ASSOCATIONS OF FRAILTY WITH IMMUNE RESPONSE TO INFLUENZA VACCINE IN ADULTS 50 YEARS OF AGE AND OLDER AND INTERRELATIONSHIPS AMONG FRAILTY, QUALITY OF LIFE INDICATORS AND SPIRITUALITY

by

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ABSTRACT

Background: Physical frailty, the multi-dimensional syndrome characterized by diminished strength, endurance, and reduced physiologic function is increasingly prevalent with advancing age. Frailty leads to increased risk of acute illness, falls, disability, hospitalization, institutionalization and mortality. The negative impact of frailty on the immune system is beyond that of aging-related changes alone. Frailty is also associated with psychosocial aspects of quality of life such as depression and stress. **Methods:** This three-paper dissertation examined the effects of physical frailty in adults ≥ 50 years of age on both immunological response to influenza vaccine (papers 1 and 2) and psychosocial factors (paper 3). **Results:** Paper 1: Frailty exists in community-dwelling adults < 65 years of age and the relationship to influenza vaccine immune response in frail younger adults differs from frail adults ≥ 65. In adults 50-64 years of age, frailty appeared to be protective in eliciting beneficial immune system response to influenza vaccine. Paper 2: Among long-term care residents ≥ 65 years, there was a differential effect of frailty on immune response to influenza vaccine by vaccine type. Frail long-term care residents, as compared to non-frail, showed overall greater odds of obtaining influenza vaccine immunogenicity protection outcomes by the high dose vaccine group than those in the standard dose group. Paper 3: Among community-dwelling adults ≥ 50 years, the

relationship between quality of life measures and frailty was moderated by spirituality. The effect of quality of life upon frailty varied by the level of spirituality; as spirituality decreased, quality of life became more important. **Conclusion:** There have been few studies that have specifically measured the influence of physical frailty on immune system response to influenza vaccination. Only one prior study has examined the role spirituality had in moderating the quality of life-frailty relationship. With an increasingly aging population and the costs associated with increased healthcare utilization, it is important to address the immunological and psychosocial aspects of health affected by physical frailty. This dissertation addresses both aspects. **Public Health Significance:** All three studies are among the first of their kind thereby adding to the literature. Important findings have emerged from this dissertation and steps are given for future research direction.

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PREFACE

This dissertation is gratefully dedicated to my God, my parents, my current and former bosses whose support for my pursuit of education allowed me to pursue my doctoral degree while working full time, my doctoral committee members and my friends. Without their support, encouragement, time, meals, loving thoughts and especially their prayers, I would not have finished this degree. Thank you, all of you, I so greatly appreciate your walking with me in this journey over the past 8.25 years.

1.0   DISSERATION OVERVIEW

This is a three-paper dissertation which addresses two main questions; the first question has two parts. Question one investigates the effect of frailty on immune system response to influenza vaccination among older adults. The first study aims to address this question in community-dwelling adults ≥ 50 years of age who received standard dose inactivated trivalent influenza vaccine. The second study aims to address this question in long-term care residents ≥ 65 years of age who were randomized to two different inactivated influenza vaccine types, high-dose or standard dose vaccine. The second question measures the impact of spirituality upon the relationship between health-related quality of life indicators of stress and depression, and frailty in community-dwelling adults ≥ 50 years of age.

A review of the literature reveals a substantial amount of research on quality of life indicators, frailty measures and spirituality in health-related studies (see Sections 4 and 5). To the best of our knowledge only one study has assessed the difference in response to standard-dose versus high-dose influenza vaccine in older adults by frailty status but that study used a much broader definition of frailty than used in this dissertation (DiazGranados CA et al., 2015). Likewise, there has been only one prior study that has assessed the influence of spirituality on the association of frailty with health-related quality of life; the measures used previously for these three variables differed substantially from those used in this dissertation.

This dissertation is organized as follows: Sections 2-5 present the literature overview relating to influenza and influenza vaccine, key drivers of immune system response, frailty and its components, health-related quality of life, and spirituality. Section 6 presents a before-and-after study nested in a cohort study which examines the effect of frailty on immunogenicity response to influenza vaccine in community-dwelling adults ≥ 50 years of age. Section 7 presents the study that examines the effect of frailty on immune response to influenza vaccine comparing frailty responses to standard-dose and high-dose vaccine among long-term-care facility residents aged ≥ 65 years in a secondary analysis of a randomized controlled trial. Section 8 presents the study that assesses the impact that spirituality has upon the relationship of health-related quality of life and frailty in community-dwelling adults ≥ 50 years of age. Section 9 summarizes the three studies and discusses key findings from each. Section 10 highlights the public health significance of the work conducted in this dissertation. Section 11 ends with directions for future research.

2.0   INFLUENZA

2.1   DEFINITION OF INFLUENZA: AN INFECTIOUS DISEASE

Influenza is a highly contagious viral respiratory illness caused by influenza viruses. The virus is a single-stranded, helically shaped, RNA virus of the family orthomyxovirus (CDC, 2015b). There are two main human antigen types, A and B, determined by the nuclear material inside the virus capsule. Influenza A and B viruses are determined by the surface glycoprotein antigens of hemagglutinin (HA), and neuraminidase (NA). Influenza type C viruses do not cause seasonal epidemics and are not studied in this dissertation.

Influenza viruses are expressed in the order of: 1) virus type, 2) geographic location of first isolation, 3) virus strain or lineage number, 4) year of isolation and, 5) virus protein antigen subtype (e.g., A/Texas/50/2012 (H3N2)). There are currently eighteen HA (H1-H18) and eleven NA (N1-N11) subtypes identified for Influenza A viruses (CDC, 2014). In humans there are three types of HA (H1, H2 and H3) which are involved in virus attachment to cells and two types of NA (type 1 and type 2) which are involved in the penetration of the influenza virus into cells. Influenza B viruses are categorized not by subtype but rather by their distinct lineages (either B/Victoria or B/Yamagata) (CDC, 2014).

Influenza occurs yearly worldwide with temporal patterns of influenza occurring mainly during the peak influenza seasonal months of December through March in the Northern

Hemisphere and May through August in the Southern Hemisphere (CDC, 2015b; Sternal J et al., 2015). Reservoirs of Influenza A include both humans and animals whereas Influenza B is known only in humans (CDC, 2015b).

Human-to-human transmission occurs primarily through large droplets that are spread by infected persons coughing and sneezing; these virus containing droplets can settle within a three foot radius (CDC, 2015b). The period of incubation varies from 1-4 days with communicability lasting in adults typically five days and in children ten days or longer (CDC, 2015b). Upon respiratory transmission, the influenza virus becomes attached to and penetrates the host's respiratory epithelial cells of the trachea and bronchi; viral replications then follow resulting in destruction of that cell (CDC, 2015b). Influenza is noted for its sudden onset and symptoms of fever/feverishness, cough, sore throat, runny/stuffy nose, muscle or body aches, headaches and fatigue. (CDC, 2017) Acute symptoms of influenza typically last up to three days (CDC, 2015b). Complications resulting from influenza illness include pneumonia, bronchitis, sinus and ear infections. Persons at highest risk for developing influenza-related complications include the elderly, people with weakened immune systems, people with chronic illness and young children (CDC, 2017)

Individuals can contract influenza repeatedly because the viruses continually undergo genetic mutations. These mutations are expressed in the surface glycoprotein antigens of HA and NA. These changes can occur either as antigenic drifts (minor changes in HA and NA surface antigens that result from pointed gene segment mutations), which can cause epidemics, or antigenic shifts (major changes in HA and NA surface antigens which occur at varying intervals and include genetic recombination), which can result in pandemics (an epidemic which occurs over a large area or worldwide) (CDC, 2015b). Once the virus mutates significantly from

the current viral strain (either by an antigenic shift or drift) that virus is then chosen as the new predominating virus for subsequent vaccines (CDC, 2015b). An example of the latest antigenic shift which resulted in an influenza pandemic was that of the 2009 Influenza A-H1N1 virus (i.e., A/California/07/2009) that was first detected in California in April of that year and by May had spread worldwide (CDC, 2010).

2.2 THE BURDEN OF INFLUENZA

The burden of influenza is substantial. Within in the United States, there are on average, 23,000 influenza-related deaths annually (Thompson WW, Shay DK, Weintraub E, & al, 2003). During the period of 1976-2007, influenza-associated deaths in the United States ranged from a low of 3,000 to a high of 49,000 people - a rate of 1.4 to 16.7 deaths per 100,000 persons, with 90% of those deaths occurring among adults 65 years of age and older (Thompson, 2010). During influenza outbreaks, attack rates of greater than 20% have been documented in long-term care facilities, even among facilities with high-vaccination coverage (Lindley MC & CB, 2015). Approximately 226,000 hospital admissions are attributed to influenza each year in the United States (McLean HQ, Meece JK, & EA, 2014). In 2007, Molinari et al., published estimates from a probalistic model using the 2003 U.S. population, which estimated that annual influenza epidemics result in: 3.1 million hospitalized days, 31.4 million outpatient visits, direct medical costs of $10.4 billion and projected lost earnings due to illness and loss of life amounting to $16.3 billion annually (Molinari NAM et al., 2007).

Influenza is unpredictable as evidenced by the variability in deaths from year to year and the overall challenge in prediction of determining which influenza vaccine strains to include in

the seasonal vaccine. Its severity varies from one season to the next due to how well the vaccine is matched to circulating influenza virus strains, seasonal mutations (antigenic drifts) of circulating viruses, underlying population immunity from past/similar exposure and from the vaccine, and how many people received the influenza vaccine - herd immunity. Death rates are also affected by the type of influenza virus that predominates in a season. Influenza A affects all age groups and results in moderate to severe illness whereas influenza B affects primarily children and is typically milder in illness severity (CDC2).

The virulence of influenza viruses is determined by the interaction of host factors (e.g., presence of target receptors on host cells, ability of the immune system to control viral replication, level of immunocompetence of host) and viral factors (e.g., ability to bind to host cells, ability of virus shedding, effectiveness of host defense mechanism response) (Kamps et al., 2006). Differences in severity amongst influenza A virus HA and NA subtypes also exist. Research has shown (Thompson, 2010) that during influenza seasons in which influenza A (H3N2) viruses are dominant, death rates are double that of seasons where influenza A (H1N1) or influenza B viruses circulate.

2.3   DETERMINING INFLUENZA VACCINE STRAINS

Influenza strains to be included in each seasonal influenza vaccine for the Northern Hemisphere are selected the February before by the World Health Organization (WHO). In collaboration with other partners and experts from the WHO Collaborating Centers Essential Regulatory Laboratories, their decision for recommending the next season's vaccine virus components is based on review of data from a worldwide network of influenza surveillance laboratories

(CDC4). In March, the U.S. Vaccines and Related Biological Products Advisory Committee, which is a part of the U.S. Food and Drug Administration (FDA), considers the recommendations made by WHO and makes its final decision regarding the composition of the vaccine for the next season for the United States (CDC4).

2.4   TYPES OF INFLUENZA VACCINES

Currently there are seven manufacturers of the yearly influenza vaccine: MedImmune, Sanofi Pasteur, AstraZeneca, Protein Sciences, ID Biomedical Corporation of Quebec, GlaxoSmithKline, and Seqirus (CDC5). Vaccines vary in the type of vaccine delivery offered, such as intramuscular (deep in the muscle), intradermal (into the skin), or intranasal (into the nose), and in the age groups they target (6-35 months, $\geq$ 36 months, 18-64 years, $\geq$ 65 years). Vaccines also vary by the number of influenza strains they include. Influenza vaccines are always composed of strains representing some lineage of influenza A (H3N2), A (H1N1), and B viruses. (CDC1). Vaccines that protect against the three main influenza viruses (H1N1, H3N2 and B) are called trivalent vaccines, those that protect against all four main influenza viruses (H1N1, H3N2 and both B Lineages) are known as quadrivalent. In 2012, the FDA approved the first use of a quadrivalent flu vaccine (MedImmune's FluMist). As of 2015, four vaccine manufacturers now offer quadrivalent influenza vaccine.

Due to antigenic drifts in circulating virus strains, influenza vaccines require changes in their compositions. At times, new influenza vaccine developments also include changes to either the way in which the vaccines are delivered or the amount of antigen included within the vaccine. For instance, the FDA approved the use of Sanofi Pasteur's Fluzone High-dose (HD)

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and Fluzone Intradermal (ID) vaccines in 2009 and 2011 respectively for the general market. Fluzone HD is purported to elicit a higher antibody response as it contains four times the antigen of the standard-dose influenza vaccine. Fluzone ID has been marketed due to its non-inferiority to the standard-dose influenza vaccine and novel new method of delivery which targets immune cells of the skin rather than those found deeper into the muscle using a microneedle the thickness of a penny. Both Fluzone ID and HD are marketed to adults, 18-64 years of age and $\geq$ 65 years, respectively. In 2015 the U.S. FDA licensed use of an adjuvanted seasonal influenza vaccine for the 2016-2017 season; titled Fluad, this vaccine is produced by Seqirus and is marketed to adults $\geq$ 65 years (CDC, 2016b).

Table 1 outlines the influenza strains included in the seasonal vaccine during each year covered by the three research papers.

Table 1. Influenza vaccine strains included over five influenza seasons covered in this dissertation

	A – H1N1	**A – H3N2**	**B**
2011-2012	California/7/2009-pdm09-like virus	Perth /16/2009-like virus	Brisbane/60/2008-like virus
2012-2013	California/7/2009-pdm09-like virus	Victoria/361/2011-like virus	Wisconsin/1/2010-like virus
2013-2014	California/7/2009-pdm09-like virus	Texas/50/2012-like virus	Massachusetts/2/2012-like virus
2014-2015	California/7/2009-pdm09-like virus	Texas/50/2012-like virus	Massachusetts/2/2012-like virus; Brisbane/60/2008-like virus
2015-2016	California/7/2009-pdm09-like virus	Switzerland/9715293/2013-like virus	Phuket/3073/2013-like virus; Brisbane/60/2008-like virus
2016-2017	California/7/2009 pdm09-like virus	Hong Kong/4801/2014-like virus	B/Brisbane/60/2008-like virus; Phuket/3073/2013-like virus

The most common way to manufacture yearly influenza vaccine is by using chicken eggs (CDC18). CDC provides private manufacturers with the seasonal vaccine viruses grown in eggs,

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following FDA regulations and then private manufacturers inject these viruses into their own fertilized chicken eggs. After a few days of allowing these vaccine viruses to replicate in the eggs, the fluid containing the virus is extracted from the eggs. For influenza shots, these influenza vaccine viruses are then inactivated and the antigen from the virus is purified. Purification and testing of the vaccine virus continues at the manufacturer and then finalized virus antigen is placed into vials for use. Each vaccine lot is tested by FDA and must be approved by the FDA before it can be released to the general market.

2.5 VACCINE IMMUNOLOGY: ELICITING RESPONSES OF INNATE AND ADAPTIVE IMMUNITY

The goal of vaccination is to elicit long-lasting immunity to viruses thereby preventing or at least reducing the severity of illness due to disease upon re-infection (Kovaiou et al, 2007). Two aspects of the immune system are engaged upon vaccination: innate immunity, a rapid but short-lasting immune response, and adaptive immunity, a longer-lasting immune response which produces long-term protection. Innate and adaptive responses, though representing two different functions of the immune system, work together to coordinate system-wide response.

The innate immune system elicits a rapid response to presenting pathogens due to its always present cells and proteins that circulate continuously throughout the body ready to quickly mobilize and fight microbes at sites of infection. Innate immune system components include: 1) physical epithelial barriers, 2) phagocytic leukocytes, 3) dendritic cells, 4) natural killer cells (NK) and 5) circulating plasma proteins. (Fisher, 2008). The innate system prevents entry of pathogens into tissues in the body, rapidly destroys pathogens that make it past this first

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line of defense and into the tissues and instructs the adaptive immune response on specific-pathogen humoral and cell-mediated immune responses (Aspinall et al, 2007).

The adaptive immune system is a slower, secondary response that elicits a much more potent immune response to pathogens. There are two different types of adaptive immunity: humoral immunity which involves B-cell lymphocytes, and cell-mediated immunity, which involves T-cell lymphocytes. Adaptive immune system components respond to the presence of pathogens by activating, proliferating, and creating potent mechanisms for neutralizing or eliminating microbes (Fisher, 2008).

Both the innate and adaptive immune systems are involved in vaccine immunogenicity. The innate system recruits antigen specific presenting cells (a type of dendritic cell), into the area of injection which then activates adaptive immune system antigen-specific B and T cell responses (Siegrist in Plotkin, 2013). Antibodies which respond to the site of infection/injection have two main purposes: to neutralize the threat and to recruit other cells and proteins to aid in antigen elimination (Fisher, 2008).

Vaccines mediate protection largely via induction of IgG serum antibodies (Siegrist in Plotkin, 2013). As opposed to other antibody isotypes which have a much shorter half-life (days), IgG antibodies have a half-life up to three weeks and therefore are used to measure vaccination antibody response (Fisher, 2008). Measuring antibody response to the influenza vaccine via serum is typically first measured around ten days post vaccination with the final serum measurement taken between twenty-one to up to thirty-five days post vaccination. Research has shown that IgG antibodies first appear in the bloodstream 10-14 days after priming with vaccination and the vaccine antibodies peak by week 6 (Siegrist in Plotkin, 2013).

2.6   DETERMINING INFLUENZA VACCINE IMMUNOGENICITY

Assay tests are required for characterization of viruses and detection of immunity (CDC, 2013). The hemagglutination inhibition (HAI) assay is the most widely used assay for detection of antibodies to influenza viruses (CDC, 2013) and likewise to influenza vaccine virus strains.

Virus nucleic acids encode surface proteins and agglutinate red blood cells (RBCs). As noted in Section 2.1, the influenza virus has an envelope protein called HA. This HA glycoprotein binds on cells and RBCs, causing the formation of a lattice; this property is known as hemagglutination (Figure 1) (Acharya T, 2014; Racaniello V, 2009). Hemagglutination forms the basis of the rapid HAI assay which is used to determine influenza virus levels present in serum samples. Conducting the HAI assay involves mixing the virus (typically 4 HA antigen units) with diluted serum containing antibodies (diluted with a buffer) and then adding in RBCs (Acharya T, 2014). RBCs either attach to the virus forming a lattice (Figure 1, line B), do not attach (Figure 1, line C), or settle irregularly or coat the tube/microtiter well; unattached RBCs sink to the bottom of the tube or microtiter well forming a compact button (Acharya T, 2014; Racaniello V, 2009).
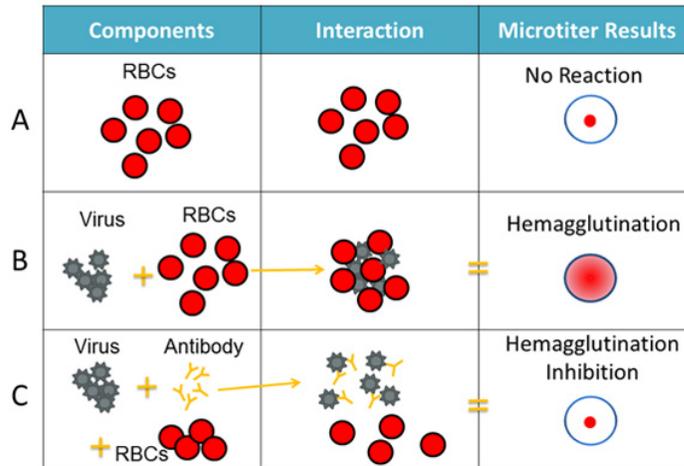
Figure 1. Hemagglutination and HI
(CDC 2013)

The underpinning of the HAI assay is that antibodies to influenza virus will prevent attachment of the virus to RBCs and thereby HAI results when antibodies are present (i.e., the lattice is not formed) (Figure 5, line C). Measuring HAI titers involves successive dilutions to the serum containing antibodies which have been mixed with the virus (to which the RBCs are then added) in order to find the minimal quantity of antibodies needed to produce a hemagglutination inhibition effect (McMullin, 1984). If there are no serum antibodies that react with the tested influenza viruses then hemagglutination will be evident in all microtiter wells; if antibodies do react to the viruses, then hemagglutination will not be observable until there is sufficient level of dilution of the antibodies (Racaniello V, 2009). The highest dilution of serum that prevents hemagglutination is called the HAI titer of the serum (Racaniello V, 2009). Serum is usually diluted 1:10 to begin the HAI assay because 10 is the lowest detectable positive titer which provides hemagglutination inhibition (Nauta et al, 2009).

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Use of HAI titers in determining correlates of protection against influenza virus was first shown in an efficacy study conducted in 1943 which demonstrated that protection in individuals was associated with higher HAI titers (Ohmit et al, 2011). The FDA uses three related serological values based upon HAI titers to quantify influenza vaccine immunogenicity: seroprotection, seroconversion and geometric mean titers (GMTs) (Nauta et al, 2009; Ohmit et al, 2011). These three serological outcomes are run separately for each vaccine strain (and/or virus) in determining levels of protection (e.g., A/H1N1, A/H3N2, B).

Seroprotection is a surrogate marker for clinical protection from influenza and is defined as an HAI titer ≥ 40 at pre-vaccination or at post-vaccination. This cut-off value has been shown to be the level at which antibodies are high enough to provide at least a 50% population level of protection if exposed to influenza (Racaniello V, 2009) (Nauta et al, 2009; Ohmit et al 2011). The interpretation of Seroprotection post-vaccination of an HAI titer ≥ 40, is that one has a population level of at least 50% protection from influenza and this is regardless of the level of HAI titer they started with pre-vaccination.

Seroconversion is a marker of the overall increased level of protection from pre- to post-vaccination. This can be measured in two ways, either as the pre-vaccination titer of less than the ratio used for the dilution (e.g., 1:10 dilution would therefore be noted as a HAI titer <10), and a post-vaccination HAI titer of ≥ 40 or, a pre-vaccination HAI titer of ≥ 10 and a four-fold rise in strain-specific HAI antibody titer between pre- to post vaccination sera (Ohmit, 2011; Ross, 2014). Seroconversion can then be interpreted as increasing from a vulnerable level (low HAI titer) at pre-vaccination to at least a 50% protected population level post-vaccination or starting at a not so vulnerable level (e.g., possible to have a pre-vaccination HAI titer of ≥ 40) and see a four-fold increase HAI titer post-vaccination.

As HAI titers tend to be non-normally distributed, it is convention to log transform them (Nauta et al, 2009). GMTs report the anti-log of the means of the log2 HAI titers and GMT ratios are the ratio of the GMT post-vaccination to that of pre-vaccination log2 titers. Overall, the higher the HAI titer, the higher the level of protection provided. The level of protection elicited by antibodies can vary with HAI titers showing higher levels of protection at times for only one or two but not necessarily all vaccine strains.

3.0   IMMUNOSENESCENCE

Immunosenescence refers to the process in which age-related dysregulation and dysfunction alters immune system response (Aw D, Silva AB, & DB, 2007). These changes affect both innate and adaptive immunity response (Ongradi J & V, 2010) resulting in a weakened ability in older adults to mount a strong and effective immune response (Gruver A, Hudson LL, & GD, 2007). As will be discussed within this section and further in Sections 4 and 5, with aging comes an aging immune system, higher levels of chronic inflammation and increased risk for frailty. All of these factors negatively impact the ability for the immune system to mount a robust response to influenza vaccine antigens.

3.1   IMMUNOSENESCENT CHANGES IN INNATE AND ADAPTIVE IMMUNITY

Natural kill cells (NK), macrophages, and neutrophils, all key components of the innate immune system which are crucial in activating the first response to sites of infection/injection, show reduced function in aging. Neutrophils, the first inflammatory cells engaged in response to inflammation or infection, (Castle, 2000), show reduced efficacy as one ages in both attracting other inflammatory cells to the site and reduced ability to devour harmful substances once they arrive (Ongradi J & V, 2010). Reduced NK cell efficiency impacts the interplay between the innate and adaptive immune systems thereby leading to a less robust adaptive immune response

(Ongradi J & V, 2010). The loss in ability of releasing key inflammatory cytokines by aged macrophage also results in a decreased immune system response (Ongradi J & V, 2010). Together, these age-related changes seen in responses within the innate immune system weaken its signaling and instructive power to the adaptive immune system which in turn impacts the ability of the adaptive immune system to mount as robust of a response as that evident in younger persons.

Significant age-related humoral adaptive immunity changes also take place. Decreases in interleukin-2 (IL-2) production (a cytokine signaling cell which activates certain white blood cells to respond to disease/infection) due to aging T-cells and the resultant impairment of T-B cell interaction has been suggested to significantly contribute to impaired humoral adaptive immune response in aged individuals (Gruver A et al., 2007). Losses of naïve B-cells being recruited to secondary lymphoid organs reduces antigen-specific B-cells which in-turn decreases the ability of the immune system to respond to novel antigens as one ages (Gruver A et al., 2007; Ongradi J & V, 2010). It has been noted that a hallmark of immunosenescence is this loss of naïve B cells (Ongradi J & V, 2010).

The cell-mediated immune system also undergoes numerous changes with aging. A key driver in reduced cell-mediated adaptive immune response due to immunosenescence is the reduction in the pool of T-cells exported from the thymus gland (Aspinall R, Del Giudice G, Effros RB, Grubeck-Loebenstein B, & S, 2007; Gruver A et al., 2007). "Production of broadly reactive T cells by the thymus and maintenance of a diverse peripheral T-cell repertoire are critical to the robustness of the human immune system" (Gruver A et al., 2007). Other cell-mediated immunosenescence changes include large numbers of T-memory cells showing characteristics of replicative senescence (Aspinall R et al., 2007; Castle, 2000).

3.2 OTHER AGE-RELATED SENESCENT CHANGES AFFECTING IMMUNITY IN OLDER ADULTS

With increased age comes increased potential of exposure to infections; some of these infections will be latent. Past and latent infections affect the aging immune system response. Prior infections shape the range of immune system response by changing the way the immune system responds to partially related or completely unrelated antigens later on (Goronzy JJ & CM, 2013); immune system changes resulting from past infections can be either helpful or harmful. Latent infections such as cytomegalovirus (CMV) seems to both hasten aging of the immune system and dampen responses to vaccines including influenza vaccine (Aspinall R et al., 2007; Goronzy JJ & CM, 2013; Kovaiou RD, Herndler-Brandstetter D, & B, 2007).

3.3 INFLAMMAGING: INCREASED CHRONIC LEVELS OF INFLAMMATION EVIDENT IN OLDER ADULTS

Inflammaging is the presence of low-grade systemic chronic inflammation seen in older persons. Inflammaging is caused by an imbalance between pro- and anti-inflammatory networks, resulting in higher circulating levels of pro-inflammatory cytokines (Franceshi C et al., 2007; Goronzy JJ & CM, 2013). Factors that contribute to inflammaging include increased adipose tissue, decreased estrogen and testosterone, subclinical infections, chronic diseases such as cardiovascular disease, and smoking (Krabbe KS, Pedersen M, & H, 2004).

Pro- and anti-inflammatory responses are important as they drive both innate and adaptive immune system response to pathogens when overcoming infections. However, when

this balance shifts to increased circulating levels of pro- and reduced levels of anti-inflammatory cytokine markers it becomes detrimental. This cytokine imbalance can result in chronic levels of circulating pro-inflammatory cells which in turn negatively impacts both innate and adaptive immune response (Franceshi C et al., 2007). Increased serum levels of pro-inflammatory cytokines interleuikin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), have been observed in the elderly (Aspinall R et al., 2007). Higher circulating levels of IL-6 and TNF-α in older adults are linked to chronic diseases (Singh T & AB, 2011) and are associated with increased risk for frailty (see Section 4.5).

3.4   CHALLENGES TO INFLUENZA VACCINE IMMUNE RESPONSE

Immunosenescence makes mounting a robust immune response to antigens present in vaccination challenging for older adults. Studies have shown that in adaptive immunity both humoral and cell-mediated influenza-specific immune system responses are lower in older adults as compared to younger adults following influenza vaccination (Gruver A et al., 2007).

Prior studies suggest that cross-reactive cell-mediated T-cell response is vital for protection against influenza in older adults (McElhaney JE, 2011; Murasko DM et al., 2002). However, in older adults, antibody response to influenza vaccine has been shown to be associated with altered T-cell function and an overall decline in cell-mediated adaptive immunity response (McElhaney JE, 2011). Reduced immune system function correlates to decreased levels of seroprotection and seroconversion in older adults post influenza vaccination.

