interest.

Assumptions for Aim 1:

1) If infection by a particular pathogen immediately prior to an ADL episode contributes to the etiology of the episode, we expect the antibody levels to increase from the ADL to the convalescent time point [58, 61, 134]. Therefore, each antibody response was defined as a positive percent change in the antibody levels from the ADL to convalescent time point.

2) If the antibody level of a pathogen is found to increase four-fold from the ADL to the convalescent time point, it is considered a component cause of ADL. Since all persons in our study had an ADL episode, they are all considered cases. Therefore, the AF among the exposed is 100% and the prevalence of a four-fold change in antibody level among the cases is equal to the population AF.

$$AF_{exp} = \frac{\#\ excess\ cases\ attributable\ to\ exposure}{\#\ exposed\ cases} = 100\%$$

$$Prevalence\ \ of\ four-fold\ change\ \ among\ the\ cases = \ AF_{pop} =$$

$$= \ \frac{\#\ excess\ cases\ attributable\ to\ exposure}{\#\ total\ cases}$$

Assumption two allows us to consider the prevalence of a four-fold change in antibody levels among the paired serum samples (ADL cases) as the AF_{pop} and is the guiding motivation for aim 1.

Our second aim was two-fold: 1) to calculate the prevalence of antibody response at different levels of antibody change (different cut-points), 2) to examine the relative pattern of pathogen contributions under different cut-point values. We used the following additional cut-points: 5%, 10%, 25% 50%, 100%, and 200%.

Two additional assumptions were made for this study:

3) The 41 lymphedema patients who gave serum samples are representative of the original cohort of 175 lymphedema patients enrolled in a lymphedema management program in Léogâne, Haiti in terms of demographics, disease severity, and duration of chronic LF systems. If willingness to give blood was related to the etiology or severity of the ADL episode, then our results may be biased.

4) We only have information from 1-3 ADL episodes among each individual in this sample population. The lymphedema patients in the cohort have been exposed to LF for many years and may have experienced many ADL episodes throughout their lives. We assume that the pathogen(s) with the greatest contribution to ADL episodes at this time point will be typical of ADL episodes in this population.

Statistical Analysis

For each antigen, we calculated an absolute change and percent change from ADL to convalescent time point:

$$Absolute\ Change\ = Antibody\ Level_{Convalescent} - Antibody\ Level_{ADL}$$

$$Percent\ Change\ = [(Antibody\ Level_{Convalescent} - Antibody\ Level_{ADL})/(Antibody\ Level_{ADL})] * 100$$

Since the distribution of antibody change in this population was not normal, we reported median values for the absolute antibody values, absolute change, and percent change along with the standard deviation and inter-quartile range (IQR). We conducted a Wilcoxon-Mann-Whitney test for the difference between mean ADL and convalescent antibody levels. We conducted an analysis of variance (ANOVA) using SAS's PROC GLM, to investigate how much of the variation in the absolute value of an antibody level was due to subject and how much was due to the different time points (ADL vs. convalescent).

We identified responses of antibody change based on a positive percent change at the following levels: 5%, 10%, 25% 50%, 100%, 200%, and 300%. For each antigen and response level we calculated the prevalence of antibody response as the number of cases showing an antibody change at the specified cut-point (i.e. those that responded for that pathogen at the specified cut-point) divided by the total number of cases in the sample (i.e. all patients, since all experienced an ADL episode).

$$Prevalence\ of\ Antibody\ Response\ = \frac{\#\ of\ cases\ responding\ at\ cut - point}{\#\ cases}$$

Under assumption 2, the prevalence of antibody response can be interpreted as a population attributable fraction only at the 300% cut-point value, representing a four-fold change.

Results

The majority of the lymphedema patients were female (83%) with a median age of 34 years (Table 7.1). Most patients presented with stage 2 or 3 lymphedema. Only 1 of the 41 patients tested positive for circulating filarial antigen (CFA) at the ADL and convalescent time point. Prior to cohort enrollment, the yearly ADL rate among lymphedema patients was 2.7 per person year. During the entire lymphedema cohort enrollment, 1995-1998, the ADL rate reported was 1.42 per person year (109 episodes over 76.81 person years). Up until the ADL episode during which serum was collected, patients had experienced an average of 3ADL episodes during their time enrolled in the lymphedema management program. The mean time between the ADL and convalescent serum sample was 17 days (range = 1-35). At the observed ADL episode during which serum was collected, about a third of patients were treated with an antibiotic, 70% had an enlarged lymph node, 62% presented with retrograde progression of their lymphedema, 75% presented with inflammation of the skin, and 81% had acute swelling at the ADL site.

To evaluate our third assumption, that the 41ADL patients who gave blood during and following an ADL episode were representative of the original cohort of 175 lymphedema patients, we compared demographic and disease severity variables between the groups (Table 7.3). There were no significant differences in the gender and age distribution, and the proportion of patients who were literate was similar across groups. In terms of disease severity, the mean duration of lymphedema, distribution of lymphedema stage, and rate of ADL episodes reported in the year prior to study enrollment were not statistically different across groups. However, the 41 ADL patients

had a statistically significant higher rate of ADL episodes during the entire study period (1995-1998): 1.42 per person year among patients who provided serum vs. 0.75 per person year among original cohort patients (p-value <0.0001).

Serum antibody levels at ADL and convalescent time points

Table 7.2 displays the median antibody levels and standard deviations for each antigen during the observed ADL episode and convalescent time point with the corresponding p-value, median difference, and median percent change. All antibody levels are measured in arbitrary units (AU). There were no significant differences between median serum antibody levels taken during the ADL episode and at the convalescent time point. The median difference between ADL and convalescent time points ranged from -39.60 for BpG1 to 0.76 for SEB, yet the IQRs were wide. The median % change of greatest magnitude was seen for the SLO antigen (-9.56%). *Trichophyton* had the smallest median percent change (-0.08%). SPEA had the greatest positive percent change (2.72%). Although the median % changes are minimal, the IQRs for each antigen are wide, indicating large variation.

An analysis of variance with absolute antibody level for each antigen as the outcome and subject as the independent variable showed R^2 values ranging from 0.83 to 0.98 (Table 7.4), indicating that a large part of the variation of antibody values was due to subject. Conversely, an ANOVA with the absolute antibody level for each antigen as the outcome and sample time (ADL vs. convalescent) as the independent variable showed low R^2 values, ranging from 0.0011 to 0.0398 (Table 7.4). We also calculated the R^2 values for time of the sample (ADL vs. convalescent) conditioning upon subject. These R^2 values were almost identical to those for subject alone.

Prevalence of Antibody Response

Figure 7.2 displays the prevalence of antibody response for the filarial antigens BpG1, BpG2, BpG3, and BpG4 at different cut-point values. All antibody responses represent an increase in serum antibody levels from the ADL to convalescent time point. Of the 52 paired serum samples, two (3.85%) showed a four-fold (300%) positive change in BpG2 levels from the ADL to convalescent time point. No paired serum samples showed a four-fold change for BpG1, BpG3, or BpG4. Looking at the relative pattern of responses at different cut-point levels, Figure 7.1 shows that BpG2 had the highest prevalence of response at all cut-point levels, followed by BpG3 at the 5% and 10% levels and BpG1 at 15%, 50%, and 100%.

