Maya Matheny
Curriculum Vitae

EDUCATION AND TRAINING

1999-2003: University of Maryland Baltimore County, Baltimore, MD
Meyerhoff Undergraduate Scholar
Degree Awarded: Bachelor of Science, Biological Sciences (Magna Cum Laude, Phi Beta Kappa)

2003-Present: University of Maryland School of Medicine, Baltimore, MD
Medical Scientist Training Program (MD/PhD program)
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HONORS AND AWARDS

University of Maryland School of Medicine
Diversity Scholarship Award, 2003
Short Term Research Training Program Travel Award, 2005

Student National Medical Association (SNMA), University of Maryland Chapter
Member of the Year Award, 2004-2005
Outstanding Service Recognition Award, 2005-2006

American Psychiatric Association
Minority Medical Student Travel Scholarship, 2006
Minority Medical Student Travel Scholarship, 2007

Sigma Gamma Rho Sorority, Inc.
Bertha Black Rhoda Fellowship, 2008
Cleo S. Higgins Higher Education Assistance Award, 2008

23rd Annual National MD/PhD Student Conference
Diversity Travel Award, 2008

National Institute on Aging
Research Supplement to Promote Diversity in Health Related Fields, 2008-2010
Supplemental award to Dr. Jay Magaziner's MERIT Award
Minority Supplement Award, 2008
National Institute on Aging Technical Assistance Workshop, 2008
Pre-doctoral Trainee, Research Training in the Epidemiology of Aging Fellowship (T32), 2011-2012

Howard Hughes Medical Institute
Meyerhoff Graduate Fellowship Program, 2009-present

American Geriatrics Society

Boston University Summer Institute in Geriatric Medicine, 2009

Co-sponsored by the National Institute on Aging and the American Geriatrics Society

American Association for Geriatric Psychiatry

Geriatric Mental Health Foundation Medical Student Scholar, 2010

Student National Medical Association, National Organization

Physician Researcher Initiative Travel Fellowship, 2012

EXTRACURRICULAR ACTIVITIES

University of Maryland Baltimore Graduate Student Association (GSA)

2005-2006: Department of Epidemiology Representative
2007-2008: Secretary
2008-2009: President

Maryland Science Center (Baltimore, MD)

2006: BodyLink Internship Program

Greater Baltimore Leadership Association

2005-2007: Public Health Committee Member, Young Professionals Chapter of the Greater Baltimore Urban League

MPRI/L-3 Communications (Windsor Mill, MD)

2007: Tutor for Language Arts and Mathematics

Baltimore County Department of Aging

2008: "Fit to a T" Bone Health Education Lectures at Baltimore County Senior Centers, in conjunction with The Baltimore Hip Studies Program

Diggs-Johnson Middle School/UMB Partnership (Baltimore, MD)

2008: Assisted middle school students with science fair project development

Morgan State University (Baltimore, MD)

2008: Lecturer, Basic Epidemiology, Health, Physical Education, Recreation and Dance (HPERD) Department

Cristo Rey Jesuit High School (Baltimore, MD)

2012: Meyerhoff Graduate Fellows Speaker Series

CONFERENCES ATTENDED

Name of Conference	City, State, Country	Date	Title of Presentation	Oral or Poster
University of Maryland School of Medicine Medical Student Research Day	Baltimore, MD, USA	9/2004	Five-Lipoxygenase Activating Protein Polymorphisms and the Risk of Cerebral Infarction in a Biracial Population: The Stroke Prevention in Young Women Study	Poster
Annual Biomedical Research Conference for Minority Students (ABRCMS)	Dallas, TX, USA	11/2004	Five-Lipoxygenase Activating Protein Polymorphisms and the Risk of Cerebral Infarction in a Biracial Population: The Stroke Prevention in Young Women Study	Poster
International Stroke Conference 2005, American Stroke Association, New Orleans, LA	New Orleans, LA, USA	2/2005	Five-Lipoxygenase Activating Protein Polymorphisms and the Risk of Cerebral Infarction in a Biracial Population: The Stroke Prevention in Young Women Study	Poster
135th Annual Meeting and Exposition of the American Public Health Association	Washington, DC, USA	11/2007	Method of Smoking Cessation and Healthcare Provider Advice	Poster
Student National Medical Association (SNMA) Region VI Conference, Howard University	Washington, DC, USA	12/2007	Method of Smoking Cessation and Healthcare Provider Advice	Poster
2nd Annual Conference for the Dissemination of Student Research, Johns Hopkins Bloomberg School of Public Health	Baltimore, MD, USA	4/2008	Association between Inflammatory Markers and Depressive Symptoms in Older	Poster

			Adults after Hip Fracture: The Baltimore Hip Studies	
23rd Annual National MD/PhD Student Conference	Keystone, CO, USA	7/2008	Association between Inflammatory Markers and Depressive Symptoms in Older Adults after Hip Fracture: The Baltimore Hip Studies	Poster
61st Annual Meeting of the Gerontological Society of America	National Harbor, MD, USA	11/2008	Association between Inflammatory Markers and Depressive Symptoms in Older Adults after Hip Fracture: The Baltimore Hip Studies", Baltimore Hip Studies Symposium	Oral
PROMISE Annual Research Symposium	College Park, MD, USA	1/2009	Association between Inflammatory Markers and Depressive Symptoms in Older Adults after Hip Fracture: The Baltimore Hip Studies	Oral
Summer Institute in Geriatric Medicine, Co-sponsored by the National Institute on Aging and American Geriatrics Society, Boston University	Boston, MA, USA	6/2009	N/A	N/A
Annual Meeting of the American Association for Geriatric Psychiatry	Savannah, GA, USA	3/2010	N/A	N/A
63rd Annual Meeting of the Gerontological Society of America	New Orleans, LA, USA	11/2010	N/A	N/A
Graduate Research Conference	Baltimore, MD, USA	4/2013	Prevalence and Persistence of Depressive	Oral

			Symptoms and Inflammatory Cytokines in Older Women after Hip Fracture	

PUBLICATIONS

Matheny, M, Cole, J, O'Connell, J, Stine, OC, Gallagher, M, Mitchell, B, Wang, J, Stern, B, Wozniak, M, Kittner S. Five-Lipoxygenase Activating Protein Polymorphisms and the Risk of Cerebral Infarction in a Biracial Population: The Stroke Prevention in Young Women Study. (Abstract) Stroke. 2005. 36(2):457.

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Matheny, M. SNMA Aids Victims of Hurricane Katrina: Reflections from the Ground. Journal of the Student National Medical Association. Spring 2006. 12(4):24.

Matheny, M, Wolpert, B, Langenberg, P, Dwyer, D, Groves, C, Zhan, M and Steinberger, E. Method of Smoking Cessation and Healthcare Provider Advice. (Abstract) American Public Health Association. 2007.

Matheny, M, Shardell M, Hicks, G, Miller R, Magaziner J, and Orwig D. Association Between Inflammatory Markers and Depressive Symptoms in Older Adults after Hip Fracture. (Abstract) The Gerontologist. 2008. 48(2):634.

Gruber-Baldini, AL, Matheny, M, Lloyd J, Chiles, N, Orwig, D, Hochberg, M, Magaziner, J. Baseline Psychosocial Differences between Men and Women after Hip Fracture. (Abstract) The Gerontologist. 2009. 49(2):347.

Matheny, ME, Miller, RR, Shardell, MD, Hawkes, WG, Lenze, EJ, Magaziner, J, Orwig, DL. Inflammatory Cytokine Levels and Depressive Symptoms in Older Women in the Year after Hip Fracture: Findings from the Baltimore Hip Studies. Journal of the American Geriatrics Society. 2011. 59(12):2249-2255.

Manuscript Submitted to the American Journal of Public Health (2008) and the Archives of Internal Medicine (2008):
Matheny, M, Wolpert, B, Langenberg, P, Dwyer, D, Groves, C, Zhan, M and Steinberger, E. (2004) Relationship of Healthcare Provider Advice and Smoking Cessation Method Used by Maryland Adults, Maryland Cancer Survey

ABSTRACT

Title of Dissertation: The Relationships between Depressive Symptoms, Inflammatory Cytokines, and Lower Extremity Function after Hip Fracture

Maya E. Matheny, Doctor of Philosophy, 2013

Dissertation Directed by: Denise L. Orwig, PhD, Associate Professor
Department of Epidemiology and Public Health

Background: Hip fractures are associated with alterations in physical, psychological, and immunologic functions in more than 1.6 million older adults who are affected annually. We aimed to examine the inter-relationships between these functional domains in a sample of older adult female hip fracture patients.

Methods: Participants were community-dwelling women aged ≥65 years, admitted to one of three Baltimore-area hospitals with a new, non-pathological fracture of the proximal femur. At baseline, 2, 6 and 12 months post-fracture, depressive symptoms were assessed with the 15-item Geriatric Depression Scale (GDS). At 2, 6, and 12 months post-fracture, serum was analyzed for interleukin-6 (IL-6) and soluble tumor necrosis factor alpha receptor 1 (sTNF-αR1), and lower extremity performance was measured by the Lower Extremity Gain Scale (LEGS). Generalized estimating equations were used to model the longitudinal relationships between variables of interest.

Results: Clinically significant levels of depressive symptoms were present in 12.5% of study participants at baseline. Persistently high depressive symptoms were significantly associated with lower sTNF-αR1 levels at 2 months ($p=0.02$) followed by an increase in sTNF-αR1 levels by 12 months ($p<0.0001$). Participants in the highest categories of IL-6

(≥5.14 pg/mL) and sTNF-αR1 (≥2421 pg/mL) had the highest GDS scores in the year post-fracture (p=0.09 for both). At 12 months post-fracture, the highest IL-6 and sTNF-αR1 categories had GDS scores that were on average 1.9 (95% confidence interval [CI]: 0.4, 3.4; p=0.01) and 1.4 (95% CI: -0.1, 3.0; p=0.07) points higher than the lowest category, respectively. Participants in the highest categories of IL-6 (≥3.69 pg/mL) and sTNF-αR1 (≥2210 pg/mL) had the lowest LEGS scores in the year post-fracture (p=0.03 for IL-6; p=0.23 for sTNF-αR1). At 2 months post-fracture, the highest IL-6 and sTNF-αR1 categories had LEGS scores that were on average 4.8 (95% CI: -8.1, -1.6; p=0.004) and 3.0 (95% CI: -6.3, 0.3; p=0.07) points lower than the lowest category, respectively.

Conclusions: Results from this study support a role for inflammation in the pathophysiology of depressive symptoms after hip fracture. The magnitude and speed of recovery of lower extremity function may depend on the level of the pro-inflammatory cytokine response.

The Relationships between Depressive Symptoms, Inflammatory Cytokines, and Lower Extremity Function after Hip Fracture

by
Maya E. Matheny

Dissertation submitted to the faculty of the Graduate School
of the University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2013

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Dedication

I would like to dedicate this dissertation to God, my family, and four individuals who have shaped my life for the better.

To the memory of my late grandmother, Lucille Sargent:
Granny -- without your life and love, I would not be here today. You saw me graduate from college and I wish you could be here today to see me finish this too, but I know you are watching over me from above.

To the memory of the late Mr. LaMont Toliver:
Mr. T. – you saw something in me as a high school senior that I did not see in myself, and you were there at every critical juncture between the ages of 17 and 30 to give your sage advice and help me keep everything in perspective. I will never forget your wisdom, sense of humor, and quiet leadership that have helped to mold the lives of hundreds of young people.

To my mother, Shirley Matheny:
Mommy -- thank you for teaching me the meaning of "finishing my course" and unconditional love. I am forever grateful for your undying support. I love you more today than yesterday!

Finally, to my son, Ryan:
Wyan Byan -- thank you for giving my life new meaning. You remind me every day to "don't give up…show determination!" and "never never never give up", and I am so very glad that I listened and did not give up. I love you through and through!

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1. Introduction

Serious medical events, such as hip fractures, have many sequelae that include depression and long-term disability among older adults. Not only do they affect the quality of life of the individuals who undergo these stressful experiences, but they also constitute a burden on the health care system, thus a public health issue. Over 310,000 persons age 65 and older suffer hip fractures each year in the United States. [1] The annual costs of these fractures are staggering, with estimated direct medical costs of $10.75 billion[2] and total costs ranging from $14 to $20 billion.[3]

With the aging trends that are currently being observed in the U.S. population, hip fracture and associated outcomes are destined to become even greater public health problems than they are today. Indeed, the proportion of the U.S. adult population age 65 and over (38.7 million in 2008) is projected to approximately double by 2030 (71 million) and further increase to 88.5 million by 2050. It is predicted that the number of hip fractures occurring in the year 2050 will rise to 700,000.

Osteoporosis, the skeletal disease in which bones become weak due to low bone mass and structural deterioration, is the major risk factor for a bone fracture. This highly prevalent disease affects an estimated 10 million Americans, with an additional 34 million estimated to have osteopenia (low bone mineral density, or BMD). Osteoporosis leads to bone fragility and increased susceptibility to fractures in any bone, though most likely in the hip, spine and wrist.[4]

There is a large gender difference in the prevalence of osteoporosis; of the 10 million Americans affected by this disease, 80% are women. As a result, a substantial

gender difference exists in the incidence of the most severe consequence of osteoporosis – hip fracture. According to the National Osteoporosis Foundation, "a woman's risk of a hip fracture is equal to her combined risk of breast, uterine and ovarian cancer, and women with a hip fracture are at a four-fold greater risk of a second one."[4]

Of great significance is the disability produced by hip fractures. Despite the advances in surgical techniques, postsurgical care, and rehabilitation, many individuals who sustained hip fracture fail to regain their prior functional ability following surgery[5]. Approximately 25 to 75% of hip fracture patients do not return to their previous level of independent living within the year following fracture[6-11]. Several factors have consistently been shown to influence recovery of physical function among the elderly after hip fracture, and they include the presence of depression[12-14] and increased levels of inflammation[15-17]. Rates of depression after hip fracture are reported to vary from 9-47%[18], and depressive symptoms and major depressive disorder manifest immediately following the fracture[19]. Depression has been identified as a risk factor for poorer recovery of function following hip fracture surgery[12,20-22], though one study found that depressive symptoms were not associated with likelihood of functional recovery[23]. These inconsistencies may have resulted from use of different measures of functional ability (walking ability, self-reported activities of daily living, instrumental ADL, physical domain of the Functional Independence Measure), as well as from "differing combinations of predictor variables and approaches to measuring these variables[5]." However, none of the prior studies have examined the relationship between depressed mood and performance of lower extremity function.

Hip fracture is an acute life event that leads to functional decline and subsequent chronic stress, which commonly results in depression among elderly patients[24]. In addition to precipitating depressed mood and other mood disorders, psychological stressors are capable of stimulating inflammatory response, both peripherally and in the brain through the sympathetic nervous system (SNS) and hypothalamo-pituitary-adrenal (HPA) axis pathways involving corticotrophin-releasing hormone (CRH) and cortisol[25]. The inflammatory cytokines that are produced have the ability to access the brain, induce inflammatory signaling pathways, and "ultimately" contribute to altered central nervous system (CNS) function (altered monoamine metabolism, increased excitotoxicity, and decreased production of relevant trophic factors) [25]. Because of the feedback loop created by psychosocial stress, CNS activity, and the inflammatory response, the relationship between the resulting depression and inflammation is hypothesized to be bidirectional[26]. Available evidence regarding the association between depression and inflammation is consistent with three possible causal pathways: depression to inflammation, inflammation to depression, and bidirectional relationships[27].

Serum levels of inflammatory cytokines increase on average with age in most populations[28-31], and elderly adults typically have high circulating levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)[32]. Inflammatory markers have also been observed to increase following trauma and surgery, including after hip fracture[33-35]. Elevations of IL-6 in old age are strongly associated with disability and mortality[30,36-38]. According to Cesari and colleagues, dysregulation of inflammatory processes may further explain age-related declines in physical function[28]. Miller and colleagues concluded that higher levels of IL-6 were

associated with poorer recovery of performance of lower extremity function among female hip fracture patients[15].

Each of the aforementioned variables (depression, inflammatory cytokine levels, and functional recovery) has been assessed in some capacity following hip fracture. It is known that depressive symptoms and inflammatory cytokine levels are (independently) risk factors for poor functional recovery post-fracture. What is not known, however, is whether depressive symptoms and inflammatory cytokine levels are associated following a hip fracture, and to which extent such associations impact lower extremity functional recovery.

To fill the gaps, we examined the relationships between depressive symptoms, serum inflammatory cytokine levels, and performance of lower extremity function after hip fracture in a sample of female hip fracture patients. We also investigated the potential bi-directionality of the depression-inflammation association. In performing the present study, we were able to integrate existing evidence and methodology from the fields of epidemiology, geriatric psychiatry, gerontology, and immunology to elucidate the mechanisms underlying morbidity, i.e., functional decline and depressive symptoms, among older adult hip fracture patients. The ultimate goal is to develop informed prevention measures to reduce morbidity among the older adult hip fracture population.

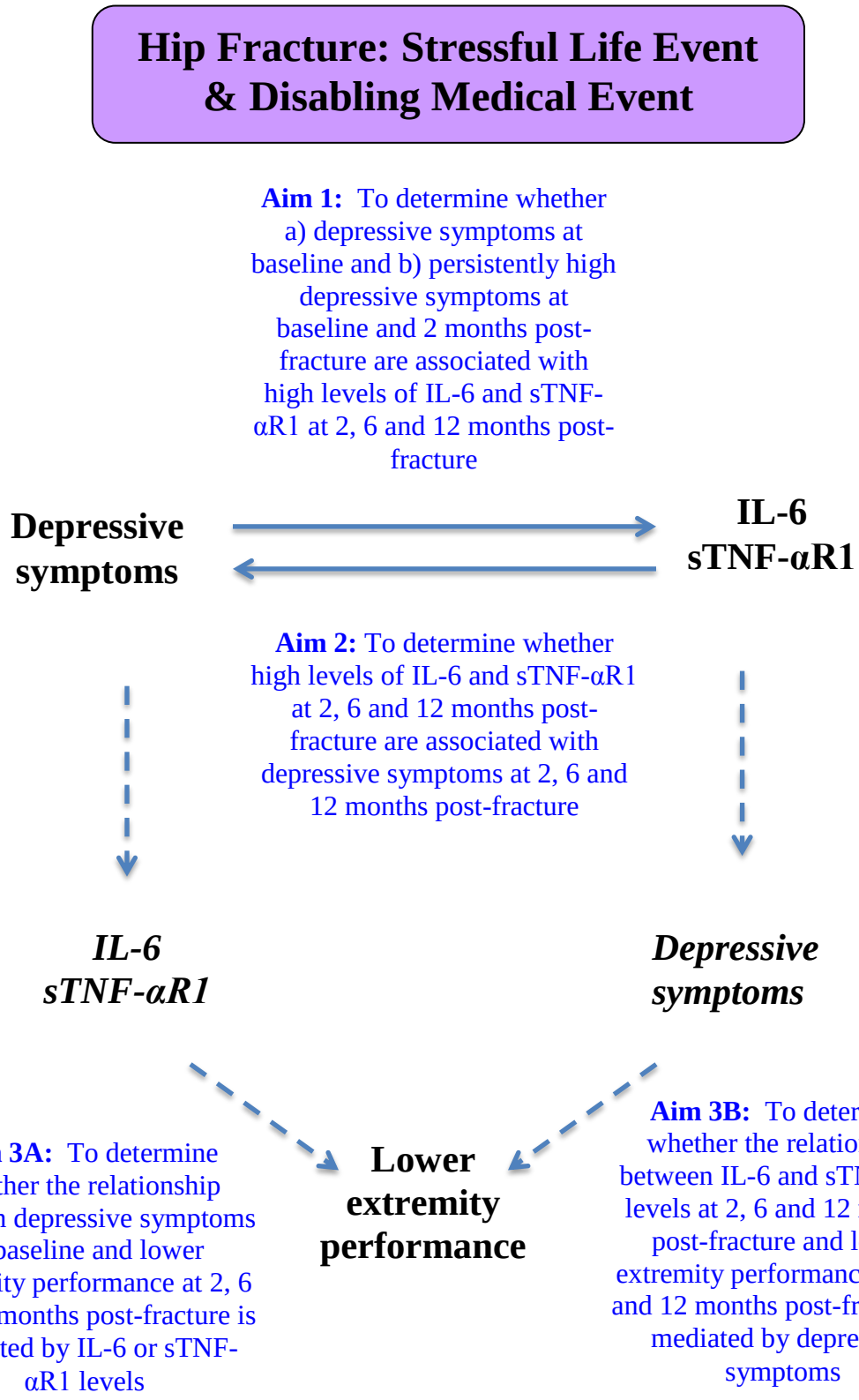
Objective and Specific Aims

The main objective of this research is to examine the various relationships between depressive symptoms, inflammatory cytokines, and lower extremity function among older women during the twelve months following surgical repair of hip fracture. The specific aims are as follows:

1. To determine if:
    a. Depressive symptoms at the time of surgical repair ("baseline") are associated with high levels of interleukin-6 (IL-6) or soluble tumor necrosis factor alpha receptor 1 (sTNF-αR1) at 2, 6 and 12 months post-fracture
    b. Persistently high depressive symptoms occurring at baseline and 2 months post-fracture are associated with high levels of IL-6 and sTNF-αR1 at 2, 6 and 12 months post-fracture
2. To determine whether high levels of IL-6 and sTNF-αR1 at 2, 6 and 12 months post-fracture are associated with depressive symptoms at 2, 6 and 12 months post-fracture
3. To examine if the relationship between:
    a. Depressive symptoms at baseline and lower extremity function at 2, 6 and 12 months post-fracture is mediated by levels of IL-6 and sTNF-αR1
    b. Levels of IL-6 and sTNF-αR1at 2, 6 and 12 months post-fracture and performance of lower extremity function at 2, 6 and 12 months post-fracture is mediated by depressive symptoms

Figure 1.1 summarizes the aims for this research and will serve as a conceptual model for the study.