A 4-year longitudinal study (years 1993-1996) assessed influenza vaccine response among healthy elderly subjects (range 78.8-80.6 years of age) living independently in continuing

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care retirement communities and young adults (range 22-39 years of age) recruited from a University campus (Murasko DM et al., 2002). Each year the change in pre- to post-vaccination seroprotection levels was significant for both age groups. The percent range across the four years for elderly subjects who were seroprotected post-vaccination was 52.5-83.3% for H1N1, 42.7-83.4% for H3N2 and 52.8-82.0% for B; ranges vary due to different vaccine strains across the four years. For the younger subjects this percent range of individuals who were seroprotected post-vaccination was 97.3-100% for H1N1, 64.0-96.1% for H3N2 and 90.0-96.6% for B. These changes in percentage of individuals who had levels high enough to be considered seroprotected pre- vs. post- vaccination for both age groups each year was significant at $P \leq$ 0.003 (Fisher's Exact test value not reported). However, these responses were significantly and consistently lower for the elderly compared to the young adults (Fisher's Exact between groups test P <0.0001-0.031 across vaccine strains, test value not reported) with these significant differences showing for H1N1 in years 2-4 and for H3N2 and B strains for years 2 and 4. The percent of individuals who had a four-fold seroconversion response each year was higher for younger persons than the elderly. Percent range of seroconversion rates across the four years among the elderly was 8.0-14.6% for H1N1, 23.5-43.3% for H3N3 and 11.7-31.1% for B. For younger adults these seroconversion percent ranges were 37.7-58.6% for H1N1, 40.0-64.5% for H3N2 and 56.0-70.6% for B. Differences between the young vs. the elderly in percent seroconversion response was significant for all four years for H1N1 and B and for H3N2 in year four (Fisher's Exact P=0.0001, test value not reported) and was significant at P=0.02 (Fisher's Exact test value not reported) for year 3 for H3N2. During this four-year study, the H1N1 strain stayed the same each year, the H3N2 saw new strains yearly and there was a new B strain in the third year. Of note, when a new vaccine strain component was added, the percent of individuals

who seroconverted was half that for the elderly compared to younger adults (30% vs. 60%) and being vaccinated yearly with the same vaccine strain resulted in a lower percent of elderly who seroconverted as compared to younger adults (10% vs. 30%).

A quantitative review of 31 studies conducted from 1986-2002 compared influenza vaccine antibody response in the elderly (58-114 years of age with mean age 68-86 years) vs. younger adults (17-59 years of age) (Goodwin K, Viboud C, & L, 2006). A weighted analysis of the probability of vaccine response, measured as seroprotection and seroconversion, was done for each vaccine strain (H1N1, H3N2, B) and regressions were run separately for each vaccine strain. Unadjusted Odds Ratios (OR) and 95% Confidence Intervals (CI) from this study for post-vaccination seroprotection levels of the elderly compared to younger adults (referent group) were: 0.47(0.40-0.55) for H1N1, 0.53 (0.45-0.63) for H3N2 and 0.58 (0.50-0.67) for B strains. Unadjusted ORs (95% CI) for seroconversion comparing the elderly to younger adults were: 0.48 (0.41-0.55) for H1N1, 0.63 (0.55-0.73) for H3N2 and 0.38 (0.33-0.44) for B strains. Comparisons of the ORs between each age group by each vaccine strain was significant at P <0.001 for the seroconversion and seroprotection regression models. The authors noted that in the adjusted multivariable regression models (accounting for living situation, health status, prior vaccination, vaccine type and dosage etc.) younger adults showed a 3-4 fold more protective response than the elderly for seroprotection post-vaccination and a 2-4 fold higher response than the elderly for seroconversion across the vaccine strains (Goodwin K et al., 2006).

3.5 STRATEGIES TO IMPROVE OLDER ADULTS' IMMUNE SYSTEM RESPONSE TO VACCINATION

Therapeutic strategies have been proposed to improve immune system response to vaccination in older adults. These include: modulation of IL-7, genetic or pharmacologic approaches to limit shortening of telomeres, physical removal of senescent cells, and more effective vaccine adjuvant types (Aspinall R et al., 2007). Adjuvants are substances added to vaccines to enhance the robustness of the vaccine antigen and therefore overall immune response. Adjuvants can act upon cellular response to either attract certain cells types to the site of injection, help cells with vaccine antigen Processing, or trap vaccine antigens to allow for a slower release and thus longer stimulation of the immune system (InvivoGen, 2011). One example of a common vaccine adjuvant that works to elicit a slower release of antigens and increased stimulation of the immune system is that of aluminum salt (Garçon N, Leroux-Roels G, & W-F, 2011). In the United States, currently aluminum salt is only included in the stockpile for one pandemic influenza vaccine (H5N1) (CDC, 2015c). In 2015, the FDA licensed use of an adjuvanted vaccine for use in the 2016-17 influenza season for those 65 years and older. This vaccine, Fluad™, contains MF59 adjuvant which is an oil-in-water emulsion of squalene oil (CDC, 2016a). Fluad™ was first marketed in Italy in 1997 and is currently licensed in 38 countries (CDC, 2016c).

As vaccination is currently the most effective way to protect against influenza and influenza-related morbidity and death, vaccine manufacturers have worked to increase the efficacy of influenza vaccines for older adults. As noted previously in Section 2, the FDA approved Sanofi Pasteur's Fluzone HD influenza vaccine for use in adults $\geq$ 65 years. Vaccine antigens present in HD influenza vaccine are the same as those in standard-dose inactivated

influenza vaccine (SD) but it contains four-times the amount of hemagglutinin (HA) (60 μg per strain vs. the standard 15 μg per strain) (DiazGranados CA et al., 2014).

Studies have shown that HD influenza vaccine is safe, well tolerated, and provides significantly higher immune system response as evidenced by serologic measures and better overall protection against laboratory-confirmed influenza than does SD influenza vaccine among adults ≥ 65 years (though studies do not specify if adults were community-dwelling, all enrolled subjects were medically stable and ambulatory) (DiazGranados CA et al., 2013; DiazGranados CA et al., 2014; Tsang P et al., 2014). However, though HD influenza vaccine provides better antibody response and reduced influenza in older adults as compared to the SD vaccine, there have been contradictory results on whether HD influenza vaccine prevents more influenza-related hospitalizations than SD vaccine. One retrospective cohort study which looked at community-dwelling veterans receiving primary care at VA medical centers found that HD provided no greater protection against influenza-related hospitalizations than did SD except among those who were 85 years and older (Richardson DM, Medvedeva EL, Roberts CB, Linkin DR, & Program, 2015). Another retrospective cohort study which reviewed Medicare data from adults across the United States found that HD influenza vaccine was significantly more effective than SD in both prevention of influenza-related medical visits and hospitalizations (Izurieta HS et al., 2015).

4.0   FRAILTY

Frailty is a multi-dimensional syndrome which is marked by losses in function and physiologic reserve (e.g., reduced walking speed due to losses of lung and/or cardiac function, and/or musculoskeletal mass) and increased vulnerability to morbidity and death (Espinoza S & JD, 2005).   Frail individuals are at increased risk of experiencing acute illness, falls, disability, hospitalization, institutionalization and mortality (Espinoza S & JD, 2005; Fried LP et al., 2001). The impact of frailty on increasing risk of morbidity and mortality has led to the development of screening tools that can be used in clinical and research settings for assessing frailty status (Morley JE et al., 2013). Though key definitional aspects of frailty have emerged over the years, there is still much dispute over the definition of frailty and the data to be used in assessing frailty in older adults (Chen X, Mao G, & SX, 2014; Gobbens RJJ, Luijkx KG, Wijnen-Sponselee MTh, & JMGA, 2009; Morley JE et al., 2013; Rodriguez-Manas L et al., 2013; Sternberg SA, Wershof Schwartz A, Karunananthan S, Bergman H, & A, 2011).

4.1   CAUSES OF FRAILTY

Multiple domains have been identified as contributing to the cycle of frailty. Physiological systems affecting frailty include inflammatory, musculoskeletal, endocrine and hematological (Walston J, 2004). Additionally, the central nervous system (Morley JE et al., 2013), genetic

makeup, environmental factors, and damage to molecular and cellular systems have been implicated (Clegg A, Young J, Iliffe S, Rikkert MO, & K, 2013). The full causes and underlying mechanisms of these systems which lead to frailty are still not fully understood (Ahmed N, Mandel R, & MJ, 2007; de Vries NM et al., 2011). Though multiple "markers" within these varying systems and the interactions among them contribute to frailty due to disruptions to the has been identified (Walston J, 2004). The columns listed in Table 2 propose the risk factors, domains and mechanisms involved in frailty.

Table 2. Pathogenesis of the frailty phenotype

Etiology/risk factors	Potential Mechanisms		Consequence of loss of Physiologic reserve	Health Outcomes
	Chronic inflammation	Intermediary systems		
Aging	Cytokines	Musculoskeletal	Weakness	Falls
Genetics	Immune cells	Endocrine	Weight loss	Disability
Lifestyle	Immune/inflammatory	Cardiovascular	Exhaustion	Dependency
Diseases	Pathway activation (e.g., IL-6, CMV and other latent infections)	Hematologic	Low activity	Death
Environments			Slowed Performance	

Adapted from Chen X, Mao G et al. (2014)

4.2   IMPACT OF IMMUNOSENESCENCE AND INFLAMMAGING ON FRAILTY

In the pathogenesis of frailty, dysregulation at the cellular level takes place in both the innate and adaptive immune systems (Li H, Manwani B, & SX, 2011). Within the innate system, significant changes in T-cell's have been observed that are greater than changes related to senescence alone (Yao X, Li H, & SX, 2011). Regarding the adaptive immune system, B-cell

repertoire diversity has been shown to be reduced especially for frail older persons; this reduction of B-cells impairs the antibody response (Goronzy JJ & CM, 2013).

As shown in Section 3, chronic inflammation has a key role in the senescent immune system. Research has shown that the imbalanced proportion of pro- to anti-inflammatory cytokines can be an indicator of frailty and mortality in older individuals (Aw D et al., 2007). The shift in older persons toward a more pro-inflammatory state is associated with a loss of muscle mass as well as increased incidence of disability and mortality (Chung HY et al., 2009). One pro-inflammatory maker in particular that has been implicated in frailty is IL-6.

High serum levels of IL-6 are associated with reduced muscle strength and power and slowed walking speed (Li H et al., 2011). IL-6 is a marker for increased risk of frailty and elevated circulating levels of IL-6 are independently associated with frailty among both elderly women (Franceshi C et al., 2007; Li H et al., 2011) and elderly men (Lai HY, Chang HT, Lee YL, & SJ, 2014). Higher levels of IL-6 in frail patients remained even after removing individuals with cardiovascular disease and diabetes, two disease states which are noted for increased inflammation (Ahmed N et al., 2007; Walston J, 2004). The pro-inflammatory maker TNF-α has also been linked to overall aging and sarcopenia (degenerative loss of skeletal muscle mass and strength associated with aging) (Ahmed N et al., 2007).

4.3   HISTORY OF USE AND DEFINITION

The term frailty first appeared in the biomedical gerontological literature in 1977, however, it wasn't until the early 2000's that the term truly gained prominence in the literature (Gilleard C & P, 2010). Previously denoted as infirmity, the denotation of frailty has now come to be seen as

distinct from that of old age, disability and co-morbidity although there is certainly overlap between these categories (Fried LP, Ferrucci L, Darer J, Williamson JD, & G, 2004; Gilleard C & P, 2010; Theou O, Rockwood MRH, Mitniski A, & K, 2012).

Despite the lack of a broader definitional consensus of frailty, during the 2012 "Frailty Consensus Conference" an operational definition on physical frailty was developed (Morley JE et al., 2013). Physical frailty is defined as:

> a medical syndrome with multiple causes and contributors that is characterized by diminished strength, endurance, and reduced physiologic function that increases an individual's vulnerability for developing increased dependency and/or death (Morley JE et al., 2013).

Measurements of physical frailty include use of questionnaires and measures of grip strength and gait speed (Fried LP et al., 2001).

4.4   TYPES OF FRAILTY MEASURES AND THE TWO PRIMARY FRAILTY VIEWPOINTS (FRIED VS. ROCKWOOD)

Many types of frailty instruments have been developed in recent years and, due to differences in how frailty is defined and therefore assessed, it can be challenging to compare the measured outcomes in various frailty studies with each other (de Vries NM et al., 2011). Frailty models which are considered to be well-validated include the: Cardiovascular Health Study (otherwise known as the Physical Frailty/Frailty Phenotype model), Study of Osteoporotic Fractures, Deficit Model (otherwise known as the Frailty Index model), FRAIL – International Academy of Nutrition and Aging, SHARE-FI, Vulnerable Elder Survey, Tillburg Frailty Index, and Groningen Frailty Indicator (Morley JE et al., 2013). Within the field of frailty research two key

investigators have emerged, Drs. Linda Fried and Kenneth Rockwood; each with a distinct definition and method for assessing frailty.

The Frailty Phenotype (FP) by Fried et al. group's (Fried LP et al., 2001) is based upon the physical definition of frailty and assesses five domains: strength, mobility, physical activity, energy and nutritional status. Each domain uses just one item for its assessment with one point possible per domain, each with specified cutoffs provided, the sum of any three or more items denotes frailty. The five FP items used are: grip strength through use of a hand dynamometer; walk time through a one-trial fifteen feet usual pace test; physical activity using the shortened form of the Minnesota Leisure Time Activity questionnaire; exhaustion through two questions from the CES-D Depression Scale; and weight loss through self-report of loss of weight over the past year and whether or not this was an intentional loss (Fried LP et al., 2001). The FP, which looks at frailty as a medical syndrome, is noted to be strong for its ease of clinical reproducibility and has been shown to be generalizable and have both criterion validity and strong internal validity (Bandeen-Roche K1 et al., 2006).

The Frailty Index (FI) by Rockwood et al.'s group, takes a multi-dimensional view of systems involved in frailty and is based on the comprehensive geriatric assessment (CGA) instrument used in clinical care. It looks at ten domains: cognitive status; mood; motivation; communication; mobility, balance; bowel function; bladder function; IADLs and ADLs; nutrition and social resources, and also, considers the number of co-morbidities present (Jones DM, Song X, & K, 2004). The FI assesses a list of 30-70 proposed frailty items and counts the total number of items for which an individual has noted deficits (Song X, A, & K, 2010). The FI is notable for its consistency in identifying frail older adults at risk of adverse health outcomes and its reproducibility of these findings across studies using the FI, even if FI deficits used

within these studies differ (30-70 potential frailty items) ((Searle SD, Mitnitski A, Gahbauer AE, Gill TM, & K, 2008).

Studies that have compared the FP and FI have noted that each instrument identifies the appropriate persons who are at risk of developing an adverse outcome, however they capture different sub-populations (de Vries NM et al., 2011). Rockwood (2007) compared his FI to Fried et al.'s FP among 2,305 adults $\geq$ 70 years and found that they correlated moderately well with each other (R=0.65) but concluded that while the FP discriminates broad levels of current risk, the FI allows future risk of adverse events to be more precisely defined than the FP, due to the greater number of deficits considered in his model (Rockwood K, Andew M, & A, 2007).

Though both the FI and FP are validated frailty measurements, due to the ease of the simplicity of a five-item measure and therefore its accessibility for research and clinical operationalization, use of the FP and various modifications to it have been more prevalent in the literature (Theou O et al., 2012). As noted in Section 7, research conducted for paper one uses the physical frailty definition and modifications to instruments used in assessing the five-item frailty criterion based upon the original FP model.

4.5   PREVALENCE OF FRAILTY

The prevalence of frailty among older adults varies based upon the definition and criterion of frailty that is used (Collard RM, Boter H, Schoevers RA, & RC, 2012). Using the definition of physical frailty, frailty is estimated to be present in 7-12% of community-dwelling adults $\geq$ 65 years and 25% in community-dwelling adults $\geq$ 85 years of age (Chen X et al., 2014). Frailty

estimates increase with age with greater than 32% frailty noted for persons aged 90 years and older (Ahmed N et al., 2007).

A recent systematic review with a meta-analysis of 21 studies of frailty conducted among community-dwelling older adults ≥ 65 years of age found an enormous range in prevalence estimates. Among these studies prevalence of frailty ranged from 4.0-59.1% with an overall pooled frailty prevalence of 10.7% (95% CI: 10.5-10.9%; 61,500 participants) and a pre-frailty (a state in between non-frail and frail) pooled estimate of 41.6% (95% CI: 41.2-42.0%; 15 studies; 53,727 participants) among persons 65 years and older (Collard RM et al., 2012). Collard et al., (2012) noted that within this range, prevalence differences existed based upon which definition and/or measures of frailty were used. Studies that utilized the physical frailty definition cited prevalence ranges of 4.0-17.0%; pooled prevalence for these studies was 9.9% (95% CI: 9.6-10.2, 15 studies; 44, 894 participants). Within studies utilizing a broader definition of frailty which included social and psychosocial components (e.g, mood, cognition, incontinence), frailty estimates ranged from 4.2-59.1% with a pooled frailty prevalence of 13.6% (95% CI: 13.2-14.0%, 8 studies; 24,027 participants) (Collard RM et al., 2012).

4.6   SEX DIFFERENCES NOTED IN FRAILTY ESTIMATES

There is a higher prevalence of frailty in women than men. Using the physical frailty definition, frailty estimates for community-dwelling adults in the United States have been estimated to be 8% for women vs. 5% for men (age not specified) (Chen X et al., 2014). In Spain, using the same physical frailty definition, the prevalence of frailty in community-dwelling adults aged ≥ 75 years was 30.9% in women vs. 9.3% in men (Fernandez-Bolanos M et al., 2008). The

authors of this study note that this marked difference in frailty estimates between the sexes may be due in part to the known sex roles still present in this age group (e.g., women as homemakers and less mobile than men which correlates to higher deficit levels on two of the five FP items). Using a frailty index scale of thirty-six variables on community-dwelling adults $\geq$ 65 years in Canada, prevalence of frailty was 25.3% (95% CI: 23.2-27.5%) for women and 18.6% (95% CI: 15.9-21.3%) for men (Song X et al., 2010). In Collard et al.'s (2012) systematic review of 21 studies of frailty, women had higher prevalence of frailty than men (9.6% vs. 5.2%; $\chi^2 = 298.9$, df=1, P <.001) (Collard RM et al., 2012). Though the populations within these studies differ, they all show higher frailty prevalence for women than men.

Sex differences also exist for frailty-related mortality though there are inconsistencies. Puts et al., (2005) used nine frailty markers (weight, peak expiratory flow, cognition, vision/hearing problems, incontinence, sense of mastery, depression and physical activity) in a study conducted in the Netherlands among community-dwelling older adults where the mean age of participants was 72.4 (SD: 8.5) years for women and 72.6 (SD: 8.6) years for men (Puts MTE, Lips P, & DJH, 2005). This study compared relative risks (RR) of dynamic frailty (difference in frailty change between two visits) and static frailty (second visit frailty measurement) on mortality. They found a higher prevalence of static frailty in women as compared to men (18% vs. 14%, P <0.01); dynamic frailty was similar between the sexes (18% vs. 17% for women vs. men, respectively). After adjusting for age and education static frailty was significantly associated with death for both women and men (RR women: 2.6; 95% CI: 1.8-3.8, P <.001 and RR men: 2.3; 95% CI: 1.7-3.2, P <.001) but that dynamic frailty was only significantly associated with mortality in women (RR women: 2.5; 95% CI: 1.77-3.74, P <.001 and RR men: 1.3; 95% CI: 0.99-1.82, P .06) (Puts et al, 2005).

Berges et al. (2009) found that among community-dwelling Mexican Americans ≥ 65 years of age (mean age 74.5 (SD: 6.06) years)), 59% who were female, physical frailty estimates were similar and not statistically different between the sexes across frailty categories for women as compared to men (44.6% vs 45.3% for non-frail, 48.3% vs. 46.0% for Pre-frail, and 7.1% vs. 8.7% for frail). However, in contrast to Puts et al., results from Berges et al. shows that associated risk of ten-year mortality was stronger for frail men than for frail women (HR men: 3.04; 95% CI: 2.16-4.28 and HR women: 1.92; 95% CI: 1.39-2.65) after adjusting for age, marital status, education, BMI, health behaviors and medical conditions (Berges IM, Graham JE, Ostir GV, Markides KS, & KJ, 2009).

4.7   FRAILTY, DISABILITY AND CO-MORBIDITIES

Frailty is related to but distinct from both disability and age-related co-morbidity (Ahmed N et al., 2007; Fried LP et al., 2004; Theou O et al., 2012). Though frailty can lead to disability and disabled persons can become frail, Fried et al. in their Cardiovascular Health Study, which utilized the physical frailty definition, noted that among 2,762 community-dwelling adults ≥ 65 years who had a comorbidity and/or disability and/or frailty, distinctions existed between these categories (Fried LP et al., 2004; Fried LP et al., 2001).

To note, of these 2,762 older adults, 5.7% were frail and disabled with no comorbidities, 46.2% were frail and had at least two comorbidities but were not disabled, 26.6% were noted as frail alone, and 21.5% were frail, disabled and had comorbid conditions. In the second wave of the Canadian Study of Health and Aging, comprised of 2,305 community-dwelling adults ≥ 65 years, Theou et al. (2012) also using the physical frailty definition reported different percentages

for each category than did Fried et al. (2004). Results from this study showed that 20.4% were frail and disabled with no comorbidities, 12.5% were frail and had ≥ 2 comorbidities but were not disabled, 3.6% were frail alone, and 63.5% were frail, disabled and had comorbidities (Theou O et al., 2012). Both studies assessed disability through use of the basic activities of daily living (ADLs) and instrumental activities of daily living (IADLs); comorbidity was defined as the presence of two or more chronic diseases. Why the distinction between frailty, disability and comorbidity? According to Fried et al. (2004) the reason to distinguish between these three concepts (and also understand when they overlap) is that each condition brings both a different prognosis and has different care-related needs.

As noted in Section 3.4, among the comorbidities that have been noted to decrease immune system response to influenza vaccine, the comorbidity of obesity has also been linked to frailty (Ahmed N et al., 2007; Blaum CS, Xue QL, Michelon E, Semba RD, & LP, 2005; Morley JE et al., 2013). The Women's Health and Aging Studies which included 599 community-dwelling women aged 70-79 years of age used the physical frailty definition and assessed the association of obesity on frailty status; obesity was defined as ≥ 30 kg/m^2 according to WHO criterion. Multivariable regression model results from this study showed that after adjusting for covariates such as age, race, education status, and other comorbidities, obesity was shown to be significantly associated with pre-frailty (in between non-frail and frail states) (OR: 2.23; 95% CI: 1.29-3.84) and frailty (OR: 3.53; 95% CI: 1.34-9.13) (Blaum CS et al., 2005).

4.8   ADDITIONAL FACTORS ASSOCIATED WITH FRAILTY: FINDINGS FROM AGING AND FRAILTY COHORTS

Kanapuru et al. (2009) described eleven large cohort studies which assessed aging and frailty in adults ≥ 65 years; total subjects enrolled in these studies ranged from 574 to 40,657 older adults. Results gleaned from these studies confirm the association of higher levels of frailty noted among women and the association of IL-6 and obesity on frailty (Kanapuru B & WB, 2009). Additional factors associated with frailty from these cohorts include: age, gender, race effects with frailty more common among African-Americans and factors such as smoking, depression and self-perceived poor or fair physical health (Kanapuru B & WB, 2009).

Espinoza and Fried (2007) delineated several reasons for these associations. Increased age may be tied to frailty through overall disruptions that occur with an aging immune system and decreased cellular functioning. Female gender may be related to sarcopenia (loss of muscle mass and strength) as women were found to have less muscle mass than age-matched men and this may confer an intrinsic risk for frailty. Race/ethnic differences may be related to differences in health status and other risk factors (such as lower SES, smoking) that may in part explain increased risk of frailty for non-white individuals. Depression has been shown to have bi-directional relationships with frailty, with each increasing the risk of the other. It is proposed that depression leads to frailty through intermediary processes of lost weight, decreased physical activity, loss of strength and overall decreased physiologic reserve. Obesity is thought to directly contribute to altered glucose metabolism and insulin insensitivity as well as activation of inflammation – all of which are physiologic alterations associated with the development of sarcopenia and frailty.

4.9   INFLUENZA VACCINATION RESPONSE AMONG FRAIL PERSONS

Few studies of influenza vaccine immunogenicity among frail older adults exist. We found only three studies which specifically measured the impact of frailty on immunogenicity to influenza vaccine using frailty measures. Only one of these studies compared differences between HD versus SD influenza vaccine. The two studies which utilized SD influenza vaccine had disparate results.

The first study (N=71) using the physical frailty definition and Fried et al. (2001) frailty phenotype measurements, demonstrated that frailty is associated with lessened immunogenicity to influenza vaccine and greater influenza-like illness among community-dwelling adults > 70 years of age (Yao X, Hamilton RG, et al., 2011). Using $\log_{10}$ transformed titers this study noted that all subjects had higher post- to pre-vaccination GMTs, however, whether or not this was a significant difference for the vaccine strains, varied by frailty status. Non-frail subjects had significantly higher post- than pre- vaccination GMTs in all three vaccine strains (H1N1: post 387±2.0 *vs.* pre 201±2.0, $P < .001$; H3N2: post 497±1.9 *vs.* pre 309±1.6, $P < .001$; and B: post 105±1.5 *vs.* pre 88±1.4, $P = .01$, respectively). Pre-frail subjects had only significantly higher post-vaccination GMTs to H1N1 and H3N2 (post 282±2.3 *vs.* pre 157±2.2, $P = .01$ and post 388±2.4 *vs.* pre 278±2.1, $P = .01$, respectively; B: post 81±1.3 *vs.* pre 78±1.6, $P = .23$). There was no significance post- to pre-vaccination GMT difference for any vaccine strain for frail subjects (H1N1: 201±2.1 *vs.* 149±1.9, $P = .43$; H3N2: 307±2.3 *vs.* 255±2.0, $P = .17$; and B: 67±2.1 *vs.* 65±2.0, $P = .33$, respectively). Overall, post-vaccination seroprotection levels were high for all three stains (94%, 92%, and 82% to H1N1, H3N2 and B strain, respectively) and were not significantly different between frailty groups.

Seroconversion (four-fold rise pre- to post vaccination) results for this study were low for all three vaccine strains and differed by frailty status. Percentage rates of seroconverting to vaccine strains for non-frail participants were 13%, 27% and 5% for H1N1, H3N2 and B vaccine strains respectively. Percentage rates of seroconverting to vaccine strains for pre-frail participants was 6% for both the H1N1 and H3N2 strain; no pre-frail participants seroconverted to the B strain. Percentage rates of seroconverting for frail participants was 6% for the H3N2 vaccine strain; no frail participants seroconverted to the H1N1 or B strains. There was a significant difference in rate of H3N2 seroconversion between non-frail and pre-frail subjects, (non-frail: 27% *vs.* pre-frail: 6%, *P*= .05, Fisher exact test value not reported). Using Jonckheere-Terpstra tests, Yao et al. (2011) also assessed the statistical significance of stepwise increase/decrease trends in GMT ratios (post GMT/pre GMT) across the three frailty groups; liner regression for a stepwise trend in decrease of GMT ratios by frailty status was used to obtain *P* values. Adjusting for age, these decreases from the non-frail and pre-frail to frail subjects in GMT ratios were: H1N1: 1.6, 1.3, 1.1, respectively, *P* = .04; H3N2: 1.9, 1.6, 1.1, respectively, *P* = .01; and B: 1.5, 1.3, 1.1, respectively, *P* = .05.

The second study (N=117) using the physical frailty definition and Fried et al. (2001) frailty phenotype measurements assessed frailty and preexisting immunity upon influenza vaccine immunogenicity in veterans ≥ 60 years of age (Van Epps P et al., 2017). The authors found that though post-vaccination seroprotection percentage rates were higher than pre-vaccination, there was no significant differences in either pre-vaccination or post-vaccination seroprotection percentage rates by frailty status for any vaccine strain (H1N1, H3N2, B). Additionally, $\log_2$ titer GMT ratios (post GMT/pre GMT) mean fold increases were similar across the frailty groups for each vaccine strain (H1N1: non-frail 2.9-fold, pre-frail 2.3-fold, frail

3.-fold; H3N2: non-frail 3.3-fold, pre-frail 5.9-fold, frail 6.4-fold; B: non-frail 3.0-fold, pre-frail 2.3-fold, frail 3.1-fold). The authors conclude that it is not frailty status but rather pre-existing immunity which predicted post-vaccination immune system response to influenza vaccine in their cohort.