Figure 7.3 displays the prevalence of antibody response for the bacterial antigens. Three paired serum samples (5.77%) experienced a four-fold change for the Strep A antigen, while none showed a four-fold change for the other bacterial antigens. Looking at the relative patterns of responses across cut-point values, the Strep A antigen for *Streptococcus A* had the highest prevalence of response followed by the SPEB and SPEA antigens for *Streptococcus A*. Figure 7.4 displays the prevalence of antibody response for the fungal antigens. Two serum samples showed a four-fold change in antibody values for the *Candida* antigen, and none showed a four-fold change for the *Trichophyton* antigen. Looking across different cut-point values, the *Candida* antigen had the highest prevalence of change at each value. Figure 7.5 displays the prevalence of antibody response for the BpG2, Strep A, and Candida antigens, those that had the highest frequency of response in each pathogen group. The prevalence of response for the Strep

A antigen is the highest at most of the cut-point values, including the 300% (four-fold) level.

Discussion

To better understand factors associated with ADL episodes among lymphedema patients in Léogâne, Haiti, we quantified antibody levels to specific pathogens during and following an ADL episode. We conceptualized our hypotheses and will discuss our results in the context of sufficient component causal models (SCCM). We calculated the prevalence of antibody responses for different pathogens thought to be associated with ADL episodes. Initially we calculated the prevalence of paired serum samples showing a four-fold positive change in antibody titers for each pathogen. We assumed that those pathogens showing a four-fold change would be considered a component cause of ADL episodes, and therefore considered those prevalence measures as population attributable fractions. Our results showed four-fold changes in antibody levels from an ADL to a convalescent time point for the following antigens: BpG2, Strep A, and Candida. However, for each of those antigens, only two or three paired samples showed a response at that level.

We then calculated the prevalence of antibody change at different cut-point levels and looked for patterns of the relative prevalence among each pathogen type (filarial, bacterial, and fungal). We hypothesize that patterns arising in the data at the lower cut-point values may be indicative of what would occur if there had been more frequent four-fold changes in antibody titer levels. Amongst the bacterial antigens, the Strep A antigen for *Streptococcus A* infection had the highest prevalence of response at all levels. It also had the highest prevalence of response compared to all other antigens tested in this study. Furthermore, among the antigens for *Streptotoccus A*, Strep A was the only one of the four that had any response at the four-fold level. Nonetheless, such changes were only

observed for a small proportion of ADL episodes and the contribution of *Streptococcus A* to ADL episodes in this cohort may be minimal compared to other unmeasured factors.

These results illustrate the SCCM 1 (Figure 7.1) indicating that infection with the bacterial pathogen *Streptococcus A* in combination with one or many unknown factors (U_1) could be sufficient to cause an episode of ADL. These findings are consistent with several other studies which found a high proportion of patients undergoing ADL to be infected with group A *Streptococcus*, leading to the conclusion that group A *Streptococcal* infection at least in part is associated with episodes of adenolymphangitis [58, 59, 61]. Of the two studies which also compared antibody levels at an ADL and convalescent time point, one found no significant rise in the antibody response to *Streptococcus A* [62], while the other found a significant rise in antistreptolysin O (ASO) titers from an ADL to convalescent time point [61]. The latter study also found that ADL patients had a higher mean ASO titer level than control patients, especially at the convalescent time point.

SCCM 2 states that at minimum, filarial worm infection in combination with a set of unknown factors U_2 must be present for an ADL episode to occur. Although we saw responses for a change in filarial antibodies at different cut-point values, the frequency of the filarial responses was less than the frequency of responses to *Streptococcus A* antigens, providing less evidence for the SCCM 2. Interestingly, the BpG2 and BpG3 filarial antigens had the highest frequencies of response change among the filarial antigens. Since IgG2 and IgG3 responses to filarial antigens are associated with carbohydrate epitopes, it is possible that these responses reflect cross reactivity stimulated by exposure to non-filarial antigens or even bacterial antigens.

The third most frequent filarial response was to the BpG1 antigen. The BpG1 response is thought to be associated with exposure to filarial L3 larvae [125], but these may not necessarily result in productive infection. Responses to BpG4, indicating adult worm infection [126], would be most consistent with the SCCM 2, yet among CFA (-) lymphedema patients, the IgG4 responses are expected to be low [29, 30] thus adult worms are not likely to be the trIgEr for ADL in this cohort. But perhaps in our cohort of CFA(-), symptomatic LF patients, responses to BpG1 may also be indicative of SCCM 2, driven by exposure to L3 larvae. Combined with other unknown factors, the anti-filarial immune response to larval exposure may be sufficient to cause ADL episodes among this group of patients. Conversely, the anti-filarial response present in this CFA(-) cohort, though minimal, may in part be driven by cross-reactivity between fungal and bacterial pathogens and not due to the presence of filarial antibodies. Others have hypothesized that the antigen-negative filarial status of some lymphedema patients may be maintained, at least in part, by recurrent fungal and bacterial infections [63].

Our study also found that among the fungal antigens tested, the prevalence of response at each cut-point value was highest for the *Candida* antigen. This evidence would support SCCM 3 (Figure 7.1) where the presence of infection by a fungal pathogen such as *Candida* in combination with other unknown factors would potentially be sufficient for an ADL episode to occur. Previous studies have found evidence for the fungal pathogens as a contributing factor in the development of ADL episodes [47, 63]. Fungal infections are often found between the toes and within deep skin folds, and can lead to inter-digital entry lesions [51]. These entry lesions can then serve as the point of entry for bacteria leading to the ADL episodes.

Although we did not observe any significant differences in the mean antibody level between the ADL and convalescent samples, the antibody levels were highly variable as indicated by the large standard deviations of the absolute measures and wide inter-quartile ranges of the relative measures. We also found that most of the variance in the antibody levels was due to differences by subject and not to timing of the sample (ADL vs. convalescent). These results may indicate that our hypotheses are incorrect or perhaps we are missing the true cause of ADL episodes in this population. The lack of variance due to the timing of samples certainly limits the conclusions that can be made from this study. The lack of antibody titer change from an ADL to convalescent time point at the 300% level may be due to several factors. Perhaps the study population in Léogâne, Haiti is regularly exposed to the pathogens of interest and may already have elevated antibody titers. Perhaps that is why only a few four-fold changes in antibody levels developed from the ADL episode to the convalescent time point. Therefore, a less than four-fold rise in antibody levels from the ADL episode to the convalescent time point may be an acceptable threshold of infection in this population.

Perhaps the lack of antibody titer change at the 300% level is due to the recurrent nature of ADL episodes. Antibody levels for the bacterial, fungal, and filarial pathogens may initially increase following an initial ADL episode and then remain high for a period of time. Instead of oscillating antibody levels between ADL episodes as we hypothesized, they may increase and then plateau at those higher levels, indicating a constant heightened immune response. In their comparison of ADL patients with healthy controls in the Dominican Republic, Vincent concluded that the mean anti-streptolysin O and anti-DNAse B titer levels of the affected persons may not fully return to normal

between episodes [61]. Furthermore, the immune response of these patients may be shifting away from humoral and towards a cellular response.