Figure 1.1 Conceptual model for study



Contribution of Research

It is the goal of this study to investigate potential associations between prevalence and persistence of depressive symptoms, inflammatory marker levels, and the performance of lower extremity function after hip fracture. Findings from these studies can be applied to methodology of future studies of depressive symptomatology and inflammatory cytokines in samples of hip fracture patients. In particular, this research may be continued to determine if depressive symptom levels and/or cytokine levels have a predictive value in mortality and morbidity in a hip fracture population. Further work in this area may also lead to identification of elderly individuals at high risk for developing depressive symptoms after hip fracture based on the severity of their inflammatory response. The relationship between depression and inflammation has important implications for treatment, as depressed patients with increased levels of inflammatory biomarkers have been found to exhibit treatment resistance[25]. Treatments for depression that target the associated increased inflammation may be of use for specific populations of hip fracture patients.

This research is novel in its objective to determine the relationships between levels of depressive symptoms, inflammatory cytokines and lower extremity function following hip fracture. In this project we are able to assess the relationships between depressive symptoms and inflammatory markers over time, because the parent study is longitudinal and data were collected throughout the year after hip fracture.

2. Background and Significance

Hip Fracture

Incidence and Public Health Importance

Hip fractures are common, costly, debilitating and deadly events in the lives of older adults. In 2008, there were approximately 341,000 emergency department visits in the US for hip fractures[39]. Based on the expected increase in the number of adults over 65 with the entrance of the "baby boomers" into the geriatric population, it is projected that there will be 437,000 hip fractures among adults over the age of 50 in 2030, and 582,000 in 2040. In addition to the significant disability and impaired quality of life that these events cause, hip fractures are also a cause of premature death in the older adult population. Approximately 20% of U.S. Medicare patients die within 6 months of sustaining their hip fracture[40]. The resulting economic burden placed on the U.S. health care system approached $20 billion per year[41].

The risk of hip fracture grows rapidly with age. Approximately 19% of women and 12% of men who reached age 85 sustained hip fractures, and nearly 30% of women and 20% of men who reached 90 years of age sustained hip fractures[39]. Based on the average life expectancy of 80 years, approximately 10% of women and 6% of men in the US will experience a hip fracture during their lifetime.

Recovery after Hip Fracture

Hip fracture can affect several of the patient's functions of daily living that include mobility, physical and instrumental task performance, cognition, and social functioning[22]. Most patients who survive the hip fracture experience have reduced mobility and are no longer able to function independently[22]. For those who do survive

the fracture, return to premorbid level of function is a major goal. Approximately 25 to 75% of hip fracture patients who were ambulatory prior to their hip fracture cannot walk without assistance and/or do not regain their previous level of independent living within the year following the event[9,11,12,42,43].

The greatest losses occur in activities requiring lower extremity function, such as walking one block, climbing stairs, and getting in and out of a bath or shower[11,22]. As many as 50% of hip fracture patients who were able to rise from a chair independently and 40% of those who were able to walk 3m (10ft) independently, prior to hip fracture, required a device or human assistance to perform these basic activities one year after the incident[22].

Depression

Public Health Importance

Depression is the leading global cause of life-years lived with disability and ranks fourth for disability-adjusted life-years worldwide, a measure of global burden of disease[44]. The World Health Organization recognized major depression as the fourth leading cause of worldwide disease in both 1990 and 2000, resulting in more disability than ischemic heart disease and cerebrovascular disease[45]. Among adults, the point prevalence of major depression ranges from 5 to 9%, and approximately 50% of depressed patients go undetected.

Epidemiology of Late-Life Depression (LLD)

Depressive symptoms and syndromes in late life are a major public health concern. The prevalence of clinically relevant levels of depressive symptoms in the elderly ranges from approximately 8% to 16%[46]; as many as 10% of elderly individuals

who are seen in primary care settings have clinically significant depression[47]. Major depression occurs in 1% to 3% of the general elderly population[48]. According to the Swedish Adoption/Twin Study of Aging, depressive symptoms increase modestly with age in both men and women, especially in older adults[49].

Symptoms of LLD

Depression may occur in the form of several different disorders, each of which can affect older adults. Major depression is diagnosed using the *DSM-IV-TR*. At least five of the following symptoms must be present nearly every day for at least two weeks and represent a change from previous functioning: depressed mood; diminished interest or pleasure in most activities (anhedonia); significant weight loss or gain, or decrease or increase in appetite; insomnia or hypersomnia; psychomotor agitation or retardation; fatigue or loss of energy; feelings of worthlessness or inappropriate guilt; diminished ability to think or concentrate, or indecisiveness; or recurrent thoughts of death or of suicide (with or without a specific plan) [50].

Dysthymia is a chronic, less severe form of depression in which depressed mood "occurs for most of the day more days than not for at least two years" and two or more of the following symptoms: poor appetite or overeating; insomnia or hypersomnia; low energy or fatigue; low self-esteem; poor concentration or difficulty making decisions; or feelings of hopelessness[50]. Adjustment disorder with depressed mood occurs in response to a specific stressor, such as placement in a long-term care facility or death of a spouse[50]. Depressive disorder not otherwise specified includes depression secondary to another medical condition or to medication[50]. Minor or subsyndromal depression is characterized

by depressive symptoms like those of major depression, but with fewer symptoms and less impairment[51].

Risk Factors for LLD

Elderly patients' experiences are unique to their life stage and can lead to late-life depressive disorders. These experiences are death of a spouse or other loved one, medical illness and injury, disability and functional decline, and lack of social contact[52]. Additional significant risk factors for depression in late-life include female gender, prior depression and sleep disturbance[53]. Negative life events in the previous three years predicted depressive symptoms, which in turn, predicted future negative life events[49].

Outcomes after LLD

Both depressive symptoms and major depression in the elderly are associated with falls and fractures[54], disability, poor physical function[55], increased perception of poor health status[55,56], increased utilization of medical services[57], and increased health care costs[24]. The most serious consequence of depression in elders is suicide, with older Caucasian males being at greatest risk[58].

Treatment for LLD

Late life depression can be successfully treated, in as many as 65% to 75% of elderly patients[59]. However, persistent impairment in functional status and in health-related quality of life is commonly observed following treatment[60]. Treatment options for depression in older adults include pharmacotherapy, psychotherapy, psychosocial interventions and electroconvulsive therapy[61].

Pharmacotherapy with antidepressant medications, such as selective serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitors (SNRIs),

tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs), can be used to treat late life depression. Caution must be taken with certain antidepressants in older adults, however, due to their adverse effects and capability of worsening comorbid medical conditions[62]. Psychotherapeutic options include cognitive behavioral therapy and interpersonal therapy. Psychosocial interventions may include increasing physical activity and social interaction[61]. Electroconvulsive therapy is reserved for severe cases of depression.

Depression after Hip Fracture

Psychosocial function after hip fracture is important. Psychiatric illness (e.g., depression, delirium and dementia) commonly occurs after hip fracture. The onset of disabling medical illness, such as hip fracture, is one of the most important risk factors for a major depressive episode, depressive symptoms and late-life depression in elderly persons[53,63-65]. Rates of depression after hip fracture vary from 9-47%[18]. The highest prevalence of depressive symptoms and major depressive disorder is noted immediately following the fracture[66].

Several events that usually follow the hip fracture experience, including pain, immobilization, hospitalization, surgery, and uncertain likelihood of recovery, qualify it to be a "depressogenic" stressor[66]. In a study of non-depressed elderly patients hospitalized for hip fracture surgery, the cumulative incidence rate of clinically significant depressive symptoms (7 or more on the 15-item version of the Geriatric Depression Scale) was 20.5%[67].

An older adult may have a diagnosis of major depressive disorder that precedes their hip fracture or depressive symptoms occurring after the fracture may be part of an adjustment reaction in response to the fracture. A careful history from the patient regarding their depressive symptoms would likely distinguish between the two. Depressive symptoms after hip fracture have been assessed using screening instruments such as the Geriatric Depression Scale, Center for Epidemiologic Studies Depression Scale, Hamilton Depression Scale, and the Patient Health Questionnaire.

Inflammation

Inflammation and Cytokines

Inflammation is defined as "the body's integrated reaction and defense against disturbances of homeostasis, particularly infections and injuries. This response is initially characterized by a local release of cytokines, soluble polypeptides responsible for the amplification and regulation of the inflammatory cascade.[68]" Cytokines are a diverse group of messenger molecules produced by immune cells (e.g. lymphocytes and macrophages) that are involved in the regulation of immune responses. These large polypeptide mediators (8-60 kDa) regulate growth, differentiation and function of many cell types. Physiologic functions of cytokines include muscle and bone tissue turnover, immunoregulation, and hematopoiesis. Cytokines can be classified into the following families: interleukins, tumor necrosis factors (TNFs), interferons (INFs), chemokines, haematopoietins, and colony-stimulating factors (CSFs). Because many cytokines are able to exert multiple actions, they may belong to more than one cytokine family.

Cytokines produced by the innate response determine the type of adaptive response. In innate immunity, cytokines are produced by macrophages and natural killer

cells, and in adaptive immunity, they are produced by T lymphocytes. There are two general categories into which cytokines are classified: anti-inflammatory and pro-inflammatory. Anti-inflammatory cytokines, such as interleukin (IL-) 4, IL-10 and IL-13, diminish the immune response by neutralizing cellular activation and counteracting the production of pro-inflammatory molecules[69]. Pro-inflammatory cytokines, such as IL-1, IL-6 and tumor necrosis factor alpha (TNF-α), are directly or indirectly involved in inflammatory processes. Their actions include stimulating immune cells to exert their effects locally or through a remote site, usually at the site of cell injury[70]. We are interested in two of these cytokines: IL-6, which activates the liver to produce acute phase proteins such as chymotrypsin, haptoglobulin and protein C, and TNF-α, which is a first-line factor in promoting and developing the inflammation pathway. The soluble tumor necrosis factor α receptor 1 (sTNF-αR1) aids in the resolution of the inflammatory response by buffering and neutralizing circulating TNF-α.

Inflammation in the Elderly

Aging is associated with increased levels of circulating cytokines[71]. Compared to younger individuals, elderly persons may have a prolonged inflammatory response with slower normalization of cytokine levels[71,72]. In healthy older adults, aging is associated with an altered acute phase response, including initial hyper-reactivity, prolonged inflammatory activity, and prolonged fever response[71,72]. Inflammation has been associated with increased morbidity and mortality in elderly persons[73,74]. Chronic inflammatory state was postulated to be detrimental by accelerating the progression of medical conditions that result in functional decline and disability[36,75]. Pro-inflammatory

cytokines may have catabolic effects on muscles, thus the hypothesis that inflammation has a direct role in the development of sarcopenia[76].

Inflammation after Hip Fracture

Inflammatory cytokine levels were reported to increase following trauma and surgery, including hip fracture[33-35]. Elderly patients have an increased and delayed IL-6 response to surgical trauma compared with young adults[35]. In the elderly hip fracture population, cytokine levels can remain elevated up to one year post-fracture with median IL-6 concentrations of 7.4 pg/mL at 12 months[15].

Relationships between Depression, Inflammation and Functional Recovery

The Cytokine Hypothesis of Depression

The cytokine hypothesis of depression implies that pro-inflammatory cytokines act as neuromodulators and that they are critical for the central mediation of the behavioral, neuroendocrine and neurochemical features of depressive disorders[69]. Administration of pro-inflammatory cytokines to animals induces "sickness behavior", which is a pattern of alterations that are very similar to behavioral symptoms of depression in humans[69]. This syndrome has many features that overlap with major depression, including anhedonia, anorexia, impaired sleep, and reduced locomotor activity[77].

Depression and Inflammation

The relationship between depression and inflammation can be best described as bidirectional. Cytokines affect the central nervous system (CNS) in various ways, including the production and enhancement of negative moods, physical symptoms including lethargy and fatigue, and a range of "sickness behaviors" from shivering to loss

of appetite[78]. There is evidence that the immune system plays a role in the neuroendocrine and behavioral features of depressive disorders[78].

Though the etiology of depression is complex, major depression has been associated with systemic immune activation[79], characterized by an increased production of pro-inflammatory cytokines (e.g., IL-1β, IL-2 and IL-6) by peripheral blood mononuclear cells (PBMC), in addition to a host of other upregulated immune components (e.g., an acute phase protein response and excessive secretion of pro-inflammatory cytokines, prostaglandins and nitric oxide)[79-82]. The increase in pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α in major depression is important because these inflammatory markers can have an impact on the CNS through several different mechanisms: disruption of the blood-brain barrier; penetration through circumventricular organs (sites in the brain containing capillaries with open junctions and abundant fenestrations); *de novo* synthesis in the CNS; and action in peripheral nerves which signal the brain[69].

Once inside the CNS, pro-inflammatory cytokines can stimulate two primary targets: 1) the serotonin transporter, which, after activation, increases serotonin (5-HT) turnover;[69,83] and 2) the hypothalamo-pituitary-adrenal (HPA) axis. HPA axis activation is frequently observed in depressive disorders. Pro-inflammatory cytokines stimulate release of hypothalamic corticotrophin-releasing hormone (CRH), which in turn stimulates the pituitary secretion of ACTH that leads to the secretion of cortisol from the adrenal glands. Both ACTH and cortisol are also reported to be elevated in major depression[84].

The role of stress in the depression-inflammation relationship

Stress has been hypothesized to explain the increased inflammation observed in patients with major depression. Both acute and chronic stresses are associated with increased production/release of pro-inflammatory cytokines and decreases in anti-inflammatory cytokines (e.g. IL-10)[85-87]. Additionally, physical and psychological stressors provoke transient increases in pro-inflammatory cytokines[88]. Both stress and administration of epinephrine elevate plasma IL-6 in animal models[89].

Population-based Studies of Depression and Inflammation

In a cross-sectional study of hospital-derived psychiatric patients aged 60 and older, persons with major depression had significantly higher levels of IL-1β (170%) than healthy comparison subjects[90]. The mean IL-1β concentration was 2.7 pg/mL (SD=2.5) for the major depression group (N=19), 1.8 pg/mL (SD=1.8) for the subsyndromal depression group (N=20), and 1.0 pg/mL (SD=1.2) for the group with no depression (N=21). In a community-based sample of older adults 70-79 years of age, Penninx and colleagues found that 145 elderly persons with depressed mood (CES-D score $\geq$16) had higher median plasma levels of IL-6 and TNF-α compared with 2879 subjects with no depressive symptoms (IL-6: 2.04 vs 1.83 pg/mL, p=.02 and TNF-α: 3.43 vs 3.16 pg/mL, p=.05)[91].

Depression and Functional Recovery after Hip Fracture

The presence and persistence of depressive symptoms are risk factors for poor functional post-fracture recovery post-fracture[12,13,92,93]. Patients with depression due to a disability require longer rehabilitation, and persistence of depressive symptoms is important to hip fracture recovery[12,13,92,93]. Mossey and colleagues compared female hip

fracture patients reporting few depressive symptoms throughout the year following hip fracture with those with persistently elevated Center for Epidemiologic Studies Depression (CES-D) scale scores. They found the former three times more likely to achieve independence in walking, nine times more likely to return to pre-fracture levels in at least five of seven physical function measures, and nine times more likely to be in the highest quartile of overall physical function than the latter[13]. Patients with symptoms that resolved by six months post-fracture had recovery patterns that are similar to those with no depressive symptoms.

Lenze and colleagues found that, in a sample of elderly patients in rehabilitation following hip fracture, those with higher depressive symptoms had lower efficiency on the motor scale of the Functional Independence Measure (FIM) [14]. This association was mediated by rehabilitation participation, in that greater depressive symptoms predicted poorer participation in rehabilitation, which, in turn, predicted lower motor FIM efficiency. Depressive symptoms were measured using the Hamilton Rating Scale for Depression (HAM-D) instrument.

In another study, Lenze and colleagues reported that over 3 years of follow-up, persistently depressed individuals showed a greater linear increase in functional disability ratings (adjusted odds ratio (OR) of 5.27 (95% confidence interval (CI) 3.03-9.16) than the temporarily depressed (adjusted OR of 2.39 (95% CI=1.55-3.69)), as compared to the not depressed groups[94]. Depressive symptoms were measured using the 10-item version of the CES-D.

Inflammation & Lower Extremity Function/Functional Recovery after Hip Fracture

Miller and colleagues examined the relationship between inflammation and functional recovery in a sample of female hip fracture patients from the Baltimore Hip Studies 3rd Cohort (BHS-3) [15]. They concluded that higher levels of IL-6 are adversely associated with recovery of lower extremity function after hip fracture. Serum interleukin-6 level (divided into tertiles) was analyzed as a potential predictor of lower extremity function in the 12 months after hip fracture. The latter was measured using the Lower Extremity Gain Scale (LEGS). Participants in the lowest tertile of IL-6 level performed better on the LEGS than those in the highest tertile ($p = .008$). At 12 months post-fracture, participants in the lowest tertile scored 5.3 points better (95% CI, 2.0-8.6) on the LEGS than those in the highest tertile ($p = .002$).

Study Significance

Our goal is to identify connections between variables that ultimately impact the recovery of function and quality of life of the hip fracture patient. The gaps in the existing literature are as follows:

It is clear that there is an upregulated immune response in the patients with major depression, particularly in late-life depressive disorders. The majority of existing studies of depressive symptoms and major depression and inflammatory cytokines including IL-1β, IL-6 and TNF-α, were conducted among older adults in either community-based samples or in a psychiatric patient population. However, the presence or absence of the relationships between depressive symptoms and inflammatory cytokine levels have not yet been demonstrated in an older adult population that has undergone a disabling

medical event, such as an incident hip fracture. This relationship could certainly be a mechanistic pathway that impacts post-fracture recovery.

The depression-functional recovery association and inflammation-lower extremity function association have been examined separately in previous studies[15,94,95], but the relationship among the three variables has not yet been assessed. Since inflammatory activation is a component of the pathophysiology of depression[81], and higher levels of inflammatory markers such as IL-6 are associated with poorer lower extremity function[15], it is possible that increased levels of inflammatory markers may serve as a mediating variable in the depressed mood-poor functional recovery association.

In this study, we will investigate if persistence of depressed mood is a potential predictor of increased inflammatory cytokine levels post-fracture. If depression and inflammation after hip fracture were associated in a bidirectional manner (addressed in Aims 1 and 2), then depressive symptoms may also mediate the association between inflammatory cytokine levels and performance of lower extremity function at later time points after hip fracture.

Prior to assessing either hypothesis of mediation in Aim 3A or 3B, the preliminary associations between depressive symptoms and lower extremity performance (3A), as well as between inflammatory cytokine levels and lower extremity performance (3B), must be confirmed in this sample. Depression has been associated with poor functional recovery in previous studies; however, self-reported measures of functional disability were used in the studies that assessed this association[94,95]. Depressive symptoms have not been studied in relation to performance measures of lower extremity function in a hip fracture population. While it has also been reported that hip fracture

patients with higher levels of inflammatory markers have worse performance of lower extremity function in older women, it is not known if this association still exists in a more robust (i.e., healthier and more cognitively intact) sample of older women who have undergone surgical repair of a hip fracture.