The third study (N=31,989), using a broader definition of frailty which included measures of disability, vision and hearing loss, impaired mobility, changes in sleep, and urinary complaints among others (14 possible frailty conditions in total; manuscript summarizes across frailty conditions and reports results for those with ≥ 3 frailty conditions) stated that for adults ≥ 65 years of age HD influenza vaccine elicited a greater immunogenicity response than did the SD vaccine (DiazGranados CA et al., 2015). Comparisons of immunogenicity response using GMTs for the group with ≥ 3 frailty conditions, showed that HD vaccine resulted in higher GMTs than SD for all vaccine strains (H1N1, H3N2, and two B strains). HD GMT for H1N1 was 429.7 (95% CI: 405-456) compared to SD GMT of 241.0 (95% CI: 225.9-257.1); for H3N2 HD GMT was 649.8 (95% CI: 596.8-707.5) vs. SD GMT of 338.2 (95% CI: 310.2-368.7); and was 145 (95% CI: 134.7-156.1) and 104.7 (95% CI: 96.8-113.2) for each HD GMT B strains vs. 105 (95% CI: 97.6-112.9) and 65.2 (95% CI: 60.7-70.1) for each SD GMT B strain. No assessments of seroprotection or seroconversion were conducted in this study.

Prior to licensure of HD influenza vaccine, a randomized controlled trial study was conducted among frail long-term care residents (deemed frail due to disability and comorbidity status and number of medications not by assessment of frailty measurements) (Roos-van Eijndhoven DG et al., 2001). This study (N=815, mean age of 83 years), assessed the antibody response to influenza vaccine among four treatment groups: 1) baseline dose of 15 μg and placebo dose at Day 84; 2) baseline dose of 15 μg vs. a booster dose of 15 μg at Day 84; 3)

baseline dose of 30 µg and placebo dose at Day 84; and 4) baseline dose of 30 µg and booster dose of 15 µg at Day 84. Results from this study found that GMTs for long-term care residents receiving a double vaccine dose (30 µg) at baseline was 15% (95% CI: 6-24%, *P*=0.001) higher as compared to residents receiving a standard dose (15 µg) and that a booster dose at Day 84 resulted in GMTs that were 14% (95% CI: 9-19%, *P*=0.001) higher as compared to those receiving a placebo dose. The authors concluded that among frail elderly either a double vaccine dose or a booster vaccine dose elicits higher antibody response than a standard dose alone.

These studies emphasize the consequences that frailty has on innate and adaptive immune response and the detrimental effects this in turn has on immunogenicity response to influenza vaccine among older adults.

5.0   HEATLH-RELATED QUALITY OF LIFE

It is well established that stress, depression and poor physical functioning have a negative impact on health-related quality of life (HRQOL) (Beaton DE & E, 2003; Daly EJ1 et al., 2010; de Frias CM & E, 2015; Gaynes BN, Burns BJ, Tweed DL, & P, 2002). People's HRQOL also suffers during influenza illness (Hollmann M et al., 2013; Nichol KL, Heilly SD, & E, 2005; Nichol KL, Heilly SJD, Greenberg ME, & E, 2009; van Hoek AJ, Underwood A, Jit M, Miller E, & WJ, 2011).   Research has shown that spirituality moderates the effect of stress on emotional and physical adjustment (Kim Y & L, 2002), religion moderates the effect of depression associated with poor physical health (Wink P, Dillon M, & B, 2005) and that spirituality also acts as a mediator between stress and psychological adjustment (Reutter KK, 2014).   This section discusses HRQOL, the effect of poor (low, or compromised) HRQOL (indicated by depression and stress) on the immune system, the impact of influenza illness on HRQOL, the relationship of spirituality on HRQOL and measurement properties of HRQOL surveys used in this dissertation.

5.1   HEALTH-RELATED QUALITY OF LIFE: DEFINITION, USE AND MEASUREMENT

The World Health Organization (WHO) stated in 1949 that "health is a state of complete physical, mental, and social well-being and not merely an absence of disease and infirmity"

(HealthyPeople.Gov, 2014). HRQOL is a multidimensional concept that seeks to understand ones' subjective perceptions of quality of life as demonstrated by physical, mental, emotional and social functioning (HealthyPeople.Gov, 2010). HRQOL seeks to "go beyond the direct measures of population health, life expectancy, and causes of death, and focuses on the impact health status has on quality of life" (HealthyPeople.Gov, 2014). Patrick and Erickson (1993) state that "health-related quality of life is the value assigned to duration of life as modified by the impairments, functional states, perceptions, and social opportunities that are influenced by disease, injury, treatment, or policy." (Feeny DH, Eckstrom E, Whitlock EP, & al., 2013)

HRQOL measurements can be broad including assessments of overall physical and mental health and social engagement or focused on certain aspects of HRQOL, such as depression, anxiety, or stress. Use of HRQOL in clinical evaluation and health research is not new and much research has been conducted over the years using HRQOL measurements. Worldwide attention was brought to the importance of HRQOL by WHO's quality-of-life Position Paper in 2005 which spoke to the need to both evaluate and improve quality-of-life for individuals (HealthyPeople.Gov, 2014). The importance of measuring HRQOL in the United States is evidenced by HRQOL improvement goals included in Healthy People 2000, 2010 and 2020 (CDC National Center for Chronic Disease Prevention and Health Promotion, 2011). These goals can be assessed using HRQOL questions being included on national surveys such as the Behavioral Risk Factors Surveillance System (BRFSS), the National Health and Nutrition Examination Survey (NHANES), and the National Health Interview Survey (NHIS) (HealthyPeople.Gov, 2014).

Spirituality, along with culture and values, can be an important aspect of overall quality of life (CDC National Center for Chronic Disease Prevention and Health Promotion, 2011).

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Greater levels of daily spiritual connectedness have been significantly correlated with reduced levels of anxiety, depression and stress, and with increased levels of optimism and perceived social support (*P* <0.01 for all correlations) (Underwood LG & Teresi JA, 2002).

HRQOL is related to both risk factors for and outcomes of disease (CDC National Center for Chronic Disease Prevention and Health Promotion, 2011). Understanding HRQOL allows physicians, researchers, and policy makers to evaluate the burden of disease and disease-related outcomes, guide interventions to improve HRQOL to lessen disease burden, and assess areas for public policy, legislation and allocation of resources (CDC National Center for Chronic Disease Prevention and Health Promotion, 2011).

5.2   DEPRESSION AND STRESS AS INDICACTORS OF POOR HRQOL

Depression is linked to worsening physical functioning including declining mobility (Milaneschi Y & Penninx BWJH, 2014). In Milaneschi and Penninx (2014) the relationship between depression and subsequent physical disability seems to be bi-directional in that each in turn increases the risk of the other. Depression decreases role functioning ability (e.g., self-care) and can exacerbate the effects of chronic illness, further reducing HRQOL (Gaynes BN et al., 2002). Stress is also associated with worsening physical functioning and with decreased overall physical health and poor mental health (de Frias CM & Whyne E, 2015).

5.3   EFFECTS OF STRESS AND DEPRESSION ON THE IMMUNE SYSTEM

Stress hormones and depression have harmful effects on the immune system, resulting in dysregulation of immune functioning, impaired vaccine response, and delayed wound healing (Kiecolt-Glaser JK & Glaser R, 2002; Webster Marketon JI & Glaser R, 2008) and reactivation of latent infections (Webster Marketon JI & Glaser R, 2008).   Increased production of inflammatory markers and ramped up cellular aging has also been seen among stressed elderly caregivers as compared to similarly-aged non-caregiving adults (Gouin JP, Glaser R, Malarkey WB, Beversdorf D, & JK, 2012; Gouin JP, Hantsoo L, & Kiecolt-Glaser JK, 2008). Depression also affects the stimulation of pro-inflammatory cytokines. Increased circulating levels of inflammatory cytokines are associated with a number of health-related conditions such as type 2 diabetes, functional decline and frailty (Kiecolt-Glaser JK & Glaser R, 2002).   In a cross-sectional study higher levels of the inflammatory marker IL-6 was associated with major depression in adults aged $\geq$ 65 years (Bremmer MA et al., 2008).   A systematic review of depression and pro-inflammatory markers in community and clinical populations corroborated the association of inflammatory markers (including IL-6) and depression and noted that there seems to be three causal pathways: inflammation to depression, depression to inflammation and bidirectional relationships between inflammation and depression with BMI seemingly serving as a mediating/moderating factor in these pathways (Bryant Howren M, Lamkin DM, & Suls J, 2009).

5.4   INFLUENZA, HRQOL AND LOWERED IMMUNE SYSTEM RESPONSE

Yearly seasonal influenza has a notable impact on HRQOL. One study reported the burden that influenza-like illness (ILI) (defined as symptoms of influenza) had on working adults aged 50-64 years of age (N=497) during the 2006-2007 influenza season (Nichol KL et al., 2009). Within this cohort 17.1% reported experiencing ILI and the mean total number of days spent feeling ill was 8.04 (SD: 6.66) days. Results showed that overall 1.60 (SD: 2.00) days were spent in bed, 3.98 (SD: 4.55) days spent out of bed but with significant decreases in normal functioning, 1.49 (SD: 1.91) days of lost work, and 4.39 (SD: 3.73) days worked while ill (reduced productivity). Additionally, 9% reported an exacerbation of an underlying medical condition due to ILI. Mean total days of illness experienced and the resulting consequences of illness such as absenteeism, decreased functional ability and presentism (working while sick) was significantly less in vaccinated adults as compared to unvaccinated. The adjusted OR for ILI between vaccinated and unvaccinated persons was: 0.48, 95% CI: 0.27-0.86) and the observed differences between those vaccinated to unvaccinated in days lost due to ILI consequences ranged from -0.28 to -1.97 (*P*=.010 to <.001).

Decreased functional status has been noted among persons who have been infected with influenza illness. A case-control study (cases had laboratory confirmed ILI) was conducted with residents from six nursing homes (N=131 cases, 127 controls) (Barker WH, Borisute H, & C, 1998). Assessments of functional status prior to an influenza outbreak with follow-up assessments at 1-2 months and 3-4 months after the outbreak were conducted via review of medical and nursing records. Functional measures included measures of ADLs. Among cases, there were no significant decreases in functional status 1-2 months after the influenza outbreak, however, there were significant decreases 3-4 months after in ADLs of independence in bathing,

dressing and mobility (P values range 0.008-0.07); controls only experienced significant decreases in mental status at both time intervals.

The influence of chronic stress on lowered immune system response to influenza vaccine has been reported. A sub-study of matched caregiver-control pairs, (from part of a larger longitudinal study comparing stress and health in older adults) compared influenza vaccine response of elderly caregivers (mean age 73.12 years, SD: 8.64) and their spouses (controls) diagnosed with Alzheimer disease or progressive dementia (Kiecolt-Glaser JK, Glaser R, Gravenstein S, Malarkey WB, & J, 1996). Caregivers were less likely to seroconvert one month after vaccination. Only 50% of caregivers seroconverted vs. 75% of the controls and this was a significant difference (χ^2=4.27, P=0.04). Caregivers reported significantly higher levels of depression than controls (F=12.46, P <0.001) but subjects did not differ with regards to influenza vaccination history, income, age, chronic illnesses, or number of medications.

Cohen et al. (2001) conducted a systematic review of the literature describing psychological stress and antibody responses to vaccination among humans (Cohen S, Miller GE, & BS, 2001). This review supports the association between psychological stress and decreased humoral adaptive immune response, particularly for secondary exposures (e.g., subsequent exposures to vaccine antigens - yearly vaccination). In this review, which included literature on influenza vaccine, the authors note that lowered immune system response was evident for persons reporting higher levels of chronic stress and that this association was found more consistently among older adults.

The association between increased levels of depression and lower influenza vaccine response has not been proven. One study (N=119) assessed IL-6 levels, depression and influenza vaccination response in older adults (mean age, 71.21, SD: 8.68) and did not find differences in

seroconversion between persons reporting greater depressive symptoms than those reporting fewer (F=1.41, *P*=0.24) (Glaser R, Robles TF, Sheridan J, Malarkey WB, & JK, 2003). Though higher levels of IL-6 were found in subjects reporting greater levels of depressive symptoms at baseline and two weeks after vaccination overall, depressive symptoms were low at both time-points. The mean number of depressive symptoms was 3.07, (SD: 3.09) at baseline and did not significantly change two weeks after vaccination (F=0.18, *P*=0.67). In additional analyses IL-6 levels at baseline and at two-weeks were not significantly related to sex, age or race but among those with lower levels of education higher levels of IL-6 was evident as two-weeks post-vaccination (F=2.89, *P*=0.03).

5.5   ASSESSING MEASUREMENT PROPERTIES OF HEALTH-RELATED QUALITY OF LIFE SURVEYS

There are two main measurement properties that need to be considered and assessed during survey design and conduction: reliability and validity. Within each of these categories there are a number of aspects to consider and ways in which to assess statistically. Instruments which measure HRQOL need to ensure that these standards are met in order to be considered as valid instruments of use.

Reliability is concerned with ensuring that an instrument is consistent in yielding the same results across time (Feeny DH et al., 2013). One way this is measured is by assessment of an instrument's internal consistency – ensuring that items of similar dimensions or domains correlate with each other and that unrelated items are not correlated with each other. Reliability also checks that the survey is consistent over time in an unchanging population (test-retest

reliability) and that the instrument is capable at producing the same rating results at different time points within the same rater (intra-rater reliability) or the same results between two different raters (inter-rater reliability). Statistically, reliability is measured in three ways. Internal consistency is checked via Cronbach's alpha, intra- and inter-rater and test-retest reliability is assessed via the intra-class correlation coefficient (ICC), for continuous items or for categorical items by the kappa statistic; scores > 70 for all three statistics are considered good for meeting reliability standards (Feeny DH et al., 2013).

Validity is the extent to which an instrument actually measures what it is designed to measure. Three criterion are assessed in validity measurements: content validity, criterion validity and construct validity (Feeny DH et al., 2013). Content validity is concerned with ensuring that the questions are appropriate and complete in covering the domains of interest to be assessed. Content validity is not measured statistically but rather uses informal analytics, such as face-validity, in addressing set questions to gauge whether or not the aspects of validity are being met (Feeny DH et al., 2013). Criterion validity measures the level of agreement between the instrument being tested against a known gold-standard. In so doing, it may also utilize the instrument's predictive validity in its assessment (e.g., whether baseline functional status is predictive of fall risk and consequent morbidity/mortality). Construct validity assesses whether the theoretical constructs on which the instrument's domains are based result in the expected associations. Convergent validity and discriminant validity, two sub-parts of construct validity, measure that similar and/or related domains yield similar results (convergent) and that the instrument is also able to distinguish between unrelated domains (discriminant) (Feeny DH et al., 2013).

5.6   MENTAL OUTCOMES STUDY SHORT-FORM SURVEY (SF-12): ITS USE IN HRQOL AND MEASUREMENT PROPERTIES

The RAND Short-Form Survey was developed for use in the Medical Outcomes Study (MOS) (an observational study of health outcomes for patients with chronic medical and psychiatric conditions) as a 36-item general health questionnaire (SF-36) (Ware JE Jr. & Sherbourne CD, 1992). Selection of survey items were pulled from those evident in health survey research over the past 20-40 years and aimed to replicate the full-length MOS parent scale to the extent possible. Many of the items included in this survey have origins in instruments used with elderly and disabled populations (Gandek B, Sinclair SJ, Kosinski M, & Jr., 2004). The SF-36 consists of eight subscales: physical functioning (PF, 10 items); role limitations because of physical health problems (RP, 4 items); bodily pain (BP, 2 items); social functioning (SF, 2 items); general mental health (physiological distress and psychological well-being) (MH, 5 items); role limitations due to emotional problems (RE, 3 items); vitality (energy/fatigue) (VT, 4 items); and overall general health perceptions (GH, 5 items). Either the individual subscales or the two summary measures compiled from these scales, the physical component summary (PCS) and mental component summary (MCS), can be used in analyses (Ware JE Jr. et al., 1995). The PCS is comprised of items of PF, RP, BP and GH; the MCS is comprised of VT, SF, RE and MH items (Gandek B et al., 1998); scales included in the PCS and MCS were chosen based upon factor analyses of correlations (Ware JE Jr. et al., 1995).

Using three diverse patient populations from the MOS study, tests of validity and reliability were conducted on each of the eight subscales (McHorney CA, Ware JE Jr., Lu R, & CD, 1994). Reliability of each scale was high (Cronbach's alpha coefficients for the scales ranged from low of 0.78 for GH to high of 0.93 for PF). Item-internal consistency across all

patient groups reported scales passing at a 97% rate. Tests of item-discriminant validity showed all scales passing at a 92% pass rate. Using multivariate analysis of variance (MANOVA), tests were conducted to assess the relative validity of the two component summary scores (Ware JE Jr. et al., 1995). Relative validity coefficients ranged from 0.20-0.94 with a median score of 0.79 for scales included in the PCS and from 0.93-1.02 with a median score of 1.02 for scales included in the MCS therefore meeting empiric validity standards.

Assessments of SF-36 reliability and validity among elderly and frail populations have been conducted with results meeting empiric standards. The Medicare Health Outcomes Survey (HOS) study evaluated the SF-36 subscales and two summary component scales among 177,714 elderly and disabled beneficiaries using 1998 baseline data (Gandek B et al., 2004). Cronbach alpha coefficients ranged from 0.83-0.93 for the subscales and were 0.94 and 0.89 for the PCS and MCS respectively. Consistency of items within the scales was met with coefficients showing substantial correlations ≥ 40. Construct validity using factor analysis assessment of the correlation between subscales and principal components with two rotations confirmed the validity of subscales comprising the PCS and MCS. Stadnyk et al. (1998) tested the SF-36 among frail elderly (57% > 80 years of age) 64% who were female; frailty was defined as being slow to regain mobility after hospitalization and included measurements of disability as assessed by ADLs and IADLs (Stadnyk K, Calder J, & K, 1998). Cronbach's alpha scores ranged from 0.72-0.91 for the eight subscales. Scales also met item convergent validity with item-scale correlation coefficient scores ≥ 40. Using a two-factor principal components analysis indicated that construct validity of the SF-36 in broadly measuring components of mental and physical health, accounting for 19% and 34% of the total variance respectively.

The Short-Form Survey-12 (SF-12) was developed by the same authors of the SF-36 using a reduced set of the items included in each of the SF-36 subscales (Ware JE Jr., Kosinski M, & SD, 1996). The SF-12 therefore consists of the same eight subscales and two summary component scales as the SF-36. Assessments of the reliability and validity of the SF-12 as compared to the SF-36 have shown that the SF-12 is a valid and reliable instrument of measuring physical and mental health. Ware et al. (1996) used data from the National Survey of Functional Health Status (NSFHS) and the MOS studies in comparisons of the two instruments (Ware JE Jr. et al., 1996). The twelve items chosen to create the SF-12 PCS and MCS from the SF-36 subscales resulted in a multiple R^2 of 0.911 prediction of the PCS-36 and 0.918 Prediction of the MCS-36 (N=2,474 from the general US population). The SF-12 PCS and MCS were highly correlated to the PCS-36 and MCS-36 with correlation coefficients of 0.95 and 0.97 respectively. Independence of the two SF-12 summary component scales was maintained (r=0.06). Test-retest reliability coefficients for the SF-12 PCS was 0.86-0.89 and coefficients were 0.76-0.77 for the SF-12 MCS. SF-12 PCS effect sizes were 0.87 and 1.30 similar to that of the SF-36 PCS scores of 0.91 and 1.45. Comparisons of the relative validity for the MCS showed coefficients of 1.07 and 0.60 for the SF-12 MCS relative to the best SF-36 scale results of 1.12 and 0.62. SF-12 MCS effect sizes were 1.68 and .93 in these tests.

5.7 PATIENT HEALTH QUESTIONNAIRE (PHQ-9): ITS USE IN HRQOL AND MEASUREMENT PROPERTIES

The Patient Health Questionnaire (PHQ) is a self-administered questionnaire based upon the primary care evaluation of mental disorders (PRIME-MD®) and evaluates depressive and other

mental health disorders commonly seen in primary care (Kroenke K & RL, 2002; Kroenke K, RL, & JBW, 2001). Due to its ease of use as a self-administered tool and similar diagnostic validity to that of PRIME-MD® (PHQ scores ≥ 10 showed an 88% sensitivity and an 88% specificity for major depression) the PHQ is commonly used for both clinical and research purposes (Kroenke K & RL, 2002; Kroenke K, RL, et al., 2001).

The PHQ-9 is a reduced nine-item version of the full PHQ utilizing just the depression section and consists of the nine criteria necessary for diagnosing depressive disorders according to the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (Kroenke K, RL, et al., 2001). DSM-IV symptoms are: depressed mood or irritability, decreased interest or pleasure, change in appetite, in sleep and in activity, fatigue or loss of energy, guilt/worthlessness, diminished concentration and suicidality (Andrews G et al., 2007). It is self-administered, assesses patient perspectives of how much they have felt bothered over the last two weeks on nine depressive symptoms (e.g., little interest or pleasure in doing things, feeling down, depressed or hopeless, feeling tired or having little energy etc.) using a four-item Likert response scale of 0=not at all, 1=several days, 2=more than half the days, and 3=nearly every day. PHQ-9 scores range from 0 to 27. Diagnosis of major depression is made if five or more of the nine symptoms have been present "more than half the days" and one of the symptoms is depressed mood or anhedonia (inability to experience pleasure in activities that were previously enjoyed); if ≤ 4 of the nine symptoms were answered "more than half the days" and either depressed mood or anhedonia is present than other types depression is diagnosed (e.g., minor depression, anxiety disorder etc.) (Kroenke K, RL, et al., 2001). Cut Points of 5, 10, 15, and 20, representing mild, moderate, moderately severe and severe depression are used. These cut points were chosen for two reasons, one, due to pragmatics (easy to remember and apply) and

two, empirically – different cut points did not significantly change the association of increasing severity and construct validity measurements (Kroenke K, RL, et al., 2001).

A validation study of the PHQ-9 was conducted with 3,000 primary care patients between 1997-1999 (Kroenke K, RL, et al., 2001). Patient characteristics were 66% female, 79% Caucasian (13% African American and 4% Hispanic), 48% were married, 54% had higher than a high school education and had a mean age of 46 years (S.D. 17). Results from this study (Kroenke K, RL, et al., 2001) show excellent internal reliability (Cronbach alpha score of 0.89). Test-retest reliability was excellent with a correlation score of 0.84. Criterion validity was assessed in 580 patients who had a structured psychiatric interview by a mental health professional blinded to PHQ-9 scores who was determining the presence or absence of major depression using standard DSM-IV diagnostic criteria. Among these patients, there was a 7% prevalence of major depression and the positive predictive value for a diagnosis of major depression ranged from 31% for a cut point of 9 on the PHQ-9 to 51% for a cut point of 15. Construct validity was assessed by comparing to the Short-Form Survey-20 (SF-20) using disability days, symptom related difficulty and utilization of health care services; analyses showed the expected relationships of depression on functional status (as evident in the literature) in that increased PHQ-9 scores resulted in decreased SF-20 scale scores. Analysis of covariance (ANOCOVA) using Bonferroni's correction for multiple comparisons shows that most pairwise comparisons between PHQ-9 and SF-20 scales were significant at $P < .05$.

Additional studies have been conducted comparing the diagnostic and psychometric properties of the PHQ-9 in primary care elderly, the general population and with patients with systemic sclerosis. Among primary care elderly (mean age of 78 years), the PHQ-9 was compared to the Geriatric Depression Scale (GDS) and the Structured Clinical Interview for

Depression (SCID) in seventy-one persons from two primary care, university-based clinics (Phelan E et al., 2010). The SCID is the criterion standard for diagnosis of DSM-IV depression in clinical research while the GDS is the instrument most commonly used in screening depression levels in geriatric care (Phelan E et al., 2010). Patient demographics were 62% female, 32% non-White, 82% had graduated high school and 42% had three or more chronic medical conditions. Using the SCID to establish depression prevalence in this sample, 12% were diagnosed with major depression and 13% with minor depression.

Phelan et al. (2010) measured the PHQ-9's sensitivity, specificity, area under the curve (AUC) using receiver operating characteristics analysis (ROC) and the likelihood ratios to the GDS (Phelan E et al., 2010). Comparisons of the sensitivity and specificity of each instrument in diagnosing major depression to that of the SCID using standardized depression cut points of $\geq$ 10 on the PHQ-9 and > 5 for the GDS, resulted in a sensitivity of the PHQ-9 of 63% with 82% specificity compared to the GDS sensitivity of 100% and specificity of 58%. Positive likelihood ratios for the two tests were 3.5 for the PHQ-9 and 2.4 for the GDS (e.g., a positive screen is 3.5 times more likely to be evident in a patient with major depression than in one without). Overall AUC scores were 0.87 (95% Confidence Interval (CI), 0.74-1.00) for the PHQ-9 and 0.81 (95% CI 0.70-0.91, P 0.551) for the GDS. Within this elderly sample (Phelan E et al., 2010), when using the PHQ-9 and the GDS to screen for both major and minor depression using the same cut points as described above, sensitivity, specificity, and positive likelihood ratios were 59%, 89% and 5.1 respectively for the PHQ-9 and 81%, 62% and 2.1 for the GDS. Overall AUC's for this broadened depression definition were 0.85 (95% CI, 0.73-0.96) for the PHQ-9 and 0.71 (95% CI, 0.55-0.87) for the GDS. These authors overall conclusions were that the PHQ-9 performs comparably well to the GDS and due to its brevity (9 items vs. the 15-item GDS) is a sound

alternative to the GDS in screening for depression among elderly persons in primary care settings.

5.8   PERCEIVED STRESS SCALE (PSS): ITS USE IN HRQOL AND MEASUREMENT PROPERTIES

The Perceived Stress Scale (PSS) developed by Cohen et al (1983), measures one's appraisal of stress experienced over the past month using a 5-item Likert response scale asking general stress-related questions (i.e. not content or situation specific). It addresses the thoughts and feelings of the degree to which one may have felt their life was unpredictable, uncontrollable and overloaded – aspects which have been noted as key components of the stress experience (Cohen S, Kamarck T, & R, 1983); higher scores are indicative of greater perceived stress. Initially developed as a 14-item questionnaire (PSS-14), the PSS is also available for use as a 10- (PSS-10) and 4-item (PSS-4) questionnaire, has been translated into 28 languages and is targeted for use with community samples having at least a 6th grade education level (Cohen, 2016; Cohen S et al., 1983). The objective of the PSS is to allow for assessment of how appraisals of stress relate to risk factors for disease or behavioral outcomes, the role moderators/mediators have on influencing stress and therefore disease/behavioral outcomes, as well as understanding the associations of other aspects (coping, social support, personality) on perceived stress (Cohen S et al., 1983).

Assessment of all three PSS instruments (PSS-4, PSS-10, PSS-14) was conducted in 1983 using a national area probability sample of 2,387 noninstitutionalized adults in the United States via telephone interview (Cohen S & GM, 1988). This probability sample included 42.4%

adults $\geq$ 45 years of age (29.1% were $\geq$ 55 years and older), 9.9% minorities (7.8% were African American) and 60.1% female. Internal consistency of the PSS instruments were relatively good; Cronbach alpha coefficients were 0.75 for the PSS-14, 0.78 for the PSS-10 and 0.60 for the PSS-4. Construct validity was done by assessments of the relationship of the PSS measures to health, other stress instruments, utilization of health services, health behaviors, life satisfaction and help-seeking. PSS scores were moderately correlated with other stress measures rating average weekly stress, comparison of current stress to levels one year ago, and life-events scale (r= -0.26 to 0.29, P <.001), and showed small to moderate correlations on self-reported health and utilization of health services (r=0.12-0.27, P <.001) (Cohen S & GM, 1988).

The PSS-4, which is the scale used in this dissertation, was developed utilizing four questions (2, 6, 7, and 14) that had the highest correlation with the full 14-item scale (Cohen S et al., 1983). The initial assessment of this 4-item scale was conducted among community-dwelling adults (N=64, mean age of 38.4 years (S.D. 11.57)) engaged in a University run smoking-cessation program. PSS-4 was assessed at baseline and at one- and three-months post treatment and was found to have reliable internal consistency (Cronbach alpha of 0.72) and moderate test-retest reliability (ICC of 0.55) over an interval of eight weeks (Cohen S et al., 1983). This scale was also found to have predictive validity with PSS-4 showing significant correlations on smoking rate at both one and three months post treatment (0.31, P <.01 and 0.37, P=0.001 respectively) (Cohen S et al., 1983).