There are several limitations in the design of this study. First, the sample size of 41 lymphedema patients is relatively small, limiting the external validity of our findings. Second, the more frequent occurrence of ADL episodes among this subset of the original cohort may have made these patients more willing to give blood. Their ADL episodes may differ etiologically than ADL episodes among the rest of the cohort so that our findings may not be representative of others that did not volunteer to give blood. Third, all antibody values are based on arbitrary units and not on a calibrated reference scale. Therefore, we cannot determine if persons in our cohort met standard antibody levels indicative of infection. We can only explore absolute and relative differences in antibody levels between the ADL and convalescent samples and assume certain levels of change indicate infection.

Furthermore, it is possible that blood was not drawn from individuals during the most florid period of their ADL episode. Patients were required to provide their own transportation to and from the clinic. Those who did willingly make the trip to the clinic to give blood may have done so after the most severe symptoms of their ADL episode had passed. If this is the case, the antibody levels measured during the ADL episode are probably an overestimate of the levels during the most florid time (assuming antibody levels increase over the period of interest). Thus, the differences in antibody levels from ADL to convalescent time point were probably an underestimate. Also, because we chose which pathogens to test a priori, we may have missed some component causes of ADL, which may contribute greatly to the ADL episodes in this study population.

Because most of the variance in the antibody levels was due to subject and not due to time, our conclusions about which pathogens contributed the most to ADL episodes in this population based on arbitrary responses of change, may not be as strong as findings from other studies in which a control population (no ADL episode) was present.

Through a descriptive analysis of the relative prevalence of antibody response at several cut-point levels of change, this study provides further, yet limited evidence for infection with *Streptococcus A* as a potential contributing factor of ADL episodes. These findings are especially important within the context of a greater focus on LF morbidity control as the infection is being eliminated in endemic areas. It adds to the current evidence and illustrates the importance of determining the causal role of bacterial and fungal pathogens and immunological antifilarial response in ADL episodes. Future and current lymphedema management programs may target the pathogens identified to be contributing to ADL episodes in this study with pathogen specific drugs or creams, with the ultimate goal of preventing ADL episodes and further lymphedema progression among affected individuals.

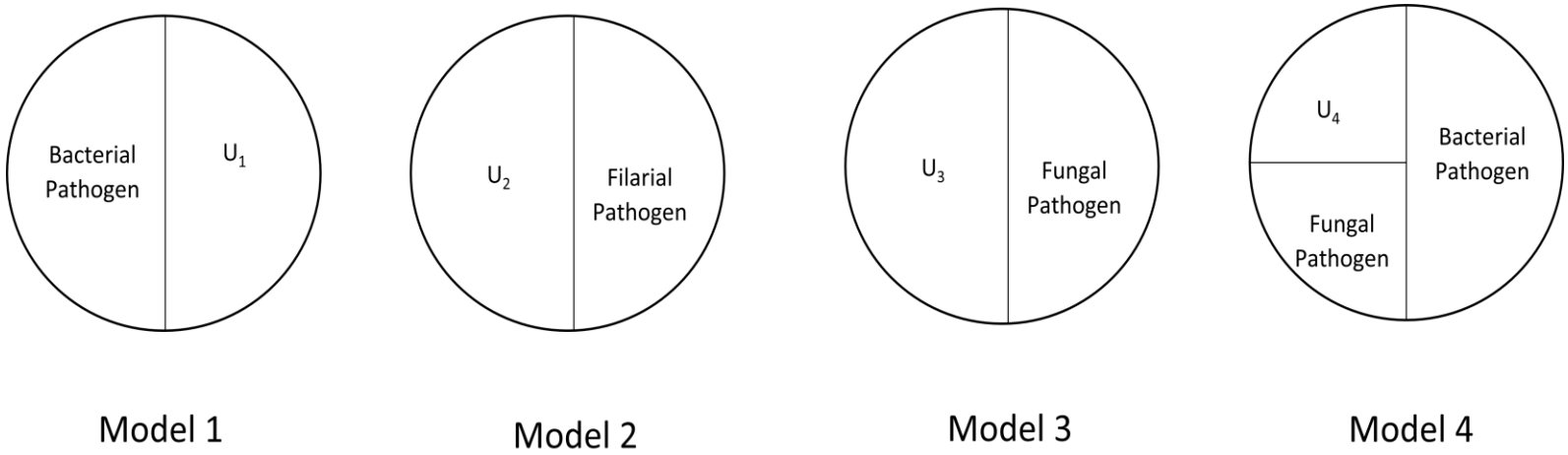

Figure 7.1. Sufficient component cause models (SCCMs) of ADL episodes among patients with lymphatic filariasis. Each SCCM represents a sufficient cause, which contains a minimal set of conditions that result in the outcome of interest. Each risk factor for the outcome is defined as a component cause and each pie represents a sufficient cause.

Table 7.1. Demographic and clinical characteristics of lymphedema patients in Léogâne, Haiti. N = 41 patients with 52 paired samples

	N (%)
Patient Characteristics (N=41)	
Female gender	34 (82.9)
Age (median, SD)	34 (15.3)
No. legs	82
Lymphedema stage	
0	24 (29.3)
1	6 (7.3)
2	22 (26.8)
3	22 (26.8)
4	8 (9.8)
CFA(+): ADL Time Point	1 (1.8)
CFA(+): Convalescent Time Point	1 (1.8)
Mean yearly rate of ADL episodes reported in year prior to cohort enrollment*	2.7
Mean yearly rate of ADL episodes reported during cohort period 1995-1998	1.42
Mean number of ADL episodes during study period up until serum sample** (range)	3.3
ADL Episode Characteristics (N=52)†	
Mean number of days between ADL and convalescent sample (range)	17 (1-35)
Treated with antibiotic during episode	14 (26.9)
Lymph node enlargement	36 (69.2)
Retrograde progression of lymphedema	32 (61.5)
Inflammation of skin (redness, pain, tenderness)	39 (75.0)
Acute swelling at ADL site	42 (80.8)

*The study period of the lymphedema management effectiveness cohort = 1995 – 1998. This number represents the mean yearly rate of ADL episodes reported in the year prior to their enrollment. N=38 (missing information on 3 patients)

**The study period of the lymphedema management effectiveness cohort = 1995 - 1998. This number represents the number of ADL episodes from patient enrollment in the cohort up until the observed ADL episode during which serum was collected.

†Serum samples were collected from 52 ADL episodes among the 41 lymphedema patients

CFA: circulating filarial antigen

Table 7.2. Serum antibody levels stratified by sample time point as determined by ELISA tests among lymphedema patients in Léogâne, Haiti. All values are in arbitrary units. N=52 paired samples.