3. Research Design and Methods

Parent Study Design

The Baltimore Hip Studies 4th Cohort (BHS-4) study was a randomized controlled trial of older adults with hip fracture who were assigned to either usual care (no intervention) or a supervised home-based exercise program that continued for 1 year after hip fracture (Exercise Plus Program) [3,96]. Recruitment for BHS-4 was initiated in November 1998 in three hospitals in the Baltimore area and ended in September 2004. Women with hip fracture were screened for eligibility within 15 days of the event. Within 22 days of the fracture, those who met entry criteria had the following: 1) a dual energy x-ray absorptiometry (DXA) test; 2) baseline performance measures; and 3) blood specimen drawn. In addition they completed a questionnaire. Only those participants who completed at least 80% of the baseline survey were randomized.

Follow-up data were collected 2, 6, and 12 months after the hip fracture. Outcomes were assessed at 2, 6, and 12 months after fracture to ensure comparability with other BHS studies with respect to the natural history of recovery. Participants were contacted monthly to ascertain information on health care use and adverse events. Institutional review board approvals were obtained from the University of Maryland Baltimore and the study hospitals, and all enrolled participants signed informed consent.

Parent Study Population

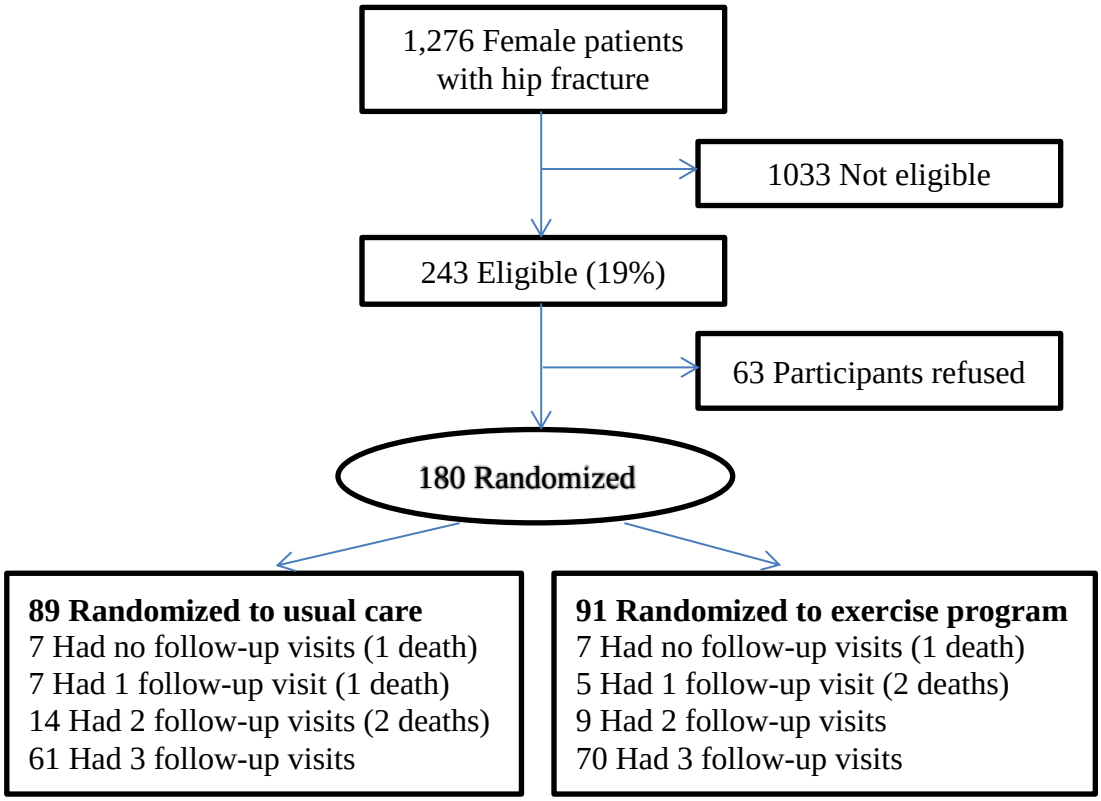
BHS-4 enrolled 180 community-dwelling women aged 65 and older and who underwent a surgical repair of a non-pathological hip fracture within 72 hours of admission to the hospital. Eligibility was determined through medical chart review, medical assessment, and cognitive function screening. Additional eligibility criteria

included ability to walk without human assistance before the fracture, a score of 20 or higher on the Mini-Mental State Examination[97] (MMSE) (i.e., cognitively intact), and medical clearance to participate from an orthopedic surgeon[3].

Exclusion criteria included pathologic fracture, cardiovascular, neurologic, and respiratory diseases that could interfere with exercising independently at home, diseases of the bone (e.g., Paget disease, osteomalacia), metastatic cancer, cirrhosis, end-stage renal disease, hardware in the contralateral hip, and conditions that increase risk of falling while exercising independently.

Because of safety concerns regarding older adults exercising independently in the home, the stringent set of study inclusion criteria resulted in only 243 (19%) of the 1,276 screened patients with hip fracture being eligible (**Figure 3.1**). Of those eligible, 180 (74%) were enrolled in the trial and randomized into two groups, intervention (n=91) and usual care (n=89). The four most common reasons for ineligibility were pre-fracture nursing home residence (24%), pre-fracture dementia or scoring below 20 on the Mini-Mental State Examination within 15 days after fracture (13%), chronic atrial fibrillation or other cardiac arrhythmia (12%), and having hardware in the contralateral hip (10%)[3].

Figure 3.1 Parent study flow chart



Design of Exercise Program

In-Home Exercise Intervention

The Exercise Plus Program consisted of an exercise component and a self-efficacy based motivational component to help optimize program adherence throughout the 12-month intervention[3,96,98,99]. The exercise program began at the end of the usual post-acute therapy period and had strength training and aerobic components. Participants were expected to exercise 5 days per week for 45 minutes each day, and the program consisted of a combination of supervised and independently performed exercise sessions. The trainers gradually increased the intensity and strength of the exercises in a standardized way. The frequency of the supervised exercise sessions decreased throughout the year as participants became more able to exercise independently.

Usual Care

The usual care (UC) group received the physician-prescribed post-fracture standard of care for patients with hip fracture in the greater Baltimore, Maryland, region at the time of study, which included relatively short hospital stays and approximately 2 to 4 weeks of rehabilitation[3,96,98,99].

Effects of Exercise Program

The intervention group reported more time spent in exercise activity during follow-up ($P<.05$). Overall, small effect sizes of 0 to 0.2 standard deviations were seen for bone mineral density measures, and no significant patterns of time-specific between-group differences were observed for muscle mass and strength, fat mass, activities of daily living, and physical and psychosocial functioning.[3]

Variables of Interest

Baseline variables were obtained prior to randomization with reference to the current, in-hospital, or pre-fracture period. Assessments were performed at participating hospitals at the time of bone mineral density testing; when this was not possible or additional sessions were needed, participants were assessed in their own residence. Outcomes were assessed 2, 6, and 12 months after hip fracture.

Demographic information and medical history, fracture type, and course of hospitalization were obtained by abstracting medical charts. Monthly telephone calls were made to participants to ascertain falls and resultant injuries, outpatient and emergency department visits, hospitalizations, and deaths.

Present Study Design and Study Sample

The present study is a secondary analysis of data from the BHS-4 study. To be included in this study, BHS-4 participants had to meet one or more of the following criteria, depending on the study aim: have had blood specimen drawn at 2, 6 and/or 12 months so that inflammatory cytokine levels could be measured (aims 1, 2 and 3); have been assessed for depressive symptoms at baseline (aims 1A and 3A) and 2 months (aim 1B); have been assessed for depressive symptoms at 2, 6 and/or 12 months (aim 2); have had LEGS measured at 2, 6 and/or 12 months (aim 3). For these analyses, data were aggregated from both treatment and usual care groups, as there was no effect of the active treatment on depressive symptoms or physical performance outcomes in the randomized controlled trial[3]. Although there was no effect of the exercise intervention on IL-6 levels, sTNF-αR1 levels were lower among exercisers[100]. Therefore, treatment group was included as a covariate in the analysis.

Study Variables

The main variables of interest for this research are depressive symptoms, inflammatory cytokines, and lower extremity performance. Measurement of these variables is described below. Each of these variables serves as a dependent variable of interest, and a table indicating the independent and dependent variables for each aim and the time point in which they were measured is included (**Table 3.1**).

Depressive Symptoms

Depressive symptoms were assessed at baseline, 2, 6 and 12 months post-fracture using the 15-item Geriatric Depression Scale (GDS-15). The GDS-15 is a series of 15 yes/no questions developed as a basic screening tool for depression in older adults[101]. Depressive symptoms are assessed as having occurred in the past week. A score of 6 or greater on the GDS-15 is indicative of clinically significant depressive symptoms, and this cutoff has a sensitivity of 82% and a specificity of 82%[102].

Inflammatory Cytokines

Blood specimens were collected from 96, 108, and 92 participants at 2, 6 and 12 months post-fracture, respectively. Sera from these participants were stored at -70°C. IL-6 and sTNF-αR1 levels were measured in serum that had been thawed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN). For IL-6, the inter-assay coefficient of variation (CV) was 3.4% and the intra-assay CV was 4.4%. For sTNF-αR1, the inter-assay CV was 3.0%, and the intra-assay CV was 5.5%.

Lower Extremity Performance

The Lower Extremity Gain Scale (LEGS) is a summary measurement of timed performance on nine tasks developed to evaluate lower extremity function in hip fracture patients[103]. Timed performance in the following tasks is measured: (1) reach for an item on the ground from a sitting position; (2) put a sock on the fractured-side foot; (3) put a shoe on the fractured-side foot; (4) rise from an armless chair; (5) walk 10 feet; (6) step up four steps; (7) step down four steps; (8) get on the toilet; and (9) get off the toilet. Performance in each of these tasks was scored on a scale of 0 to 4, resulting in a total score of 0 to 36. A higher LEGS score indicates better performance.

Baseline Covariates

The medical record was reviewed at baseline to obtain the patient's age and the number of medical comorbidities. Comorbidities were assessed using the Charlson Comorbidity Index. Height (in meters) and weight (in kilograms) were used to calculate body mass index (BMI) (kg/m^2). Treatment group was based on whether the patient had been randomly assigned to the exercise or control groups for the trial. Cognitive status was assessed at baseline using the Mini-Mental State Examination (MMSE) [97].

Table 3.1 Independent and dependent variables and variable type, by study aim

Aim	Independent variable	Variable type	Time point	Dependent variable	Variable type	Time point
1A	GDS score	continuous	Baseline	IL-6 sTNF-αR1	continuous	2, 6 and 12 months
1B	Persistent GDS score $\geq$ 6	dichotomous	Baseline and 2 months	IL-6 sTNF-αR1	continuous	2, 6 and 12 months
2	IL-6 level group (low, middle, high) sTNF-αR1level group (low, middle, high)	categorical	2, 6 and 12 months	GDS score	continuous	2, 6 and 12 months
3A	GDS score	continuous	Baseline	LEGS score	continuous	2, 6 and 12 months
3B	IL-6 level group (low, middle, high) sTNF-αR1 level group (low, middle, high)	categorical	2, 6 and 12 months	LEGS score	continuous	2, 6 and 12 months

Sample Size Calculations and Power Analysis

Aim 1

For Aim 1A, participants had to have both a GDS score at baseline and a serum cytokine measurement at one or more study follow-up visit(s) to be included in this analysis. There were 136 participants with IL-6 and sTNF-αR1 results who were included in the analysis (**Table 3.2**).

Table 3.2 Sample size calculation for Aim 1A

Aim 1A: Baseline GDS score and cytokine measured at:	IL-6	sTNF-αR1
3 visits	51	53
2 visits	54	53
1 visit	31	30
Total number of participants	***136***	***136***

For Aim 1B, Participants had to have both a GDS score measured at baseline and at two months, and a serum cytokine measurement at the second of those visits or a subsequent follow-up visit (i.e., baseline and two-month GDS score with two-, six- or twelve-month cytokine measurement). There were 124 participants who had results for IL-6 and sTNF-αR1 that were included in the analysis (**Table 3.3**).

Table 3.3 Sample size calculation for Aim 1B

Aim 1B: Baseline and 2-month GDS scores and cytokine measured at:	IL-6	sTNF-αR1
3 visits	51	53
2 visits	47	46
1 visit	26	25
Total number of participants	***124***	***124***

We used a mean concentration for IL-6 of 6.56 pg/mL (standard deviation of 8.33) that was found in a community-dwelling cohort of older adults[104], and an alpha level of 0.05, to calculate the power to detect differences in cytokine levels with a paired t-test (**Table 3.4;** PS Software, v.3.0.43). With a sample size of 136 for Aim 1A, this study had 60% power to detect a difference in IL-6 concentration of 1.59 pg/mL and 90% power to detect a difference in IL-6 concentration of 2.33 pg/mL. With a sample size of 124 for Aim 1B, this study had 60% power to detect a difference in IL-6 concentration of 1.67 pg/mL and 90% power to detect a difference in IL-6 concentration of 2.45 pg/mL.

Table 3.4 Power analysis for Aim 1A and 1B

	Power (%)			
Sample Size	**60**	**70**	**80**	**90**
124 (1B)	1.67	1.87	2.11	2.45
136 (1A)	1.59	1.79	2.02	2.33

Aim 2

Participants had to have both a serum cytokine measurement and GDS score at one or more follow-up visit(s) to be included in this analysis, and 134 participants fulfilled these criteria (**Table 3.5**).

Table 3.5 Sample size calculation for Aim 2

Aim 2: Both cytokine and GDS score at:	IL-6	sTNF-αR1
3 visits	51	53
2 visits	53	52
1 visit	30	29
Total number of participants	***134***	***134***

We used a mean 12-month GDS score of 5.5 (standard deviation of 3.5) that was found in a hip fracture cohort[105] and an alpha level of 0.05 to calculate the power to detect differences in GDS scores with a paired t-test (**Table 3.6**; PS Software, v.3.0.43).

With a sample size of 134, this study had 60% power to detect a mean difference in GDS score of 0.68 points and 90% power to detect a mean difference in GDS score of 0.99 points.

Table 3.6 Power analysis for Aim 2

	Power (%)			
Sample Size	**60**	**70**	**80**	**90**
134	0.68	0.76	0.85	0.99

Aim 3

For Aim 3A, participants had to have both a GDS score and LEGS score at one or more study follow-up visit(s) to be included in this analysis, and 155 participants fulfilled these criteria (**Table 3.7**).

Table 3.7 Sample size calculation for Aim 3A

Aim 3A: Baseline GDS score and LEGS score at:	Number of participants
3 visits	107
2 visits	38
1 visit	10
Total number of participants	***155***

For Aim 3B, participants had to have both a cytokine measurement and LEGS score at one or more study follow-up visit(s) to be included in this analysis, and 136 participants fulfilled these criteria (**Table 3.8**).

Table 3.8 Sample size calculation for Aim 3B

Aim 3B: Both cytokine and LEGS score measured at:	IL-6	sTNF-αR1
3 visits	49	50
2 visits	52	53
1 visit	35	33
Total number of participants	***136***	***136***

We used a mean 12-month LEGS score of 25.0 (standard deviation of 8.0) found in a hip fracture cohort[15] and an alpha level of 0.05 to calculate the power to detect a difference in LEGS scores with a paired t-test (**Table 3.9**; PS Software, v.3.0.43). With a sample size of 155 for Aim 3A, this study had 60% power to detect a mean difference in LEGS score of 1.43 and 90% power to detect a mean difference in LEGS score of 2.10 points. With a sample size of 136 for Aim 3B, this study had 60% power to detect a mean difference in LEGS score of 1.53 points and 90% power to detect a mean difference in LEGS score of 2.24 points. The clinically meaningful difference in LEGS scores is 2 to 3 points[106].

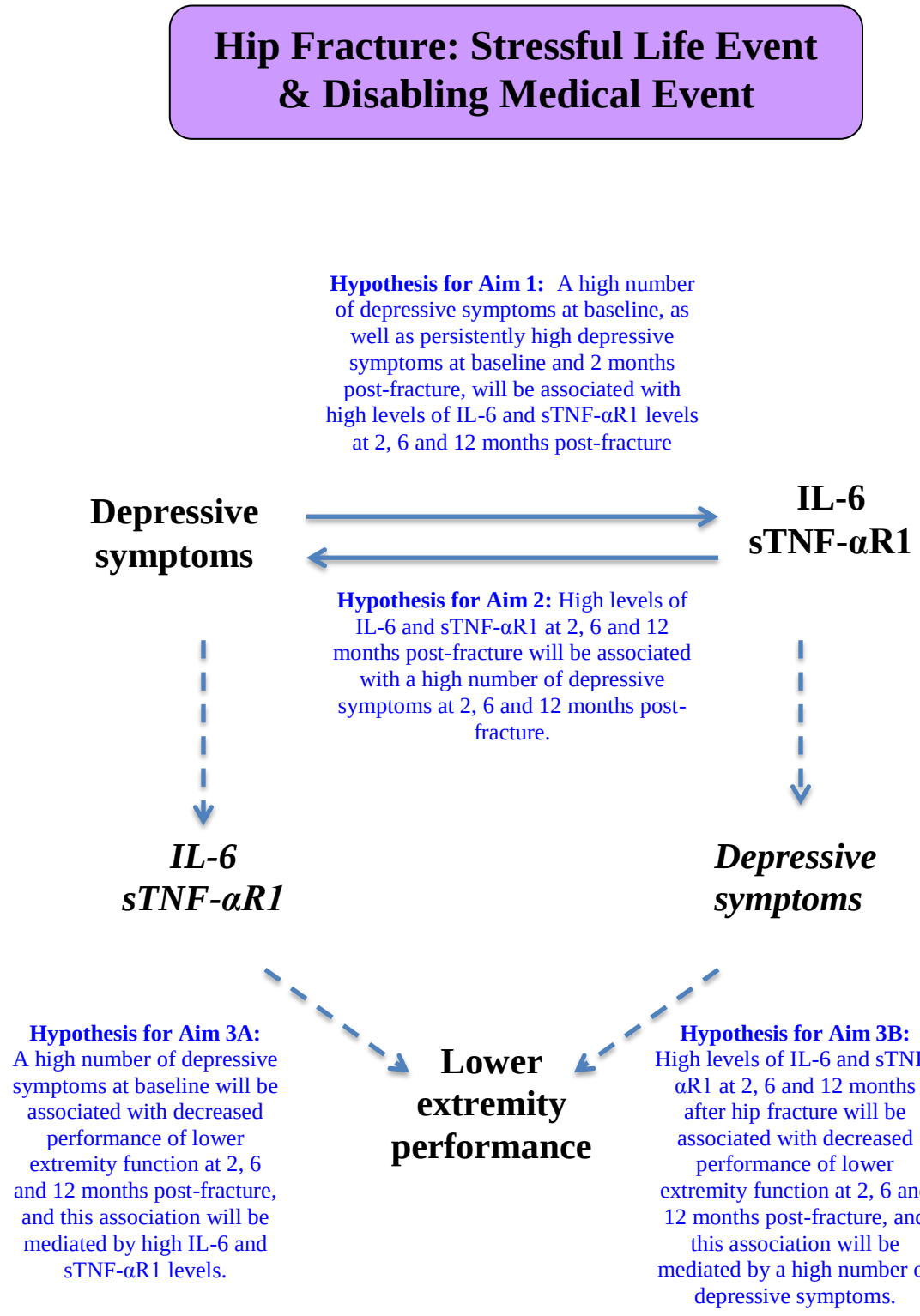
Table 3.9 Power analysis for Aims 3A and 3B

	Power (%)			
Sample Size	**60**	**70**	**80**	**90**
136 (3B)	1.53	1.72	1.94	2.24
155 (3A)	1.43	1.61	1.81	2.10

Conceptual Model and Hypotheses

Figure 3.2 illustrates the conceptual model for the study, along with the hypothesis for each study aim.

Figure 3.2 Conceptual model for study with hypotheses for each aim



Statistical Analysis

Descriptive analyses were performed to describe the characteristics of the study population and distributions of GDS scores, IL-6 concentrations, sTNF-αR1 concentrations, and LEGS scores. This included means and standard deviations for continuous variables, percentages for categorical variables and scatter plots for each independent and dependent variable over time. Participant characteristics were described for the overall study population. Spaghetti plots were constructed to graphically portray changes in GDS scores, cytokine concentrations and LEGS scores over time. Histograms were generated to determine whether the dependent variables were normally distributed at each time point. Bivariate analyses (i.e., Pearson correlations) were performed to assess time-specific correlations between variables and to determine which variables to include in the multivariable analyses.