A recent systematic review of the literature cited nineteen studies which used PSS instruments in assessments of stress; of these, seven studies included the PSS-4 (Lee, 2012). Results show that internal reliability is good with Cronbach alpha coefficients of 0.60-0.82; three of the six studies had values >0.70, two studies showed values of 0.67 and 0.68. Criterion

validity was assessed in three studies with one study showing weak to moderate correlations to other stress measures, another study reported high correlation to the Short-Form Survey 36 mental component score (r= -0.70) and moderate correlation to the physical component score (r= -0.23), and the third study noted moderate correlation to the PSS-10 (r=0.63), *P* values for these correlations were not provided in Lee (2012).

As is evidence by work done by Cohen et al. (1983 and 1988) and noted in this systematic review by Lee (2012), the PSS-4 is not as robust a scale as the PSS-10 or the PSS-14 due perhaps to the limited size of the scale. However, Cohen et al. (1983) noted that though the PSS-4 is less internally reliable than the full PSS-14, the factor structure and the predictive validity of the PSS-4 is good and therefore it still provides a valuable assessment of perceived stress that can be used with populations where a shorter survey may be better suited (Cohen S & GM, 1988; Cohen S et al., 1983).

5.9   DAILY SPIRITUAL EXPERIENCE SCALE (DSES): ITS USE IN HRQOL AND MEASUREMENT PROPERTIES

The Daily Spiritual Experience Scale (DSES) was developed as a follow-up to the 1994 working group co-funded by the Fetzer Institute and the National Institute on Aging which developed the domains that became the Brief Multidimensional Measurement of Religiousness/Spirituality (BMMRS) (Underwood, 2006). The BMMRS, which was a multi-dimensional assessment to glean understanding on the varying facets of religiousness/spirituality, included the DSES (Fetzer Institute, 2003; Underwood, 2006). A shortened version of the DSES was included in the

General Social Survey (GSS) conducted in the United States in 1997-1998 (Fetzer Institute, 2003), the full-length version of the DSES was included in the GSS in 2004 (Underwood, 2006).

The DSES is a sixteen-item self-administered questionnaire which seeks to capture the everyday, ordinary spiritual experiences of recognizing and relating to the divine or transcendent, and how these experiences in turn inform daily life (Underwood, 2011). The DSES intentionally overlaps between religiousness and spirituality (Underwood, 2006, 2011). The distinction between religion and spirituality has evolved over recent years. Currently as evident in the literature, religion can be understood as referring to the visible, institutional, public and collective whereas spirituality denotes aspects of the internal, private, subjective and personal (Reutter KK, 2014; Zinnbauer BJ et al., 1997). Though many of the questions on the DSES are theistic in nature (i.e., "God"), the introduction makes clear that if the term "God" is not constructive, to substitute whatever word brings to mind the "divine or holy" (Underwood, 2011). This introduction was added after review of the DSES among agnostics, atheists, Buddhists, Christians, Jews, Hindus, and Muslims during a WHO working group on Spiritual Aspects of Quality-of-life meeting. At this meeting, it was evident that all but those belonging to the Buddhist tradition were accepting of the word "God" as this word could be internally translated into other terms denoting concept of the divine for non Judeo-Christians (Underwood LG & Teresi JA, 2002).

The sixteen items assess daily experience via a 6-item response Likert Scale (many times a day to never or almost never) covering concepts of connection (feeling God's Presence and connection to all of life), joy (feel joy which lifts one out of daily concerns), strength and comfort (find strength and comfort in religion or spirituality), peace, divine help (ask for God's help in the midst of daily activities), divine guidance (feel guided by God in the midst of daily

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activities), perceptions of divine love (feel God's love directly or feel God's love through others), awe (spiritually touched by the beauty of creation), thankfulness/appreciation (thankful for blessings), compassionate love (selfless caring for others and acceptance of others even when they do things that are considered wrong), and union and closeness (desire to be close to God or the divine and measuring how close one feels to God) (Underwood, 2006). In ensuring the content validity in creation of the DSES, Underwood held focus groups and in-depth interviews with people of many religious traditions, reviewed spirituality scales used in the literature, pulled from a wide variety of theological, religious and spiritual writings, tested the meaning behind what the items were actually tapping in one-on-one open-ended interviews and tested item wording among professionals from many religions during a WHO working group (Underwood LG & Teresi JA, 2002).

Reliability and validity of the 16-item DSES showed to be sufficient in a study conducted with 233 middle aged women (mean age 46.76, SD=2.74) who were part of the Chicago site Study of Women Across the Nation (SWAN); 60% were White, 54% were Catholic, 26% were Protestant and 8% identified as Other religion (Underwood LG & Teresi JA, 2002). Internal consistency reliability using Cronbach's alpha was 0.94. Assessment of zero-ordered correlations showed that the DSES had moderate to high inter-correlations (r=0.60-0.80). Within this SWAN sample, the items of "find strength in religion/spirituality" and "find comfort in religion/spirituality" were collinear (r=0.96) and the authors recommend assessing this pattern in future study samples and eliminating one of the items if collinearity is evident. Construct validity assessed using an exploratory principal components analysis found that the scale was unidimensional and a further test using an oblique rotation resulted in all but two items loading highly on the first factor with load scores ranging from 0.69-0.93. The authors' note that a 2-

item scale is not desirable and in the case of this analysis was not meaningful as it only captured

8% of the variance (Underwood LG & Teresi JA, 2002).

Reliability and validity was also ascertained among U.S. adults using the 16-item DSES

in the 2004 GSS (Ellison CG & D, 2008). Among this GSS sample (number ranged from 854 to

1,318 respondents) which included a weight variable to account for non-response as well as the

total number of adults within a household, Cronbach alpha's internal consistency reliability

coefficient was 0.96. Construct validity of the DSES using principal components analysis was

similar to the Chicago SWAN study with load scores ranging from 0.50-0.90.

5.10   THE LINK BETWEEN SPIRITUALTY AND HRQOL

Underwood and Teresi (2002) suggest that health is impacted by ones daily spiritual experience.

For instance, psychological stress may be reduced for those indicating they experience daily

God's presence and guidance and feelings of anxiety and depression may be reduced for those

who daily note experiences of love, comfort and spiritual peace (Ellison CG & D, 2008;

Underwood LG & Teresi JA, 2002). There is a vast amount of literature denoting links between

constructs of religiousness/spirituality and health outcomes, particularly for mental and physical

health with most showing positive health outcomes for those engaged in religious and/or spiritual

practices (Ellison CG & D, 2008). A review of the breadth of studies conducted in this area can

be seen in Koenig et al. (2012) *Handbook of Religion and Health* which includes 3,300 studies

measuring associations between religion/spirituality and health (Koenig HG, King DE, & VB,

2012).

A number of studies assessing the role of spirituality on health outcomes have used the DSES instrument (Boswell GH, Kahana E, & P, 2006; Ellison CG & D, 2008; Greenfield EA, Vaillant G, & Marks NF, 2007; Koenig HG, George LK, Titus P, & Meador KG, 2004; McCauley J, Tarpley MJ, Haaz S, & SJ, 2008). Studies of the DSES on HRQOL including that of depression and stress, have mixed results. In a cross-sectional study McCauley et al. (2008) measured DSE, pain, energy and fatigue, depression and perceptions of health among 99 adults > 50 years of age in a primary care practice who reported having ≥ 1 chronic health condition. Results from this study (62% women, 50% African American, mean age of 65.8 (SD: 9.6) years, 74% with hypertension, 54% with arthritis and 24% with heart disease)) show that ≥ 70% of this sample reported experiencing DSE most to many times of day (range across DSES items was 70%-96%). Among those with greater comorbid conditions, more frequent DSE was reported (r= -0.295, P=0.003). In analyses adjusting for number of chronic conditions, sex, race, age, and pain, reports of more frequent DSE was significantly associated with less depression and greater energy, (ρ= -0.282, P=0.007 and ρ= -0.272, P=0.009, respectively).

In a two-year longitudinal study of 221 adults ≥ 65 years of age (76% female, 82% Caucasian, 2.1 (SD: 1.2) chronic conditions and mean age of 80.2 (SD: 5.3) years))) analyzing spirituality, religiousness and healthy lifestyle on subjective physical well-being and chronic illness, higher levels of DSE contributed to greater physical well-being and had a counter-balancing effect in the stress deterrent model (Boswell GH et al., 2006). Specifically, in an OLS regression model which evaluated the association of chronic illness, DSE, public and private religiosity, healthy diet and physical activity on subjective physical well-being adjusting for age, race, sex, education, income, a unit increase in DSE resulted in increased subjective well-being (β=0.164, P <0.05) holding all other variables constant.

Ellison and Fan (2008) used DSES results included in the 2004 GSS conducted among U.S. adults to measure the association between DSE on five psychological outcomes: psychological distress, happiness, excitement with life, satisfaction with self and optimism about the future. Within this sample (N=854 to 1,318 respondents) 52% were female, 11% were African American, and 58% were $\geq$ 40 years of age. Zero-inflated Poisson regression results indicated that a unit increase in DSE related to people having a 31% higher odds of experiencing no distress (OR=1.31, P <0.05) after adjusting for a number of demographic variables (e.g., age, sex, race, education, income, marital status, religious attendance) but this association did not hold for total number of distressed days experienced (OR=0.91, NS) holding other variables constant. Ordered logistic regression results show that DSE was significantly, positively associated with being optimistic, happy, satisfied with self and excited with life. For a one unit increase in DSE and holding all other variables constant (e.g., demographic variables as listed above) the odds of being happy increased 28% (OR=1.28, P <0.001), the odds of being excited with life increased 40% (OR=1.40, P <0.001), the odds of being satisfied with self increased 64% (OR=1.64, P <0.0001) and the odds of being optimistic about the future increased 71% (OR=1.71, , P <0.0001). The authors note that in subgroup analyses looking at effects of DSE on the five outcomes by sex, race, age and SES, no meaningful pattern was found and of the five significant statistical interactions that were seen (out of a total fifty assessed) these would be non-significant if adjusting for multiple comparisons.

6.0 PAPER 1: FRAILTY AND IMMUNE SYSTEM RESPONSE TO FLU VACCINE IN COMMUNITY-DWELLING ADULTS ≥ 50 YEARS OF AGE

The effect of frailty on HAI response to influenza vaccine among community-dwelling adults ≥ 50 years of age

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6.1   ABSTRACT

The immune response to vaccine antigens is less robust in older adults because of changes in the aging immune system. Frailty, the multi-dimensional syndrome marked by losses in function and physiological reserve, is increasingly prevalent with advancing age. Frailty accelerates this immunosenescence but the consequence of frailty on immune response specific to influenza vaccine among older adults, is mixed. An observational, prospective study of 114 adults was conducted in the fall of 2013 to assess the association of physical frailty with immune response to standard dose influenza vaccine in community-dwelling adults $\geq$ 50 years of age. Participants were stratified by age (<65 years and $\geq$65 years), and vaccine strain (Influenza A/H1N1, A/H3N2 and B) was analyzed separately adjusting for body mass index and baseline $\log_2$ hemagglutination inhibition (HI) titers. Overall, immune responses were lower among those $\geq$65 years of age than those <65 years. Among those $\geq$65 years there were no significant differences between frail and non-frail individuals in seroprotection or seroconversion for any influenza strain. Frail individuals <65 years of age compared with non-frail individuals were more likely to be seroprotected and to seroconvert postvaccination. Linear regression models show the same pattern of significant differences between frail and non-frail for those <65 years but no significant differences between frailty groups for those $\geq$65 years. Additional research may elucidate the reasons for the differences observed between younger frail and non-frail adults.

6.2   INTRODUCTION

The burden of annual influenza is substantial. Approximately 226,000 hospital admissions (McLean HQ et al., 2014) and 23,000 deaths (Thompson WW et al., 2003) are attributed to influenza each year in the United States (U.S.); 90% of deaths occur among adults 65 years of age and older (Thompson, 2010). Annual influenza epidemics are estimated to result in: 3.1 million hospitalized days, 31.4 million outpatient visits, \$10.4 billion in direct medical costs and \$16.3 billion in lost earnings (Molinari NAM et al., 2007). Thus, influenza vaccination is recommended for everyone ≥6 months of age (Grohskopf LA et al., 2015a).

Older adults are less able to mount a robust immune response to antigens present in vaccines because of age-related changes in the immune system. For example, studies have shown lower humoral and cell-mediated immune system responses specific to influenza vaccination in older adults compared with younger adults (Gruver A et al., 2007). Moreover, antibody responses to influenza vaccine in older adults is associated with altered T-cell function and an overall decline in cell-mediated adaptive immunity response (McElhaney JE, 2011). Advancing age is also associated with increasing prevalence of frailty, the multi-dimensional syndrome marked by losses in function and physiological reserve (Espinoza S & JD, 2005). Frailty has been shown to accelerate immunosenescence, such that individuals determined to be frail have been shown to mount lower immune responses to antigen stimulation (Gruver A et al., 2007). Physical frailty, characterized by diminished strength, endurance, and reduced physiologic function (Morley JE et al., 2013), leads to increased risk of acute illness, falls, disability, hospitalization, institutionalization and mortality (Espinoza S & JD, 2005; Fried LP et al., 2001).

Relatively few studies of influenza vaccine immunogenicity among frail older adults exist. We found only two studies that specifically measured the impact of frailty on immune response to influenza vaccine using physical frailty measures. One study demonstrated that physical frailty is associated with lessened immunological response to influenza vaccine and greater influenza-like illness among community-dwelling adults >70 years of age (Yao X, Hamilton RG, et al., 2011). The other found no difference in post-vaccination geometric mean titer ratios between frail and non-frail groups of veterans aged ≥62 years (mean age=81 years) (Van Epps P et al., 2017). To our knowledge, similar studies of adults younger than 65 years of age have not been conducted. The purpose of this study was to examine the effect of physical frailty on immune response to influenza vaccine in community-dwelling adults ≥50 years of age and determine if those responses differed by age.

6.3   PATIENTS AND METHODS

Study design and participants

This was an observational prospective study of adults ≥50 years of age who were recruited from three family practices and the University of Pittsburgh community during the 2013-2014 influenza season (September-November 2013) using nonprobability convenience sampling. To be eligible, participants had to self-report prior season receipt of influenza vaccine, have no known egg allergies or Guillian Barré syndrome and not have already received but intended to receive the standard dose influenza vaccine for the current season. Participants were ineligible if they had an immunocompromising condition or were on immunosuppressant drugs, a history of allograft, or were cognitively impaired. Participants provided written informed consent prior to study initiation. The University of Pittsburgh Institutional Review Board approved this study.

Data collection

Baseline data were collected via interview with direct entry by the research assistants into REDCap™ (a secure, online database management system) (Harris PA et al., 2009). Baseline demographics included sex, race, ethnicity, self-reported age, presence (yes/no) and type (1 vs. 2) of diabetes, and smoking status. Height and weight from the electronic medical record (EMR) if available, or from self-report were used to calculate Body Mass Index (BMI). BMI was calculated as [weight (lb.) ÷ (height (in.)^2 X 703]; categorical obesity was defined as BMI ≥30. Questions on depression (9-item Patient Health Questionnaire), stress (4-item Perceived Stress Scale), Socioeconomic status (SES) (MacArthur Scale of Subjective Social Status, scored 0 low to 9 high) and overall health state (EQ-5D VAS, scored 0 low to 100 high) were also obtained at baseline.

Frailty

Physical frailty was measured at the Day 21 post-vaccination visit using a 4-item summed frailty score based on weakness, self-reported exhaustion, walking time and physical activity. Grip strength measured weakness, using a Layfayette hydraulic hand dynamometer (Model J00105, Lafayette Instrument Company, Lafayette, IN). Three measurements were taken on each hand while the participant was seated with his/her elbow flexed at 90° and shoulder adducted and neutrally rotated with the forearm and wrist held in a neutral position (Baker NA, Moehling KK, Desai AR, & NP, 2013). The average of these measurements for each side was then calculated; grip strength values for each side were age- and gender- adjusted to U.S. norms (Mathiowetz V et al., 1985).

The Short-Form Survey-12 (SF-12) (version 2, 4-week recall) was used to assess exhaustion (vitality scale), walking time (physical function scale) and physical activity (physical component summary score). Use of this instrument for these physical frailty components has been demonstrated in a systematic review of modifications to Fried et al's. (2001) frailty phenotype (Kanapuru B & WB, 2009; Theou O et al., 2015). Each of the SF-12 frailty components was adjusted to U.S. population norms (Maruish & DeRosa, 2009) using QualityMetric Health Outcomes™ 4.5 Scoring Software (Lincoln, RI).

The four frailty components were used as T-scores. Scores for any of the four components at or below the 25th percentile for this cohort were determined to be a deficit (Fried LP et al., 2001; Puts MTE et al., 2005). A 2-level categorical frailty variable was created by counting the number of deficits across the four components, with <2 deficits indicating non-frailty and ≥2 deficits indicating frailty. Missing values were allowed for one frailty component

and were imputed with zero (Fried LP et al., 2001; Theou O et al., 2012); participants with two or more missing frailty components were dropped from analysis.

Biological samples and laboratory methods

Non-fasting whole blood samples were obtained on participants at baseline (pre-) and 21 days post influenza vaccination (range 19-35 days) using serum tubes with clot activator and silicone coated interior additive (BD Vacutainer, REF 367820) and held at room temperature until centrifugation to separate serum. Aliquoted serum samples were frozen at -80°C until assayed. Following Centers for Disease Control and Prevention (CDC) standardized protocols, sera were tested in HAI assays against each vaccine strain included in the 2013-14 influenza vaccine using V-shaped 96-well microtiter plates, measuring the ability of antibodies to inhibit agglutination of hemagglutinin to turkey erythrocytes. HAI titers were the reciprocal dilution of the last well that contained inhibited agglutination; all tests were conducted in duplicate. Positive and negative serum controls were included in each plate. Outcome measures were $\log_2$ Geometric Mean Titers (GMTs), seroprotection and seroconversion. Seroprotection was defined as an HAI titer ≥1:40 post-vaccination at Day 0 and at Day 21. Seroconversion was defined as a 4-fold rise in HAI titer post-vaccination given a pre-vaccination of ≥10.

Influenza vaccine

After the blood draw at the baseline visit, all participants received an intramuscular injection of the 2013-14 seasonal trivalent influenza vaccine containing influenza strains A/H1N1/California/7/2009-pdm09-like virus, A/H3N2/ Texas/50/2012-like virus and B/Massachusetts/2/2012-like virus.

Statistical analyses

All analytical procedures were performed using SAS® 9.3 (Cary, NC). Due to the skewness of the HAI titers at Day 0 and Day 21 they were transformed using the $\log_2$ method. GMTs were computed by first calculating the means and 95% Confidence Intervals (CI) of the $\log_2$ HAI titers for each time point and then calculating the anti-log of those values.

Summary statistics of demographics and immunological response (seroconversion, seroprotection, GMTs) were conducted across all participants and by frailty status within age groups (<65 years and ≥65 years) using Chi-square/Fisher Exact tests for categorical variables and ANOVA/Kruskal Wallis for continuous variables. Proportions are reported for categorical variables and means and standard deviations or median and quartiles one and three are reported for continuous variables.

Differences in rates of seroconversion and seroprotection within age groups by frailty status were tested using Chi-square tests. Differences in GMTs within age groups by frailty status were tested using t-tests.

Logistic regression (seroconvesion and seroprotection at Day 21) and Linear regression ($\log_2$ transformed Day 21 antibody titers) models run separately by age group for each vaccine strain and by each outcome assessed the association of frailty with immunological response to influenza vaccination. Adjustment covariates were added to models based upon their univariate relationship to the outcomes, their effect on frailty estimates, and those noted to be associated with frailty. Initial models adjusted for sex, race, smoking status, obesity, depression, baseline health status (characteristics noted to be associated with frailty), SES and baseline HAI titers.

Due to small event sizes for the outcomes, covariates that were non-significant ($P > 0.05$) and showed no evidence of effect modification on frailty, no substantial change to frailty P-value

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estimates and no substantial change in overall model fit statistics, were removed from the final models. Emphasis was put on creating parsimonious models that were consistent across strains and outcomes. Final models were adjusted for BMI and baseline HAI titers.

Statistical significance of two-sided tests was set at type I error (alpha) equal to 0.0083 (0.05/6) after adjusting for multiple comparisons using Bonferroni correction. Nominal *P*-values of <0.05 are also reported.

6.4   RESULTS

Of the 114 enrolled, 8 participants were missing ≥2 of the frailty indicator components, leaving a total sample size for analysis of 106. Characteristics of the participants are presented in Table 3. Overall, participants were predominantly female (75%), White (56%), had a median age of 62.3 years (57.3-67.6), self-reported a median SES score of 5 (4-7), 53% reported baseline health at ≥80%, indicated average levels of stress (median 3), and 68% had low levels or no depressive symptoms. Thirty-four percent of the cohort were diabetic and over half (55%), were obese. Frail and non-frail participants ≥65 years did not differ in demographic or health characteristics. Conversely, among those <65 years of age, frail individuals as compared with non-frail individuals reported a significantly lower health state (64% <80% vs. 38% <80%) and lower SES (5; 3-6 vs. 5; 5-7).

Table 4 shows the percent of individuals in each frailty category by age group who seroconverted (top), were seroprotected at baseline and Day 21 (middle), as well as GMTs for each group (bottom). There were no significant differences between frail and non-frail individuals who were ≥65 years old.

Among those <65 years of age, statistically significant differences were seen between the frailty categories.   Notably, a greater percent of frail persons as compared to non-frail seroconverted to the A/H1N1 (34% vs. 8%, $P=0.008$) and A/H3N2 (59% vs. 22%, $P=0.002$) vaccine strains. Higher percentages of being seroprotected at Day 21 for each vaccine strain were evident for frail persons as compared to the non-frail in this age category and these differences were statistically significant for each strain (A/H1N1: 79% vs. 40%, $P=0.002$; A/H3N2: 86% vs. 62%, $P=0.03$; B: 90% vs. 68%, $P=0.04$). At Day 21, GMTs were higher for

the frail compared with the non-frail; these between-group differences were significant for A/H1N1 13.0 vs. 4.8, $P=<0.001$) and A/H3N2 (14.0 vs. 6.8, $P=0.01$) vaccine strains.

Table 5 provides logistic regression results for the outcome of seroprotection 21 days post-vaccination for each vaccine strain. Among persons <65 years of age, frailty was positively associated with post-vaccination seroprotection with frail individuals having greater odds of being seroprotected than non-frail individuals, adjusting for obesity and baseline $\log_2$ HAI titers. This was significant after multiple comparison adjustment for the A/H1N1 strain (OR: 8.79, 95% CI: 1.78-43.31, $P=0.008$). Adjusting for multiple comparisons, the overall effect of frailty on post-vaccination seroprotection levels did not vary by age group.

Logistic regression models for the outcome of seroconversion for each vaccine strain are shown in Table 5. Frailty was positively associated with seroconversion among those <65 years of age with frail individuals having greater odds of seroconverting than non-frail persons. This was significant after multiple comparison adjustment for the A/H3N2 (OR: 5.85, 95% CI: 1.86-18.40, $P=0.003$) vaccine strain. The overall effect of frailty on seroconversion status varied by age group and was significant after multiple adjustment comparison for the A/H3N2 ($P=0.005$) vaccine strain.

Linear regression models for each vaccine strain are shown in Table 5. Among those <65 years of age, frail individuals as compared to non-frail persons had small, approximately a ½ fold, but significant (using multiple comparison adjustment) increases in HAI post-vaccination titer levels for A/H1N1 and A/H3N2 vaccine strains (beta 0.55, $P=<0.001$ and beta 0.50, $P=0.005$ respectively). The effect of frailty on post-vaccination HAI titers varied by age and was significant after multiple comparison adjustment for the A/H3N2 ($P=0.002$) vaccine strain.

Although greater than half of our cohort was considered to be obese, obesity was only a nominally significant predictor of being seroprotected post-vaccination for the A/H1N1 vaccine strain (P=0.02) for those <65 years of age. SES and baseline health among frail persons <65 years of age was not significantly associated with immune system outcomes to influenza vaccine nor did they substantially change frailty estimates (data not shown). There was no evidence of an interaction between the predictor frailty and obesity or baseline $\log_2$ HAI titers for either post-vaccination seroprotection or seroconversion status.

6.5   DISCUSSION

To our knowledge, this is the first analysis conducted that assessed the effect of physical frailty on influenza vaccine immune response that includes community-dwelling persons younger than 65 years of age and the only study that stratifies these effects by age. One study which assessed vulnerability, a concept similar to frailty, among community-dwelling adults ≥50 years of age, found no consistent pattern of the effect of frailty on immunological response to the 2008-2009 influenza vaccine (Talbot HK et al., 2012).   Vulnerability in their cohort was significantly associated with seroconversion for the A/H1N1 vaccine strain only; only 10% of the cohort had high vulnerability scores and models did not stratify by age (Talbot HK et al., 2012).

Frailty in adults ≥65 years of age has been shown to result in lower immunological responses to influenza vaccine compared to non-frail persons (Yao X, Hamilton RG, et al., 2011). Interestingly, in our cohort, the opposite picture was seen for persons <65 years of age with frailty being a significant predictor of post-vaccination seroprotection status for the A/H1N1 vaccine strain, for the A/H3N2 vaccine strain for seroconversion status and for the A/H1N1 and A/H3N2 vaccine strains for $\log_2$ post-vaccination HAI titers.

Previously denoted as infirmity, frailty is now viewed as distinct from old age, disability and co-morbidity although there is overlap among these categories (Fried LP et al., 2004; Gilleard C & P, 2010; Theou O et al., 2012). Frailty is a multidimensional concept that involves a number of biological systems: nervous, endocrine, immune and musculoskeletal (Rodriguez-Mana L & Sinclair AJ, 2014) and is marked by losses in function and strength (Espinoza S & JD, 2005). The physical frailty definition is built around declines in mobility, strength, endurance, nutrition and physical activity (Espinoza S & JD, 2005; Fried LP et al., 2001). Of our 4-item frailty measure, the greatest median difference for both age groups was seen in the SF-12

physical component score (PCS) which is a summary report of broad physical health status. Lower PCS scores indicate greater limitations in physical functioning and role participation caused by physical problems, poor general health and higher levels of bodily pain (Maruish ME & Turner-Bowker Dm, 2009).

The associations among stress, SES and health are robust; indeed, a primary explanation of the association between low SES and poor health is exposure to stress (Matthews, Gallo, & Taylor, 2010). Stress is associated with worsening physical functioning, with decreased overall physical health and poor mental health (de Frias CM & E, 2015). Stress promotes immune dysfunction including increasing levels of inflammation and reducing the immune response to vaccines (Cohen S et al., 2001; Godbout & Glaser, 2006). In turn, chronic levels of circulating pro-inflammatory cells negatively affect both innate and adaptive immune response (Franceshi C et al., 2007) and certain pro-inflammatory cytokines (e.g., IL-6 and TNF-α) have been associated with increased risk of frailty (Ahmed N et al., 2007; Franceshi C et al., 2007). Surprisingly, the significantly lower levels of self-reported SES and baseline health in our cohort among frail persons <65 years of age was not significantly associated with immune system outcomes to influenza vaccine nor did they substantially change frailty estimates (data not shown).

Higher levels of chronic inflammation have also been associated with increased adipose tissue (Krabbe KS et al., 2004). Obesity in middle-age adults has been shown to initially result in higher fold increase of antibodies to influenza vaccine but to decline substantially with a 3-4 fold reduction in antibody response within 12 months of vaccination (Sheridan PA et al., 2012). Since the pandemic influenza outbreak in 2009, obesity has been considered an independent risk factor for both increased influenza-related morbidity and mortality (Sheridan PA et al., 2012). Obesity has also been linked to frailty (Ahmed N et al., 2007; Blaum CS et al., 2005; Morley JE

et al., 2013). Obesity was accounted for in each model; there was no evidence of effect modification of obesity on frailty (results not shown).

Strengths and Limitations

We used a 4-item physical frailty score. It is possible that our frailty sample size would have been higher for one or both age groups had a fifth frailty item been included, as has been noted in other research (Theou O et al., 2015).