	ADL		Convalescent				
Indicator	**Median**	**SD**	**Median**	**SD**	**P-value***	**Median Absolute Difference (IQR)**	**Median % Change (IQR)**
BpG1	1733.50	2934.21	1605.00	2629.26	0.8581	-39.60 (-209.5, 219.0)	-2.03 (-21.27, 13.30)
BpG2	707.85	2424.22	781.00	1767.44	0.9249	0.32 (-78.28, 131.0)	1.21 (-20.17, 25.86)
BpG3	57.13	140.64	50.42	219.96	0.6310	-1.29 (-7.14, 14.21)	-1.95 (-17.22, 20.22)
BpG4	88.83	1365.53	71.32	918.75	0.9786	-0.80 (-10.32, 10.10)	-2.84 (-10.40, 7.27)
Pseudomonas	38.14	76.05	36.86	66.32	0.7470	-1.63 (-5.51, 2.53)	-6.28 (-16.06, 6.97)
SEB	307.40	406.47	321.30	448.25	0.9041	-1.10 (-35.5, 33.1)	-0.49 (-12.14, 10.79)
SPEA	99.40	67.35	108.80	99.43	0.7264	0.76 (-15.28, 18.61)	2.72 (-15.23, 34.30)
SPEB	28.70	23.57	25.15	22.96	0.7061	-0.95 (-8.03, 5.49)	-3.16 (-30.80, 29.10)
SLO	132.55	123.81	135.80	126.78	0.6845	-12.05 (-38.52, 13.55)	-9.56 (-26.83, 16.58)
Strep A	130.40	154.73	150.05	146.57	0.9456	0.29 (-25.22, 32.15)	1.73 (-26.07, 32.53)
Candida	8.61	15.53	8.95	15.58	0.6420	-0.01 (-0.71, 1.54)	-0.63 (-10.38, 18.59)
Trichophyton	67.30	45.91	64.70	46.32	0.9172	-0.20 (-7.02, 9.04)	-0.08 (-9.14, 12.45)

* Wilcoxon-Mann-Whitney p-value for difference between mean ADL and convalescent antibody levels
IQR = inter-quartile range

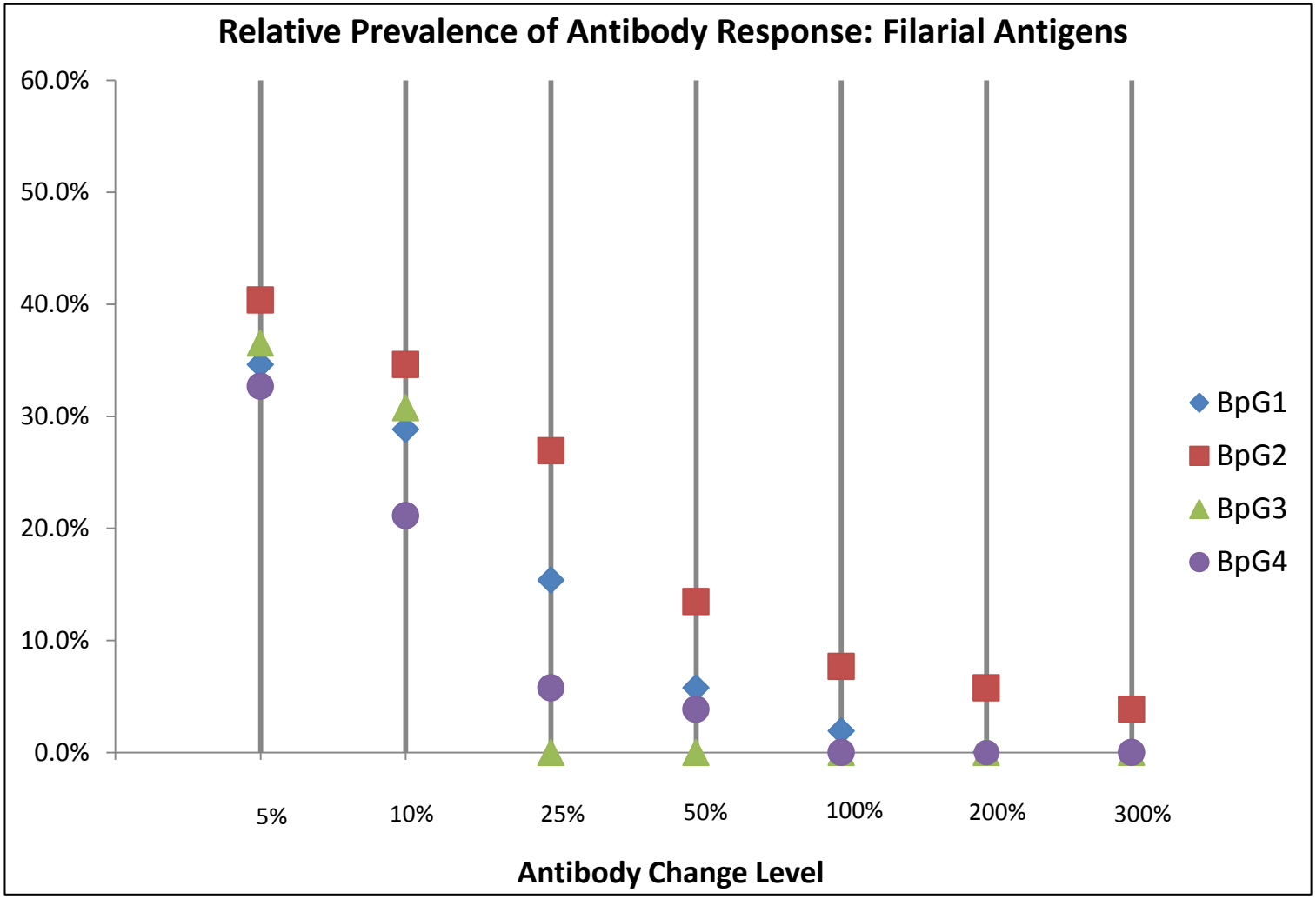

Figure 7.2. Prevalence of antibody response at different levels of antibody change for filarial antigens BpG1, BpG2, BpG3, and BpG4 among a cohort of 52 paired serum samples from patients enrolled in a lymphedema program in Léogâne, Haiti. All antibody change levels represent a positive percent increase in antibody titer from the ADL to convalescent time point.