Generalized estimating equations (GEE) were used to model the longitudinal relationships between independent and dependent variables over the year after hip fracture. The GEE analytic method uses data from all visits with both the independent and dependent variables measured and enables performing longitudinal analyses when some study visits are missing. This method can account for possible correlations in repeated measures over time and is suitable for exploring differences in values measured at different times. Generalized F tests were used to compare dependent variables over time. Time-specific t-tests were performed to compare dependent variables at each time point. The models were adjusted for age, BMI, Charlson Comorbidity Index score, MMSE score, and treatment group (exercise versus control). Analyses were performed using SAS, version 9.2 (SAS Institute, Inc., Cary, NC).

Analytic Strategy by Aim

The results from Aims 1A and 1B are included in Chapter Four, entitled "Prevalence and Persistence of Depressive Symptoms and Inflammatory Cytokines in Older Women in the Year after Hip Fracture".

Aim 1A

To determine whether depressive symptoms at baseline are associated with IL-6 and sTNF-αR1 levels at 2, 6, and 12 months post-fracture

Hypothesis 1A

Among female elders who have undergone surgical repair of hip fracture, those with a high number of depressive symptoms at baseline will have high levels of IL-6 and sTNF-αR1 at 2, 6 and 12 months post-fracture.

Statistical Model for Aim 1A

[$Cytokine_T$] = a + (β1*GDS) + (β2*Time) + (β3*GDS*Time) + (β4*Covariates)

$Cytokine_T$: Serum cytokine concentration at time T, where T=2, 6 or 12 months

GDS: GDS Score at time T, where T=baseline

Time: T0= 2 months (reference), T1= 6 months, T2= 12 months

Covariates: Age, BMI, Charlson Comorbidity Index score, MMSE, treatment group

Hypothesis Testing for Aim 1A

To determine if there is an association of GDS scores with cytokine concentrations over time, a longitudinal analysis testing the following hypotheses can be done using a Generalized Estimating Equation (GEE), two-sided test, alpha level of 0.05:

H_o: (β1*GDS) = 0: There is no association between GDS score and cytokine level.

H_o: (β2*time) = 0: Mean cytokine levels do not change over time.

H_o: (β3*GDS*time) = 0: The relationship between GDS score and cytokine level does not change over time.

Aim 1B

To determine whether persistently high depressive symptoms at baseline and 2 months after hip fracture are associated with IL-6 and sTNF-αR1 levels at 2, 6, and 12 months post-fracture

Hypothesis 1B

Among female elders who have undergone surgical repair of hip fracture, those with persistently high depressive symptoms at baseline and 2 months after surgery will have higher inflammatory cytokine levels at 2, 6 and 12 months, as compared to those without persistently high depressive symptoms.

Statistical Model for Aim 1B

$[Cytokine_T]$= a + (β1*DepressionT1T2) + (β2*Time) + (β3*DepressionT1T2*Time) + (β4*Covariates)

$Cytokine_T$: Serum cytokine concentration at time T, where T= 2, 6 or 12 months

Depression category (T1 = baseline, T2 = 2 months):

-No depression (reference), Persistent depression (“Depression T1T2”)

Time: T= 2 months (reference), 6 months or 12 months

Covariates:

Age, Charlson Comorbidity Index score, BMI, MMSE, treatment group

Hypothesis Testing for Aim 1B

To determine if there is an association of persistently high depressive symptoms with cytokine concentrations over time, the following hypotheses can be tested using GEE, two-sided test, alpha level of 0.05:

H_o: (β1*DepressionT1T2) = 0: The relationship between persistently elevated GDS score and cytokine levels does not differ from the not persistently elevated GDS score and cytokine level relationship.

H_o: (β2*time) = 0: Mean cytokine levels do not change over time.

H_o: (β3*DepressionT1T2*time) = 0: The relationship between persistently elevated GDS score and cytokine level does not change over time.

The results from Aim 2 are included in Chapter Five, entitled "Inflammatory Cytokines and Depressive Symptoms in Older Women in the Year after Hip Fracture".

Aim 2

To determine whether levels of IL-6 and sTNF-αR1 at 2, 6 and 12 months post-fracture are associated with depressive symptoms at 2, 6 and 12 months post-fracture

Hypothesis 2

Among female elders who have undergone surgical repair of hip fracture, those with high levels of IL-6 and sTNF-αR1 at 2, 6 and 12 months after hip fracture will have a higher number of depressive symptoms at 2, 6 and 12 months post-fracture.

Statistical Model for Aim 2

$[GDS_T]$ = a + (β1*Cytokine level group) + (β2*Time) + (β3*Cytokine level group*Time) + (β4*Covariates)

GDS_T: GDS Score at time T, where T=2, 6 or 12 months

Cytokine: Serum cytokine level group – low (reference), middle, high

Time: T0= 2 months, T1= 6 months, T2= 12 months

Covariates: Age, BMI, Charlson Comorbidity Index score, MMSE, treatment group

Hypothesis Testing for Aim 2

To determine if there is an association of serum cytokine level group with GDS scores over time, a longitudinal analysis testing the following hypotheses can be addressed using a Generalized Estimating Equation (GEE), two-sided test, alpha level of 0.05:

H_o: (β1*Cytokine level $group_T$) = 0: There is no association between serum cytokine level group and GDS scores.

H_o: (β2*time) = 0: Mean GDS scores are constant over time.

H_o: (β3*Cytokine level $group_T$*time) = 0: The relationship between serum cytokine level group and GDS scores does not change over time.

The results from Aims 3A and 3B are included in Chapter Six, entitled "Depressive Symptoms, Inflammatory Cytokines and Lower Extremity Performance in Older Women in the Year after Hip Fracture".

Aim 3A

To examine the relationships between depressive symptoms at baseline and performance of lower extremity function at 2, 6 and 12 months post-fracture

Hypothesis 3A

A high number of depressive at baseline will be associated with decreased performance of lower extremity function at 2, 6 and 12 months post-fracture.

Statistical Model for Aim 3A

$[LEGS_T] = a + (\beta1*GDS_T) + (\beta2* Time) + (\beta3*GDS_T*Time) + (\beta4*Covariates)$

$LEGS_T$: Lower Extremity Gain Scale (LEGS) score at time T, where T = 2, 6 and 12 months

GDS_T: GDS Score at time T, where T = baseline and 2 months

Time: T0= 2 months (reference), T1= 6 months, T2= 12 months

Covariates: Age, BMI, comorbidity, MMSE, treatment group

Hypothesis Testing for Aim 3A

To determine if there is an association of GDS scores with LEGS scores over time, a longitudinal analysis testing the following hypotheses can be addressed using a Generalized Estimating Equation (GEE), two-sided test, alpha level of 0.05:

H_o: $(\beta1*GDS_T) = 0$: There is no association between GDS scores and LEGS scores.

H_o: $(\beta2*Time) = 0$: Mean LEGS scores are constant over time.

H_o: (β3*GDS_T*Time) = 0: The relationship between GDS scores and LEGS scores does not change over time.

Aim 3A.1

To determine whether high levels of IL-6 and sTNF-αR1 post-fracture mediate (or account for) the association between high numbers of depressive symptoms and decreased performance of lower extremity function

Hypothesis 3A.1

High levels of IL-6 and sTNF-αR1 post-fracture will mediate the association between high numbers of depressive symptoms and decreased performance of lower extremity function.

Statistical Analysis for Aim 3A.1

The analysis for Aim 3A.1 will be similar to Aim 3A, with the addition of cytokine level as a potential mediating variable. This aim will only be explored if an association exists for Aim 3A.

Statistical Model for Aim 3A.1

$[LEGS_T] = a + (\beta1*GDS_T) + (\beta2*Time) + (\beta3*GDS_T*Time) + (\beta4*Covariates \& Cytokine_T)$

Hypothesis Testing for Aim 3A.1

To determine if serum cytokine level mediates the association of GDS scores with LEGS score over time, a longitudinal analysis testing the following hypothesis can be addressed using a Generalized Estimating Equation (GEE), two-sided test, alpha level of 0.05:

H_o: (β4*Covariates & $Cytokine_T$) = 0: There is no difference in the GDS score-LEGS score relationship by cytokine level over time.

Aim 3B

To examine the relationships between levels of IL-6 and sTNF-αR1at 2, 6 and 12 months post-fracture and performance of lower extremity function at 2, 6 and 12 months post-fracture

Hypothesis 3B

High levels of IL-6 and sTNF-αR1 at 2, 6 and 12 months post-fracture will be associated with decreased performance of lower extremity function at 2, 6 and 12 months.

Statistical Model for Aim 3B

$[LEGS_T] = a + (\beta 1 * \text{Cytokine level group}_T) + (\beta 2 * \text{Time}) + (\beta 3 * \text{Cytokine level group}_T * \text{Time}) + (\beta 4 * \text{Covariates})$

$LEGS_T$: Lower Extremity Gain Scale (LEGS) score at time T, where T = 2, 6 and 12 months

Cytokine level $group_T$: Serum cytokine level group – low (reference), middle, high

Time: T0= 2 months, T1= 6 months, T= 12 months

Covariates:

Age, BMI, comorbidity, MMSE, treatment group

Hypothesis Testing for Aim 3B

To determine if there is an association of serum cytokine concentration with LEGS scores over time, a longitudinal analysis testing the following hypotheses can be addressed using a Generalized Estimating Equation (GEE), two-sided test, alpha level of 0.05:

H_o: (β1*Cytokine level group$_T$) = 0: There is no association between serum cytokine level group and LEGS scores.

H_o: (β2*Time) = 0: Mean LEGS scores are constant over time.

H_o: (β3*Cytokine level group$_T$*Time) = 0: The relationship between serum cytokine level group and LEGS scores does not change over time.

Aim 3B.1

To determine whether depressive symptoms mediate the association between levels of IL-6 and sTNF-αR1 and performance of lower extremity function

Hypothesis 3B.1

High depressive symptom levels will mediate the association between high levels of IL-6 and sTNF-αR1 and decreased performance of lower extremity function.

Statistical Analysis for Aim 3B.1

The analysis for Aim 3B.1 will be similar to that of Aim 3B, with the addition of GDS score as a potential mediating variable. This aim will only be explored if an association exists for Aim 3B.

Statistical Model for Aim 3B.1

$[LEGS_T] = a + (\beta1*\text{Cytokine level group}_T) + (\beta2* \text{Time}) + (\beta3*\text{Cytokine level group}_T*\text{Time}) + (\beta4*\text{Covariates \& } GDS_T)$

$LEGS_T$: Lower Extremity Gain Scale at time T, where T = 2, 6 and 12 months

$Cytokine_T$: Cytokine level group at time T, where T = 2, 6 and 12 months

Covariates: Age, BMI, Charlson Comorbidity Index score, MMSE, and treatment group

GDS_T: GDS Score at time T, where T = 2, 6, or12 months

Hypothesis Testing for Aim 3B.1

To determine if GDS scores mediate the association of serum cytokine concentrations with LEGS score over time, a longitudinal analysis testing the following hypothesis can be addressed using a Generalized Estimating Equation (GEE), two-sided test, alpha level of 0.05:

H_o: (β2*Covariates & GDS_T) = 0: There is no difference in cytokine level group-LEGS score relationship by GDS score over time.

4. Prevalence and Persistence of Depressive Symptoms and Inflammatory Cytokine Levels in Older Women in the Year after Hip Fracture

ABSTRACT

Background – Depressive symptoms are a common occurrence after hip fracture. Systemic immune activation, including an increase in pro-inflammatory cytokine levels, has been associated with the pathophysiology of depression. However, the relationship between depression and inflammation has not been studied in the context of the hip fracture patient.

Methods – We used the fourth cohort of the Baltimore Hip Studies (BHS-4), in which women age 65 and older with surgical repair of a non-pathologic incident hip fracture were recruited from three Baltimore area hospitals for a randomized controlled trial of an in-home exercise intervention, to select patients who had the Geriatric Depression Scale (GDS) measured at baseline and an inflammatory cytokine [interleukin (IL-) 6 and soluble tumor necrosis factor alpha receptor 1 (sTNF-αR1)] measured at 2, 6 or 12 months post-fracture for the present study. Generalized estimating equations were used to model the relationships between GDS score at baseline and cytokine concentration at 2, 6, and 12 months post-fracture, as well as between persistently elevated GDS score group and cytokine concentration at 2, 6, and 12 months post-fracture.

Results – Clinically significant levels of depressive symptoms were present in 12.5% of study participants (N=136) at baseline. Baseline GDS scores were associated with higher levels of IL-6 and sTNF-αR1 at 2, 6 and 12 months postfracture, though not statistically significant. At 2 months, participants with persistently elevated GDS scores had

significantly lower sTNF-αR1 levels than those without persistently elevated GDS scores (β=-383 pg/mL; 95% CI: -722, -45; p=0.02), as well as greater increases in sTNF-αR1 levels by 12 months (p<0.0001).

Conclusions **–** Persistence of depressive symptoms may play an important role, not only in physical but also in physiologic function after hip fracture.

4.1 INTRODUCTION

Hip fracture is an acute life event that leads to functional decline and subsequent chronic stress, which commonly results in depression among elderly patients[24]. Some research has suggested that depressive symptoms after hip fracture are highest in the immediate post-fracture period, with a fairly rapid decline in the subsequent 2 months, likely representing an acute stress response[13,18,19,22]. By four to six months post-fracture, most patients have reached their recovery plateau in depressive symptoms[22].

On the other hand, depressive symptoms are associated with systemic immune activation, characterized by increased production of pro-inflammatory cytokines (e.g., interleukin 1 beta (IL-1β), IL-2, IL-6, and tumor necrosis factor alpha) by peripheral blood mononuclear cells, in addition to a host of other upregulated immune components (e.g., an acute phase protein response and excessive secretion of pro-inflammatory cytokines, prostaglandins and nitric oxide) [80-82]. Evidence from the field of psychoneuroimmunology suggests that complex, multi-directional interactions among the immune, central nervous and endocrine systems, initiated by physical and psychological stressors, explain the increased production and release of pro-inflammatory cytokines observed in patients with major depression[26,70,86].

The pro-inflammatory cytokines IL-1β, IL-6 and TNF-α have been identified by many studies as important components in the pathophysiology of depressive symptoms[107]. In one recent meta-analysis, positive associations were reported between IL-1β, IL-6 and CRP and depression in clinically depressed and community-based adults of varying ages[27]. In a second meta-analysis of 24 cytokine studies, subjects who met diagnostic criteria for major depressive disorder had elevated levels of IL-6 and TNF-α

compared to control subjects[108]. Several large scale epidemiologic studies have examined the prospective relationships between markers of systemic inflammation and depressive symptoms in young adult populations[109,110]. However, findings in older adults are limited[91,111,112], and there are no studies in elderly persons who have had a hip fracture and may be at increased risk for depression.

An increase in and persistence of depressive symptoms post-fracture as well as increased inflammatory cytokine levels[15] have independently been linked to poor recovery of function after hip fracture. Therefore, investigating the potential coordinated response between these variables is important to elucidate mechanisms underlying functional impairment in the hip fracture patient.

We propose to: 1) characterize the prevalence of depressive symptoms in a sample of hip fracture patients who were cleared to participate in an in-home exercise intervention during the 12-month post-fracture recovery period; 2) determine whether depressive symptoms that occur early in the recovery period are associated with inflammatory cytokine levels later in the recovery period; and 3) determine whether depressive symptoms that persist (i.e., occur at the time of fracture and 2 months thereafter) are associated with inflammatory cytokine levels over the year after hip fracture.

4.2 METHODS

Study Design

We selected our study patients from the fourth cohort in the Baltimore Hip Studies (BHS-4), a randomized clinical trial which tested the feasibility of the Exercise Plus Program compared to usual care in hip fracture patients, described elsewhere[3,96].

Briefly, participants were enrolled within 15 days of the hip fracture. Baseline measurements (questionnaire, performance measures and blood draw) and self-reported pre-fracture data were collected within 22 days post-hip fracture and follow-up data were collected at 2, 6 and 12 months post-fracture.

For this analysis, data were aggregated from both treatment and usual care groups. In the randomized controlled trial, there was no effect of the active treatment on depressive symptoms[3].Although there was no effect of the exercise intervention on IL-6 levels, sTNF-αR1 levels were lower among exercisers[100]. Therefore, treatment group was included as a covariate in the analysis.

Participants

Eligibility was determined through a medical chart review, medical assessment, and cognitive screen. BHS-4 enrolled 180 community-dwelling female hip fracture patients 65 and older who had a non-pathologic fracture within 72 hours of admission and surgical repair of the fracture. Additional eligibility criteria included ability to walk without human assistance prior to the fracture, no medical conditions contraindicated with exercise, and a score of $\geq$20 on the Mini-Mental State Examination (MMSE).[97] Due to safety concerns of older adults exercising independently in the home, the stringent set of study inclusion criteria resulted in only 243 of the 1,276 (19%) screened hip fracture patients being eligible; and 74% of eligible women agreed to participate in the trial.

Variables of Interest

Depressive Symptoms

Baseline and two-month depressive symptoms were assessed using the 15-item Geriatric Depression Scale (GDS). The GDS-15 is a series of 15 yes/no questions

developed as a basic screening tool for depression in older adults[101]. Depressive symptoms are assessed as having occurred in the past week. A score of 6 or greater on the GDS-15 is indicative of clinically significant depressive symptoms, and this cutoff has a sensitivity of 82% and a specificity of 82%[102].

Inflammatory Cytokine Levels

At the 2-, 6- and 12-month evaluations, blood specimens were drawn from 96, 108, and 92 participants, respectively, and the sera were stored at -70°C. Serum IL-6 and sTNF-αR1 levels were measured using commercially available enzyme-linked immuno-sorbent assay (ELISA) kits *(R&D systems, Minneapolis, MN).* For IL-6, the inter-assay coefficient of variation (CV) was 3.44% and the intra-assay was 4.37%. For sTNF-αR1, the inter-assay CV was 2.98% and the intra-assay was 5.47%.

Baseline Covariates

The medical record was reviewed to obtain the participant's age and the number of medical comorbidities. Height and weight were used to calculate body mass index (BMI). The treatment group variable was based on whether the patient had been randomly assigned to the exercise or control group. Cognitive status was assessed using MMSE[97].

Analytic Strategy

Depending on the study aim, we included participants who fulfilled different criteria as shown in **Table 4.1**. **Tables 4.2** and **4.3** describe the total number of study participants based on whether cytokine levels were measured at one or more study visits for Aims 1A and 1B, respectively.

Table 4.1 Inclusion criteria by study aim

	Number of Participants Included	Inclusion Criteria		
		GDS at baseline	GDS at 2 months	IL-6 or sTNF-αR1 at 2, 6 or 12 months
Aim 1A: Association between baseline GDS and 2-, 6-, and 12-month cytokine level	136	X		X
Aim 1B: Association between persistently elevated GDS and 2-, 6-, and 12-month cytokine level	124	X	X	X

Table 4.2 Total number of study participants for Aim 1A

Aim 1A: Baseline GDS score and cytokine measured at:	IL-6	sTNF-αR1
3 visits	51	53
2 visits	54	53
1 visit	31	30
Total number of participants	***136***	***136***

Table 4.3 Total number of study participants for Aim 1B

Aim 1B: Baseline and 2-month GDS scores and cytokine measured at:	IL-6	sTNF-αR1
3 visits	51	53
2 visits	47	46
1 visit	26	25
Total number of participants	***124***	***124***

Generalized estimating equations (GEE)[113] were used to model the longitudinal relationships between the following variables: a) GDS scores at baseline and inflammatory cytokine concentration over the year after hip fracture and b) persistently high depressive symptoms and inflammatory cytokine levels. The GEE analytic method uses data from all visits with both a GDS and cytokine measure and enables performing

longitudinal analyses when some study visits are missing. This method can account for possible correlations in repeated measures over time and is suitable for exploring differences in values measured at different times. Generalized F tests were used to compare IL-6 and sTNF-αR1 levels over time. Time-specific t-tests were performed to compare IL-6 and sTNF-αR1 levels at each time point. The models were adjusted for age, BMI, Charlson Comorbidity Index score, MMSE score, and treatment group (exercise versus control). Analyses were performed using SAS, version 9.2 (SAS Institute, Inc., Cary, NC).