The relatively small size of the sample was a limitation as it prevented the inclusion of all potential confounders in the final analyses and may have reduced power to detect significant differences in sample characteristics between the frail and the non-frail especially in the age stratified analyses. It is also possible that the absence of a significant moderating effect of obesity on the association of frailty with the outcomes was due to small numbers of obese and frail individuals within each age group. Sensitivity analyses including confounders of sex, race, smoking status, depression and baseline health found no evidence of effect modification of these covariates on frailty status for either age category nor were there significant changes with these included covariates on frailty estimates.

The factors that may limit the generalizability of these results are also its strengths. Although the racial distribution was dissimilar to the U.S. adult population, the large number of African-Americans in our sample demonstrated the ability to recruit and enroll minority populations in research studies and allowed us to assess racial differences in our outcomes. Furthermore, a larger proportion of the study group had diabetes or another chronic condition. While patients with diabetes were intentionally oversampled, the prevalence of diabetes and

other high risk conditions among adults <65 years of age may have contributed to the relatively high prevalence of frailty observed.

Though not all older people become frail, research with larger sample sizes might allow for further examinations of frailty by age to understand why physical frailty seems to have a positive effect on immune response to influenza vaccine in adults aged 50-64 years of age but not in those 65 and older, and to determine which specific factors may be driving this association.

6.6   TABLES

Table 3. Paper 1: Demographics overall and by frailty status stratified by age groups‡

Characteristics	< 65 years (N=66)			≥ 65 years (N=40)			Overall (N=106)
	Frail (N=29)	Non-frail (N=37)	*P* value[a]	Frail (N=18)	Non-frail (N=22)	*P* value[a]	
Age, Median (q1, q3)	57.3 (54.4-61.8)	58.3 (56.1-61.0)	0.28	70.5 (68.1-74.0)	68.0 (66.6-73.6)	0.24	62.3 (57.3-67.6)
White race, N (%)	15 (52)	19 (51)	0.98	12 (67)	13 (59)	0.75	59 (56)
Non-Hispanic, N (%)	29 (100)	36 (97)	1.00	17 (94)	21 (95)	1.00	103 (97)
Female, N (%)	23 (79)	28 (76)	0.78	12 (67)	17 (77)	0.50	80 (75)
Diabetic, N (%)	11 (38)	12 (32)	0.64	7 (39)	6 (27)	0.44	36 (34)
Current smokers, N (%)	11 (38)	7 (19)	0.09	3 (18)	2 (9)	0.64	23 (22)
BMI, N (%)							
<30 (not obese)	8 (28)	19 (51)	0.05	6 (33)	13 (59)	0.13	47 (45)
≥30 (obese)	21 (73)	18 (49)		12 (67)	9 (41)		57 (55)
Socioeconomic Scale, Median (q1, q3)[b]	5 (3-6)	5 (5-7)	0.02*	5 (4-7)	6 (5-7)	0.34	5 (4-7)
EQ VAS Health Scale, (median split), N %)[c]							
<80% at baseline	18 (64)	14 (38)	0.03*	10 (59)	7 (32)	0.09	49 (47)
≥80% at baseline	10 (36)	23 (62)		7 (41)	15 (68)		55 (53)
Perceived Stress Scale, Median (q1, q3)	5 (2-7)	4 (1-7)	0.27	2 (2-5)	2 (0-4)	0.18	3 (1-6)
PHQ-9 Depression, N (%)							
None to minimal depression	15 (52)	26 (70)	0.12	12 (71)	18 (82)	0.47	71 (68)

Table 3 Continued

(score 0-9)							
Mild to severe depression (score 10-27)	14 (48)	11 (30)		5 (29)	4 (18)		34 (32)
Frailty Items, Median, (q1, q3)[d]							
Grip Strength T-score, M(SD)	48.7 (11.9)	51.8 (8.8)	0.22	50.6 (12.2)	58.5 (11.7)	0.05	52.2 (11.3)
SF-12 Vitality T-Score	49.1 (39.2-49.1)	58.9 (49.1-58.9)	<.0001	49.1 (39.2-49.1)	58.9 (49.1-58.9)	<.0001	49 (49-59)
SF-12 Physical Functioning T-Score	41.3 (33.5-41.3)	49.2 (49.2-57.1)	<.0001	41.3 (33.5-41.3)	57.1 (49.2-57.1)	<.0001	49 (41-57)
SF-12 PCS T-Score	38.3 (36.2-41.1)	51.8 (45.9-57.0)	<.0001	37.8 (31.2-41.0)	52.1 (50.8-56.7)	<.0001	46 (39-53)

‡ Numbers may not add to 100% due to rounding

a- *P* values for tests: Chi-square/Fisher's Exact for categorical variables, Anova/Kruskal Wallis for continuous variables

b- Socioeconomic scale range is 1-9 where: 1=Worst off, 5=Middle, and 9=Best off

c- EQ-VAS Health scale range is 0-100 where 0=Worst imaginable health state and 100=Best imaginable health state (at baseline)

d- Physical Frailty items: Grip strength (weakness), SF-12: Vitality scale (exhaustion), Physical Functioning scale (walking time), Physical Component Summary score (physical activity health)

* significant at *P* value <0.05

Table 4. Paper 1: HAI responses to 2013-2014 vaccine strains, overall and by frailty status stratified by age groups

Immunological response measure	< 65 years (N=66)			≥ 65 years (N=40)			Overall (N=106) No. (%)
	Frail (N=29) No. (%)	Non-frail (N=37) No. (%)	*P value*[a]	Frail (N=18) No. (%)	Non-frail (N=22) No. (%)	*P value*[a]	
Seroconversion (4-fold rise at Day 21)							
H1N1 A/California/07/2009	10 (34)	3 (8)	0.008*	5 (28)	6 (27)	1.00	24 (23)
H3N2 A/Texas/50/2012	17 (59)	8 (22)	0.002*	2 (11)	7 (32)	0.15	34 (32)
B-Yamagata lineage B/Massachusetts/2/2012	11 (38)	8 (22)	0.15	5 (28)	7 (32)	0.78	31 (29)
Seroprotection Day 0 (HI titer ≥ 1:40)							
H1N1 A/California/07/2009	9 (31)	12 (32)	0.90	4 (22)	7 (32)	0.72	32 (30)
H3N2 A/Texas/50/2012	8 (28)	13 (35)	0.51	4 (22)	3 (14)	0.68	28 (26)
B-Yamagata lineage B/Massachusetts/2/2012	11 (38)	13 (35)	0.81	8 (44)	7 (32)	0.41	39 (37)
Seroprotection Day 21 (HI titer ≥ 1:40)							
H1N1 A/California/07/2009	23 (79)	15 (40)	0.002*	8 (44)	14 (64)	0.22	60 (57)
H3N2 A/Texas/50/2012	25 (86)	23 (62)	0.03*	9 (50)	12 (55)	0.77	69 (65)
B-Yamagata lineage B/Massachusetts/2/2012	26 (90)	25 (68)	0.04*	12 (67)	17 (77)	0.50	80 (75)
Immunological response measure	< 65 years (N=66)			≥ 65 years (N=40)			Overall (N=106) Mean (95% CI)
	Frail (N=29) Mean (95% CI)	Non-frail (N=37) Mean (95% CI)	*P value*[a]	Frail (N=18) Mean (95% CI)	Non-frail (N=22) Mean (95% CI)	*P value*[a]	
Geometric Mean Titers D0							
H1N1 A/California/07/2009	4.3 (2.8-6.6)	3.2 (2.2-4.6)	0.29	2.1 (1.4-3.1)	2.9 (1.8-4.6)	0.28	3.2 (2.6-3.9)
H3N2 A/Texas/50/2012	3.4	3.4	1.00	3.6	2.5	0.22	3.2

Table 4 Continued

	(2.5-4.6)	(2.5-4.6)		(2.4-5.4)	(1.7-3.6)		(2.7-3.8)
B-Yamagata lineage B/Massachusetts/2/2012	4.0 (2.8-5.7)	4.0 (2.7-5.9)	1.00	3.9 (2.3-6.3)	4.3 (2.8-6.5)	0.76	4.0 (3.3-4.9)
Geometric Mean Titers D21							
H1N1 A/California/07/2009	13 (8.4-19.8)	4.8 (3.6-6.6)	<.001*	5.9 (3.5-10.0)	6.4 (3.9-10.5)	0.81	6.9 (5.6-8.6)
H3N2 A/Texas/50/2012	14 (9.7-19.9)	6.8 (4.6-10.0)	0.01*	6.1 (3.7-10.0)	6.6 (4.4-9.9)	0.80	8.1 (6.5-10.0)
B-Yamagata lineage B/Massachusetts/2/2012	13 (9.1-18.2)	9.0 (6.3-12.8)	0.16	7.7 (4.5-13.3)	10.3 (7.3-14.5)	0.36	9.9 (8.2-12.1)

Seroconversion: 4-fold rise in post vaccination titer at Day 21 given Day 0 titer ≥ 10; Seroprotection: HI titer ≥ 40;

a- *P* value for tests: Chi-square/Fisher's Exact test (Seroconversion and Seroprotection D0 and D21); T-test (Geometric Mean Titers);

* significant at *P* value <0.05

Table 5. Paper 1: Multivariable Regressions: Associations of frailty on Seroprotection Day 21, Seroconversion, and Log$_2$ Day 21 HAI titers to 2013-2014 vaccine strains stratified by age group (adjusted for BMI and baseline log$_2$ titers)

Variable	< 65 years (N=66)				≥ 65 years (N=40)				P value for age difference
	Odds Ratio	95% Confidence Interval	P value		Odds Ratio	95% Confidence Interval	P value		
H1N1 A/California/07/2009 – Seroprotection Day 21[a]									
Frail vs. not frail	8.79	1.78 – 43.31	0.008**		0.54	0.12 – 2.52	0.43		0.009*
H3N2 A/Texas/50/2012 – Seroprotection Day 21[a]									
Frail vs. not frail	6.00	1.25 – 28.75	0.02*		0.36	0.06 – 1.99	0.23		0.014*
B/Massachusetts/2/2012 – Seroprotection Day 21[a]									
Frail vs. not frail	7.79	1.15 – 52.61	0.03*		0.46	0.05 – 4.67	0.51		0.05
H1N1 A/California/07/2009 - Seroconversion[b]									
Frail vs. not frail	6.86	1.55 – 30.37	0.011*		0.75	0.17 – 3.30	0.70		0.045*
H3N2 A/Texas/50/2012 - Seroconversion[b]									
Frail vs. not frail	5.85	1.86 – 18.40	0.003**		0.29	0.05 – 1.73	0.17		0.005**
B/Massachusetts/2/2012 - Seroconversion[b]									
Frail vs. not frail	2.68	0.81 – 8.78	0.107		0.61	0.14 – 2.68	0.51		0.221
H1N1 A/California/07/2009 – Log$_2$ D21 antibody titers[c]									
Frail vs. not frail	0.55	0.15	<0.001**		0.06	0.25	0.82		0.06
H3N2 A/Texas/50/2012 - Log$_2$ D21 antibody titers[c]									
	0.50	0.17	0.005**		-0.26	0.17	0.15		0.002**

Table 5 Continued

Frail vs. not frail									
B/Massachusetts/2/2012 - Log$_2$ D21 antibody titers[c]									
Frail vs. not frail	0.29	0.14	0.05		-0.17	0.14	0.23		0.04*

* significant at *p* value <0.05

** significant after adjusting for multiple comparisons 0.05/6, *p* value 0.0083

a- Event sizes for Seroprotecting at Day 21: H1N1 (N=38 <65 years; N=22 ≥65 years); H3N2: (N=48 <65 years; N=21 ≥65 years); B: (N=51 <65 years; N=29 ≥65 years)

b- Event sizes for Seroconverting: H1N1 (N=13 <65 years; N=11 ≥65 years); H3N2: (N=25 <65 years; N=9 ≥65 years); B: (N=19 <65 years; N=12 ≥65 years)

c- Linear regression equation: Log$_2$ D21 HAI titer = $B_0 + B_{1*Frail} + B_{2*BMI} + B_{3*log2\ baseline\ titer} + \varepsilon$

7.0   INTRODUCING PAPER 2

Ninety percent of influenza-related deaths in the United States occur in adults 65 years of age and older (Thompson 2010). Adults ≥65 years are also 10 to 30 times more likely than younger adults to experience acute respiratory failure attributed to influenza disease (Pilkinton MA and HK 2015) and complications due to influenza among older adults include higher rates of pneumonia, strokes and heart attacks (McElhaney JE 2011).

As described in the literature review and evident in Paper 1, frailty in adults 65 years and older tends to result in lower immune system response to influenza vaccination. To elicit a stronger immunological response to vaccine antigens and confer higher levels of protection against influenza disease, high dose influenza vaccine is available for adults ≥65 years of age. High dose vaccine has been shown to provide superior immunological response as well as increased efficacy against laboratory-confirmed influenza disease (DiazGranados CA, Dunning AJ et al. 2014) as compared to standard dose vaccine in older adults.

The parent study for this 2nd paper, which was a randomized controlled trial, demonstrated superior immunological response elicited by high dose vaccine among long-term care residents (Nace DA, Lin CJ et al. 2015). The purpose of this 2nd paper therefore was to determine if physical frailty among adults ≥65 years of age living in LTCs was associated with a differential immune response to influenza high dose and standard dose vaccine.

7.1 PAPER 2: FRAILTY AND IMMUNE SYSTEM RESPONSE TO FLU VACCINE IN LONG-TERM CARE RESIDENTS ≥ 65 YEARS OF AGE

The effect of frailty on antibody response to high dose and standard dose influenza vaccine among long-term care residents ≥65 years of age

7.2   ABSTRACT

Objective

To quantify the association of frailty with immune response to two different influenza vaccines in long-term care residents ≥65 years of age.

Methods

This was a secondary analysis using data from a randomized controlled trial (RCT) which enrolled long-term care residents during two influenza seasons 2011-2012 and 2012-2013 and examined the superiority and non-inferiority of high dose versus standard dose vaccine. Blood draws were performed pre- and 30 days post-vaccination each season for determining hemagglutination inhibition titers (HAI). Frailty was defined as walking speed <0.8 meters/second.

Results

Frailty had no impact upon likelihood of seroprotecting, seroconverting, or in post-vaccination $\log_2$ HAI titer levels in adjusted multivariable regressions for either vaccine group for the 2011-2012 season. For the 2012-2013 season in the standard dose group, frailty was nominally significant for the H3N2 vaccine strain with frail persons having lower adjusted odds of seroprotecting than non-frail persons; no significant frailty differences were seen in the high dose group for any vaccine strain. For the 2012-2013 season, frailty had no impact upon likelihood of seroconverting or in post-vaccination $\log_2$ HAI titer levels in adjusted multivariable regression for either vaccine group. However, when comparing the effect of frailty across the vaccine groups, we see significant differences (at multiple comparison adjustment and nominally) with

frail compared to non-frail individuals showing overall greater odds of seroprotecting and seroconverting in the high dose vaccine group than those in the standard dose group, particularly during the 2012-2013 season.

Conclusions

Significant differences were seen between vaccine types in the association of frailty with immunologic response among this cohort of long-term care residents ≥65 years of age. Future research should account for greater heterogeneity in study population and larger samples sizes.

7.3   INTRODUCTION

Influenza disease results in substantial morbidity and mortality costs. In the United States alone, approximately 226,000 hospital admissions (McLean HQ et al., 2014) and 23,000 deaths are attributed to influenza each year (Thompson WW et al., 2003); 90% of deaths occur in adults 65 years of age and older (Thompson, 2010). Adults ≥65 years are also 10 to 30 times more likely than younger adults to experience acute respiratory failure attributed to influenza disease (Pilkinton MA & HK, 2015). Moreover, complications due to influenza among those ≥65 years include higher rates of pneumonia, strokes and heart attacks (McElhaney JE, 2011). Due to the high burden of disease, influenza vaccination is recommended for everyone ≥6 months of age (Grohskopf LA et al., 2015b).

  With increased chronological age, comes senescence of the immune system and a decreased immune response to vaccine antigens. Studies have shown lower humoral and cell-mediated immune system responses specific to influenza vaccination in older adults compared with younger adults (Gruver A et al., 2007), with altered T-cell function and overall declines in cell-mediated adaptive immunity (McElhaney JE, 2011). It has been well documented that influenza outbreaks can result in attack rates >20% even among well-vaccinated long-term care (LTC) facilities (Lindley MC & CB, 2015), which is substantially greater than the 2.0-7.5% attack rates reported in the general population during the 2016-17 influenza season (CDC, 2016d).

  To elicit a stronger immunological response to vaccine antigens and confer higher levels of protection against influenza disease, high dose (HD) influenza vaccine, is available for adults

≥65 years of age. HD vaccine provides four times the vaccine antigen levels found in standard dose (SD) vaccine and has been shown to provide superior immunological response as well as increased efficacy against laboratory-confirmed influenza disease (DiazGranados CA et al., 2014). The superior immunological response elicited by high dose vaccine has also been noted in LTC residents (Nace DA et al., 2015).

In addition to immunosenescence, increasing age is associated with frailty. Frailty, is a multi-dimensional syndrome marked by losses in function and physiological reserve (Espinoza S & JD, 2005). Reduced physiological reserve, exhibited by slower gait speed, is a noted characteristic of frailty (Chen X et al., 2014; Clegg A, Young J, Iliffe S, Olde Rikkert M, & K, 2013). This reduced reserve is associated with increased risk of functional disability, falls, hospitalization, institutionalization, and is a predictor of all-cause mortality (Cummings SR, Studenski S, & L, 2014; Hornyak V, VanSwearingen JM, & JS, 2012).

Functional status has been documented to be an important confounder in estimates of influenza vaccine efficacy in reducing all-cause mortality (Chan TC et al., 2013; Jackson LA et al., 2006). Yet, DiazGranados et al. (2015), using a broad measure of frailty that included measures of functional status of adults ≥65 years of age, found that high dose vaccine elicited greater efficacy and higher serologic response to that of standard dose, irrespective of functional status (DiazGranados CA et al., 2015).

The purpose of this study was to determine if frailty, defined as gait speed of <0.8 meters/second, among adults ≥65 years of age living in LTC facilities was associated with a differential response to influenza HD and SD vaccine.

7.4   METHODS

Study design and participants

This study is a secondary analysis of data from an RCT that assessed the noninferiority and superiority of HD to SD influenza vaccine among 187 LTC residents (*Clinical Trials.gov* NCT01654224).   Detailed methods have been reported previously (Nace DA et al., 2015).   In brief, adults ≥65 years of age were recruited in 2011-2012 and 2012-2013 from 15 LTC facilities which included a mix of nursing homes, assisted-living or personal-care homes and independent living homes throughout greater Pittsburgh, PA.   Eligible participants demonstrated functional disability with a need for full or partial assistance in at least one activity of daily living (ADL) or at least two instrumental activities of daily living (IADL).   Participants were excluded if they had a life expectancy of <6 months, an allergic reaction to influenza vaccine or to eggs, a history of Guillain-Barré syndrome, current immunosuppression or expected immunosuppression within 6 months or use of immunosuppressant medications.

Based on a computer generated 1:1 randomization assignments, participants were allocated to either an SD or HD vaccine group at enrollment.   Neither the investigator evaluating the laboratory measurements, nor the enrolled participants were aware of their group assignment.

The Institutional Review Boards of the University of Pittsburgh and the Pennsylvania Department of Health approved the RCT study.   All participants (or their legal healthcare proxy) provided written informed consent prior to study procedures.

Data collection

Baseline data were collected via paper forms and later entered by the research assistants into REDCap™ (a secure, online database management system) (Harris PA et al., 2009). Demographics included age, sex, race, ethnicity, educational and marital status, current health state, smoking status, chronic diseases, current medications, and vaccination history. Functional disability status was assessed using ADLs and IADLs (higher scores indicate greater functionality, scores range from 0 to 14). Gait speed measured the time to walk 4 meters at usual pace allowing for a 2-meter run-in and cool-down period; walking aids were allowed. Other baseline measurements included a clinical assessment of height, weight and temperature. Categorical Body Mass Index (BMI) was defined as <25 and $\geq$25 kg/m^2. The type of LTC residence was recorded for each participant.

Determination of Frailty

Gait speed was calculated using the formula of distance/time. From this calculation, frailty was defined as walking <0.8 meters per second. This cut-point has been noted to indicate increased risk of poor health outcomes including that of mortality (Abellan van Kan G et al., 2009; Cruz-Jentoft AJ et al., 2010; Studenski S et al., 2011). A 2-level categorical variable was then created to indicate <0.8 as frail versus $\geq$0.8 as non-frail.

Biological samples and laboratory methods

Non-fasting whole blood samples were obtained on participants at baseline (pre-) and 30 days (±14 days) post- influenza vaccination. Tubes were refrigerated at 4ºC and taken to the processing laboratory daily. Aliquoted serum samples were frozen at -80°C until assayed.

Following Centers for Disease Control and Prevention (CDC) standardized protocols, sera were tested in hemagglutination inhibition (HAI) assays against each vaccine strain included in the 2011-2012 and 2012-2013 influenza vaccine using V-shaped 96-well microtiter plates, measuring the ability of antibodies to inhibit agglutination of hemagglutinin to turkey erythrocytes. HAI titers were the reciprocal dilution of the last well that contained inhibited agglutination; all tests were conducted in duplicate. Positive and negative serum controls were included in each plate.

Outcome measures were $\log_2$ Day 30 HAI titers, seroprotection and seroconversion. Seroprotection was defined as an HAI titer $\geq 1:40$ at Day 0 and at Day 30. Seroconversion was defined as a 4-fold rise in HAI titer post-vaccination given a pre-vaccination of ≥ 10.

Influenza vaccine

Following the baseline blood draw, all participants received via intramuscular injection the seasonal trivalent influenza vaccine. For the 2011-2012 season, the vaccine contained influenza strains of A/H1N1/California/7/2009-pdm09-like virus, A/H3N2/Perth/16/2009-like virus and B/Brisbane/60/2008-like virus. For the 2012-2013 season, influenza vaccine strains included that of A/H1N1/California/7/2009-pdm09-like virus, A/H3N2/Victoria/361/2011-like virus and B/Wisconsin/1/2010-like virus.

Statistical analyses

All analytical procedures were performed using SAS® 9.3 (Cary, NC). Geometric mean titers (GMTs) were computed by first calculating the means and 95% Confidence Intervals (CI) of the $\log_2$ HAI titers for each time point and then calculating the anti-log of those values.

Summary statistics of demographics and immunological response (seroconversion, seroprotection, GMTs) were conducted for all participants and by frailty status within vaccine groups (standard dose and high dose) using chi-square/Fisher exact tests for categorical variables and t-tests for continuous variables. Proportions are reported for categorical variables, and means and standard deviations are reported for continuous variables. Due to the skewness of the HAI titers at Day 0 and Day 30, they were transformed using the $\log_2$ function at each timepoint.

The association between frailty and immunological response to influenza vaccine within vaccine type were examined using logistic regression (seroconversion and seroprotection) and linear regression ($\log_2$ transformed Day 30 antibody titers). Models were run separately for each vaccine strain by each outcome each year.

As the emphasis was to create parsimonious models that were consistent across years, strains, and outcomes, model building used the outcome measure of post-vaccination seroprotection due to the literature reporting greater percentages of individuals who seroprotect as compared to serconvert. Initially, each association was examined accounting for *a priori* confounders of age, sex, race, BMI, heart disease and lung disease. Additional adjustment covariates were tested in the models if they were associated with the outcome in univariate analysis ($P<0.20$) or if significant differences for that variable were seen between the vaccine groups ($P<0.05$). Covariates were retained if they remained independently associated with the outcome ($P<0.05$). Final models for each outcome measure included covariates of sex, baseline $\log_2$ titers, educational level, and level of LTC type of home.

Sensitivity tests examined the influence of sex on the association between frailty and the outcome measure of post-vaccination seroprotection for the H1N1 vaccine strain in the 2012-2013 season in unadjusted and adjusted models.

Statistical significance of two-sided tests was set at type I error (alpha) equal to 0.0083 (0.05/6) after adjusting for multiple comparisons using Bonferroni correction. Nominal *P*-values are reported and tests with nominal significance (*P*<0.05) are reported.

7.5   RESULTS

Twenty-nine participants missing gait speed at baseline were excluded from analysis resulting in a total sample size of 158.   Twenty-seven participants were enrolled for both years. Characteristics of the participants are presented in Table 6.   Overall participants were Non-Hispanic White (100%), female (68%), educated (62% with college/bachelors/graduate school), were a mean age of 86.8 (±5.5) years, and reported good to excellent health at baseline (72%). Eighteen percent of the cohort was diabetic and over half (55%) were overweight or obese. Sixty-eight percent was living in independent care homes at time of enrollment.   Functional levels mean ADL score was 12.35 (±2.87) out of 14 and mean IADL score was 8.73 (±3.73) out of 14. Mean gait speed was 0.67 meters/second.

Significant differences were seen between frailty groups within each vaccine type. Notably, ADL and IADL scores were lower for frail as compared to non-frail in both vaccine groups. For frail SD recipients, ADL scores were 11.7±3.2 vs. 13.43±2.0 for the non-frail, $P=0.004$ and IADL scores were 7.61±3.6 vs. 11.36±2.8, $P<.0001$ for frail vs. non-frail. For frail HD recipients ADL scores were 11.68±3.2 vs. 13.62±0.9 for the non-frail, $P<.001$ and IADL scores were 7.21±3.7 vs. 10.72±2.5, $P<.0001$ for frail vs. non-frail. Frail persons were also more likely in both vaccine groups to be taking a greater number of medications (SD frail vs. non-frail: 9.76±4.2 vs. 7.46±4.3, $P=0.02$; HD frail vs. non-frail: 9.47±3.6 vs. 7.38±4.3, $P=0.03$) and to not be living independently as compared to non-frail (SD frail vs. non-frail: 63% vs. 89%, $P=0.01$; HD frail vs. non-frail: 51% vs. 86%, $P=0.001$). A greater percentage of frail persons than non-

frail were ≥85 years of age (78% vs. 50%, *P*=0.01) in the standard dose group. A greater percentage of frail than non-frail persons were female (83% vs. 31%, *P*=<.0001) in the high dose group.

Table 7 reports the percent of individuals in each frailty category by vaccine group who seroconverted and were seroprotected at baseline and Day 30 for each influenza vaccination season. There were no significant differences in seroprotection or seroconversion status between frail and non-frail individuals for either year in either vaccine group.

Table 8 provides the mean and 95% Confidence Interval (CI) for GMTs for each frailty category by vaccine group during each influenza vaccination season. During the 2011-2012 season, a significant difference was seen in the high dose vaccine group for the H3N2 vaccine with frail persons showing higher GMTs at baseline (14.9, 8.8-25.2) than non-frail persons (5.8, 4.8-7.1) after adjusting for multiple comparisons (*P*=0.004). For the 2012-2013 season, a significant difference was seen in the high dose vaccine group for the H1N1 vaccine with frail persons showing higher post-vaccination GMTs (63.5, 41.0-98.3) than non-frail persons (24.6, 15.5-39.1) persons after adjusting for multiple comparisons (*P*=0.008). In addition, within the standard dose group, non-frail persons had nominally higher baseline GMTs for the B vaccine strain (13.1, 8.4-20.4) than frail persons (7.7, 6.4-9.2) (*P*=0.04).

Logistic regression models for the outcome of post-vaccination seroprotection are shown in Table 9. Models, stratified by vaccine type, reflect the association of frailty on the outcome for each vaccine strain included in that influenza vaccination season while adjusting for sex, educational level, independent living status and baseline $\log_2$ HAI titers.

There were no significant differences in post-vaccination seroprotection status between frailty categories for either vaccine group during the 2011-2012 season. However, the overall

effect of frailty on seroprotection status at Day 30 was nominally significant between vaccine types (*P*=0.04) for the H3N2 strain, with the Odds Ratio (OR) for SD 0.06 (95% CI: 0.002-1.90) and OR for HD 3.28 (95% CI: 0.21-51.08). For the 2012-2013 season, a nominally significant difference was seen in the SD group for the H3N2 vaccine strain, with frail persons having lower odds of being seroprotected than non-frail persons (OR: 0.14, 95% CI: 0.02-0.85, *P*=0.03). No significant differences were seen between frailty categories in the high dose group for this season. The difference of the effect of vaccine type on the association between frailty and post-vaccination seroprotection was significant after adjusting for multiple comparisons for the H3N2 strain (*P*=0.004) and nominally significant for the B strain (*P*=0.02); SD (OR: 0.86, 95% CI: 0.21-3.43) vs. HD (OR: 0.90, 95% CI: 0.15-5.30).