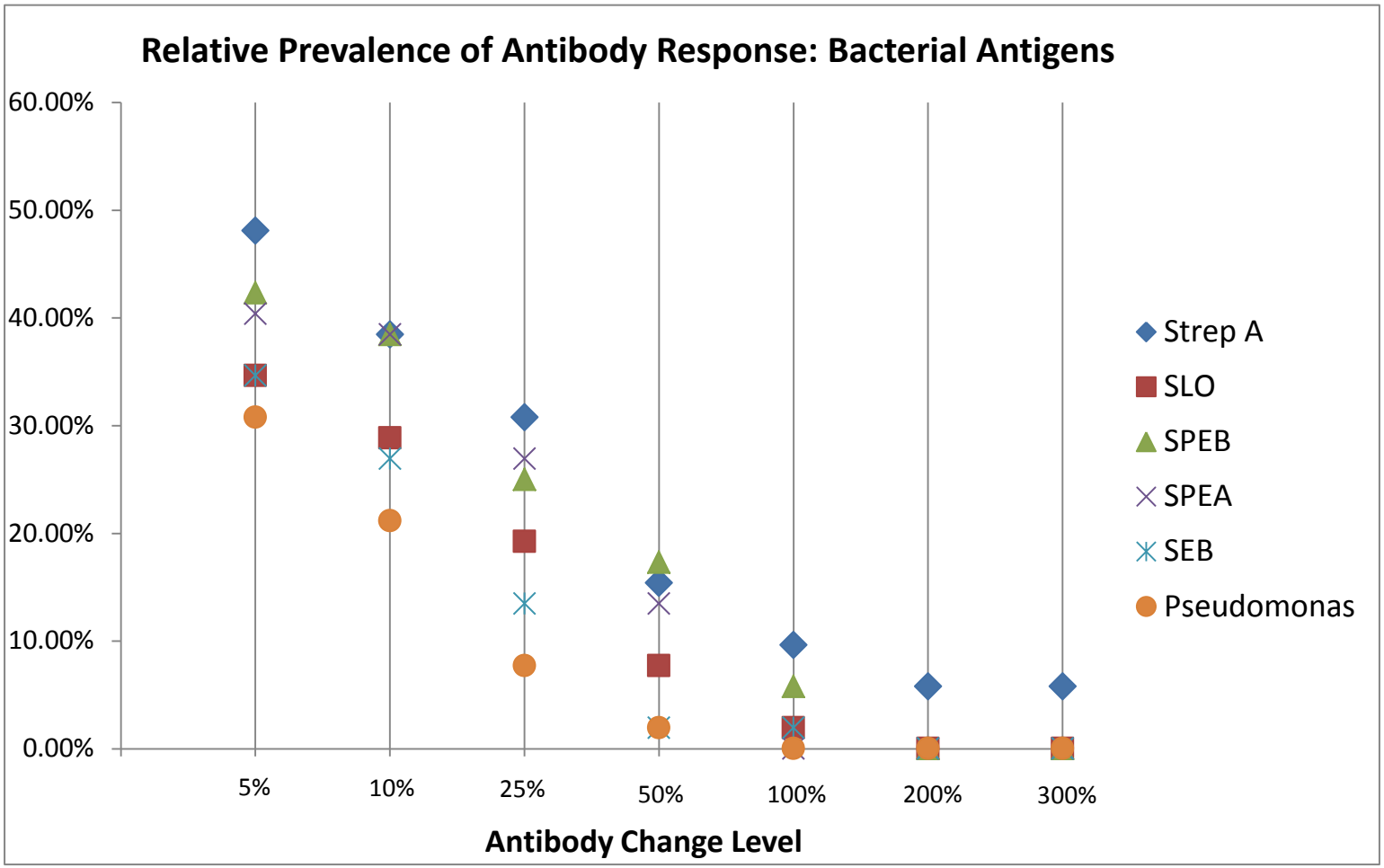

Figure 7.3. Prevalence of antibody response at different levels of antibody change for the bacterial antigens Strep A, SLO, SPEB, SPEA, SEB, and Pseudomonas among a cohort of 52 paired serum samples from patients enrolled in a lymphedema program in Léogâne, Haiti. All antibody change levels represent a positive percent increase in antibody titer from the ADL to convalescent time point.

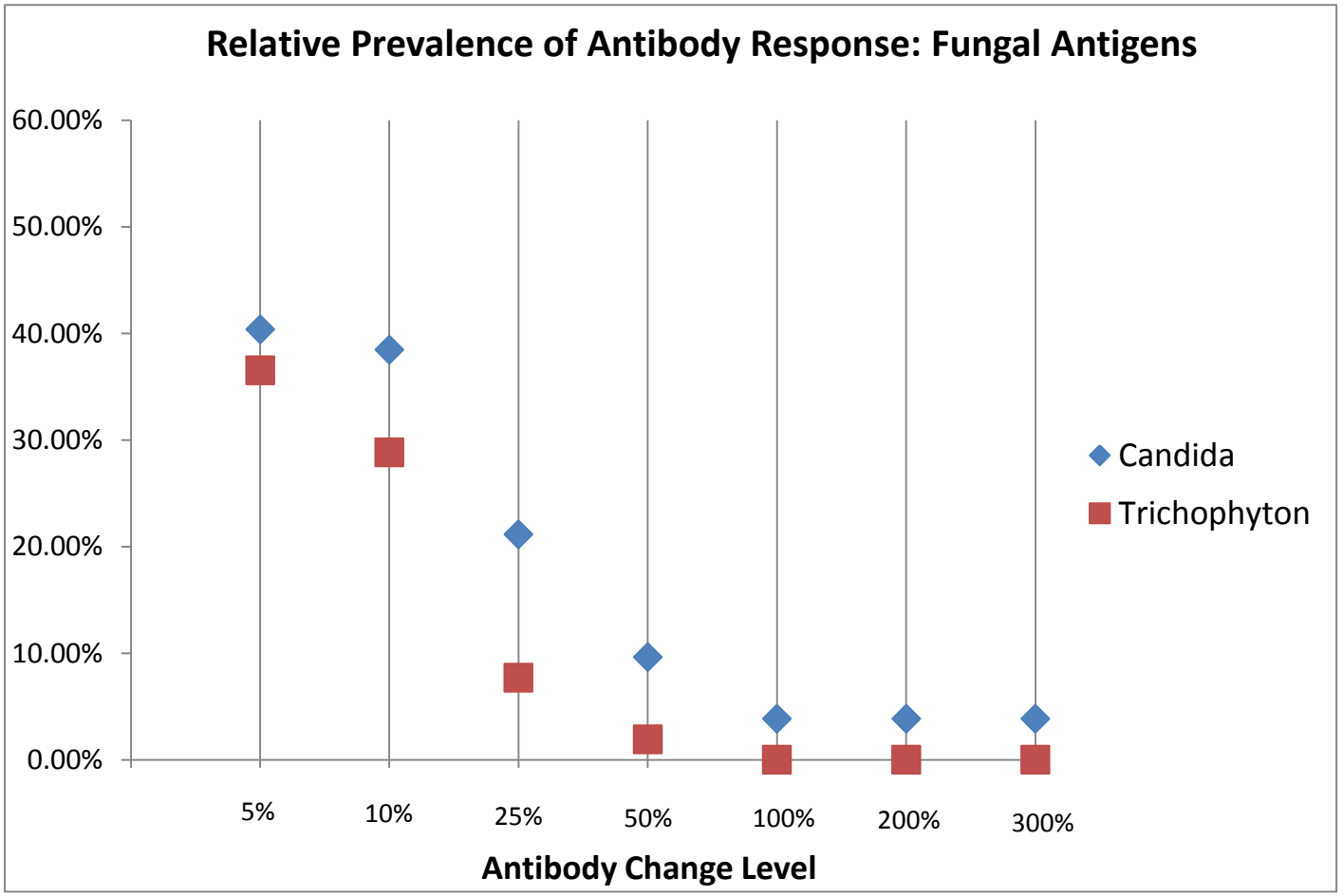

Figure 7.4. Prevalence of antibody response at different levels of antibody change for the fungal antigens Candida and Trichophyton among a cohort of 52 paired serum samples from patients enrolled in a lymphedema program in Léogâne, Haiti. All antibody change levels represent a positive percent increase in antibody titer from the ADL to convalescent time point.