4.3 RESULTS

Demographic and medical characteristics of the study sample at baseline are summarized in **Table 4.4** for the overall sample. The study sample consisted of 136 female hip fracture patients, with a mean age of 81.4 years (*SD* = 6.2). At baseline, participants had a mean BMI of 23.8 kg/m^2 (*SD* = 4.2), Charlson Comorbidity Index of 1.0 (*SD* = 1.3), and MMSE score of 27 (*SD* = 2.5). The sample was representative of hip fracture patients in terms of age, race, and fracture type, but the patients had fewer comorbid medical conditions and were cognitively intact as required by the study inclusion criteria.

At baseline, the prevalence of clinically significant depressive symptoms (GDS $\geq$ 6) was 12.5%, and GDS scores ranged from 0 to 14, with mean (SD) of 2.3 (2.5). Demographic and medical characteristics were similar among participants with and without high depressive symptoms at baseline (data not shown).

Table 4.4 Demographic and Medical Characteristics of the Study Participants at Baseline

	Overall N=136
Age, mean (SD), years	81.4 (6.2)
White, No. (%)	131 (96.3)
Married, No. (%)	47 (34.6)
Anesthesia risk rating, mean (SD)	2.7 (0.5)
Body mass index (kg/m^2), mean (SD)	23.8 (4.2)
Charlson comorbidity index score, mean (SD)	1.0 (1.3)
Fracture type, No. (%)	
Intertrochanteric	55 (40.4)
Subcapital	74 (54.4)
Subtrochanteric	7 (5.2)
Geriatric Depression Scale score*, mean (SD)	2.3 (2.5)
Length of hospital stay, mean (SD), days	4.0 (1.4)
Mini-Mental Status Examination score, mean (SD)	27.0 (2.5)
Post-operative complications, No. (%)	
Confusion or disorientation	6 (4.4)
Pulmonary embolism	1 (0.7)
Renal failure	1 (0.7)
Shock post-op	2 (1.5)
Urinary tract infection	4 (2.9)
Wound hematoma/bleeding	3 (2.2)
Other complications	37 (27.2)

kg/m^2 = kilograms per meter squared

*Range for Geriatric Depression Scale: 0-15; maximum score in this sample: 14

The median and interquartile ranges for IL-6 and sTNF-αR1 are displayed in **Figures 4.1** and **4.2**, respectively. Serum cytokine levels were highest at baseline, and declined at 2 months, with a more gradual decline in median levels thereafter.

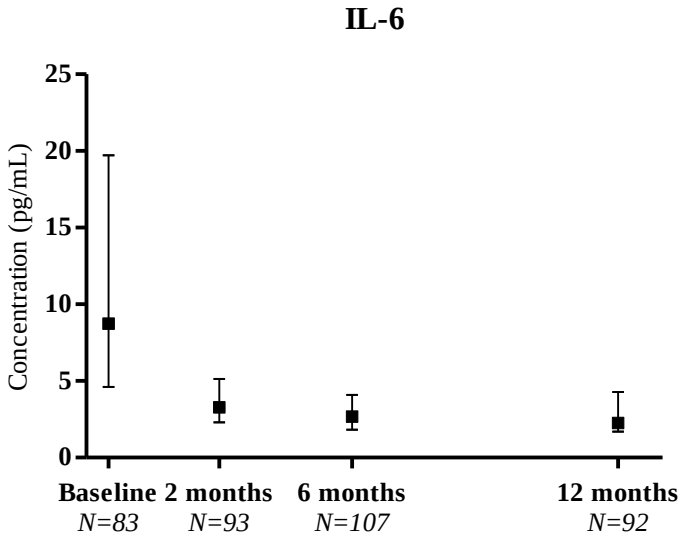


Figure 4.1 Median value and interquartile range for serum IL-6 concentration at each time point

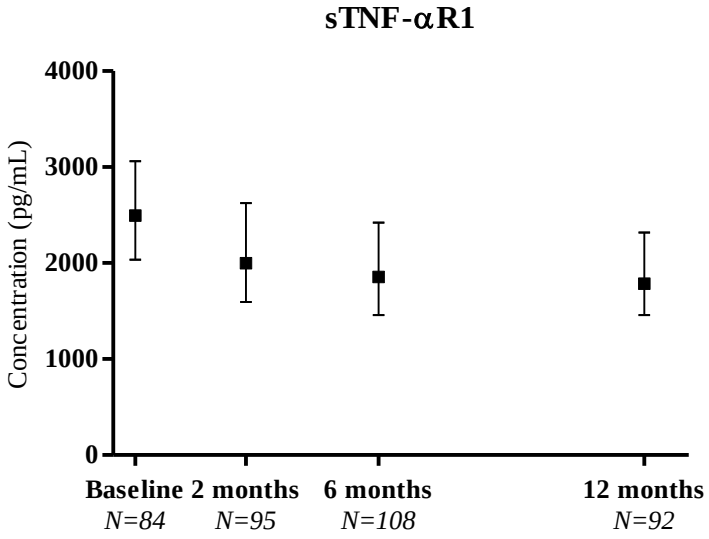


Figure 4.2 Median value and interquartile range for serum sTNF-αR1 concentration at each time point

Figures 4.3 and 4.4 illustrate the adjusted associations between baseline GDS score and inflammatory cytokine levels at 2, 6, and 12 months after hip fracture. The associations presented in these graphs represent the difference in inflammatory cytokine concentration per unit of GDS score and the 95% confidence interval. After adjusting for covariates, study participants with higher baseline GDS scores had higher average levels of IL-6 (**Figure 4.3**) and sTNF-αR1 (**Figure 4.4**). All cross-sectional 2-, 6-, and 12-month point estimates were positive for IL-6 and sTNF-αR1 at all three follow-up time points, though not statistically significant. The global associations, assessing longitudinal relationships with inflammatory cytokine level including time x GDS score interaction, were not statistically significant for IL-6 (p=0.39) or sTNF-αR1 (p=0.69).

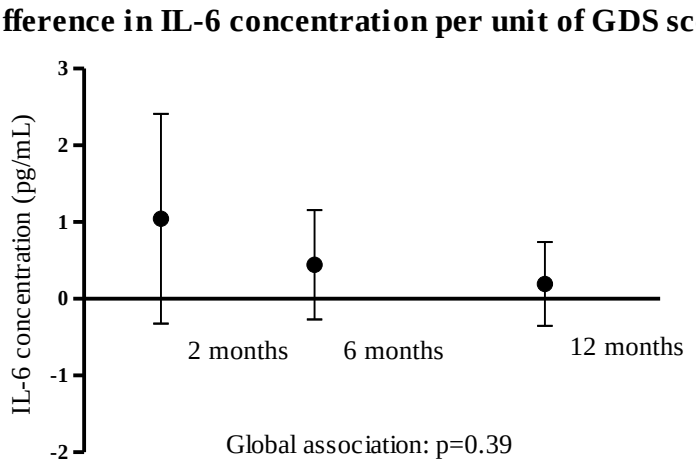


Figure 4.3 Difference in IL-6 concentration per unit of GDS score at baseline

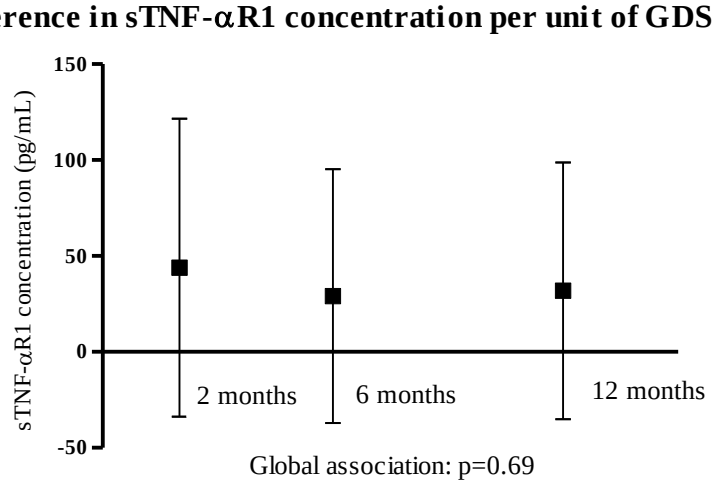


Figure 4.4 Difference in sTNF-αR1 concentration per unit of GDS score at baseline

Of the 124 study participants with a GDS score measured at baseline and two months who also had a cytokine measured at 2, 6 or 12 months, 12 participants (9.7%) had clinically significant depressive symptoms (GDS≥6) at baseline and two months post-fracture. **Figures 4.5 and 4.6** illustrate the difference in inflammatory cytokine concentration between participants with and without persistently high GDS scores, along with the 95% confidence interval. After adjusting for covariates, participants with persistently high GDS scores did not have consistently higher or lower IL-6 (**Figure 4.5**) or sTNF-αR1 (**Figure 4.6**) levels than participants without persistently high GDS scores. The association between persistently high GDS scores and sTNF-αR1 concentration was strongest at 2 months post-fracture, such that participants with persistently high GDS scores had significantly lower mean sTNF-αR1 concentration than participants without persistently high GDS scores. The global association, assessing longitudinal relationships with inflammatory cytokine level including time x persistence interaction, was not statistically significant for IL-6 (p=0.75) but was statistically significant for

sTNF-αR1 (p<0.0001), indicating that participants with persistently elevated GDS scores had lower levels of sTNF-αR1 initially but their levels increased at a faster rate. There were positive changes in sTNF-αR1 levels over time among those with persistently high GDS scores (p for time x persistence interaction <0.0075); that is, participants with persistently high GDS scores had greater increases in sTNF-αR1 over time.

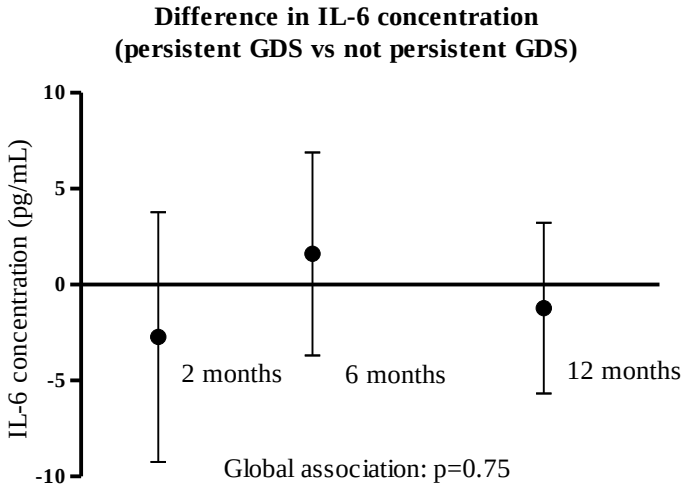


Figure 4.5 Difference in IL-6 concentration between participants with and without persistently high GDS scores

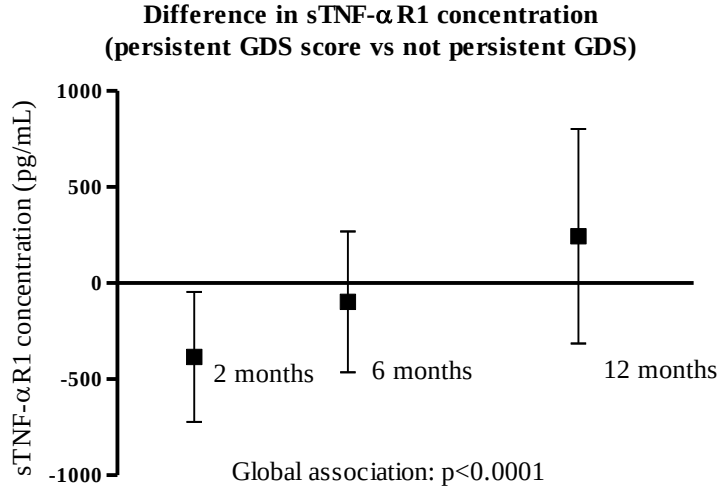


Figure 4.6 Difference in sTNF-αR1 concentration between participants with and without persistently high GDS scores

To test the robustness of these data against the influence of outliers, a sensitivity analysis was conducted excluding the highest cytokine values (IL-6 >84 pg/mL and sTNF-αR1 >5400 pg/mL). The direction of the association between baseline GDS and IL-6 concentration at 2, 6 and 12 months changed from positive to negative for the 12-month cross-sectional association. Neither the direction nor statistical significance of the cross-sectional or longitudinal relationship changed at any other time point for either of the cytokines with the exclusion of these potential outliers from the analysis. This was also observed for the association between persistence of high GDS scores and cytokine concentrations.

4.4 DISCUSSION

This is the first study to assess an association between depressive symptoms and inflammatory cytokine levels after hip fracture. Outside of community-based samples of healthy participants and psychiatric-derived patient populations, the depression-inflammation association has usually been studied in patients with cardiac disease or cancer[27]. We found a trend toward a positive association between baseline GDS scores and cytokine levels during the year after hip fracture. Study participants with persistently elevated GDS scores had significantly lower sTNF-αR1 levels at 2 months, with greater increases in sTNF- αR1 levels over time than those without persistently elevated GDS scores. There was no consistently meaningful association between persistently elevated GDS scores and IL-6 levels. Despite the low mean GDS score at baseline, the prevalence of clinically significant depressive symptoms (12.5%) in our study sample is consistent with earlier reports of depression following hip fracture[18].

We found no statistically significant associations between depressive symptoms at baseline and IL-6 concentrations over the year after fracture. It is interesting to note that, at all post-fracture time points, median levels of IL-6 were lower than the levels measured in a previous hip fracture cohort from the Baltimore Hip Studies.[15] Lower levels of inflammatory markers could be a reflection of health status of the participants in this study. The stringent inclusion criteria applied to the 4th BHS cohort[3] resulted in a sample with fewer comorbid conditions and greater cognitive function than among the typical hip fracture patient population. In a sample more representative of the latter, we might have seen different results for the association between depressive symptoms and IL-6 in the post-fracture recovery period.

Exercise is a critical component of hip fracture rehabilitation, and it is also associated with fewer depressive symptoms and a reduction in inflammation associated with aging[114-116]. Although, in our study, the exercise intervention performed by a group of participants was not associated with a difference in GDS scores, the capacity of the patient to perform the exercise intervention may have affected the physiologic outcome of interest (i.e., inflammatory cytokine levels). Indeed, a prior study from our group found that levels of sTNF-αR1 were lower among the group participating in the exercise intervention[100]. Hip fracture patients who qualified for participation in the study and were able to perform the exercise intervention may have led healthier lifestyles prior to the fracture (e.g., diet, exercise, use of antioxidant supplements).

More important than just the occurrence of depressive symptoms at a single time point after hip fracture may be the persistence of depressive symptoms. These findings indicate that hip fracture patients with persistently high GDS scores have a different

inflammatory cytokine profile than those low scores over the post-fracture recovery period, particularly for sTNF-αR1. We expected to find higher levels of both inflammatory markers at 2, 6, and 12 months in study participants with persistently elevated depressive symptoms. We were surprised that the persistent depression group had lower levels of sTNF-αR1 at 2 and 6 months, though the increase in sTNF-αR1 levels over time was consistent with our hypothesis. These results may have been strongly influenced by the small number of study participants with persistently elevated GDS score group (n=12) compared to participants without persistently elevated GDS scores (n=112). Alternatively, these findings could be the result of random variation.

These results support the hypothesis that depression may precede inflammation. Indeed, a meta-analysis of studies of the association between depression and C-reactive protein, IL-6 and IL-1 suggested that there was evidence supporting three causal pathways: depression to inflammation, inflammation to depression, and bidirectional relationships[27].

The importance of screening for depressive symptoms throughout the post-fracture recovery period cannot be understated. Although the GDS is not a diagnostic instrument, it is appropriate for screening and identification of older patients who may be at risk for developing depressive disorders. Patients with depression due to disability require longer rehabilitation, and presence and persistence of depressive symptoms are risk factors for poor functional recovery post-fracture[13,92,95]. Our findings indicate that the presence and persistence of depressive symptoms impact, not only the patients' physical functional recovery, but also their physiologic function. This might explain why depressed patients with increased levels of inflammatory biomarkers have been found to

exhibit treatment resistance[25]. A better understanding of this chronic immune dysregulation after hip fracture is needed, in order to develop new treatment that targets both depressive symptoms and functional recovery.

This study has several limitations. The study population was only comprised of white women, making findings less generalizable to women of other ethnic backgrounds and men. The women who participated in this study were also much healthier and more cognitively intact than the general hip fracture population.

Because depression has been linked to all-cause mortality in older adults, it is important to study biological factors that may be associated with depressive symptomatology in at-risk older populations. It is not clear whether the presence or persistence of depressive symptoms contribute to the increased risk of death for hip fracture patients in the first year following the event. However, further research in this area may help to understand these factors.

5. Inflammatory Cytokine Levels and Depressive Symptoms in Older Women in the Year after Hip Fracture: Findings from the Baltimore Hip Studies[1]

ABSTRACT

OBJECTIVES: Determine whether interleukin 6 (IL-6) or soluble tumor necrosis factor α receptor 1 (sTNF-αR1) are associated with depressive symptoms in the year after hip fracture

DESIGN: Prospective cohort

SETTING: Three Baltimore-area hospitals

PARTICIPANTS: Community-dwelling women aged ≥65 years, admitted with a new, non-pathological fracture of the proximal femur (n=134).

MEASUREMENTS: At 2, 6 and 12 months postfracture, serum was analyzed for IL-6 and sTNF-αR1, and depressive symptoms were measured using the 15-item Geriatric Depression Scale (GDS). Generalized estimating equations were used to model the longitudinal relationship between IL-6 and sTNF-αR1 and GDS score. We also examined whether lower extremity function, as measured by Lower Extremity Gain Scale (LEGS), explained the relationship between IL-6, sTNF-αR1 and GDS score.

RESULTS: Patients in the highest categories of IL-6 (≥5.14 pg/mL) and sTNF-αR1 (≥2421 pg/mL) had the highest GDS scores in the year postfracture (p=0.09 for both). At

[1] M.E. Matheny, R.R. Miller, M.D. Shardell, W.G. Hawkes, E.J. Lenze, J. Magaziner, D.L. Orwig

As submitted to the Journal of the American Geriatrics Society

12 months postfracture, those in the highest IL-6 and sTNF-αR1 categories had GDS scores that were on average 1.9 (95% confidence interval [CI]: 0.4, 3.4; p=0.01) and 1.4 (95% CI: -0.1, 3.0; p=0.07) points higher than those in the lowest category, respectively. Adjusting for LEGS score, the mean difference in GDS scores for highest versus lowest IL-6 categories was 1.6 (95% CI: 0.2, 3.0; p=0.02) points at 12 months.

CONCLUSION: Results from these exploratory analyses support a role for inflammation in the pathophysiology of depressive symptoms after hip fracture. Depressive symptoms in the context of elevated cytokines may represent a sickness syndrome that is chronic in some individuals. Further research should establish the cause and effect of this relationship as well as long-term correlates.

5.1 INTRODUCTION

As the world's population ages, the incidence of hip fractures is predicted to increase worldwide. Globally, more than 1.6 million fractures occur annually, and in the United States, over 350,000 persons aged 65 years and over are affected by hip fractures[117,118]. The patients who survive the hip fracture and surgical repair experience tremendous levels of disability, with 25 to 75% of patients who were ambulatory prior to fracture no longer able to walk without assistance or regain their previous level of independent living within the year following fracture[8,9,11].

In addition to the physical disability resulting from hip fractures, there are also psychological and immune sequelae. The onset of disabling medical illness, such as hip fracture, is one of the most important risk factors for a major depressive episode or depressive symptoms in elderly persons[53,63]. Rates of depression after hip fracture are reported to vary from 9-47%[18], and depressive symptoms and major depressive disorder are the highest immediately following the fracture[19].

On the physiologic level, inflammatory cytokine levels have been observed to increase following trauma and surgery, including after hip fracture[33-35]. Elderly patients have an increased IL-6 response to surgical trauma compared with young adults[35]. In the elderly hip fracture population, cytokine levels may remain high up to one year postfracture with median interleukin-6 (IL-6) concentrations of 7.05 pg/mL at 12 months[15].