Logistic regression models for the outcome of seroconversion are shown in Table 10. Models, stratified by vaccine type, show the association of frailty on the outcome for vaccine strains included in each influenza vaccination season while adjusting for sex, educational level, independent living status and baseline $\log_2$ HAI titers.

There were no significant differences in seroconversion status between frailty categories for either vaccine group during the 2011-2012 or 2012-2013 seasons. However, the association between frailty and seroconversion status varied by vaccine type and was nominally significant for all three vaccine strains for the 2012-2013 season. The difference for H1N1 was *P*=0.02 with the OR for SD (0.35, 95% CI: 0.07-1.92) vs. HD (OR: 1.10, 95% CI: 0.18-6.81). For H3N2 this difference was *P*=0.01 with the OR for SD (0.47, 95% CI: 0.12-1.85) vs. HD (OR: 1.10, 95% CI: 0.24-5.12). This difference for B was *P*=0.03 with the OR for SD (1.43, 95% CI: 0.32-6.30) vs. HD (OR: 0.62, 95% CI: 0.11-3.41). The effect of this difference between vaccine types is in the same direction for H1N1 and H3N2 with larger ORs noted for frail as compared to non-frail for

those receiving HD vaccine, however, the opposite direction in effect is seen for the B vaccine strain with a larger OR for frail as compared to non-frail for those in the SD vaccine group. No differences in effect between vaccine types were seen for the 2011-2012 season.

Linear regression models assessed the association of frailty on $\log_2$ post-vaccination HAI titers adjusting for sex, educational level, independent living status and baseline $\log_2$ HAI titers (Table 11). There were no significant differences in post-vaccination $\log_2$ HAI titers between frailty categories for either vaccine group during the 2011-2012 or 2012-2013 seasons. The overall effect of vaccine type on the association between frailty and post-vaccination HAI titer levels was not significant.

Sensitivity analyses which examined the unadjusted effect and comparative step-wise effects of sex on the adjusted association between frailty and post-vaccination seroprotection for the H1N1 vaccine strain in 2012-2013 found no change in interpretation (direction or significance of variables) between models.

7.6 DISCUSSION

The parent study was the first to evaluate the superiority and non-inferiority of high dose influenza vaccine to that of standard dose in a randomized clinical trial among LTC residents ≥65 years of age (Nace DA et al., 2015). They found that high dose vaccine produced significantly higher GMTs for all vaccine strains except that of H1N1 in 2012-2013 (Nace DA et al., 2015). Other studies conducted among community-dwelling adults ≥65 years also have noted this superior efficacy and immunologic response of high dose compared to standard dose influenza vaccine, (DiazGranados CA et al., 2014) even after adjusting for number of high-risk comorbidities and frailty/functionality conditions (DiazGranados CA et al., 2015).

Previously denoted as infirmity, frailty is now seen as distinct from old age, disability and co-morbidity although there is overlap among these categories (Fried LP et al., 2004; Gilleard C & P, 2010; Theou O et al., 2012). Frailty is a multidimensional concept that involves a number of biological systems: nervous, endocrine, immune and musculoskeletal (Rodriguez-Mana & Sinclair, 2014) and is marked by losses in function and strength (Espinoza & Walston, 2005). Among frail individuals lower immune system responses to antigen stimulation have been noted (Gruver A et al., 2007). Given the high morbidity and mortality burden of influenza disease, particularly in LTC facilities (Lindley MC & CB, 2015), it is important to assess the immunologic response of high dose and standard doze influenza vaccine in older adults when comparing across frailty statuses.

Gait speed, a noted frailty marker, is one of the five items used in Fried et al's. (2001) frailty phenotype measure (Fried LP et al., 2001). Yao et al. (2011) using the frailty phenotype measure, reported that frailty among community-dwelling adults >70 years of age was associated with lower levels of seroprotection and seroconversion status to influenza vaccine (Yao et al., 2011). However, in a study of veterans ≥62 years of age (median age 81 years), using the frailty phenotype measure, there were no significant differences in post-vaccination GMT levels between frail and non-frail groups (Van Epps P et al., 2017).

To our knowledge this is the first study to assess the differences between frailty groups on immunologic response to two different influenza vaccine types among LTC residents ≥65 years of age in two subsequent influenza vaccination seasons. In this study, there were no significant differences between frailty groups on seroconversion for either vaccine type in either year. Though post-vaccination GMTs in the high dose group for the H1N1 vaccine strain were significantly higher for the frail as compared to the non-frail in 2012-2013, no significant differences between frailty groups for post-vaccination $\log_2$ HAI titer response were evident for either vaccine type in either year. The only significant difference in frailty status on immunological response seen in our data was lower levels of seroprotection for frail as compared to non-frail for the standard dose H3N2 vaccine strain in 2012-2013. The reason this frailty difference was seen only for the H3N2 strain for this vaccine type is not clear.

Immunosenescence, chronically high levels of circulating inflammatory cytokines (inflammaging), and the overall declines evident in both innate and adaptive immunity that come with aging reduce vaccination response in older adults (Aspinall R et al., 2007; McElhaney JE et al., 2012). To help address this aging impairment, influenza vaccines have been enhanced to elicit greater immune system response.

Results from this study show little to no difference between frail and non-frail LTC residents in influenza immunologic response within each vaccine type. However, significant differences (one after adjusting for multiple comparisons and five nominally) were seen *between* vaccine types on the association of frailty for both seroprotection and seroconversion. Though all but one association was nonsignificant within vaccine type, comparisons of Odds Ratios reflect that, on the whole, frailty produced greater odds of being seroprotected and seroconverted to vaccine strains for those within the HD vaccine group, whereas frailty decreased odds of seroprotecting and seroconverting within the SD vaccine group. These findings fall in line with a recent study which found that HD vaccine was superior over SD in reducing hospitalization due to influenza-like illness for frail long-stay nursing home residents ≥65 years of age (Gravenstein S et al., 2017). The significance of our findings between vaccine types suggest that HD vaccine may be superior to SD for frail LTC residents; this finding is consistent with that reported by DiazGranados et al. (2015) who reported greater efficacy and serologic response of HD vs. SD in adults ≥65 years regardless of their functional status (DiazGranados CA et al., 2015).

Strengths and Limitations

This secondary analysis was conducted on participants enrolled in the first RCT conducted in LTC facilities to assess the difference in immune response between two difference influenza vaccines across two vaccination seasons. This, therefore, is the first analysis to test the effect of frailty of LTC residents ≥65 years of age on influenza vaccine immune response between frailty groups within two different influenza vaccines.

The challenges of recruitment in LTC populations has been documented (Nace DA et al., 2015). The small number of participants enrolled each year, particularly in the 2011-2012 season, may have affected the ability to detect any significant differences between frailty groups within vaccine type. This is likely as evidenced by the small cell sizes for the frailty groups; the original study was not powered to address the objective of this secondary analysis. Study participants were 100% Non-Hispanic Whites enrolled in one geographic location. This limits the generalizability of the study and might have precluded the ability to detect frailty immunogenicity response differences that may be present if examined among other racial or ethnic groups.

Conclusion

Overall, lower odds for both seroprotection and seroconversion were noted for frail LTC residents as compared to non-frail residents for those receiving SD vaccine, whereas higher odds were seen for frail as compared to non-frail LTC residents for those receiving HD vaccine; these differences within vaccine type were only nominally significant for the H3N2 vaccine strain in 2012-13 for SD recipients. Significant differences were seen between vaccine types on the association of frailty for both seroprotection and seroconversion. Future research in this area should account for greater heterogeneity in study population and larger sample sizes.

7.7   TABLES

Table 6. Paper 2: Demographics overall and by frailty status, stratified by vaccine type‡

Characteristics	Standard Dose (N=82)			High Dose (N=76)			Overall (N=158)[b]
	Frail (N=54)	Non-frail (N=28)	P value[a]	Frail (N=47)	Non-frail (N=29)	P value[a]	
Age, mean ± SD	86.96 ± 5.6	84.57 ± 5.8	0.07	88.19 ± 4.6	86.14 ± 6.0	0.10	86.75 ± 5.5
Age ≥ 85 years, N (%)	42 (77.8)	14 (50.0)	0.01*	38 (80.9)	19 (65.5)	0.13	113 (71.5)
Non-Hispanic White, N (%)	54 (100)	28 (100)	1.00	47 (100)	29 (100)	1.00	158 (100)
Female, N (%)	41 (75.9)	18 (64.3)	0.27	39 (83.0)	9 (31.0)	<.0001*	107 (67.7)
Diabetic, N (%)	11 (20.4)	5 (17.9)	0.79	8 (17.0)	5 (17.2)	1.00	29 (18.4)
Education, N (%)							
≤ High school	24 (45.3)	8 (28.6)	0.14	22 (46.8)	5 (17.2)	0.008*	59 (37.6)
College/bachelors/graduate	29 (54.7)	20 (71.4)		25 (53.2)	24 (82.8)		98 (62.4)
BMI, mean ± SD	26.94 ± 4.83	26.89 ± 4.98	0.96	25.68 ± 4.9	25.00 ± 3.8	0.51	26.20 ± 4.74
BMI, N (%)							
<25 (underweight-normal)	19 (35.2)	12 (42.9)	0.50	23 (48.9)	17 (58.6)	0.41	71 (44.9)
≥25 (overweight-obese)	35 (64.8)	16 (57.1)		24 (51.1)	12 (41.4)		87 (55.1)
Health status, N (%)							
Excellent/good	38 (70.4)	22 (78.6)	0.43	29 (61.7)	24 (82.8)	0.05	113 (71.5)
Fair/poor/cannot answer	16 (29.6)	6 (21.4)		18 (38.3)	5 (17.2)		45 (28.5)
ADL score, mean ± SD	11.70 ± 3.2	13.43 ± 2.0	0.004*	11.68 ± 3.2	13.62 ± 0.9	<.001*	12.35 ± 2.87
IADL score, mean ± SD	7.61 ± 3.6	11.36 ± 2.8	<.0001*	7.21 ± 3.7	10.72 ± 2.5	<.0001*	8.73 ± 3.73
Heart disease, N (%)	24 (44.4)	12 (42.9)	0.89	18 (38.3)	16 (55.2)	0.15	70 (44.3)
Lung disease, N (%)	9 (16.7)	3 (10.7)	0.74	7 (14.9)	1 (3.7)	0.24	20 (13.0)
Number of medications, mean ± SD	9.76 ± 4.2	7.46 ± 4.3	0.02*	9.47 ± 3.6	7.38 ± 4.3	0.03*	8.82 ± 4.18
Independent living, N (%)	34 (63.0)	25 (89.3)	0.01*	24 (51.1)	25 (86.2)	0.001*	108 (68.4)
Frailty Item							
Gait speed, mean ± SD	0.67 ± 0.24		-	0.67 ± 0.30		-	0.67 ± 0.27
< 0.8 m/sec, N (%)	54 (65.9)		<0.01*	47 (61.8)		0.04*	101 (63.9)
≥ 0.8 m/sec, N (%)	28 (34.2)			29 (38.2)			57 (36.1)

‡ Numbers may not add to 100% due to rounding
a- *P* values for tests: Chi-square/Fisher's Exact for categorical variables, t-test for continuous variables
b- Baseline gait speed data were missing for 29 subjects
* significant at *P* <0.05

Table 7. Paper 2: Seroconversion and Seroprotection responses to vaccine strains, overall and by frailty status, stratified by vaccine type

Immunological response measure	Standard Dose N=25			High Dose N=23			Overall N=48
2011-2012	Frail (N=15) No. (%)	Non-frail (N=10) No. (%)	*P* value[a]	Frail (N=14) No. (%)	Non-frail (N=9) No. (%)	*P* value[a]	No. (%)
Seroconversion (4-fold rise at Day 30)							
H1N1: A/California/7/2009	3 (20.0)	2 (20.0)	1.00	7 (50.0)	5 (55.6)	1.00	17 (35.4)
H3N2: A/Perth/16/2009	1 (6.7)	2 (20.0)	0.54	5 (35.7)	3 (33.3)	1.00	11 (22.9)
B: B/Brisbane/60/2008	0 (0)	0 (0)	-	3 (21.4)	0 (0)	0.25	3 (6.3)
Seroprotection Day 0 (HI titer ≥ 1:40)							
H1N1: A/California/7/2009	5 (33.3)	4 (40.0)	1.00	6 (42.9)	2 (22.2)	0.40	17 (35.4)
H3N2: A/Perth/16/2009	1 (6.7)	0 (0)	1.00	2 (14.3)	0 (0)	0.50	3 (6.3)
B: B/Brisbane/60/2008	2 (13.3)	2 (20.0)	1.00	2 (14.3)	5 (55.6)	0.07	11 (22.9)
Seroprotection Day 30 (HI titer ≥ 1:40)							
H1N1: A/California/7/2009	7 (46.7)	5 (50.0)	1.00	11 (78.6)	5 (55.6)	0.36	28 (58.3)
H3N2: A/Perth/16/2009	2 (13.3)	3 (30.0)	0.36	10 (71.4)	3 (33.3)	0.10	18 (37.5)
B: B/Brisbane/60/2008	3 (20.0)	2 (20.0)	1.00	6 (42.9)	4 (44.4)	1.00	15 (31.3)
	N=57			N=53			N=110
2012-2013	Frail (N=39) No. (%)	Non-frail (N=18) No. (%)	*P* value[a]	Frail (N=33) No. (%)	Non-frail (N=20) No. (%)	*P* value[a]	No. (%)
Seroconversion (4-fold rise at Day 30)							
H1N1: A/California/7/2009	2 (5.1)	4 (22.2)	0.07	10 (30.3)	3 (15.0)	0.33	19 (17.3)
H3N2: A/Victoria/361/2011	6 (15.4)	6 (33.3)	0.17	15 (45.5)	9 (45.0)	0.97	36 (32.7)
B: B/Wisconsin/1/2010	7 (18.0)	3 (16.7)	1.00	14 (42.4)	8 (40.0)	1.00	32 (29.1)

Table 7 Continued

Seroprotection Day 0 (HI titer ≥ 1:40)							
H1N1: A/California/7/2009	22 (56.4)	8 (44.4)	0.40	15 (45.5)	8 (40.0)	0.70	53 (48.2)
H3N2: A/Victoria/361/2011	1 (2.6)	2 (11.1)	0.23	2 (6.1)	0 (0)	0.52	5 (4.6)
B: B/Wisconsin/1/2010	1 (2.6)	2 (11.1)	0.23	5 (15.2)	0 (0)	0.14	8 (7.3)
Seroprotection Day 30 (HI titer ≥ 1:40)							
H1N1: A/California/7/2009	27 (69.2)	13 (72.2)	0.82	21 (63.6)	9 (45.0)	0.18	70 (63.6)
H3N2: A/Victoria/361/2011	7 (18.0)	8 (44.4)	0.05	17 (51.5)	10 (50.0)	0.91	42 (38.2)
B: B/Wisconsin/1/2010	9 (23.1)	8 (44.4)	0.10	18 (54.5)	8 (40.0)	0.30	43 (39.1)

Seroconversion: 4-fold rise in post vaccination titer at Day 30 given Day 0 titer ≥ 10; Seroprotection: HI titer ≥ 40

a- P value for tests: Chi-square/Fisher's Exact test

* significant at P value <0.05

Table 8. Paper 2: Geometric Mean Titer responses to vaccine strains, overall and by frailty status, stratified by vaccine type

Immunological response measure	Standard Dose			High Dose			Overall
	N=25			N=23			N=48
2011-2012	Frail (N=15) Mean (95% CI)	Non-frail (N=10) Mean (95% CI)	P^a	Frail (N=14) Mean (95% CI)	Non-frail (N=9) Mean (95% CI)	P^a	Mean (95% CI)
Geometric Mean Titers D0							
H1N1: A/California/7/2009	14.5 (8.0-26.1)	18.7 (7.3-47.7)	0.64	23.2 (12.1-44.7)	13.6 (7.4-24.9)	0.28	17.3 (12.3-24.4)
H3N2: A/Perth/16/2009	6.6 (4.2-10.4)	6.2 (4.6-8.2)	0.83	14.9 (8.8-25.2)	5.8 (4.8-7.1)	0.004 **	8.1 (6.3-10.3)
B: B/Brisbane/60/2008	10.5 (6.8-16.1)	15.2 (9.2-25.1)	0.29	15.6 (8.6-28.4)	25.2 (13.3-47.8)	0.31	15.0 (11.4-19.8)
Geometric Mean Titers D30							
H1N1: A/California/7/2009	23.0 (12.1-43.5)	28.3 (9.5-84.6)	0.73	92.8 (46.8-184.2)	46.7 (15.5-140.8)	0.28	41.2 (26.6-63.7)
H3N2: A/Perth/16/2009	9.1 (4.8-17.2)	10.7 (5.8-20.0)	0.74	46.4 (28.2-76.3)	18.5 (8.6-39.8)	0.05	17.3 (12.1-24.8)
B: B/Brisbane/60/2008	12.6 (8.5-18.6)	15.2 (10.0-23.0)	0.54	28.3 (18.6-43.1)	27.2 (14.3-51.9)	0.92	19.2 (15.0-24.5)
	N=57			N=53			N=110
2012-2013	Frail (N=39) Mean (95% CI)	Non-frail (N=18) Mean (95% CI)	P^a	Frail (N=33) Mean (95% CI)	Non-frail (N=20) Mean (95% CI)	P^a	Mean (95% CI)

Table 8 Continued						
Geometric Mean Titers D0						
H1N1: A/California/7/2009	35.3 (23.4-53.3)	26.2 (14.9-45.9)	0.42	25.7 (16.2-40.8)	18.7 (10.6-32.8)	0.40	27.2 (21.3-34.8)
H3N2: A/Victoria/361/2011	6.2 (5.3-7.3)	6.6 (4.8-9.0)	0.73	7.0 (5.6-8.7)	7.9 (6.3-9.8)	0.51	6.8 (6.1-7.5)
B: B/Wisconsin/1/2010	7.7 (6.4-9.2)	13.1 (8.4-20.4)	0.04 *	9.0 (6.7-12.1)	6.6 (5.5-7.9)	0.09	8.5 (7.4-9.8)
Geometric Mean Titers D30						
H1N1: A/California/7/2009	54.1 (37.2-78.8)	40.0 (21.7-73.3)	0.40	63.5 (41.0-98.3)	24.6 (15.5-39.1)	0.008 **	46.8 (37.1-59.2)
H3N2: A/Victoria/361/2011	12.8 (9.2-17.9)	19.2 (11.6-32.0)	0.19	23.2 (15.4-34.9)	23.0 (15.2-34.7)	0.99	18.2 (14.8-22.4)
B: B/Wisconsin/1/2010	16.2 (12.5-21.0)	23.3 (14.5-37.5)	0.15	29.8 (23.0-38.6)	21.4 (15.5-29.7)	0.13	21.7 (18.5-25.5)

a - value for tests: T-test

* significant at P value <0.05

** significant after adjusting for multiple comparisons 0.05/6=0.0083

Table 9. Table 2: Multivariable Logistic Regression: Association of frailty with Seroprotection at Day 30 to 2011-2012 and 2012-2013 vaccine strains, stratified by vaccine type[a]

Immunological response measure	Standard Dose				High Dose				
	N=25				N=23				
2011-2012	Odds Ratio	95% Confidence Interval	*P* value[b]		Odds Ratio	95% Confidence Interval	*P* value[b]		*P* value for vaccine difference
H1N1: A/California/7/2009									
Gait speed: frail vs. non-frail	0.58	0.04-8.30	0.69		2.00	0.21-19.25	0.55		0.20
H3N2: A/Perth/16/2009									
Gait speed: frail vs. non-frail	0.06	0.002-1.90	0.11		3.28	0.21-51.08	0.40		0.04*
B: B/Brisbane/60/2008									
Gait speed: frail vs. non-frail	1.04	0.04-25.86	0.98		2.64	0.23-30.56	0.44		0.49
	N=57				N=53				*P* value for vaccine difference
2012-2013	Odds Ratio	95% Confidence Interval	*P* value[b]		Odds Ratio	95% Confidence Interval	*P* value[b]		
H1N1: A/California/7/2009									
Gait speed: frail vs. non-frail	0.55	0.08-3.83	0.54		0.98	0.12-7.90	0.98		0.64

Table 9 Continued								
H3N2: A/Victoria/361/2011								
Gait speed: frail vs. non-frail	0.14	0.02-0.85	0.03*		1.20	0.22-6.58	0.83	0.004**
B: B/Wisconsin/1/2010								
Gait speed: frail vs. non-frail	0.86	0.21-3.43	0.83		0.90	0.15-5.30	0.91	0.02*

a- Models ran separately for each vaccine type and adjusted for sex, education level, independent living status and baseline $\log_2$ titers

* significant at *p* value <0.05

** significant after adjusting for multiple comparisons 0.05/6=0.0083

b- Event sizes for Seroprotecting at Day 30:

   2011-2012: H1N1 (N=12 SD; N=16 HD); H3N2: (N=5 SD; N=13 HD); B: (N=5 SD; N=10 HD)

   2012-2013: H1N1 (N=40 SD; N=30 HD); H3N2: (N=15 SD; N=27 HD); B: (N=17 SD; N=26 HD)

Table 10. Paper 2: Multivariable Logistic Regression: Association of frailty with Seroconversion at Day 30 to 2011-2012 and 2012-2013 vaccine strains, stratified by vaccine type[a]

Immunological response measure	Standard Dose N=25				High Dose N=23				
2011-2012	Odds Ratio	95% Confidence Interval	*P* value[b]		Odds Ratio	95% Confidence Interval	*P* value[b]		*P* value for vaccine difference
H1N1: A/California/7/2009									
Gait speed: frail vs. non-frail	0.32	0.02-5.46	0.43		0.76	0.09-6.55	0.80		0.12
H3N2: A/Perth/16/2009									
Gait speed: frail vs. non-frail	0.19	0.01-3.51	0.26		1.51	0.14-16.12	0.74		0.07
B: B/Brisbane/60/2008									
Gait speed: frail vs. non-frail	-	-	-		7.83	0.21-292.78	0.27		-
	N=57				N=53				*P* value for vaccine difference
2012-2013	Odds Ratio	95% Confidence Interval	*P* value[b]		Odds Ratio	95% Confidence Interval	*P* value[b]		
H1N1: A/California/7/2009									
Gait speed: frail vs. non-frail	0.35	0.07-1.92	0.23		1.10	0.18-6.81	0.92		0.02*
H3N2: A/Victoria/361/2011									
Gait speed: frail vs. non-frail	0.47	0.12-1.85	0.28		1.10	0.24-5.12	0.90		0.01*

Table 10 Continued

B: B/Wisconsin/1/2010								
Gait speed: frail vs. non-frail	1.43	0.32-6.30	0.64	0.62	0.11-3.41	0.58		0.03*

a- Models ran separately for each vaccine type and adjusted for sex, education level, independent living status and baseline $\log_2$ titers

* significant at *p* value <0.05

** significant after adjusting for multiple comparisons 0.05/6=0.0083

b- Event sizes for Seroconversion:

   2011-2012: H1N1 (N=5 SD; N=12 HD); H3N2: (N=3 SD; N=8 HD); B: (N=0 SD; N=3 HD);

   2012-2013: H1N1 (N=6 SD; N=13 HD); H3N2: (N=12 SD; N=24 HD); B: (N=10 SD; N=22 HD)

Table 11. Paper 2: Multivariable Linear Regression: Association of frailty on Log$_2$ D30 antibody titers to 2011-2012 and 2012-2013 vaccine strains, stratified by vaccine type[a]

Immunological response measure	Standard Dose N=25				High Dose N=23				
2011-2012	**Beta**	**Standard Error**	***P* value**		**Beta**	**Standard Error**	***P* value**		***P* value for vaccine difference**
H1N1: A/California/7/2009									
Gait speed: frail vs. non-frail	-0.50	0.51	0.35		0.84	1.07	0.45		0.65
H3N2: A/Perth/16/2009									
Gait speed: frail vs. non-frail	-0.67	0.58	0.27		0.51	0.63	0.43		0.56
B: B/Brisbane/60/2008									
Gait speed: frail vs. non-frail	-0.10	0.32	0.76		0.89	0.46	0.07		0.39
	N=57				N=53				***P* value for vaccine difference**
2012-2013	**Beta**	**Standard Error**	***P* value**		**Beta**	**Standard Error**	***P* value**		
H1N1: A/California/7/2009									
Gait speed: frail vs. non-frail	0.13	0.25	0.60		0.54	0.45	0.24		0.06
H3N2: A/Victoria/361/2011									
Gait speed: frail vs. non-frail	-0.34	0.37	0.36		-0.09	0.52	0.87		0.42
B: B/Wisconsin/1/2010									
Gait speed: frail vs. non-frail	0.04	0.36	0.91		0.14	0.37	0.71		0.27

Linear regression equation: Log_2 D30 HAI titer $= B_0 + B_{1*Gaitspeed} + B_{2sex} + B_{3education} + B_{4independentcare} + B_{5*log2\ baseline\ titer} + \varepsilon$

a- Models ran separately for each vaccine type and adjusted for sex, education level, independent living status and baseline log_2 titers

* significant at *p* value <0.05

** significant after adjusting for multiple comparisons 0.05/6=0.0083

8.0   INTRODUCING PAPER 3

In the process of preparing to analyze the effect that frailty had upon immune system response in older adults, I became interested in the psychosocial factors noted to be associated with frailty in the literature. As reported in Section 4.8, depression and stress, which I refer to in this 3rd study as quality of life, were highly associated with frailty. While reviewing the literature I also came across an article which highlighted results from a systematic review of 3,300 quantitative studies which reported mostly positive effects that religion and spirituality had upon mental health including depression and stress.

These relationships among religion/spirituality, depression and stress led me to the question of whether spirituality can modulate the association between depression or stress and frailty in older adults. Therefore, this 3rd study takes a different approach than that of the first two studies. Within this study I utilize a similar population to that used in the 1st study, a racially diverse sample of community-dwelling older adults 50 years of age and older.

8.1   PAPER 3: AFFECT OF SPIRITUALITY UPON THE DEPRESSION AND STRESS FRAILTY RELATIONSHIP

Title: The relationship between depression, stress and frailty and the role of spirituality among community-dwelling adults ≥ 50 years of age

Keywords: depression, stress, spirituality, frailty, adults

8.2   ABSTRACT

Introduction

Depression and stress are noted to be associated with frailty. Religion and spirituality (R/S) have been shown to be associated with reduced depression, stress-resilience and psychological well-being. This study examines whether spiritualty moderates the relationship of depression and stress with frailty.

Methods

This is a cross-sectional analysis of 110 community-dwelling adults ≥ 50 years of age enrolled in a prospective cohort study of influenza vaccine immune system response in the fall of 2015. Depression and stress were combined into a 3-level quality of life (QoL) indicator variable to denote high QoL, mid QoL, and poor QoL. Final logistic regression models tested the relationship between quality of life and frailty and whether spirituality modified this relationship in three multivariate models (base, parsimonious, fully adjusted). The most parsimonious model was chosen based upon the Akaike Information Criterion (AIC) value.

Results

This was a racially diverse (41% African American), female (72%) cohort that reported high levels of spirituality, low levels of stress and low levels of depression. Spirituality significantly moderated the association of QoL with frailty in all three regression models (*P* ranged 0.008 to 0.03) for those with mid QoL: base (Model 1): β= -1.97, *P*=0.003; parsimonious (Model 2): β= -2.07, *P*=0.006; fully adjusted (Model 3): β= -2.08, *P*=0.009. Of the additional demographic and

health covariates adjusted for in the full model, only age was a significant independent predictor of frailty. Odds Ratios (OR) and 95% Confidence Intervals (CI) from Model 2 showed that the effect of QoL upon frailty varied by level of spiritualty. As spiritualty decreased, the importance of having good QoL to prevent frailty became evident; this was significant only for those at the lowest levels of spiritualty (ref=high QoL; mid QoL - OR: 8.75; 95%CI: 1.20-64.09; poor QoL – OR: 8.23; 95% CI: 1.34-50.74).

Conclusion

Future research in this area should include larger sample sizes, a longitudinal design and include measures of R/S coping as well as other psychosocial factors noted to be associated with stress resilience and reduced stress-induced depression.