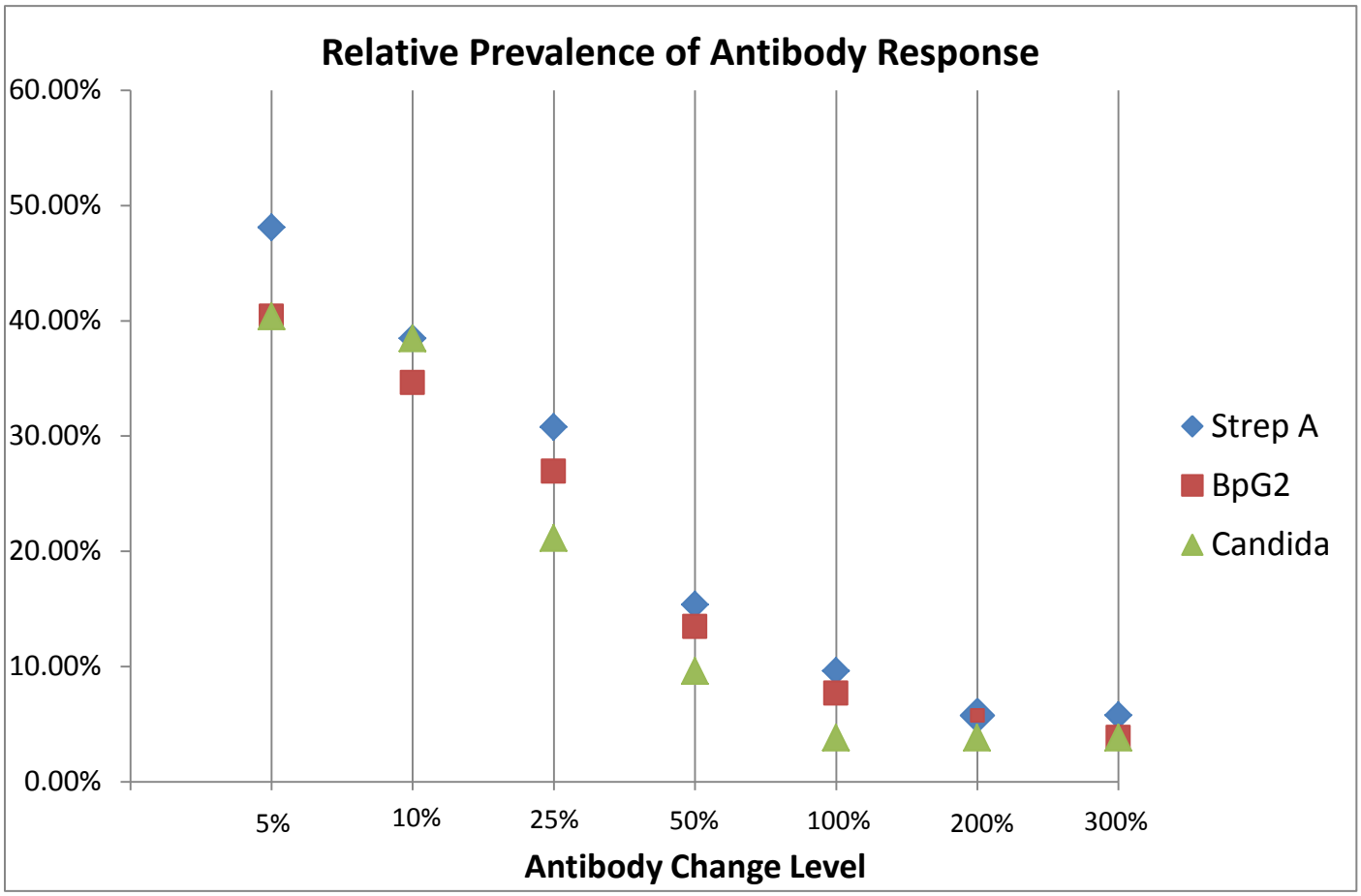

Figure 7.5. Prevalence of antibody response at different levels of antibody change for the Strep A, BpG2, and Candida antigens among a cohort of 52 paired serum samples from patients enrolled in a lymphedema program in Léogâne, Haiti. All antibody change levels represent a positive percent increase in antibody titer from the ADL to convalescent time point.

APPENDIX: SUPPLEMTNAL TABLES FOR CHAPTER 7

Table 7.3. Demographic and clinical characteristics of lymphedema patients in Léogâne, Haiti, comparing the entire cohort to the ADL cohort.

Patient Characteristics (N=175)	**Entire Cohort (n=175)**	**ADL Cohort (n=41)**	**P-value**
Female gender	145 (82.9)	34 (82.9)	0.85
Age (median, SD)	38 (15.96)	34 (15.3)	0.09
% Literate	87 (49.7)	18 (43.9)	0.79
Mean (range) duration of lymphedema upon entry into study (years)	11.3 (<1-50)	10.8 (<1-37)	0.77
No. legs	350	82	
Lymphedema stage			
0 (No Edema)	85 (24.3)	24 (29.3)	0.1169
1	38 (10.8)	6 (7.3)	
2	129 (36.9)	22 (26.8)	
3	85 (24.3)	22 (26.8)	
4	13 (3.7)	8 (9.8)	
Yearly rate of ADL episodes reported in year prior to cohort enrollment **	2.06	2.7	0.1368
Yearly rate of ADL episodes reported during cohort period 1995-1998**	0.75	1.42	<0.0001

*The study period of the lymphedema management effectiveness cohort = 1995 – 1998. This number represents the mean yearly rate of ADL episodes reported during their enrollment. N=38 (missing information on 3 patients)

**The study period of the lymphedema management effectiveness cohort = 1995 - 1998. This number represents the number of ADL episodes from patient enrollment in the cohort up until the observed ADL episode during which serum was collected.

†Serum samples were collected from 52 ADL episodes among the 41 lymphedema patients

CFA: circulating filarial antigen

Table 7.4. Variation of serum antibody levels due to subject, time, and time given subject among lymphedema patients in Léogâne, Haiti. N=104 samples.

Indicator	**R^2 Subject**	**R^2 Time**	**R^2 Time given Subject**
BpG1	0.93	0.00008	0.0011
BpG2	0.87	0.00224	0.0166
BpG3	0.90	0.00164	0.0215
BpG4	0.96	0.00111	0.0270
Candida	0.90	0.00141	0.0137
Pseudomonas	0.98	0.00163	0.0628
SEB	0.99	0.00003	0.0026
SPEA	0.92	0.01080	0.0465
SPEB	0.83	0.00252	0.0148
SLO	0.95	0.00204	0.0398
Strep A	0.89	0.00014	0.0013
Trichophyton	0.98	0.00008	0.0037

Chapter 8: Summary, Future Directions, and Implications

Summary of Findings:

The Global Programme to Eliminate Lymphatic Filariasis (GPELF) is working to eliminate transmission of lymphatic filariasis by the year 2020. As mass drug administration (MDA) programs to interrupt transmission begin to conclude in many endemic countries, there will still be millions of patients with the chronic conditions of lymphatic filariasis. With this shift and the Global Programme's recent recommendation that all LF-endemic countries provide access to morbidity management programs, it is important to understand the efficacy and effectiveness of lymphedema management programs, to find predictors of compliance to program activities, and to provide further insight into the etiology of ADL episodes in order to prevent future episodes and lymphedema progression.

The first dissertation study found a 35% decrease in the 30-day rate of ADL episodes among a cohort of LF patients enrolled in a lymphedema management program in Odisha State, India over a 2-year follow-up period. The lowest rate of ADL episodes was observed after 6 months of program enrollment. Those with inter-digital entry lesions had a higher ADL rate throughout the study. Those with advanced lymphedema at baseline (stages 4-7) also had a higher rate of ADL. Study one also demonstrated that patients enrolled in the lymphedema management program experienced a moderate decrease in lymphedema stage; with the majority of lymphedema regression occurring among patients with stages 3 and 4 transitioning to stages 1 and 2. The percentage of patients whose lymphedema progressed to a more severe stage from one time point to the next also decreased over the study period.