Depressive symptoms are associated with systemic immune activation, characterized by increased production of pro-inflammatory cytokines (e.g., interleukin 1 beta (IL-1β), IL-2, IL-6, and tumor necrosis factor alpha) by peripheral blood

mononuclear cells, in addition to a host of other upregulated immune components (e.g., an acute phase protein response and excessive secretion of pro-inflammatory cytokines, prostaglandins and nitric oxide) [80-82]. Evidence from the field of psychoneuroimmunology suggests that complex, multi-directional interactions among the immune, central nervous and endocrine systems, initiated by physical and psychological stressors, explain the increased production and release of pro-inflammatory cytokines observed in patients with major depression[26,70,86].

The pro-inflammatory cytokines IL-1β, IL-6 and TNF-α have been identified by many studies as important components in the pathophysiology of depressive symptoms[107]. In one recent meta-analysis, positive associations were reported between IL-1β, Il-6 and CRP and depression[27]. In a second meta-analysis of 24 cytokine studies, subjects who met diagnostic criteria for major depressive disorder had elevated levels of IL-6 and TNF-α[108]. Several large-scale epidemiologic studies have examined the prospective relationships between markers of systemic inflammation and depressive symptoms in adult populations[109,110]. However, findings in older adults are limited, and there are no studies in elderly persons who have had a hip fracture and may be at increased risk for depression.

Studies of inflammatory cytokine levels and depressive symptoms are important in the field of hip fracture research, as both have been studied independently in relation to functional recovery after hip fracture. Increased levels of inflammation have been associated with poor recovery of function in hip fracture patients. Miller and colleagues concluded that higher serum levels of IL-6 were associated with poorer recovery of performance of lower extremity function among female hip fracture patients in the year

following fracture[15]. They also suggested that reduced muscle strength may better explain the poorer recovery of lower extremity function in patients with higher levels of IL-6[16].

This study was an exploratory analysis conducted to estimate the relationship between levels of inflammatory cytokines and depressive symptoms in the year after hip fracture surgery. Additionally, lower extremity performance was examined as a potential mediator of the relationship between postfracture inflammatory cytokine levels and depressive symptoms. The present findings present a focused extension of prior work linking inflammation to depression, which would be of interest to researchers/clinicians who study age-related disorders.

5.2 METHODS

Study Design

Patients were drawn from the fourth cohort in the Baltimore Hip Studies (BHS-4), a randomized clinical trial which tested the feasibility of the Exercise Plus Program compared to usual care in hip fracture patients[3,96]. The BHS-4 study was a two-arm randomized clinical trial with hip fracture patients assigned to either usual care (no intervention) or a supervised home-based exercise program that continued for one year post-hip fracture (The Exercise Plus Program). The exercise program began at the end of the usual post-acute therapy period and contained both strength training and aerobic components. Participants were expected to exercise 5 days per week for 45 minutes each day, and the program consisted of a combination of supervised and independently performed exercise sessions. The intensity and strength of the exercises were increased gradually by the trainers in a standardized way. The frequency of the supervised exercise

sessions decreased throughout the year as participants became more able to exercise independently. For this analysis, data were aggregated from both treatment and usual care groups, and, in the randomized controlled trial, there was no effect of the active treatment on depressive symptoms[3]. Recruitment was initiated in 3 hospitals in the Baltimore area in November 1998 and ended in September 2004. Participants were enrolled within 15 days of the hip fracture. Baseline measurements and pre-fracture data (questionnaire, performance measures and blood draw) were collected within 22 days post-hip fracture and follow-up data were collected at 2, 6 and 12 months postfracture.

Participants

Eligibility was determined through a medical chart review, medical assessment, and cognitive screen. BHS-4 enrolled 180 community-dwelling female hip fracture patients 65 and older who had a non-pathologic fracture within 72 hours of admission and surgical repair of the fracture. Additional eligibility criteria included ability to walk without human assistance prior to the fracture, no medical conditions contraindicated with exercise, and a score of $\geq$20 on the Mini-Mental State Examination (MMSE)[97]. Due to safety concerns of older adults exercising independently in the home, the stringent set of study inclusion criteria resulted in only 243 of the 1,276 (19%) screened hip fracture patients being eligible; however, 74% of eligible women enrolled in the trial. The final sample for this analysis consisted of the 134 unique BHS-4 participants who had both serum cytokine concentration measurements and Geriatric Depression Scale measurements at one or more follow-up visits (2, 6, and 12 months). Institutional Review Board approvals were obtained from the University of Maryland, School of

Medicine as well as the study hospitals, and all enrolled subjects provided their own informed consent.

Measures

Depressive Symptoms and Cytokine Measurement

Two, 6- and 12-month depressive symptoms were measured using the 15-item Geriatric Depression Scale (GDS) [101]. At the 2-, 6- and 12-month evaluations, serum was collected on 96, 108, and 92 participants, respectively. Sera from these participants were stored at -70C. IL-6 and sTNF-αR1 levels were measured in serum that had been previously thawed using commercially available enzyme-linked immuno-sorbent assay (ELISA) kits *(R&D systems, Minneapolis, MN).* For IL-6, the inter-assay CV was 3.44% and the intra-assay CV was 4.37%. For sTNF-αR1, the inter-assay CV was 2.98% and the intra-assay CV was 5.47%.

Covariates

The medical record was reviewed to obtain the patient's age and the number of medical comorbidities. Height and weight were used to calculate body mass index. Treatment group was based on whether the patient had been randomly assigned to the exercise or control groups for the trial. Cognitive status was assessed at baseline using the Mini-Mental State Examination (MMSE) [97]. Because elders with worse function may be more likely to manifest depressive symptoms[119,120], the Lower Extremity Gain Scale[103] (LEGS) score during the follow-up period was also included as a covariate. The LEGS is a summary measurement of timed performance in nine tasks developed to evaluate lower extremity function in hip fracture patients. Performance in each of these tasks was scored

on a scale of 0–4, resulting in a total score between 0 and 36. A higher LEGS score indicates better performance.

Analytic Strategy

Participants had to have both a serum cytokine measurement and GDS score at one or more study follow-up visit(s) to be included in this analysis. The analytic sample size consisted of 134 unique participants. For IL-6, this was comprised of 51 participants with both a cytokine and GDS measured at all three follow-up visits, 53 with both a cytokine and GDS measured at two follow-up visits, and 30 with both a cytokine and GDS measured at only one follow-up visits (134=51+53+30). For sTNF-αR1, this was comprised of 53 participants with both a cytokine and GDS measured at all three follow-up visits, 52 with both a cytokine and GDS measured at two follow-up visits, and 29 with both a cytokine and GDS measured at only one follow-up visit (134=53+52+29).

Serum levels of IL-6 and sTNF-αR1 at the baseline evaluation are more likely to reflect a normal physiological response to the trauma of hip fracture and subsequent surgical repair, and not a prolonged inflammatory process, thus the analysis focused on the relationship between cytokines and GDS at the follow-up visits (2-, 6-, and 12-months post-enrollment) [71,72]. This approach is consistent with previous work from our group[15]. Patients were divided into groups based on their serum IL-6 and sTNF-αR1 levels over the 2-, 6-, and 12-month time points of the follow-up period (IL-6: low group: IL-6 $\leq$ 2.42, middle group 2.42 < IL-6 < 5.14, and high group $\geq$ 5.14 pg/mL; and sTNF-αR1: low group: sTNF-αR1 $\leq$ 1734, middle group: 1734 < sTNF-αR1 < 2421, high group: sTNF-αR1 $\geq$ 2421 pg/mL). These cut-points represent the IL-6 and sTNF-αR1 tertile levels of all the observations over the 2 to 12 month follow-up period, therefore

ensuring that approximately equal numbers of observations were included in each cytokine group over the follow-up period[15,16]. These categories are not interpreted as time-specific tertiles; however, this approach ensured a sufficient sample size in each category at each time-point while accurately accounting for patients' changes in cytokine levels.

Generalized estimating equations[113] (GEE) were used to model the longitudinal relationship between IL-6 and sTNF-αR1 category at the 2, 6 and 12 month follow-up evaluations and GDS scores. The GEE analytic method uses data from all visits with both a GDS and cytokine measure and enables performing longitudinal analyses when some study visits are missing. This method can account for possible correlations in repeated measures over time and is suitable for exploring differences in values measured at different times. Generalized F tests were used to compare GDS scores over time between those in the midlevel and high IL-6 and sTNF-αR1 categories to the lowest (reference) IL-6 and sTNF-αR1 group, respectively. Time-specific t-tests were performed to compare IL-6 and sTNF-αR1 group scores at each time point. The models were adjusted for age, Charlson Comorbidity Index score, MMSE score, treatment group (exercise versus control). In order to examine the role of lower extremity function as a potential mediator of the relationship between inflammation and depressive symptoms additional models adjusted for lower extremity performance as measured by the LEGS score. Analyses were performed using SAS, version 9.1 (SAS Institute, Inc., Cary, NC).

5.3 RESULTS

Demographic and medical characteristics of the study sample are summarized in **Table 5.1**. As the table shows, the sample was representative of hip fracture patients in terms of age, race, and fracture type, but relatively healthier and more cognitively intact as required by the study inclusion criteria. The majority of post-operative complications were listed as "other" (27%), though 4% of participants experienced confusion or disorientation, 3% experienced urinary tract infections and 2% had wound hematomas/bleeding.

After adjusting for covariates, patients in the highest categories of IL-6 and sTNF-αR1 had marginally higher average GDS scores than the lowest categories during the follow-up period, but the result was not statistically significant at the alpha=0.05 level (p=0.09 for both). GDS scores did not differ significantly over the follow-up period between patients in the middle and lowest categories of IL-6 (p=0.78; **Figure 5.1**) or sTNF-αR1 (p=0.68; **Figure 5.2**). The difference in mean GDS scores between high and low IL-6 groups was greatest at 12 months postfracture; when adjusting for covariates, on average those in the highest IL-6 group scored 1.9 (95% CI 0.4, 3.4; p=0.01) points higher on the GDS (**Figure 5.3**). For sTNF-αR1, the difference in GDS score between high and low sTNF-αR1 groups was also greatest at 12 months postfracture, when adjusting for covariates, on average those in the highest sTNF-αR1 group scored 1.4 (95% CI -0.1, 3.0; p=0.07) points higher on the GDS (**Figure 5.4**).

Table 5.1 Demographic and Medical Characteristics at Baseline (N=134)

Age, mean (SD), y	81.7 (6.7)
White, No. (%)	130 (96.3)
Married, No. (%)	47 (34.8)
Anesthesia risk rating, mean (SD)	2.7 (0.5)
Body mass index (kg/m^2), mean (SD)	23.8 (4.2)
Charlson comorbidity index score, mean (SD)	1.1 (1.3)
Fracture site, No. (%)	
Intertrochanteric	55 (40.7)
Subcapital	73 (54.1)
Subtrochanteric	5 (5.2)
Geriatric Depression Scale score*, mean (SD)	2.3 (2.5)
Length of hospital stay, mean (SD)	4.0 (1.4)
Lower Extremity Gain Scale score at 2 months†, mean (SD)	23.3 (6.5)
Mini-Mental Status Examination score, mean (SD)	27.0 (2.5)
Post-operative complications, No. (%)	
Confusion or disorientation	6 (4.4)
Pulmonary embolism	1 (0.7)
Renal failure	1 (0.7)
Shock post-op	2 (1.5)
Urinary tract infection	4 (3.0)
Wound hematoma/bleeding	3 (2.2)
Other complications	37 (27.4)

kg/m^2 = kilograms per meter squared

*Range for Geriatric Depression Scale: 0-15

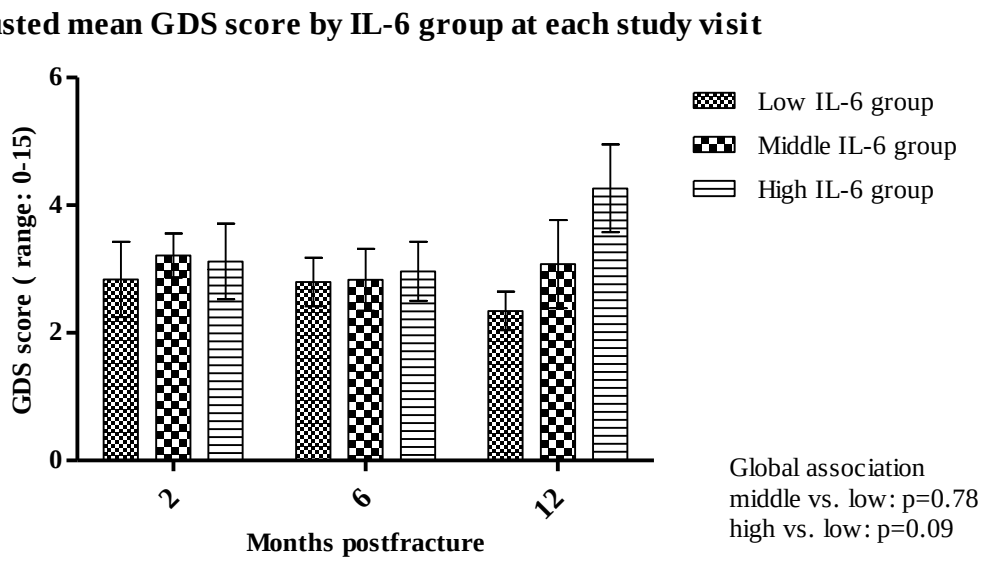


Figure 5.1 Adjusted mean GDS score by IL-6 group at each study visit

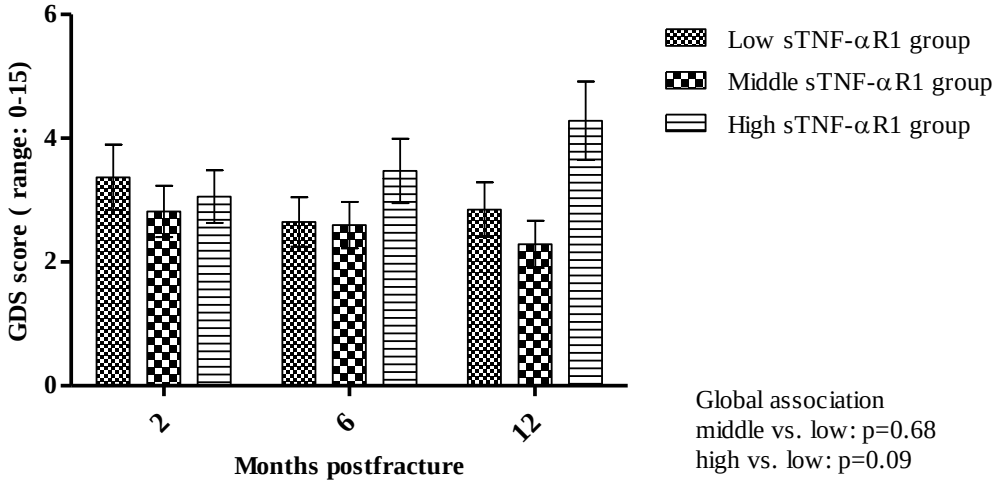


Figure 5.2 Adjusted mean GDS score by sTNF-αR1 group at each study visit

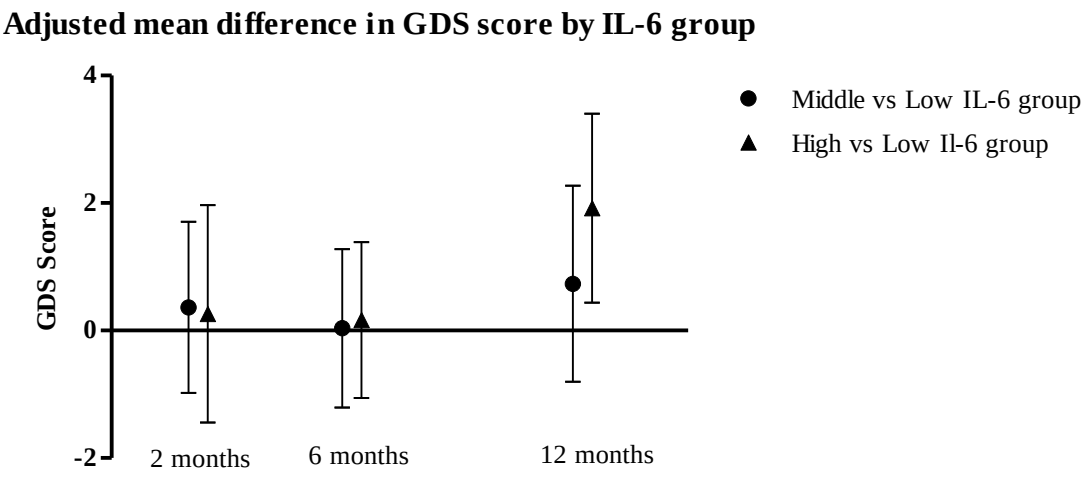


Figure 5.3 Adjusted mean difference in GDS score by IL-6 group at each study visit

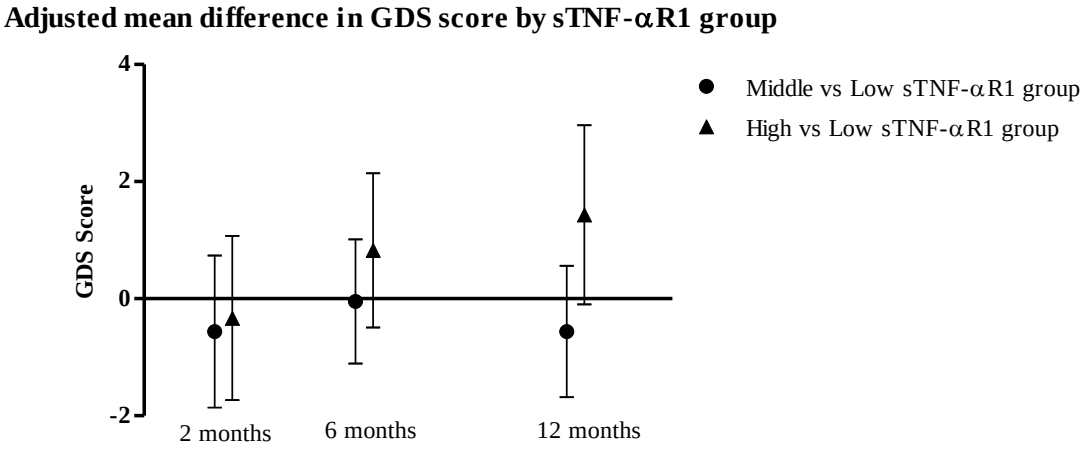


Figure 5.4 Adjusted mean difference in GDS score by sTNF-αR1 group at each study visit

When additionally adjusting for Lower Extremity Gain Scale (LEGS) score, the difference in mean GDS scores between high and low IL-6 groups was attenuated by 16% (**Figure 5.5**). When adjusting for covariates, on average those in the highest IL-6 group scored 1.6 (95% CI 0.2, 3.0; p=0.02) points higher on the GDS at 12 months postfracture. GDS scores did not differ significantly between middle and low IL-6

groups (p=0.28), between high and low sTNF-αR1 groups (p=0.39) or between middle and low sTNF-αR1 groups (p=0.32) (**Figure 5.6**).