8.3 INTRODUCTION

Attendant to advancing age is a complex interplay of immunological, physical, physiological, behavioral, and environmental changes. These changes are associated in varying degrees with physical and mental impairment that increase risk for chronic disease, susceptibility to infectious disease, reduced cognition and poor affect. One frequent syndrome found among aging individuals is frailty. Frailty, is a multi-dimensional syndrome marked by losses in function and physiological reserve (Espinoza S & JD, 2005), influenced by disruptions to homeostatic mechanisms across physiological systems (such as inflammatory, skeletal muscle, endocrine, and hematologic), the central nervous system, and molecular and cellular systems (Morley JE et al., 2013; Walston J, 2004). Frail individuals are at increased risk for experiencing falls, disability, hospitalization, institutionalization, morbidity and death (Espinoza S & JD, 2005; Fried LP et al., 2001). Although not all older adults become frail, the risk of frailty increases with age. Prevalence of physical frailty among community-dwelling adults $\geq$ 65 years ranges from 4-17% (Collard RM et al., 2012).

Depression and stress are known to be related to frailty in older adults. The relationship between depression and frailty is bi-directional, with each increasing the risk of the other (Soysal P et al., 2017). Additionally, depression is associated with impairments in physical, social, and role functioning (Bromberger JT & T., 2009). Depression is typically categorized as clinical depression, minor (subsyndromal) and major depressive disorder (MDD), or as elevated depressive symptom levels.

Among community-dwelling adults ≥ 65 years of age, prevalence of clinically significant depressive symptoms have been reported to be 15%, whereas prevalence of MDD ranges from 1-5% (Fiske A, Loebach Wetherell J, & Gatz M, 2009). Prevalence of MDD is higher among women than men of all ages (Alexopoulos GS, 2005; Fiske A et al., 2009); there are few differences by ethnicity or race, although SES factors are noted to be an important risk factor for depression (Fiske A et al., 2009).

Depression onset is most frequent in young adults. However, a substantial minority of individuals experience a later onset. Late onset of depression is defined as the first episode of depression taking place in later adulthood (≥ 65 years). Late onset subthreshold depression (minor/subsyndromal depression combined) is 2-3 times more prevalent than MDD among adults ≥ 65 years of age (Meeks T, Vahia I, Lavressky H, Kulkarni G, & Jeste D, 2011). Prevalence rates of subthreshold depression in the general population (not age specific) ranges from 2.9-9.9% in primary care and 1.4-17.2% in community settings (Rodriguez MR, Neuvo R, Chatterji S, & JL., 2012). Among adults > 65 years, 16% of primary-care patients have reported subthreshold depression (Alexopoulos GS, 2005). Risk factors for late onset subthreshold depression (minor/subsyndromal) include increased medical burden, female gender, decreased social support and neurological illnesses (Meeks T et al., 2011).

Adults with subthreshold depression are at high risk for developing MDD. A study of 622 primary care patients ≥ 60 years of age reported that, compared to those with no depression, patients meeting criteria for minor or subsyndromal depression had a 5.5-fold increased risk for developing MDD one year later, independent of demographic characteristics (Lyness JM et al., 2006).

Contributors to late onset MDD depression include brain structural changes, stressful life events (e.g., changes in finances and living situation, bereavement, loss of social roles), changes in health (physical, cognitive), chronic medical diseases, and limitations to daily activities among others (Alexopoulos GS, 2005; Fiske A et al., 2009).

Perceived stress has also been associated with poor mental and physical health and decreased physical functioning in mid- and late-life (de Frias CM & E., 2015). A number of studies (The National Academies, 2004) have shown that stress is associated with morbidity and mortality. Stress has been defined as "environmental demands that tax or exceed the adaptive capacity of an organism, resulting in biological and psychological changes that may be detrimental and place the organism at increased risk for disease [or disability]" (The National Academies, 2004). Perceived stress reflects the individual's experience of environmental demands being excessive and often out of his/her control.

Women report higher levels of perceived stress than men, older adults report lower levels of stress than do younger adults (Warttig SL, Forshaw MJ, South J, & AK, 2013) and African Americans, Asians and Hispanics report higher levels of stress compared to Caucasians (The National Academies, 2004). Higher levels of cortisol which are considered stress hormones are associated with higher levels of reported perceived stress (Ezzati A et al., 2014). Dysregulation of cortisol has recently been posited as underlying the musculoskeletal pathophysiology of frailty (Johar H et al., 2014). The associations among stress, SES and health are robust. Studies report that persons of lower SES experience both more frequent negative life events and greater acute and chronic stressors than higher SES individuals (Matthews et al., 2010). A primary explanation of the association between low SES and poor health is exposure to stress (Matthews et al., 2010).

As stress and depression seem to have wide-ranging effects on the health and well-being of older adults, significant research has been undertaken to explore the factors that may mitigate the effects of depression and stress on physical health and frailty. Three main categories have emerged when examining the protective effects of psychosocial factors which appear to buffer older adults against developing depression or experiencing depressive symptoms in the context of stressful life events and biological changes: 1) resources (cognitive, health, SES), 2) psychological strategies and social support used to manage them, and 3) the role of meaningful engagement (including that of religion) (Fiske A et al., 2009).

This study will focus on spirituality as a moderator of the association of stress/depression with physical frailty in older adults. The constructs of religion and spirituality (R/S) are closely related. Religion has been defined as referencing an external, institutionalized, group affiliated, organized system of doctrinal beliefs, practices and rituals related to the transcendent or Divine, whereas spirituality represents a more personal, internal search for and discovery of the transcendent or Divine which may or may not develop out of a religious identity or affiliated community (Bailly N & Roussiau N, 2010; Hill PC & Pargament KI, 2003; Koenig HG, 2012). A broad definition of spirituality is "that which gives meaning and purpose to one's life and connectedness to the significant and sacred" (Bremault-Phillips et al., 2015).

An association of R/S levels with age has been reported with older adults reporting higher R/S than younger adults (Bailly N & Roussiau N, 2010; Skarupski KA, Fitchett G, Evans DA, & Mendes de Leon CF, 2010). Gender and racial differences also exist. Women report higher levels of R/S than men and African Americans report increased R/S compared to other races (Skarupski KA et al., 2010).

The associations between R/S and physical and mental health have been well established. As of 2010 there were 3,300 quantitative studies examining the effects of R/S on mental and physical health of which over 80% have focused on mental health (Koenig HG, 2012). In a systematic review by Koenig (2012), 73% to 93% of studies reported direct, positive associations between R/S and positive emotions such as well-being/happiness (326 studies), hope (40 studies), optimism (32 studies), meaning and purpose (45 studies) self-esteem (69 studies) and sense of control during challenging life circumstances (21 studies), (Koenig HG, 2012). Of 444 studies that examined R/S and depression, significant inverse relationships with depression were reported for 61% and only 6% found that R/S was significantly positively associated with higher levels of depression (Koenig HG, 2012). Of 31 studies that assessed the relationship or effect of R/S on stress hormones (cortisol, epinephrine, and norepinephrine), 74% reported significant protective associations/effects of R/S and none noted negative associations/effects on stress hormone levels (Koenig HG, 2012). The association between R/S and psychological well-being has been shown to be stronger for older adults than younger adults (Bailly N & Roussiau N, 2010).

These relationships among R/S, depression and stress lead to the question of whether R/S can modify the association between depression or stress and frailty in older adults. Only one study has examined the relationships among spirituality, psychosocial well-being (PWB) and frailty (Kirby SE, Coleman PG, & Daley D, 2004). PWB was based on six factors: environmental mastery, personal growth, positive relations with others, purpose in life, self-acceptance and autonomy. The investigators found among 233 British adults ≥ 65 years old living in warden-controlled retirement housing, that frailty was negatively associated with PWB, spirituality was positively associated with PWB and there was a significant modifying effect of

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spirituality upon frailty which reduced the negative effect of frailty upon total PWB. The authors concluded that spirituality may be a resource for maintaining PWB.

The aim of this study was to determine if spirituality modifies the relationship between a combined depression/stress quality of life indicator and frailty among community-dwelling adults ≥ 50 years of age. Our hypothesis was that among community-dwelling adults ≥ 50 years of age, the association between poor and mid-level quality of life and frailty would be attenuated for those reporting higher levels of spirituality.

8.4 METHODS

Study design and participants

This is a cross-sectional analysis of an observational prospective study of community-dwelling adults ≥ 50 years of age enrolled in a study which analyzed pre- and post- immune system response to the 2015-2016 influenza vaccine. Participants were recruited from three primary care practices and the University of Pittsburgh community in the fall of 2015 using nonprobability convenience sampling. Eligibility for the parent study included at least one prior season's receipt of influenza vaccine, no known allergies to the vaccine or vaccine components, and intent to receive the 2015-2016 influenza vaccine. Persons who had an immunocompromising condition or were on immunosuppressant drugs, had impaired cognition, or a history of allograft transplants were ineligible. The University of Pittsburgh's IRB approved this study and all participants provided written informed consent prior to study procedures.

Measures

Demographics

Sex, race, ethnicity, date of birth, socioeconomic status (SES) (MacArthur Scale of Subjective Social Status, scored 0=low to 9=high), presence (yes/no) and type (1 vs. 2) of diabetes, smoking status, and overall health state (EQ-5D VAS, scored 0=low to 100=high) were collected at enrollment. Height and weight, obtained from the electronic medical record (EMR), if available, or from self-report were used to calculate body mass index (BMI), calculated as [weight (lb.) ÷

(height (in.)^2 X 703]. Religious affiliation was assessed using the question, "what is your present religion, if any?" and corresponding response categories from those outlined in the Pew U.S. Religious Landscape Survey (Pew Research Center, 2017).

Depression

Depression was measured using the 9-item Patient Health Questionnaire (PHQ-9). The PHQ-9 assesses how much an individual has felt bothered by each of nine depressive symptoms on a likert scale over the last two weeks; scores range from 0-27 with higher scores indicating greater depression. The PHQ-9 consists of the nine criteria necessary for diagnosing depressive disorders according to the American Psychiatric Association Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition (DSM-IV) (Kroenke K, Spitzer RL, & JBW, 2001).

To distinguish between level of depression severity, the PHQ-9 uses score ranges for cut points. Scores ranging from 0-4 denote minimal depression, scores 5-9 mild depression, scores 10-14 moderate depression, scores 15-19 moderately severe depression and scores 20-27 reflect severe depression (Kroenke K, Spitzer RL, et al., 2001). For clinical use, this instrument records scores of 0-4 to be none to minimal depression with "no treatment needed," and scores 5-27 noted as mild to severe depression with treatment recommended varying from "watchful and retest" to "immediate use of pharmaceuticals" (Kroenke K & RL, 2002). A PHQ-9 score of $\geq$ 10 has an 88% sensitivity and 88% specificity for MDD (Kroenke K, Spitzer RL, et al., 2001). Recently, scores of $\geq$ 8 on the PHQ-9 have been shown to have > 80% sensitivity and specificity for MDD (Manea L, Gilbody S, & McMillan D, 2012). Lower cut-points with similar levels of specificity and sensitivity have also been used for capturing MDD; cut points vary by geographic

location and age of the population studied (Chen I-P et al., 2016; Han C et al., 2008; Lamers F et al., 2008). The PHQ-9 has been validated for use in older adults (Phelan E et al., 2010).

Following guidelines for scores outlined for clinical use and treatment, as our interest was to capture the distinction between no depression versus the range of minor depression to MDD, the PHQ-9 was dichotomized to create a 2-item binary variable using the cut-point of scores ≤ 4 (none to minimal depression) vs. scores ≥ 5 (mild to severe depression).

Stress

Stress was measured using the 4-item Perceived Stress Scale (PSS-4). The PSS-4 is a global stress measure that uses a 5-item Likert scale to assess one's appraisal of stress experienced over the past four weeks. It measures the degree to which an individual may have felt his/her life was unpredictable, uncontrollable and overloaded – aspects noted as key components of the stress experience (Cohen S et al., 1983). Scores range from 0-16 with higher scores indicating greater levels of perceived stress. Numerous studies have confirmed the reliability and validity of this instrument across a variety of settings, ages and languages (Cohen S et al., 1983; Lee, 2012; Warttig SL et al., 2013). The factor structure and predictive validity of the PSS-4 compares to the original PSS 14-item scale (Cohen S & GM, 1988; Cohen S et al., 1983).

There are no established cut-points for the PSS-4 scale. Instead scores are typically compared to a normative value (Warttig SL et al., 2013). However, a recent study using the PSS to assess stress in community-dwelling adults ≥ 70 years of age, utilized weighted quartiles with the first quartile denoting lowest levels of stress serving as the reference group (White RS, Jiang J, Hall CB, & Katz MJ, 2014). Studies using the PSS scales have shown that stress levels decline with age, with lowest perceived stress levels reported among persons ≥ 65 years of age

(Warttig SL et al., 2013). Following the example of White et al. (2014), a 2-item binary variable for PSS-4 was created using the weighted quartile q1 score of ≤ 4 to denote low stress vs. > 4 to denote high stress.

Quality of Life

Quality of life (QoL) is a multidimensional concept that includes ones' subjective perceptions of QoL as demonstrated by physical, mental, emotional, and social functioning (HealthyPeople.Gov, 2010). QoL can include broad measures of overall assessments for each domain or can focus specifically on certain types of QoL within domains. Two aspects that are important components of QoL are perceptions of stress and depressive symptoms.

Within this sample, stress and depression were found to be highly correlated. Therefore, a QoL variable was developed by combining depression and stress categories as follows: high quality of life (ref=0) was defined as those who were neither depressed nor stressed, or stressed only; mid-quality of life was defined as those who were depressed only; and poor quality of life was defined as those who were both depressed and stressed. Due to the low number of stressed-only persons within our sample and upon review of the slopes of the interaction between QoL, spirituality and the outcome variable frailty indicating that depression was the driving factor of the relationship, stressed only persons were included with the neither depressed nor stressed (see Figure 2, Appendix 2).

Spirituality

Spirituality was measured using the Daily Spiritual Experience Scale (DSES). The DSES has been proven reliable and valid in a number of studies (Ellison CG & D, 2008; Underwood, 2011;

Underwood LG & Teresi JA, 2002). The DSES is a 16-item self-administered questionnaire which captures the everyday, ordinary spiritual experiences of recognizing and relating to the divine or transcendent, and how these experiences in turn, inform daily life (Underwood, 2011). Many of the questions are theistic in nature (i.e., "God"), however, the introduction makes clear that if the term "God" is not a helpful construct, to substitute whatever word brings to mind the "divine or holy" (Underwood, 2011).

Responses for the first 15-items which use a 6-item Likert scale, were coded from 1 (never) to 6 (many times a day) so that higher scores reflect greater spirituality (Underwood, 2006). Based upon the recommendation provided by the scales author, the 16th item, which assesses one's overall relational closeness with God, uses a 4-item Likert scale, is reverse coded and adjusted to fit the 6-item scale used for the other questions (Ellison CG & D, 2008; Underwood, 2006). All 16 items were then summed for each respondent; summed scores can range from a low of 16 to a high of 96. For use in analyses, DSES scores were averaged across the 16 items with scores ranging from 1 to 6 (Underwood, 2006); this score was then centered at its mean for use as an interaction term in analyses.   A 3-item categorical spirituality score was also created based on the distribution of scores in this cohort. This 3-item score used tertial cut points rounded to whole numbers to note high DSES, mid-DSES, and low DSES.

Interleukin-6 (IL-6)

Non-fasting blood was collected into red-top (serum) tubes (Vacutainer, Becton-Dickinson). Samples remained at room temperature until transported to the processing laboratory within 4 hours of draw; during transport samples were placed into a cold cooler. Samples were brought to room temperature prior to centrifugation (1500g for 15 minutes at 4ºC). Aliquots of 1mL were

stored at -70ºC until analysis. IL-6 (200 µl of sample) was measured in duplicate, using a commercial high sensitive ELISA kit (R&D Systems, Minneapolis, MN, cat# HS600) following standard procedures. Due to the skewness of IL-6 values they were transformed using the $\log_2$ method.

Frailty

Physical frailty was measured using a 4-item summed frailty score based on weakness, self-reported exhaustion, walking time and physical activity. Grip strength measured weakness using a hydraulic hand dynamometer (Model J00105, Lafayette Instrument Company, Lafayette, IN) following standard protocol (Baker NA et al., 2013). Three measurements were taken on each hand. The average for each side was calculated and grip strength values were age- and gender adjusted to U.S. norms (Mathiowetz V et al., 1985).

The Short-Form Survey-12 (SF-12) (version 2, 4-week recall) was used to assess exhaustion (vitality scale), walking time (physical function scale) and physical activity (physical component summary score). Each SF-12 component was adjusted to U.S. population norms (Maruish & DeRosa, 2009) using QualityMetrics Health Outcomes™ 4.5 Scoring Software (Lincoln, RI). The use of this instrument for assessing physical frailty has been reported in a systematic review of modifications to Fried et al.'s. (2001) frailty phenotype (Kanapuru B & WB, 2009; Theou O et al., 2015).

The four frailty components were used as T-scores. Scores for any of the four components at or below the 25th percentile for the cohort were determined to be a deficit (Fried LP et al., 2001). The number of deficits across the four components were counted and a 2-level categorical variable was created with < 2 deficits indicating non-frailty and ≥ 2 deficits

indicating frailty. Missing values were allowed for one frailty component (Fried LP et al., 2001; Theou O et al., 2012); participants with two or more missing frailty components were dropped from analysis.

Statistical analyses

All analytical procedures were performed using SAS® 9.3 (Cary, NC). Summary statistics of demographics were conducted for overall and by frailty status using chi-square/Fisher exact tests for categorical variables and t-tests/Wilcoxon for continuous variables. Proportions are reported for categorical variables and means and standard deviations or medians (Q1, Q3) are reported for continuous variables. The association of quality of life and spirituality with frailty was examined using logistic regression.

Initial univariate analyses tested the association of the independent variables, hypothesized moderator and the *a priori* confounders of age, race, gender, smoking status, BMI and IL-6 with the outcome frailty. Covariates associated with the outcome ($P<0.20$) were retained in multivariate models.

Three multivariate models were conducted: Model 1 – main effects of quality of life and spirituality and quality of life*spirituality interaction term on frailty; Model 2 - model 1 plus additional covariates included in the parsimonious model; Model 3 – model 2 plus covariates known to be associated with frailty (fully saturated model). The most parsimonious model was based upon the AIC value. A test for interactions was conducted for each demographic covariate separately with quality of life and with spirituality using the model 1 as the base.

Statistical significance of two-sided tests was set at type I error (alpha) equal to 0.05.

8.5   RESULTS

Description of sample

Eight participants missing ≥ 2 frailty items were excluded from analysis resulting in a total sample size of 110. Characteristics of the participants are presented in Table 12. Overall participants were predominantly Caucasian (58%), female (72%), obese with mean BMI of 32.0 ± 7.6, and had $\log_2$ IL-6 levels at a mean of 1.8 ± 1.0. Mean age for this cohort was 65 ± 7.1 years. Participants reported low levels of perceived stress with a mean score of 3.7 and reported low levels of depressive symptoms with a total PHQ-9 median score of 5.0 (44% of the cohort fell into the 0-4 range denoting minimal depression; 46% had scores 5-9 showing mild depression). An average SES was noted with mean score at 5.5 and 55% reported $\geq 75\%$ health score at baseline. Spirituality was high in this cohort with median DSES of 73.0 which is comparable to normative range for U.S. adults ≥ 18 years of age (Underwood, 2006). Forty-four percent of the cohort self-identified as Protestant with the majority of the remainder reporting Catholic as their religious affiliation.

Comparisons of frail and non-frail characteristics

Table 12 shows that significant demographic differences existed between frailty groups. A greater percentage of frail as compared with non-frail persons were African American and were current smokers. Frail persons also reported lower SES, poorer health, higher levels of depressive symptoms and had higher $\log_2$ IL-6 levels than non-frail persons.

Relationship of additional included covariates

Table 13 indicates the associations between demographic variables, quality of life, spirituality and frailty included in the parsimonious Model 2. Quality of life was weakly but significantly, negatively associated with health. Health was also weakly but significantly, negatively associated with frailty. Those who reported greater health showed higher mean SES scores than those with poorer health. SES was moderately and significantly inversely related to $\log_2$ IL-6 levels. Significant differences were seen in mean values of spirituality by QoL group with highest mean spirituality reported for those with mid quality of life.

Regression models

Table 14 reports the logistic regression beta coefficients, standard error, adjusted R^2, c and *P* values for each of the final models. All three models show that the interaction term between quality of life and spirituality was significant (Model 1: *P*=0.008; Model 2: *P*=0.02; Model 3: *P*=0.03). Within each of these models, the interaction term of spirituality and quality of life shows mid-quality of life performing differently from that of high and poor quality of life. Only the interaction term of spirituality and mid-quality of life was significant (Model 1: β= -1.97, *P*=0.003; Model 2: β= -2.07, *P*=0.006; Model 3: β= -2.08, *P*=0.009). None of the added covariates in Model 2 had significant coefficients. With the additional covariates included in Model 3, the interaction between quality of life and spirituality remained significant and age became significant (age, β=0.09, *P*=0.03).

Table 15 reports the unadjusted percentages of being frail for each quality of life level within each of the tertile DSES categories. There was no consistent pattern of categorical spirituality upon quality of life for unadjusted percent of frailty.

The Odds Ratio (OR) and 95% Confidence Intervals (CI) using Model 2 (with continuous DSES) report the comparison effect of mid and poor quality of life vs. high quality of life upon frailty at each tertile DSES level of high, mid, and low spirituality (refer to Table 16). The effect of quality of life on frailty varied by the level of spirituality. Within both high and middle levels of spirituality, there was no significant relationship between mid and poor quality of life (vs. high QoL) upon frailty. However, for those with low levels of spirituality, both mid and poor quality of life resulted in much higher odds of frailty than for those with high quality of life (mid QoL, OR: 8.75; 95% CI: 1.20-64.09; poor QoL, OR: 8.23; 95% CI: 1.34-50.74).

Appendices

Table 17 (Appendix 1) provides means and standard deviations by frailty status for the mean DSES score used in analyses and for each of the DSES 16-item components. There were no differences between frailty groups in any of the DSES item scores.

Figure 2 (Appendix 2) shows the interaction plot of continuous DSES upon depression and stress using a 4-level QoL measure and frailty. This 4-level QoL measure consisted of: neither depressed or stressed, stressed only, depressed only, both depressed and stressed. As noted previously in methods, review of this plot led to a 3-level QoL measure being used in models as the interaction among the QoL measures, spirituality, and frailty showed that depression was the driving factor of the spirituality and QoL-frailty relationship and there were only 9 individuals in the stressed only category (not shown).

Figure 3 (Appendix 3) shows the final interaction plot of continuous DSES upon depression and stress (e.g., the chosen 3-level QoL measure) and frailty. The slopes within this interaction plot reveal differences in the effect of spirituality upon quality of life which varied by

robust vs. frail states. Non-frail persons with mid quality of life had high spirituality scores but within this QoL level, spirituality scores were lower for frail persons. For high and poor quality of life, spirituality scores were higher for frail than for those who were non-frail.

8.6   DISCUSSION

This is one of only two studies which have examined whether spirituality moderates the quality of life-frailty relationship. Our sample of older adults was diverse with 41% African American. Substantial chronic morbidity was evident with 37% self-reporting diabetes.   Twenty-four percent of the cohort were current smokers which is substantially above not only national U.S. estimates of 15.1% but also higher than that reported for the state of Pennsylvania (16.4-20%), higher than those reported nationally across all racial groups (7-22%) as well as that noted for adults $\geq$ 65 years of age (8.4%) (CDC, 2015a). This predominately female cohort self-reported average SES, good health, high spirituality and low levels of stress and depression.

It is not surprising that higher $\log_2$ IL-6, lower SES and poorer self-rated health were more prevalent in frail as compared to non-frail persons as these factors are known to be associated with frailty (Espinoza S & Fried LP, 2007). Nor is it surprising that health and quality of life, health and SES, and SES and $\log_2$ IL-6 were significantly associated. Dunlop et al. (2003) noted the increased health burden of chronic disease, functional limitations, and health behaviors (such as smoking) that African American adults aged 54-65 years bore compared to the Caucasian respondents (Dunlop DD, Song J, Lyons JS, Manheim LM, & Chang RW, 2003). The authors remarked that once these health conditions and SES were taken into account, standardized depression prevalence rates were lowered 24% among African Americans in that sample, making their overall depression rates significantly lower than that of Caucasians

(Dunlop DD et al., 2003). Consistent with other studies of depression in African Americans and Caucasians (Somervell PD, Leaf PJ, Weissman MM, Blazer DG, & Livingston Bruce M, 1989).

As seen in Table 14, the overall direction of the beta coefficients for the DSES x quality of life interaction terms are similar across all three models, though slightly closer to zero once accounting for additional covariates. The c-statistic for each of the three models was reasonable to good and the overall model variance explained was highest for the fully saturated model. These results indicate that even after covariates known to be associated with frailty are controlled for, spirituality modifies the effect of quality of life upon frailty. The significance of this association was evident for those with mid-quality of life (e.g., depressed only persons); results indicate that those with mid-quality of life performed differently than those with high or poor quality of life.

Spirituality scores varied by level of quality of life with the highest mean DSES score evident for those with mid quality of life (mean scores not reported). A review of Table 15 shows that there is no consistent effect of categorical spirituality by quality of life on the unadjusted percent of persons who are frail. Adjusted ORs and 95% CIs from the most parsimonious Model 2, which included DSES as a continuous measure, show that the effect of quality of life on frailty varied by the level of spirituality (Table 16). As spirituality decreased, quality of life became more important as a predictor of frailty. The overall trend of the ORs are in line with our hypothesis, however, the significance of the effect of quality of life upon frailty was only evident for those with low levels of spirituality – highest odds of frailty for those with mid and poor quality of life (vs. high QoL).

The results of this study are similar to those noted by Kirby et al. (2004), who found that spirituality significantly moderated the relationship of frailty with psychological well-being by

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reducing the negative effect of frailty upon PWB (Kirby SE et al., 2004). The authors reported that this moderating effect of spirituality was consistent with literature noting that spirituality is often used as a resource and that R/S has been found to have both direct and indirect positive effects upon well-being. Other studies have noted a beneficial effect of R/S upon mental health (Koenig HG, 2012). Of 70 prospective cohort studies which examined the relationship between R/S and depression, 56% saw quicker remission of depression or overall lower levels of depression for those with greater R/S (Koenig HG, 2012).

Koenig (2001) proposes four reasons to explain the positive associations found between R/S and mental and physical health (Koenig H, 2001). Firstly, R/S provides a context within which both positive and negative experiences are given meaning and this meaning allows circumstances to be appraised through a different, perhaps more optimistic lens. Secondly, R/S beliefs and practices often evoke positive emotions (joy, gratitude, awe) which in turn may help alleviate the stressors encountered in daily life. Thirdly, religion provides an undergirding of ritualistic practices which sanctify and thereby ease major life transitions as well as provide space for the community to rally around supporting those enduring difficult seasons. Lastly, religious beliefs provide rules which guide social norms for that which is seen as acceptable or deviant behavior and thereby if followed, provide a context which often reduces negative behaviors.

The ability to re-frame, to re-assess in a positive light, to accept, to persevere and to grow from challenges have been noted as key psychosocial factors that are found in stress-resilient individuals undergoing highly stressful circumstances (Southwick SM, Vythilingam M, & Charney DS, 2005). In a review of the literature on psychosocial and neurobiological factors associated with resilience to stress and stress-induced depression, R/S is listed as one of these

key psychosocial factors (Southwick SM et al., 2005). The authors of this review note that R/S is an important component that should be included within cognitive behavioral therapeutic interventions for persons either suffering from, or at-risk of developing stress-induced depression.

As evident from graphical representation of the interaction of spirituality upon quality of life on frailty (Figure 3, Appendix 3), mid and poor quality of life persons who were frail had higher mean spirituality scores than those who were non-frail, whereas the inverse was seen for those with mid quality of life (e.g., depressed only persons). Research on R/S and coping have found that R/S can have both positive and negative effects on health (Pargament KI, Smith BW, Koenig HG, & Perez L, 1998).

Some studies have noted worse physical health among those who were more spiritual or religious and postulated that greater levels of stress and poorer health prompt some individuals to turn to R/S for comfort and hope (e.g., the stressor response model) and therefore greater R/S may be a response to increased disability and chronic pain (Rippentrop A-E, Altmaier EM, Chen JJ, Found EM, & Keffala VJ, 2005). Poorer mental health has been related to negative religious coping in some studies with persons who report negative religious coping often citing feelings of being abandoned by God, feeling punished by God, questioning God's power and avoiding reliance on God (Maltby J & Day L, 2004; Rippentrop A-E et al., 2005).