Overall compliance to program techniques increased over the course of the study, with a peak at 6 months. The study found that reported difficulty accessing soap and antifungal cream were negatively associated with compliance. Overall compliance to lymphedema management appeared to have little to no association with the frequency of ADL episodes among those without entry lesions, while among those with entry lesions compliance was significantly associated with an increase in the rate of ADL episodes. Looking at the lymphedema management techniques separately, compliance to washing the limb with soap was significantly associated with a decreased rate of ADL episodes in most disease groups. Compliance to antifungal cream among those with inter-digital entry lesions was not associated with the rate of ADL. Compliance to wearing footwear outside had little to no association with the rate of ADL episodes. We found that those who are compliant to all management techniques were slightly less likely to progress to a more advanced stage of lymphedema, yet this finding was not statistically significant. Compliance to soap was significantly negatively associated with lymphedema progression.

Study one indicates that a community-based lymphedema management program is beneficial for lymphedema patients for both acute and chronic morbidity and these benefits can be sustained over a two year time period. The study also illustrates that compliance to lymphedema techniques can be improved if patients are provided the proper resources such as soap and antifungal cream.

Using a marginal structural model (MSM) to deal with a time-varying exposure and confounder, study two found an increased occurrence of anti-fungal cream use over time was slightly associated with a decreased frequency of ADL episodes at a subsequent

time point. The rate ratio was only significant at the 18-month time point where an increased occurrence of anti-fungal cream use over 12 months was associated with a 23% decreased frequency of ADL episodes after 24 months. Compare this to an estimated 7% increase using a crude model and a 14% decrease using a traditionally adjusted model.

As expected, the MSM estimates were consistently further from the null than those of the traditionally adjusted regression. We suspect the traditional models may be controlling away some of the effect of anti-fungal cream by controlling for inter-digital entry lesions, an intermediate variable on the proposed causal pathway. Assuming no unmeasured confounding and correctly specified treatment, censoring, and marginal structural models, we interpret the estimated effects as causal and conclude there is a slightly protective effect of anti-fungal cream on the frequency of ADL episodes.

Study three quantified antibody levels to pathogens that potentially contribute to ADL episodes during and after an episode among lymphedema patients in Léogâne, Haiti. We also sought to calculate the prevalence of antibody responses for different pathogens thought to be associated with ADL episodes. Initially we calculated the prevalence of paired serum samples showing a four-fold positive change in antibody titers for each pathogen. We assumed that those pathogens showing a four-fold change would be considered a component cause of ADL episodes, and therefore considered those prevalence measures as population attributable fractions. Our results showed four-fold changes in antibody levels from an ADL to a convalescent time point for the following antigens: BpG2, Strep A, and Candida. However, for each of those antigens, only two or three paired samples showed a response at that level.

We then calculated the prevalence of antibody change at different antibody change levels and looked for patterns of the relative prevalence among each pathogen type (filarial, bacterial, and fungal). We hypothesized that patterns arising in the data at the lower antibody change values would be indicative of what would occur if there had been more frequent four-fold changes in antibody titer levels. Amongst all bacterial, fungal, and filarial antigens tested, the Strep A antigen for *Streptococcus A* infection had the highest prevalence of response at all cut points. Through this descriptive analysis of the relative prevalence of antibody response at several levels of change, study three provides further, yet limited evidence for infection with *Streptococcus A* as a potential contributing factor of ADL episodes.

Study Limitations

The main limitation of this dissertation is the lack of a control group in all three studies. In studies one and two, the lymphedema management program was offered to all lymphedema patients in the study. The studies did not include a subset of comparable patients who did not receive the community-based lymphedema program because it may be viewed as unethical to withhold knowledge of lymphedema management techniques to those with lymphedematous limbs. Because of this limitation, we note the possibility that the decrease in the rate of ADL episodes over the study period may have occurred regardless of the intervention. Future studies of lymphedema management programs could implement a "step-wedge" study design. This design enrolls patients in morbidity management programs on a rolling basis so that all willing persons can obtain access to the resources. However, data are continually collected during the study period and patients are followed-up while they are not enrolled in the morbidity program. This

provides a “non-exposed” group from which comparisons of ADL episodes or lymphedema stage can be made with those currently enrolled in the morbidity management program. These types of designs are beginning to be used in studies of podoconiosis, another neglected tropical disease that causes severe morbidity and lymphedema (personal communication, Gail Davey). As MDA programs begin to conclude and more resources become available for studies of morbidity management programs, this type of study design will become more feasible.

Another major limitation of studies one and two was the lack of prospective follow-up, specifically with regards to the reporting of ADL episodes and frequency of lymphedema management activities. Furthermore, our patient follow-up was performed at unequal time intervals, complicating the interpretation of our findings. An ideal study design would involve prospective follow-up of both of these aspects along with independent observation of the episodes and lymphedema management techniques. This would allow us to further elucidate the temporal sequence of the use of anti-fungal cream and soap on the occurrence of subsequent ADL episodes. Unfortunately, the prospective follow-up requires more frequent patient visits and more study staff for which our study’s funds did not allow. Future studies should prioritize the prospective data collection and as well as equal time intervals in their funding and planning.

Like studies one and two, study three did not have a control group, patients who did not have an episode of ADL, limiting the conclusions we could make from our work. Future studies of the contributing pathogens to ADL episodes should also recruit patients who are not undergoing an ADL episode to provide a pair of serum samples at the same two points in time as a person undergoing an episode (ADL and convalescent time

points). This would allow one to make much stronger conclusions based on statistical tests comparing cases to controls.

In addition to lacking a control group, for most of the patients, study three only collected serum samples from one ADL episode. The conclusions made from this study can only be made about that specific episode, which may differ from findings if more episodes had been included. Furthermore, most of the patients in study three had been suffering the chronic effects of LF for many years, so this was most likely not their first ADL episode. Therefore, we did not have baseline antibody titers to compare with the ADL titers. It would be ideal to have paired serum samples from multiple ADL episodes among patients with early lymphedema, so as to explore antibody levels early in the chronic phase of LF. Another major limitation of study three was that all antibody values were based on arbitrary units and not on a calibrated reference scale. Therefore, we could not determine if persons in our cohort met standard antibody levels indicative of infection. We could only explore absolute and relative changes in antibody levels between the ADL and convalescent samples and assume certain levels of change indicate infection. Ideally, we would have had the raw antibody levels and antigen test information from which we could have used a validated infection threshold.

Study Strengths

Despite the limitations previously discussed, this dissertation research has several strengths. Studies one and two followed lymphedema patients enrolled in the morbidity management program for two years. Compared to previously published studies of lymphedema management programs, this is a rather longer period of patient follow-up. We also were able to stratify our findings regarding ADL episodes and lymphedema

progression by the severity of disease including the presence of inter-digital entry lesions and baseline lymphedema status (advanced vs. early). Furthermore, this is the first study to explore the association between compliance to specific lymphedema management techniques and the rate of ADL episodes and lymphedema progression. This will help future programs to potentially prioritize lymphedema management activities.