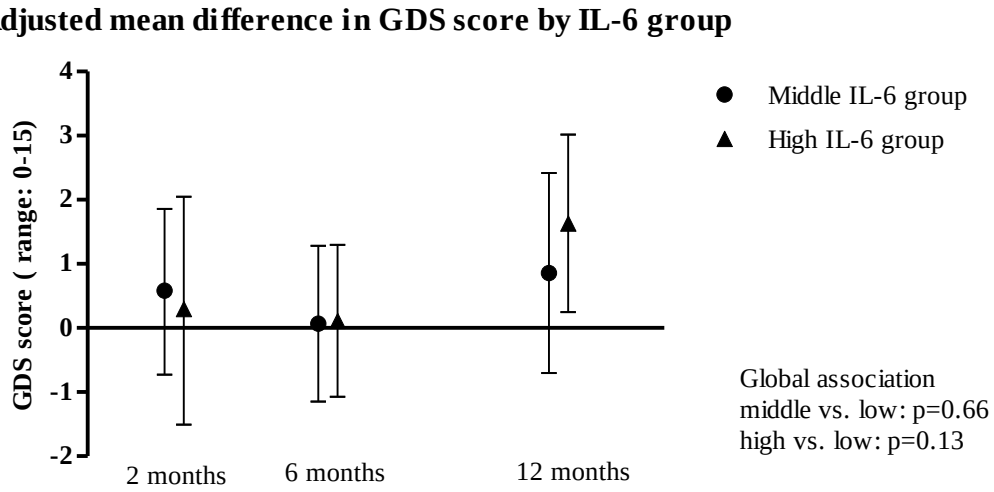


Figure 5.5 Adjusted mean difference in GDS score by IL-6 group at each study visit additionally controlling for LEGS

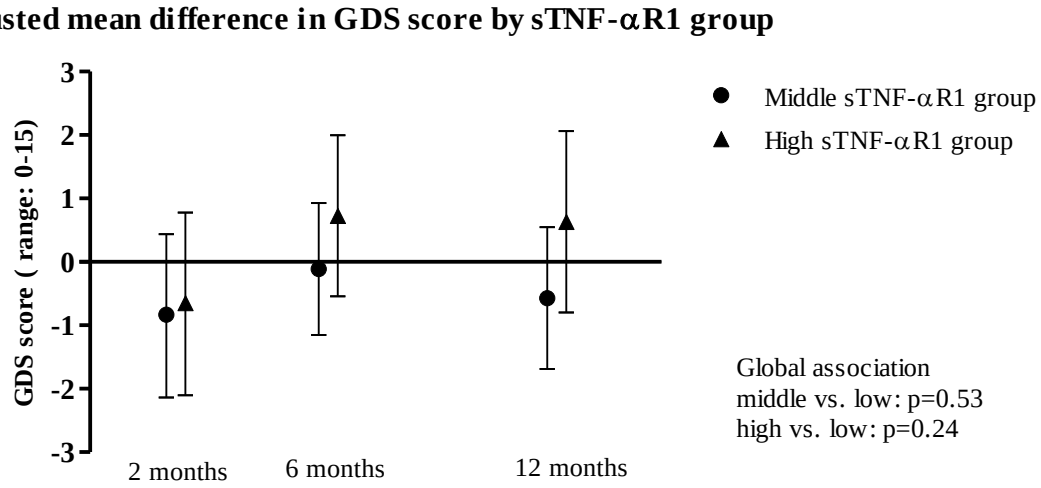


Figure 5.6 Adjusted mean difference in GDS score by sTNFα-R1 group at each study visit additionally controlling for LEGS

5.4 DISCUSSION

In the current analyses, we examined whether inflammatory cytokine levels were associated with depressive symptom levels, measured by GDS scores, in the year following hip fracture. We found that hip fracture patients in the highest group of inflammatory cytokine levels for both IL-6 and sTNF-αR1 had higher levels of depressive symptoms than those in the lowest group, particularly at 12 months postfracture. After adjusting for lower extremity performance, measured by LEGS score, GDS scores for hip fracture patients in the highest IL-6 category were attenuated but remained significantly higher than those in the lowest IL-6 category at 12 months postfracture.

Some research has suggested that depressive symptoms after hip fracture are highest in the immediate post-fracture period, with a fairly rapid decline in the subsequent 2 months, likely representing a relatively acute stress response.[13,18,19,22] By four to six months postfracture, most patients have reached their recovery plateau in depressive symptoms[22]. In contrast, our results suggest that chronically high levels of inflammation are predictive of persistently elevated depressive symptoms for up to a year postfracture, suggesting a different and more chronic pathophysiological process. Depressive symptoms in the context of elevated cytokines might represent a chronic sickness syndrome and not reaction to disability.

Findings such as these could also point to biological mechanisms underlying the occurrence of depressive symptoms after hip fracture. Physical and psychological stressors stimulate transient increases in pro-inflammatory cytokine levels, with most pronounced effects on IL-6[88,121]. Physical and psychological stressors also stimulate

hypothalamo-pituitary-adrenal (HPA) axis and sympathetic nervous system activity, leading to the production of catecholamines and corticosteroids[88,121-123]. Stress hormones can directly modulate immune function (e.g., cytokine production) by binding to their receptors on the surface of immune cells, or by other indirect mechanisms which dysregulate immune activity[26]. Cytokines that are produced peripherally are also able to cross the blood-brain barrier and stimulate HPA activity by way of increased corticotrophin-releasing hormone production from the median eminence of the hypothalamus[26,48,124,125].

An avenue of future research would be to examine postfracture levels of related endogenous mediators (e.g., markers of HPA axis activity such as adrenocorticotropic hormone or cortisol) to help uncover the biological mechanisms underlying the relationship between inflammation and depressive symptoms after hip fracture. Since loss of physical function is one of the most significant consequences of a hip fracture, results of studies that help to uncover biological mechanisms underlying poor recovery of function after hip fracture are of great importance. Because elders with worse function may be more likely to manifest depressive symptoms[119,120,126], we adjusted for lower extremity performance in this analysis. After doing so, the association between higher levels of IL-6 and GDS scores was attenuated by higher scores (i.e., better performance) on the LEGS. This suggests that the association of IL-6 levels and depressive symptoms may be partially explained by lower extremity functional recovery. The exact nature of the interplay among all three variables (depressive symptoms, inflammatory cytokine levels and functional recovery) remains to be elucidated. However, the results agree with a vast literature supporting a relationship between depressive symptoms and functional

recovery in older adults[20,95,127], and our results suggest that prolonged inflammation is an important variable in this relationship. These results must be viewed in the context of the following limitations. The GDS is not a diagnostic instrument, though it is appropriate for screening and identification of older patients who may be at risk for developing depressive disorders. Regarding the study sample, only 19% of hip fracture patients identified for the study were eligible[3].

Hip fracture patients enrolled in this study were quite robust compared to the general hip fracture population, in that they were more cognitively intact, suffered from fewer comorbid conditions, and must have been able to perform the study's exercise intervention, thus making the findings from this sample less generalizable to the hip fracture population. Also, the majority of patients included in these analyses were white women and thus we are unable to assess whether similar associations exist in men or in women of other ethnicities. A final limitation was the inclusion of hip fracture patients with MMSE scores as low as 20; due to the lack of validity on the MMSE, participants with a score of 20 may have significant cognitive decline if investigated using more sensitive cognitive measures. Because MMSE is an important confounder, we included it in the analysis. Even with MMSE scores as low as 20, these patients had cognitive function that was good enough to participate in the year-long exercise trial. Additionally, we would like to generalize to a population of female hip fracture patients with cognitive decline. Indeed, chronic inflammation post hip fracture might predict cognitive impairment as well as depressive symptoms.

Findings such as these underscore the importance of routine screening, evaluation and treatment of depression and depressed mood in rehabilitation settings during the

postfracture recovery period. Identifying subgroups of hip fracture patients with higher levels of depressive symptoms is important because of the impact of depression on level of recovery and length of rehabilitation. Patients with depression due to disability require longer rehabilitation, and prevalence and persistence of depressive symptoms are risk factors for poor functional recovery postfracture[13,92,95]. Finally, the relationship between depression and inflammation also has important implications for treatment, as depressed patients with increased levels of inflammatory biomarkers have been found to be more likely to exhibit treatment resistance[25]. A better understanding of this chronic immune dysregulation after hip fracture is needed, in order to develop new treatment targets for both depressive symptoms and functional recovery. Further research should establish the cause and effect of this relationship as well as long-term correlates.

6. Depressive Symptoms, Inflammatory Cytokines and the Performance of Lower Extremity Function in Older Women in the Year after Hip Fracture

ABSTRACT

Background- Impairment in physical performance is one of the most disabling outcomes following a hip fracture. Previous studies found that depressive symptomatology and elevated interleukin-6 (IL-6) levels are associated with poor physical performance after hip fracture. It is not clear, however, whether soluble tumor necrosis factor alpha receptor 1 (sTNF-αR1), another pro-inflammatory cytokine, is associated with lower extremity performance, and what type of relationships exist between inflammatory cytokine levels, depressive symptoms and lower extremity performance.

Methods- Study participants (N=136) were women ≥65 y who underwent surgical repair of a non-pathologic incident hip fracture. The scores for Geriatric Depression Scale (GDS) and Lower Extremity Gain Scale (LEGS) and serum levels of inflammatory cytokines (IL-6 or sTNF-αR1) were analyzed for potential associations, using generalized estimating equations.

Results- There was no significant association between GDS score at baseline and LEGS score over the year after hip fracture. After adjusting for covariates, participants with the highest levels of IL-6 (≥3.69 pg/mL) and sTNF-αR1 (≥2210 pg/mL) had LEGS scores that were on average 4.8 (95% confidence interval [CI]: -8.1, -1.6; p=0.004) and 3.0 (95% CI: -6.3, 0.3; p=0.07) points lower than the LEGS scores among participants with the lowest cytokine levels.

Conclusions- High levels of IL-6 and sTNF-αR1 are associated with poor lower extremity performance after hip fracture. It is possible that the speed of lower extremity function recovery is directly related to the magnitude of the pro-inflammatory cytokine response.

6.1 INTRODUCTION

The hip fracture patient typically experiences a period of decline in physical[9,11,12,42,43] and psychological[19] function, along with a heightened inflammatory response[33-35]. Recovery patterns for each of these domains vary over the first year post-fracture. Some researchers have suggested that depressive symptoms after hip fracture are highest in the immediate post-fracture period, with a fairly rapid decline in the subsequent 2 months, likely representing a relatively acute stress response[13,18,19,22]. By four to six months post-fracture, most patients reach their recovery plateau in depressive symptoms[22], but lower extremity physical function tends to be impaired throughout the entire year post-fracture[22]. Inflammatory cytokine levels, which are highest immediately post-fracture, can remain elevated for as long as 12 months post-fracture[15].

Depressive symptoms and physical function and how they relate to each other and to the adverse outcomes one year following hip fracture have been studied[105]. Depressive symptoms have been consistently associated with poor recovery of physical function post-fracture[12,13,20-22,92,93], though some results were conflicting[23]. Regardless of whether the finding was significant, none of the previous studies have examined functional ability using an instrument developed specifically to assess the performance of lower extremity function in the hip fracture patient.

Furthermore, Miller and colleagues examined the relationship between inflammation and functional recovery after hip fracture in a sample of female hip fracture patients from the Baltimore Hip Studies 3rd Cohort (BHS-3).[15] They found that participants with the lowest tertile of IL-6 level performed better on the Lower Extremity Gain Scale than did those in the highest tertile (p =.008), and at 12 months post-fracture, they scored 5.3 points better (95% confidence interval, 2.0-8.6; p =.002). A clinically meaningful difference on the LEGS is 2 to 3 points[106]; therefore, the authors concluded that higher levels of IL-6 are adversely associated with recovery of lower extremity function after hip fracture.

To further explore these relationships, we examined the associations among all three variables – depressive symptoms, inflammatory cytokines, and performance of lower extremity function. Because depression and inflammation may be associated in a bi-directional manner, it was of interest to assess whether each of these two variables played a role in their individual associations with lower extremity performance.

6.2 METHODS

Study Design

Patients were selected from the fourth cohort in the Baltimore Hip Studies (BHS-4), a randomized clinical trial which tested the feasibility of the Exercise Plus Program compared to usual care in hip fracture patients[3,96]. Briefly, participants were enrolled within 15 days of the hip fracture. Baseline measurements and pre-fracture data (questionnaire, performance measures and blood draw) were collected within 22 days post-hip fracture and follow-up data were collected at 2, 6 and 12 months post-fracture.

Participants

Eligibility for the BHS-4 was previously described[3]. Participants had to have both a GDS score at baseline and LEGS score at one or more study follow-up visit(s) to be included in the analysis of the relationship between depressive symptoms at baseline and lower extremity performance. Participants had to have both a cytokine measurement and LEGS score at one or more study follow-up visit(s) to be included in the analysis of the relationship between inflammatory cytokine category and lower extremity performance.

Variables of Interest

Depressive Symptoms

Depressive symptoms were measured at baseline using the 15-item Geriatric Depression Scale (GDS). The GDS-15 is a series of 15 yes/no questions developed as a basic screening tool for depression in older adults.[101] Depressive symptoms are assessed as having occurred in the past week. A score of 6 or greater on the GDS-15 is indicative of clinically significant depressive symptoms, and this cutoff has a sensitivity of 82% and a specificity of 82%.[102]

Inflammatory Cytokines

At the 2-, 6- and 12-month evaluations, blood specimens were drawn from 96, 108, and 92 participants, respectively, and the sera were stored at -70°C. Serum IL-6 and sTNF-αR1 levels were measured using commercially available enzyme-linked immuno-sorbent assay (ELISA) kits *(R&D systems, Minneapolis, MN).* For IL-6, the inter-assay coefficient of variation (CV) was 3.44% and the intra-assay was 4.37%. For sTNF-αR1, the inter-assay CV was 2.98% and the intra-assay was 5.47%.

Lower Extremity Performance

The Lower Extremity Gain Scale (LEGS) is a summary measurement of timed performance on nine tasks developed to evaluate lower extremity function in people with hip fracture[103]. Performance in each of these tasks was scored on a scale of 0 to 4, resulting in a total score of 0 to 36. A higher LEGS score indicates better performance. A difference of 2 to 3 points on the LEGS is considered clinically meaningful[106].

Baseline Covariates

The medical record was reviewed at baseline to obtain the patient's age and the number of medical comorbidities. Height and weight were used to calculate body mass index. Treatment group was based on whether the patient had been randomly assigned to the exercise or control groups for the trial. Cognitive status was assessed at baseline using the Mini-Mental State Examination[97] (MMSE).

Analytic Strategy

Depending on the study aim, we included participants who fulfilled different criteria as shown in **Table 6.1**. **Tables 6.2** and **6.3** describe the total number of study participants based on whether cytokine levels were measured at one or more study visits for Aims 3A and 3B, respectively.

Table 6.1 Inclusion criteria, by study aim

	Number of Participants Included	Inclusion Criteria		
		GDS at baseline	IL-6 or sTNF-αR1 at 2, 6 or 12 months	LEGS score at 2, 6 or 12 months
Aim 3A: Association between baseline GDS and 2-, 6-, and 12-month LEGS score	155	X		X
Aim 3B: Association between cytokine level group and 2-, 6-, and 12-month LEGS score	136		X	X

Table 6.2 Total number of study participants for Aim 3A

Aim 3A: Baseline GDS score and LEGS score at:	Number of participants
3 visits	107
2 visits	38
1 visit	10
Total number of participants	***155***

Table 6.3 Total number of study participants for Aim 3B

Aim 3B: Both cytokine and LEGS score measured at:	IL-6	sTNF-αR1
3 visits	49	50
2 visits	52	53
1 visit	35	33
Total number of participants	***136***	***136***

Serum levels of IL-6 and sTNF-αR1 at the baseline evaluation are more likely to reflect a normal physiological response to the trauma of hip fracture and subsequent surgical repair, and not a prolonged inflammatory process, thus we focused on the relationship between cytokines and LEGS scores at the follow-up visits (2-, 6-, and 12-months post-enrollment)[71,72]. This approach is consistent with previous work from our

group[15]. Study participants were divided into groups based on their serum IL-6 and sTNF-αR1 levels over the 2-, 6-, and 12-month time points of the follow-up period (IL-6: low group: IL-6 $\leq$ 2.09, middle group 2.09 < IL-6 < 3.69, and high group $\geq$ 3.69 pg/mL; and sTNF-αR1: low group: sTNF-αR1 $\leq$ 1607, middle group: 1607 < sTNF-αR1 < 2210, high group: sTNF-αR1 $\geq$ 2210 pg/mL). This categorization is based on the IL-6 and sTNF-αR1 tertile levels of all the observations over the 2 to 12 month follow-up period, therefore ensuring that approximately equal numbers of observations were included in each cytokine group over the follow-up period[15,16]. These categories are not interpreted as time-specific tertiles; however, this approach ensured a sufficient sample size in each category at each time-point while accurately accounting for patients' changes in cytokine levels.

Generalized estimating equations (GEE)[113] were used to model the longitudinal relationship between the following variables: a) GDS scores at baseline and LEGS scores at 2, 6 and 12 months after hip fracture and b) IL-6 and sTNF-αR1categories at the 2, 6 and 12 month follow-up evaluations and LEGS scores. The GEE analytic method uses data from all visits with both cytokine level and LEGS score and enables performing longitudinal analyses when some study visits are missing. This method can account for possible correlations in repeated measures over time and is suitable for exploring differences in values measured at different times. Generalized F tests were used to compare LEGS scores over time between those in the middle and high IL-6 and sTNF-αR1 levels to the low (reference) IL-6 and sTNF-αR1 level group. Time-specific t-tests were performed to compare IL-6 and sTNF-αR1 group scores at each time point. The models were adjusted for age, BMI, Charlson Comorbidity Index score, MMSE score,

and treatment group (exercise versus control). Mediation was assessed by additionally including the potential mediating variable in the statistical model. Mediation was only assessed if an association existed between the independent and dependent variable. Analyses were performed using SAS, version 9.2 (SAS Institute, Inc., Cary, NC).

6.3 RESULTS

Demographic and medical characteristics of the study sample at baseline are summarized in **Table 6.4** for the overall sample. The study sample consisted of 155 female hip fracture patients, with a mean age of 81.5 years (*SD* = 6.2). At baseline, participants had a mean BMI of 24.0 kg/m^2 (*SD* = 4.3), Charlson Comorbidity Index of 1.2 (*SD* = 1.3), and MMSE score of 26.9 (*SD* = 2.6). The sample was representative of hip fracture patients in terms of age, race, and fracture type, but the patients had fewer comorbid medical conditions and they were cognitively intact as required by the study inclusion criteria.

Table 6.4 Demographic and Medical Characteristics of the Study Participants at Baseline

	Overall N=155
Age, mean (SD), y	81.5 (6.4)
White, No. (%)	149 (96.1)
Married, No. (%)	51 (32.9)
Anesthesia risk rating, mean (SD)	2.7 (0.5)
BMI (kg/m^2), mean (SD)	24.0 (4.3)
Charlson comorbidity index score, mean (SD)	1.2 (1.3)
Fracture type, No. (%)	
Intertrochanteric	66 (42.6)
Subcapital	82 (52.9)
Subtrochanteric	7 (4.5)
GDS score*, mean (SD)	2.5 (2.6)
Length of hospital stay, mean (SD),days	4.1 (1.4)
LEGS score at 2 months, mean (SD)[#]	22.4 (7.4)
MMSE score, mean (SD)	26.9 (2.6)
Post-operative complications, No. (%)	
Allergic reaction	1 (0.7)
Confusion or disorientation	6 (3.9)
Myocardial infarction	1 (0.7)
Pulmonary embolism	1 (0.7)
Renal failure	1 (0.7)
Shock post-op	2 (1.3)
Urinary tract infection	7 (4.5)
Wound hematoma/bleeding	3 (1.9)
Wound infection	1 (0.7)
Other complications	39 (25.2)

kg/m^2 = kilograms per meter squared

*Range for GDS (Geriatric Depression Scale): 0-15; maximum score in this sample: 14

[#]Range for LEGS (Lower Extremity Gain Scale): 0-36; maximum score in this sample: 36

Figure 6.1 displays the median (interquartile range) for LEGS scores at each study visit. LEGS score was lowest at 2 months and improved over the follow-up period, with higher scores at both the 6 and 12 month examinations.

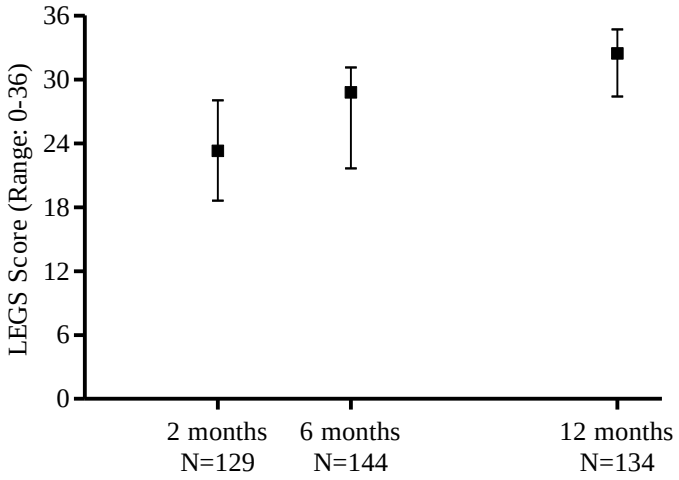


Figure 6.1 Median value and interquartile range for Lower Extremity Gain Scale at each time point

Depressive Symptoms and Lower Extremity Function

Figure 6.2 illustrates the adjusted associations between GDS score at baseline and LEGS scores at 2, 6, and 12 months after hip fracture. The associations presented in these graphs represent the difference in LEGS score per unit of GDS score and the 95% confidence interval. After adjusting for covariates, there were no consistent meaningful relationships between baseline GDS score and LEGS score. Because there was no cross-sectional or longitudinal association between GDS score at baseline and LEGS score, mediation of this relationship by IL-6 or sTNF-αR1 level at 2 months was unlikely.