A longitudinal study conducted among medically ill elderly persons, noted that type of religious coping (positive or negative) was significantly predictive of both spiritual outcome and changes in both mental and physical health with positive coping patterns associated with health improvement and negative coping with health declines (Pargament KI, Koenig HG, Tarakeshwar N, & Hahn J, 2004). Specifically, within this population, negative religious coping was

associated with reduced independence in daily living, poorer cognition, worse quality of life and greater depressed mood (Pargament KI et al., 2004). Among elderly patients being treated for depression, religious practice and religious coping were both cross-sectionally associated with depression outcomes and greater levels of positive religious coping was also significantly related to lower six-month depression scores independent of demographic characteristics and baseline depression scores (Bosworth HB, Park K-S, McQuoid DR, Hays JC, & Steffens DC, 2003).

Studies which have utilized the DSES survey have noted that higher DSES was independently associated with greater psychological well-being (positive affect, personal growth, purpose in life, self-acceptance, environmental mastery, etc.) (Greenfield EA, Vaillant GE, & Marks NF, 2009) and that DSES taps factors important for well-being which is above and beyond those noted to be influenced by religious practices (Ellison CG & D, 2008). Studies using the DSES to examine associations of spiritualty upon depression have mixed results. One study reported that higher levels of DSES were associated with lower levels of depression in women aged 18-78 years of age independent of demographic and psychosocial factors of anxiety and social support suggesting a protective role of spirituality and that this was above and beyond the variance explained by the mediating role of social support (Bennett KS & Shepherd JM, 2012). Another study noted that the link between spiritualty and depression was indirect and mediated by optimism, volunteering and social support among racially diverse adults $\geq$ 45 years of age (Mofidi M et al., 2007). As has been noted earlier, the nature of the relationship of R/S on depression is complex (Baetz M, Griffin R, Bowen R, Koenig HG, & Marcoux E, 2004).

With an increasingly aging population and the concomitant costs associated with increased medical utilization, it is important to address the psychosocial aspects at play within the physical frailty phenotype. Both cross-sectional and longitudinal research has shown that

depression is associated with frailty (Espinoza S & Fried LP, 2007) and that there appears to be a bi-directional relationship between depression and frailty with each in turn, increasing the risk of the other (Soysal P et al., 2017). Fried et al., the author of the physical frailty phenotype (Fried LP et al., 2001), states that there is a biologically plausible explanation for how depression or depressive symptoms lead to frailty. They note that the side effects that often come along with depression - weight loss and declined activity, can lead to decreased muscle mass and strength and reduced reserve making one more vulnerable to acute illnesses (Espinoza S & Fried LP, 2007). The stress hormone cortisol has been recently cited as being the factor underlying the musculoskeletal pathophysiology of frailty (Johar H et al., 2014).

A recent meta-analysis which examined the association between frailty and quality of life among community-dwelling adults ≥ 60 years of age, reported that frailty (and prefrailty) was consistently inversely associated with both worse physical and mental quality of life (Kojima G, Iliffe S, Jivraj S, & Walters K, 2016). The authors suggested that interventions targeting frailty may have additional benefit of increased quality of life. Conversely, R/S is one psychosocial component that may have beneficial effects upon quality of life which in turn may positively impact health and help with the continuation of a more robust state.

Strengths and Limitations

To our knowledge this is one of only two studies which have been conducted in older persons that have examined the relationship of spirituality upon quality of life in regard to frailty. Our results, though utilizing different measures, saw a similar beneficial moderating effect of spirituality upon the quality of life-frailty association and our sample was racially diverse.

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This is a cross-sectional analysis therefore it is limited in its ability to make inferences on the directionality of spirituality and quality of life. It is quite possible that bi-directional relationships exist with R/S and quality of life and within the designs of this study, the direction cannot be untangled. We did not assess R/S coping measures or other psychosocial factors beyond R/S noted as important for stress resilience and stress-induced depression. Though R/S has been cited as a key psychosocial factor, there may be other factors which contribute to stress resilience and positive (or negative) coping that may contribute to depression and frailty.

Additionally, beyond diabetes, we did not ascertain other medical conditions, therefore, health conditions which may be related to quality of life and frailty measures were not able to be accounted for within our models and residual confounding may exist.

Conclusion

R/S has been noted as an important factor in promoting stress resilience, reducing stress-induced depression and overall promoting better mental health. Depression and stress are two psychosocial factors known to be associated with frailty. This study suggests that spirituality has an important moderating effect upon the relationship of quality of life and frailty. Future research in this area should include larger sample sizes, a longitudinal design and measures of R/S coping, as well as other psychosocial factors noted as being key aspects of stress resilient individuals.

8.7 TABLES

Table 12. Paper 3: Demographics overall and by frailty status‡

	Overall (N=110)	Frail (N=39)	Non-frail (N=71)	*P* value[a]
Characteristics				
Age, Mean ±SD	65.20 ± 7.1	66.2 ± 7.2	64.7 ± 7.0	0.30
White race, N (%)	64 (58.2)	18 (46.2)	46 (64.8)	0.06
African American race,[b] N (%)	45 (41)	21 (54)	22 (31)	**0.02***
Female, N (%)	79 (71.8)	28 (71.8)	51 (71.8)	1.00
Diabetic, N (%)	41 (37.3)	17 (43.6)	24 (33.8)	0.31
Current smokers, N (%)	26 (23.9)	15 (38.5)	11 (15.7)	**0.01***
BMI, Mean ±SD	32.0 ± 7.6	33.6 ± 7.5	31.1 ± 7.5	0.10
Socioeconomic Scale, Mean ±SD[c]	5.5 ± 1.8	5.0 ± 1.9	5.7 ± 1.7	**0.04***
EQ VAS Health Scale, (median split), N (%)[d] ≥ 75% at baseline	59 (54.6)	16 (41.0)	43 (62.3)	**0.03***
Perceived Stress Scale, Mean ±SD	3.7 ± 2.7	4.4 ± 3.1	3.4 ± 2.4	0.07
PHQ-9 Depression, Median (q1, q3)	5.0 (3.0, 7.0)	6.0 (4.0, 9.0)	5.0 (3.0, 6.0)	**0.02***
PHQ-9 Depression, N (%)				
None to minimal depression (0-4)	48 (44)	14 (36)	34 (48)	0.23
Mild to severe depression (5-27)	62 (56)	25 (64)	37 (52)	
Log$_2$ IL-6, Mean ± SD	1.8 ± 1.0	2.2 ± 0.8	1.6 ± 1.1	**0.001***
Total DSES score, Median (q1, q3)	73.0 (55.0, 81.0)	71.0 (62.0, 81.0)	74.0 (51.0, 81.0)	0.74
Protestant Religious Affiliation, N (%)	48 (43.6)	20 (51.3)	28 (39.4)	0.23

‡ Numbers may not add to 100% due to rounding
a- *P* values for tests: Chi-square/Fisher's Exact for categorical variables, T-test/Wilcoxon for continuous variables
b-Other racial categories represented: Asian, N=1 and Native Hawaiian/Pacific Islander, N=1
c- Socioeconomic scale range is 1-9 where: 1=Worst off, 5=Middle, and 9=Best off
d- EQ-VAS Health scale range is 0-100 where 0=Worst imaginable health state and 100=Best imaginable health state (at baseline)
* significant at *P* value <0.05

Table 13. Table 3: Tests of association between key variables (N=110)

Tested variables	DF	Test statistic value	*P* value
Quality of life vs. frailty	110	0.13[a]	0.14
Quality of life vs. health	108	-0.22[a]	**0.01***
Frailty vs. health	108	-0.21[a]	**0.03***
Quality of life vs. age	109	1.27[b]	0.28
Quality of life vs. SES	109	1.13[b]	0.33
Quality of life vs. spirituality	106	5.07[b]	**0.008***
Quality of life vs. IL-6	109	0.45[b]	0.64
Frailty vs. age	109	1.11[b]	0.30
Frailty vs. SES	109	4.47[b]	**0.04***
Frailty vs. spirituality	106	0.43[b]	0.51
Frailty vs. IL-6	109	10.86[b]	**0.001***
Health vs. age	107	0.12[b]	0.73
Health vs. SES	107	4.45[b]	**0.04***
Health vs. spirituality	104	0.31[b]	0.58
Health vs. IL-6	107	3.84[b]	0.05
SES vs. age	110	0.02[c]	0.83
SES vs. spirituality	107	-0.03[c]	0.78
SES vs. IL-6	110	-0.30[c]	**0.001***
Spirituality vs. IL-6	107	0.05[c]	0.63
Spirituality vs. age	107	-0.12[c]	0.22
IL-6 vs. age	110	0.15[c]	0.11

Associations of variables included in table are those in the Step 2 Model
a-Test used: Kendall's Tau Rank Correlation, Tau
b-Test used: ANOVA, F value
c-Test used: Pearson's Correlation, Rho
 * significant at *P* <0.05
High QoL(reference group)=not depressed not stressed, or stressed only; Mid-QoL=depressed only; Poor QoL=both depressed and stressed

Table 14. Paper 3: Results of multivariate logistic regression analysis of quality of life, spirituality and demographics as predictors of frailty

	Model 1			Model 2			Model 3		
	β	SE	P	β	SE	P	β	SE	P
Intercept	-1.24	0.44	0.005	-4.14	2.54	0.10	-7.71	3.20	0.02
DSES	1.35	0.55	0.01*	1.43	0.61	0.02*	1.36	0.64	0.03*
QoL			0.12			0.13			0.09
High	0.00	REF	-	0.00	REF	-	0.00	REF	-
Mid	0.82	0.60	0.17	0.78	0.68	0.25	0.80	0.71	0.26
Low	1.22	0.60	0.04	1.38	0.69	0.05	1.60	0.74	0.03
Interaction term			0.008*			0.02*			0.03*
DSES*high-QoL	0.00	REF	-	0.00	REF	-	0.00	REF	-
DSES*mid-QoL	-1.97	0.65	0.003*	-2.07	0.75	0.006*	-2.08	0.80	0.009*
DSES*poor-QoL	-1.11	0.64	0.08	-1.09	0.69	0.11	-1.09	0.72	0.13
Age				0.06	0.04	0.14	0.09	0.04	0.03*
SES				-0.25	0.15	0.10	-0.20	0.16	0.22
Health $\geq$ 75% (ref=<75)				-0.56	0.49	0.26	-0.55	0.52	0.29
Log_2 IL-6				0.47	0.27	0.08	0.38	0.29	0.20
Male (ref=female)							0.58	0.58	0.32
African American (ref=non A.A.)							0.44	0.60	0.46
BMI							0.02	0.03	0.72
Smokers (ref=non/past)							1.07	0.60	0.08
R^2		0.193			0.350			0.396	
c		0.701			0.800			0.823	

*P <0.05

High QoL(reference group)=not depressed, not stressed, or stressed only; Mid-QoL=depressed only; Poor QoL=both depressed and stressed

DSES=spirituality; score is centered at its mean for all three models

Table 15. Paper 3: Unadjusted percentage of being frail by spirituality/quality of life categories

Spirituality	Quality of Life	Number of people	Number Frail	Unadjusted percentage of being frail (%)
High	High	12	6	50%
	Mid	18	4	22%
	Poor	3	2	66%
Mid	High	21	8	38%
	Mid	6	3	50%
	Poor	13	7	54%
Low	High	14	0	0%
	Mid	8	4	50%
	Poor	12	4	33%

Spirituality: DSES using tertial cut-points (from mean DSES score)
High QoL(reference group)=not depressed not stressed, or stressed only; Mid-QoL=depressed only; Poor QoL=both depressed and stressed

Table 16. Paper 3: Model 2 Odds Ratio and 95% Confidence Intervals for the effect of quality of life upon frailty holding daily spiritual experience constant at tertial values

Spirituality (DSES)	Quality of Life (QoL)	aOdds Ratio	95% Confidence Intervals
Highest levels	High	1.00	-
	Mid	0.39	0.11 – 1.46
	Poor	1.61	0.36 – 7.23
Middle levels	High	1.00	-
	Mid	1.10	0.34 – 3.60
	Poor	2.77	0.75 – 10.20
Lowest levels	High	1.00	-
	Mid	8.75	1.20 – 64.09
	Poor	8.23	1.34 – 50.74

Model 2 uses continuous DSES in model

High QoL(reference group)=not depressed, not stressed, or stressed only; Mid-QoL=depressed only; Poor QoL=both depressed and stressed

Table 17. Paper 3 Appendix 1: Summary statistics (Mean ± Std.) for the daily spiritual experience scale (DSES) across frailty status

	Frail (N=39)	Non-frail (N=71)	*P* value*
Total mean score DSES	4.47 ± 1.19	4.31 ± 1.22	0.51
Q1. Presence	4.62 ± 1.53	4.25 ± 1.52	0.24
Q2. Connection	4.53 ± 1.24	4.40 ± 1.23	0.63
Q3. Joy	4.22 ± 1.57	4.25 ± 1.52	0.91
Q4. Strength	4.49 ± 1.41	4.46 ± 1.42	0.92
Q5. Comfort	4.41 ± 1.42	4.47 ± 1.42	0.82
Q6. Peace	4.35 ± 1.38	4.24 ± 1.38	0.68
Q7. Ask for help	4.51 ± 1.46	4.13 ± 1.61	0.23
Q8. Feel guided	4.42 ± 1.63	3.94 ± 1.75	0.18
Q9. Direct love	4.46 ± 1.56	4.12 ± 1.63	0.30
Q10. Others love	4.23 ± 1.59	4.13 ± 1.51	0.77
Q11. Beauty	4.43 ± 1.46	4.49 ± 1.29	0.85
Q12. Thankful	4.69 ± 1.33	4.85 ± 1.19	0.54
Q13. Selfless caring	4.64 ± 1.17	4.35 ± 1.38	0.29
Q14. Accept others	4.14 ± 1.25	4.17 ± 1.28	0.88
Q15. Desire closer union	4.67 ± 1.35	4.25 ± 1.48	0.17
Q16. Feel close to God	4.50 ± 1.48	4.47 ± 1.48	0.92

Range for individual DSES questions: 1(low) to 6(high) – higher scores indicate greater daily spiritual experience
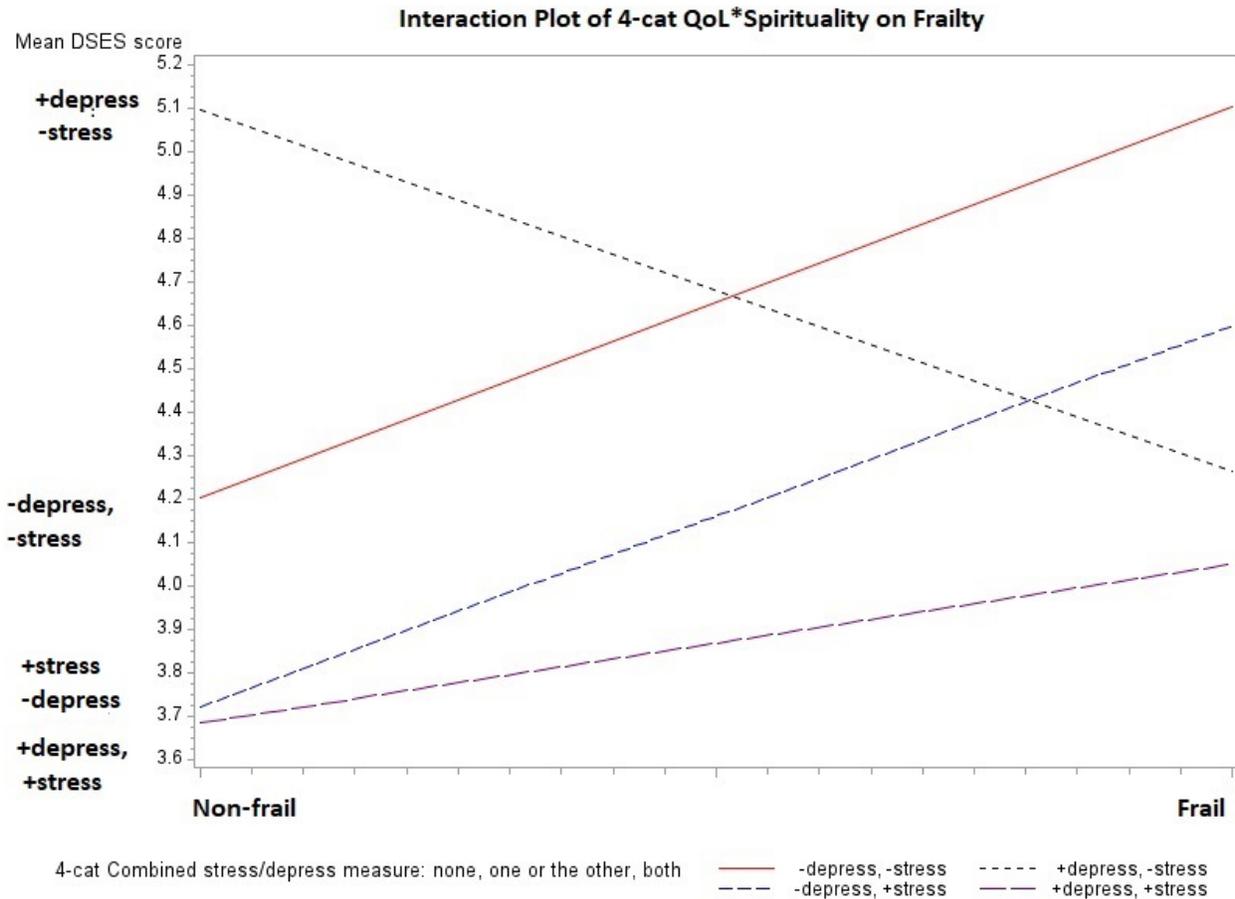
* *P* <0.05 using T-test

Figure 2. Paper 3 Appendix 2: Graphical representation of the interaction between spirituality and depression and stress upon frailty

A graphical review of the interaction between a 4-item quality of life variable (neither depression nor stress; depression only; stress only; both depression and stress) and spirituality on frailty revealed that stress only mattered if one was depressed, and that the slope of the line for those with stress only was similar to that of no depression or stress. Based upon this graph, a 3-item categorical quality of life variable was created and used in analyses (high QoL: not depressed not stressed, or stressed only; mid QoL: depressed only; poor QoL: both depressed and stressed).
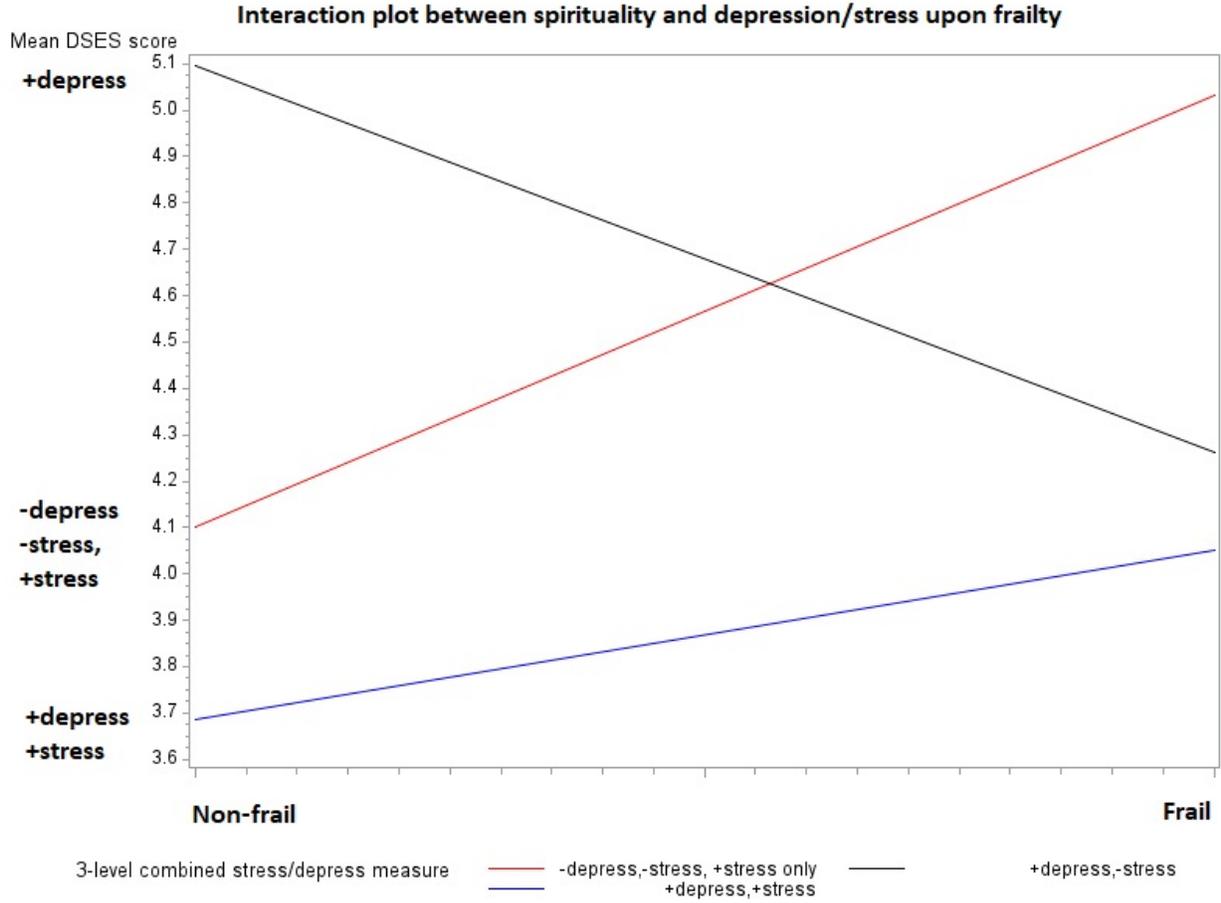
Figure 3. Paper 3 Appendix 3: Graphical representation of the interaction between spirituality and depression and stress upon frailty using the QoL variable included in models

High quality of life: not depressed not stressed, or stressed only; mid quality of life: depressed only; poor quality of life: both depressed and stressed

9.0   DISCUSSION

Physical frailty, the multi-dimensional syndrome characterized by diminished strength, endurance, and reduced physiologic function (Morley JE et al., 2013), is increasingly prevalent with advancing age. The cost of frailty is significant. It is a known risk factor for adverse events including increased risk of acute illness, falls, disability, hospitalization, institutionalization and mortality (Espinoza S & JD, 2005; Fried LP et al., 2001).

With increased chronological age, comes senescence of the immune system and a decreased immune response to vaccine antigens. Frailty's negative impact upon the immune system however, is noted to be beyond that of aging-related changes alone (Yao X, Li H, et al., 2011). Frailty has also been shown to accelerate immunosenescence (Gruver A et al., 2007), though the literature shows mixed results of the effect of physical frailty upon immune system response to influenza vaccine for community-dwelling adults ≥ 62 years of age.

Regarding psychosocial aspects of health, the literature notes bi-directional relationships between frailty and depression wherein each factor increases the risk of the other (Soysal P et al., 2017). Dysregulation of the stress hormone cortisol has recently been posited as the key factor underlying the musculoskeletal pathophysiology of frailty (Johar H et al., 2014), and higher levels of self-reported perceived stress are associated with increased levels of cortisol (Ezzati A et al., 2014).

This three-paper dissertation examined the effects of physical frailty on both immunological response (papers 1 and 2) and psychosocial factors (paper 3) in adults $\geq$ 50 years of age.

Paper 1, which compared the effect of physical frailty on immune system response to influenza vaccine among community-dwelling adults $\geq$ 50 years of age stratifying by age ($<$ 65 years and $\geq$ 65 years), showed evidence that frailty existed in those $<$ 65 years of age. Indeed, frailty appeared to be protective of immune system response to influenza vaccine for adults 50 – 64 years of age. Within this younger age group, frailty was a significant predictor of post-vaccination seroprotection status for the A/H1N1 vaccine strain, of seroconversion status for the A/H3N2 vaccine strain, and of $\log_2$ post-vaccination HAI titers for both the A/H1N1 and A/H3N2 vaccine strains.

Paper 2, which examined the effect of physical frailty on immune system response to two different influenza vaccine types (high dose vs. standard dose) among long-term care residents $\geq$ 65 years of age, showed no evidence of frailty having an impact on immunological response *within* either vaccine group. However, when comparing the effect of frailty *across* the standard dose and high dose vaccine groups, significant differences were evident for both post-vaccination seroprotection and seroconversion status, particularly during the second year (2012-2013 season). Frail long-term care residents as compared to non-frail showed overall greater odds of being seroprotected and seroconverted in the high dose vaccine group than those in the standard dose group.

Paper 3, which examined whether spirituality moderates the relationship of depression and stress (defined as quality of life (QoL)) with physical frailty among community-dwelling adults $\geq$ 50 years of age, indeed found evidence for spirituality's moderating role in this

relationship. This moderating role was evident even within a cohort who self-reported low levels

of stress and depression and high levels of spirituality. Logistic regression models showed that

spirituality significantly moderated the association of QoL with frailty and that this effect of QoL

upon frailty varied by level of spiritualty. As spiritualty decreased, QoL became more important

in predicting frailty; this was significant only for those at the lowest levels of spiritualty

10.0 PUBLIC HEALTH SIGNIFICANCE

There have been very few studies that have specifically measured the association of physical frailty on immune system response to influenza vaccination. Paper 1 is the first analysis conducted that assessed the effect of physical frailty on influenza vaccine immune response that includes community-dwelling persons younger than 65 years of age and it is the only study that stratifies these effects by age. As evident within this first paper, physical frailty exists in adults < 65 years of age and the relationship to influenza vaccine response in frail younger adults differs from frail adults ≥ 65 years of age. Paper 2 is the first study to assess the differences between frailty groups on immunologic response to two different influenza vaccine types (high dose vs. standard dose) among long-term care residents ≥65 years of age and does so in two consecutive influenza vaccination seasons.   As evident within this second paper, the relationship to influenza vaccine immune response in frail vs. non-frail long-term care residents showed differential effects by vaccine type. Paper 3 is only the second study to assess if spirituality modifies the relationship of quality of life to frailty. Modifying effects of spiritualty were seen even among a sample that reported low levels of stress and depression; as spirituality decreased quality of life became more important.

11.0   FUTURE RESEARCH DIRECTIONS

This dissertation examines important questions related to the impact of physical frailty upon immune system response and quality of life indicators. Research with larger sample sizes might allow for further examinations of frailty by age to understand why physical frailty seems to have a positive effect on immune response to influenza vaccine in adults aged 50-64 years of age but not in those 65 and older, and to determine which specific factors may be driving this association. With larger sample sizes it may be possible to chart the course of immune response decline from middle to older age and find its tipping point. It may also provide insight into the ideal age to initiate use of high dose influenza vaccine for frail persons < 65 years of age, thereby helping reduce the high morbidity and hospitalization burden of influenza disease and its secondary-infections through use of a vaccine proven to stimulate higher immunological response in adults ≥ 65 years of age.

Larger sample sizes and greater heterogeneity are important for studies seeking to assess immunological differences by influenza vaccine type. With adjuvanted vaccine now available for adults ≥ 65 years of age in the United States, it is important to examine the superiority and non-inferiority of high dose vs. adjuvanted vs. standard dose, using randomized clinical controlled study design to assess these differences. Such studies should include sufficient numbers of racially diverse participants as well as assessments of frailty to allow for detection of

frailty immunogenicity response differences that may be present if examined among other racial or ethnic groups of long-term care residents.

Along with larger sample sizes, a longitudinal design, inclusion of other quality of life instruments, and collecting measures of religious/spiritual (R/S) coping mechanisms and co-morbidities known to be associated with both quality of life and frailty are important in future studies assessing the association of R/S upon quality of life and frailty. It has been suggested that interventions targeting frailty have the additional benefit of increased quality of life (Kojima G et al., 2016). Conversely, R/S is an important psychosocial component with beneficial effects upon quality of life; R/S may therefore positively impact health, leading to the continuation of a more robust health state. Understanding the types of R/S coping mechanisms used when stressed or depressed would provide a greater understanding of the helpfulness or harmfulness (depending upon the coping style) of R/S upon quality of life and frailty, helping to address questions of who benefits and who is most at risk.

12.0   CONCLUSION

With an increasingly aging population and the concomitant costs associated with increased medical utilization, it is important to understand the immunological and psychosocial aspects affected by physical frailty. This dissertation addresses both factors and evaluates both older adults and adults < 65 years of age, who are not typically included in frailty studies. All three studies are among the first of their kind and therefore add significantly to the literature. Important findings have emerged from these three papers and steps are given for future research direction.

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