Study two is the first to explore the effect of cumulative anti-fungal cream use on the rate of ADL episodes, and to do so considering the effects of a time-varying confounder and a time-varying exposure. Looking at the sum of cream use over time allowed us to elucidate the potential cumulative effects of cream use. Although our findings were mostly insignificant, this was the first study to find a slight association between use of anti-fungal cream to treat lesions and a decrease in the rate of ADL episodes. Future prospective studies without as great a potential for recall bias, but using the same techniques and causal hypotheses may find more significant effects. Study two also attempted to adjust for potential selection bias due to censoring by using inverse probability of censoring weights (IPCWs).

Study three was unique in that it framed a traditional immunologic analysis within the context of sufficient component cause models (SSCM). SCCMs are well known in the epidemiology field, but are rarely applied to other fields. This study will allow the immunology and neglected tropical disease field experts the opportunity to consider ADL episodes within the framework of SCCMs and may bridge the gap between epidemiology and immunology. Additionally in study three, we were able to explore antibody levels for a wide range of bacterial, fungal, and filarial pathogens using several different types of antigen tests.

Future Directions

Future studies of lymphedema management programs are needed in other LF-endemic countries. These could better elucidate the temporal sequence between lymphedema management techniques and ADL episodes by applying equal intervals to follow-up. Given available funds, they should also incorporate prospective follow-up of lymphedema patients, especially when collecting information on ADL episodes and frequency of lymphedema management activities. Independent observation of lymphedema management techniques will be necessary to reduce recall bias. Physician or nurse confirmation of an ADL episode should be sought and detailed information regarding the start and end times of each ADL episode should be collected along with information about detailed symptoms and location of the episode.

Future studies of lymphedema management programs may also consider the "step-wedge" study design in order to collect information from a "non-exposed" group of patients not currently enrolled in a lymphedema management program. This study design would entail a gradual roll-out of the lymphedema management program over the course of the study period. By the end of the roll-out, all lymphedema patients enrolled in the study would have been exposed to the program, yet it is rolled-out in different geographic areas at different time points. Data would be collected from all individuals during the entire study period so that at certain points, some groups serve as the "non-exposed" group. One can then compare the ADL rate of the exposed group (where program roll-out has already occurred) to an unexposed group (where program roll-out has not yet occurred) at specific time points throughout the study period. By the end of follow-up however, all patients will have been exposed to the program. This design will allow one

to make more definitive conclusions regarding the effectiveness of lymphedema management programs.

Future studies exploring pathogens thought to contribute to ADL episodes should ensure the inclusion of lymphedema patients who are not experiencing an ADL episode. The study could collect two serum samples from each case and control, one during the case's ADL episode and one following the case's ADL episode. One can then compare the change in antibody levels of the cases to controls and provide more conclusive evidence for which pathogens most likely contribute to ADL episodes. Future studies may also choose to focus on patients with early lymphedema, in order to collect serum from patients experiencing their first ADL episode. There should also be careful choice of antigens that are more specific to certain antibodies in order to avoid the potential for cross-reactivity that may be driving apparent antibody levels. Finally, in addition to the antibody response, future studies may want to consider the cytokine immunologic response and other immunologic biomarkers when exploring the etiology of ADL episodes.

Implications

As the Global Programme to Eliminate Lymphatic Filariasis (GPELF) reaches end points of mass drug administration (MDA), there will be a continued need for the implementation of and access to morbidity management programs. This dissertation provides further evidence for the efficacy of community-based lymphedema management programs for patients dealing with the chronic manifestations of lymphatic filariasis. It also provides more detail on which lymphedema management techniques are most beneficial for acute and chronic outcomes. Furthermore, it offers insight into the

potential contributing factors for ADL episodes, the prevention of which is critical to stop further lymphedema progression. The dissertation demonstrates the benefits of scaling up community-based lymphedema management programs, which is important since GPELF has recommended that all LF endemic countries provide access to morbidity management services [107].

This dissertation elucidates the effectiveness of specific lymphedema management techniques that should be noted for the planning and implementation of future programs. The results from study one suggest a greater emphasis on the use of soap for washing the affected limb in lymphedema management programs. Results also suggest a focus on the procurement of soap and anti-fungal cream for all lymphedema management patients in order to improve overall compliance and compliance specifically to washing with soap and water. Although the evidence is not as strong as with soap, this dissertation also highlights the potential effectiveness of the cumulative use of anti-fungal cream over a period of time on decreasing the rate of ADL episodes. Given the limitations of study two's design (i.e. measurement error, loss of statistical power due to censoring) more work is needed to further elucidate the effectiveness of anti-fungal cream in decreasing the rate of ADL episodes. Like ours, this work should consider the potential for time-varying confounding by inter-digital entry lesions as well as the time-varying nature of anti-fungal cream use and employ the appropriate analysis techniques.

Although there were differences in program effectiveness by disease severity group, we still support the broad implementation of lymphedema management efforts within national LF programs. This work demonstrates the benefit of broad community-based lymphedema management programs implemented by volunteers or community

health workers, which have the potential to reach large numbers of lymphedema patients at a relatively low cost. We also recognize the need for referral care for patients with complicated lymphedema and more severe disease and support the integration of broad-based and specialized lymphedema care.

This dissertation highlights the importance of morbidity management programs, which may be applied more broadly as an aspect of other neglected tropical disease (NTD) elimination programs. Of the NTD elimination programs, only lymphatic filariasis and trachoma programs incorporate morbidity management into their strategy. Other NTD programs such as leprosy, schistosomiasis, and Chagas disease would also benefit from morbidity management programs incorporated into their global strategies. This work sheds light on the importance of morbidity management programs in an LF-endemic setting and may have implications for their use in other NTD programs.

This work also brings to light the potential for integrated limb care. In this LF-specific context, our work demonstrated the efficacy of a lymphedema management program and for some specific techniques such as soap and anti-fungal cream it demonstrated their effectiveness as well. There are numerous infectious and chronic diseases such as diabetes, Buruli Ulcer, leprosy, venous insufficiency, mycoses, and podoconiosis that cause swollen limbs. Numerous countries, including India and Haiti, are endemic for several diseases which affect the limbs. Integrated limb care programs, where patients in areas endemic for a multitude of these diseases may come to receive care, provide an opportunity to provide needed services for many with lymphedema or limb wounds from various causes. Integrated limb care provides the global public health field an opportunity for efficient management of chronic conditions such as lymphedema

as well as an opportunity to move morbidity management forward on a global scale. Others have suggested the incorporation of a cohesive global construct for lymphedema care that transcends both geographic and socio-economic boundaries in both developed and developing countries [135].

This dissertation research extends the literature by demonstrating the efficacy of a community-based lymphedema management program over a 2-year follow-up period for both ADL episodes and lymphedema progression. It also demonstrates an association between compliance to specific program techniques, such as soap use, and a reduced rate of ADL episodes. Our exploration of the effect of cream use on the rate of ADL episodes implemented marginal structural models, not previously used in the lymphatic filariasis field. Although we did not find a strong significant association between cream use and ADL episodes, we note that the limitations of the study design especially the loss of statistical power and measurement error may be biasing the results. Our investigation of contributing factors to ADL episodes among a cohort of lymphedema patients in Haiti provides limited yet further evidence for *Streptococcus A* as a contributing pathogen, supporting previous literature on the subject. This dissertation extends the breadth of epidemiologic methodologies used within the field of lymphatic filariasis morbidity. The findings are important for the planning and implementation of future morbidity management programs for patients suffering from lymphatic filariasis and potentially for patients suffering the effects of other neglected tropical diseases.

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