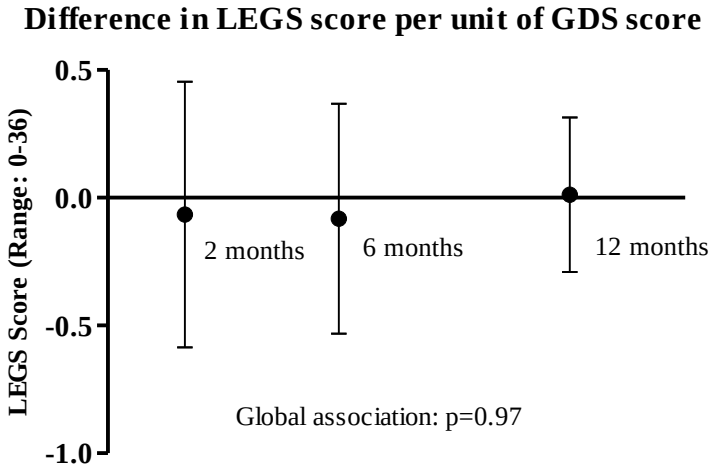


Figure 6.2 Difference in LEGS score per unit of GDS score

IL-6 and Lower Extremity Function

After adjusting for covariates, participants with the highest levels of IL-6 had significantly lower average LEGS scores than those with the lowest levels (p=0.03) during the follow-up period (**Figure 6.3**). LEGS scores did not differ significantly over the follow-up period between participants in the middle and lowest categories of IL-6 levels (p=0.11). The difference in mean LEGS scores between high and low IL-6 groups was greatest at 2 months post-fracture; when adjusting for covariates those in the highest IL-6 levels group scored 4.8 (95% CI -8.1, -1.6; p=0.004) points lower on the LEGS (**Figure 6.4**) as compared with those in the lowest levels group. Although a significant association was found between IL-6 group and LEGS score longitudinally and at 2 months, mediation of this relationship by GDS score at 2 months was not present due to a lack of association between IL-6 group and GDS score at 2 months.

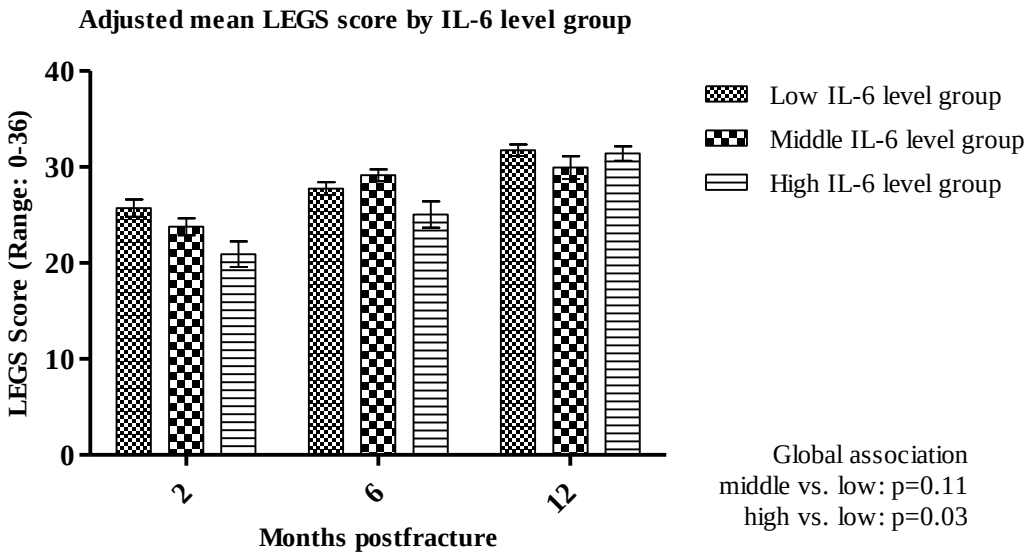


Figure 6.3 Adjusted mean LEGS scores by IL-6 level group at each study visit

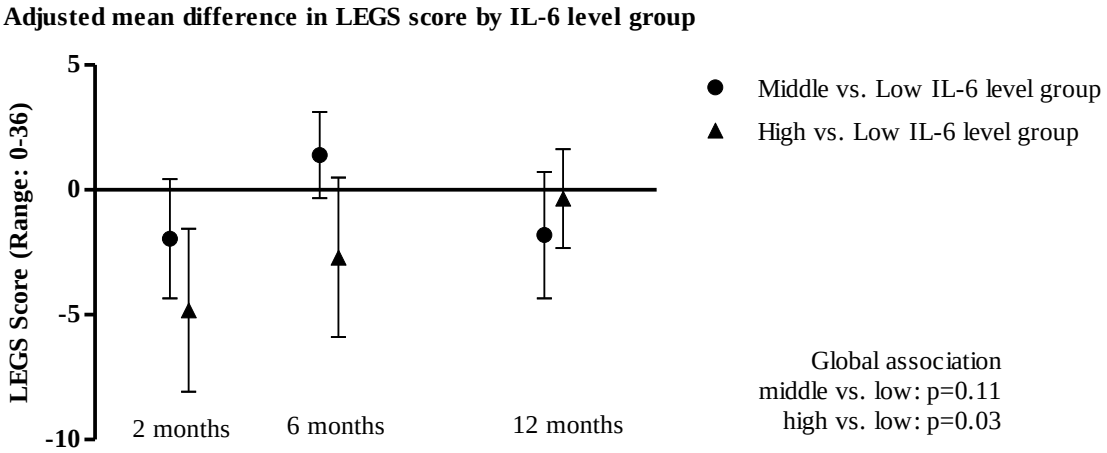


Figure 6.4 Adjusted mean difference in LEGS score by IL-6 level group at each study visit

sTNF-αR1 and Lower Extremity Function

After adjusting for covariates, LEGS scores did not differ significantly over the follow-up period between the high and low (p=0.35) or the middle and low (p=0.23) level categories of sTNF-αR1 (**Figures 6.5**). Adjusted mean LEGS scores were lower at all three time points for the high as compared to low, as well as middle compared to low sTNF-αR1 level groups. The difference in LEGS score between high and low sTNF-αR1 level groups was greatest at 2 months post-fracture; when adjusting for covariates, on average, those in the highest sTNF-αR1 level group scored 3.0 (95% CI -6.3, 0.3; p=0.07) points lower on the LEGS (**Figure 6.6**) as compared to the low sTNF-αR1 level group.

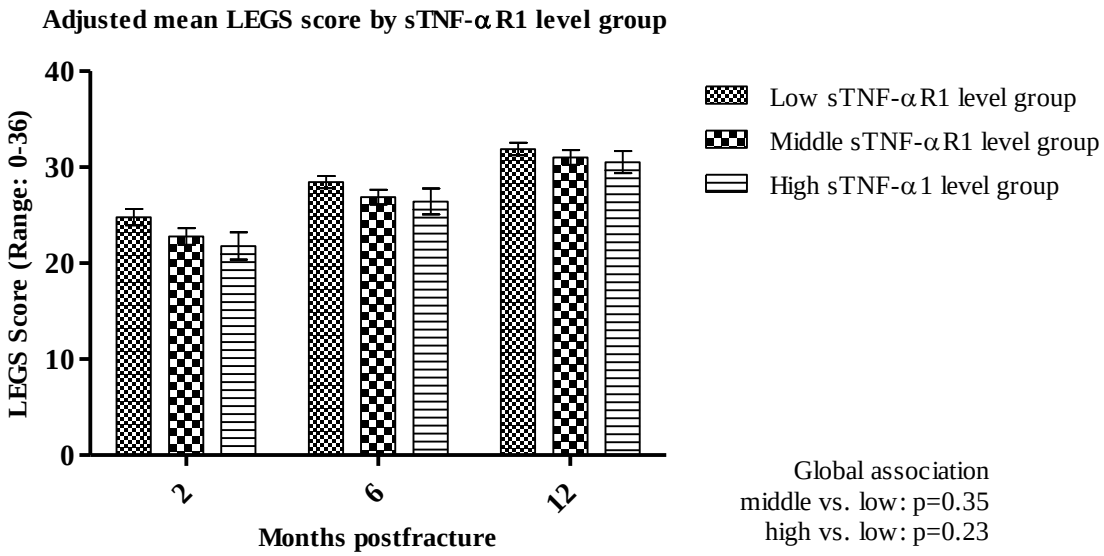


Figure 6.5 Adjusted mean LEGS scores by sTNF-αR1 level group at each study visit

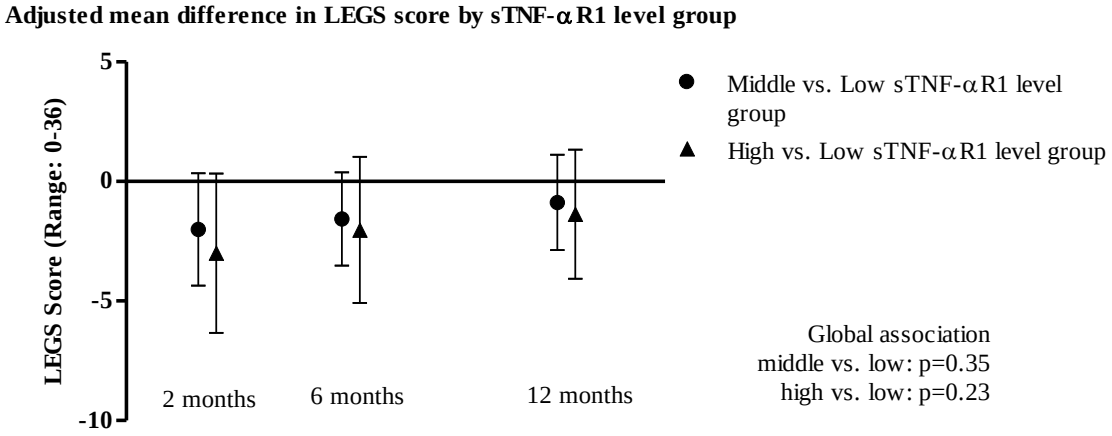


Figure 6.6 Adjusted mean difference in LEGS score by sTNF-αR1 group at each study visit

6.4 DISCUSSION

We found that during the year post hip fracture participants in the group with high IL-6 concentration had significantly lower LEGS scores than those in the group with low IL-6 concentration; and the difference in LEGS scores between the two groups was greatest at the 2-month examination. There was a trend toward a dose-dependent inverse association between groups categorized by sTNF-αR1 levels and LEGS score, though not statistically significant. Furthermore, we found no association between GDS scores at baseline measured and LEGS scores at 2, 6 or 12 months after hip fracture, hence no relationship to be mediated by IL-6 or sTNF-αR1 level at 2 months. Although there were significant cross-sectional and longitudinal relationships between the high IL-6 levels and LEGS scores, there was no correlation between IL-6 group and GDS score at the 2-month examination, which would be necessary to demonstrate mediation of this relationship by 2-month GDS score[128]. There was also no correlation between sTNF-αR1

group and GDS score at 2 months, which prevented further assessment of mediation of the relationship between sTNF-αR1 group and LEGS score over the follow-up period.

Depression is an important risk factor for poor post-fracture outcomes. It is known to negatively impact the hip fracture patient's participation in rehabilitation programs, which can subsequently affect functional outcomes[14]. One possible explanation for our failure to find an associations between depression and lower extremity function among our study participants is that the latter's depressive symptoms were not severe enough to interfere with their physical function recovery, which is a potential limitation of this study.

There were interesting trends in the relationships between cytokine levels and lower extremity function. The low and high IL-6 level groups eventually recovered lower extremity function to the same point (i.e., negligible difference in adjusted mean LEGS scores between groups at 12 months). However, the high IL-6 level group started at a significantly lower level of function than the low level group and thus made greater strides in its recovery over time. Lower extremity function for the mid-level IL-6 group reached its recovery plateau by the 6-month visit, whereas the low and high IL-6 level groups continued to show increases in LEGS scores through the 12-month visit. The middle level group had maximum functional levels at 12 months post-fracture that were lower than those of either the low or the high level group. Despite the middle group's recovery to a lesser magnitude of function, moderate inflammatory activity may be a marker of a speedier recovery of lower extremity function.

There was a trend toward a dose-dependent inverse relationship between sTNF-αR1 group and LEGS scores. The low group of sTNF-αR1 had the highest adjusted

mean LEGS scores at all study visits, and the high sTNF-αR1 group had the lowest adjusted mean LEGS scores at all study visits. There was no plateau of recovery, as was seen with IL-6. All three sTNF-αR1 groups' adjusted mean LEGS scores increased at each study visit.

This is the first study to examine the relationship between sTNF-αR1 levels and the performance of lower extremity function after hip fracture as measured by the LEGS. A prior study from our group has examined the relationship between sTNF- αR1 levels and Six-Minute Walking Distance[17]. Results from this study also confirm the inverse association between IL-6 and lower extremity performance found by Miller and colleagues[15]. Together, these results further highlight the importance of regulated pro-inflammatory cytokine activity in the post-fracture recovery period. Inflammation plays a critical role in the regeneration of bone after fracture or injury. If the acute inflammatory response does not resolve within the first week of the injury or is abnormally elevated, the injured bone may not heal properly. This becomes evident as decreased load-bearing capability, which could ultimately impact the patient's ability to recover the function of their lower extremity[15,129].

Recovery from an incident hip fracture can be limited or enhanced by the inflammatory response in the first 12 months of the post-fracture recovery period. Interventions targeting physical, psychological and physiologic rehabilitation within the first two months post-fracture will likely positively impact the trajectory of the hip fracture patient's recovery over the entire first year after fracture.

7. Discussion

This chapter will provide a summary and interpretation of findings of the dissertation, followed by the implications of the results and conclusions from this research.

7.1 Summary of Findings

Table 7.1 provides an overview of the findings.

Table 7.1 Summary of results

Independent variables	***Dependent variables***			
	GDS score at 2, 6, 12 months	IL-6 concentration at 2, 6, 12 months	sTNF-αR1 concentration at 2, 6, 12 months	LEGS score at 2, 6, 12 months
GDS score at baseline		*Positive association* at 2,6,12 months (N.S.)	*Positive association* at 2,6,12 months (N.S.)	No association
Persistently high GDS score (≥6 at baseline *and* 2 months) vs. not persistently high GDS score		No consistently meaningful association	***Negative association*** Persistently high GDS group had lower sTNF-αR1 levels at 2 months (-383 pg/mL; p=0.02) -Longitudinal: p<0.0001	
IL-6 level group *high* *middle* *low (reference)*	***Positive association*** High IL-6 level group scored 1.9 points higher than low level group on GDS at 12 months (p=0.01) -Trend for longitudinal association (p=0.09)			***Negative association*** High IL-6 level group had lower LEGS scores than low level group -Greatest at 2 months (-4.8 points; p=0.0004) -Longitudinal: p=0.03(high v low)
sTNF-αR1level group *high* *middle* *low (reference)*	*Positive association* High sTNF-αR1 level group scored 1.4 points higher on GDS than low level group (p=0.07) -Trend for longitudinal association (p=0.09)			*Negative association* High sTNF-αR1 level group had lower LEGS scores than the low level group -Greatest at 2 months (-3.0 points; p=0.07) -Longitudinal:N.S.

Prevalence of Depressive Symptoms

Participants in this study presented at baseline with a range of depressive symptoms, and a mean GDS score of 2.3. Overall, 12.5% of them were experiencing clinically significant symptom. Without knowledge of pre-fracture affective status, it is difficult to tell whether post-fracture depressive symptomatology is attributable to the hip fracture or an extension of a pre-existing, more chronic depression.

Depression and Inflammation

Available evidence regarding the association between depression and inflammation is consistent with three possible causal pathways: depression to inflammation, inflammation to depression, and bidirectional relationships[27]. It is not clear which of these mechanisms predominates in the context of hip fracture, making this the first study to examine various relationships between depressive symptoms and IL-6 and sTNF-αR1 levels in the older adult hip fracture patient.

Consistent with the rationale that pro-inflammatory cytokines can contribute to the development of depressive symptoms[91,111,112,130,131], we assessed the relationship between groups of IL-6 and sTNF-αR1 levels (high-middle-low levels) and depressive symptoms (GDS scores) over the year post-fracture in a sample of hip fracture patients. We found that participants with the highest levels of IL-6 and sTNF-αR1 had higher GDS scores than those with the lowest levels, at the 12-month examination. Our results suggest that chronically high levels of inflammation are predictive of persistently elevated depressive symptoms for up to a year post-fracture, indicating a different and potentially chronic pathophysiological process. High GDS scores at 12 months post-

fracture in the context of elevated cytokines may represent a chronic sickness syndrome, as opposed to a reaction to disability.

Evidence also exists for a downstream increase in inflammatory cytokine activity in the context of depressive disorders[79,81,132]. Therefore, we assessed the associations of baseline GDS scores with IL-6 and sTNF-αR1 levels at 2, 6 and 12 months post-fracture, expecting that high scores would be associated with elevated inflammatory cytokine levels. Though none of the cross-sectional associations were statistically significant, all of the point estimates were positive at each time point, indicating an increase in cytokine levels per unit of GDS score. There was no evidence of a longitudinal association between GDS scores at baseline and IL-6 or and sTNF-αR1 levels over the year post-fracture.

In order to explore the effect of depressed mood occurring at multiple post-fracture examination times, we assessed the relationship between the persistence of baseline high GDS scores at 2 months post-fracture and the levels of IL-6 and sTNF-αR1 at 2, 6 and 12 months post-fracture. We expected that participants with persistently high GDS scores would have elevated levels of IL-6 and sTNF-αR1 over the year post-fracture, compared to participants without persistently high scores. We found the former to have a significantly lower adjusted mean level of sTNF-αR1 than the latter at 2 months, but not at 12-month where the levels were reversed; the persistently depressed group had higher levels of sTNF-αR1 than the group without persistently high GDS scores. This result was therefore indicative of a delayed increase in the sTNF-αR1 response, as opposed to the consistently high inflammatory response over the entire year

post-fracture that we expected. There was no evidence of an association between persistently high depressive symptoms and IL-6 levels.

At this point, it is not completely clear why there was an association between persistence of depressive symptoms and sTNF-αR1 levels but not IL-6 levels. Both IL-6 and TNF-α are pro-inflammatory cytokines; however, they are produced at different points during the inflammatory response and from different sources. In our study, IL-6 and sTNF-αR1 concentrations were moderately and positively correlated at 2, 6, and 12 months (data not shown), indicating some evidence of a relationship between the two cytokines but not as strong as we expected.

Elevated IL-6 levels have consistently been found to be associated with depression in older adults in prior studies[91,111,112,130]. It is possible that levels of TNF-α and its receptor may be more sensitive biomarkers of depression in the hip fracture population of older adults. This finding is important in light of recent data showing that anti-inflammatory agents may have antidepressant properties. Indeed, TNF inhibitors such as Etanercept and Infliximab have been shown to reduce depressive symptoms in patients with psoriasis and treatment resistant depression, respectively[133]. Based on the findings from our study, older adults in the hip fracture population who have persistently high GDS scores may also benefit from these anti-inflammatory therapies with antidepressant efficacy.

It is becoming clearer that transiently elevated GDS scores (e.g., occurring solely at "baseline" or immediately post-fracture) may not be clinically relevant in an acutely ill person because of spontaneous remission. Persistence of depressive symptoms has previously been established as an important predictor of poor physical function in the hip

fracture patient[13]. Our findings add that physiologic function (specifically, inflammatory cytokine levels) may be different in hip fracture patients with persistently high depressive symptoms.

The relationship between depression and inflammation is difficult to disentangle, particularly in the context of the older adult who has undergone surgical repair of hip fracture. This special population has several "competing forces" that could work synergistically to contribute to elevated levels of inflammatory cytokines. High inflammatory marker levels could be due to the pro-inflammatory state that occurs as a person ages[28-32]; in fact, the majority of the genes that are upregulated during late life are genes that regulate inflammatory processes[134-136]. There could also be an aberrant inflammatory reaction that lasts longer than is needed to promote healing of the bone fracture[129]. Additionally, following a hip fracture, the muscle in the affected side atrophies and is replaced by fat which acts similarly to visceral fat and is pro-inflammatory[16]. Finally, there could be a contribution of inflammatory activity associated with a particular depressive symptom profile or other comorbid conditions. Knowledge of pre-fracture inflammatory cytokine levels could help to establish a true baseline level of inflammation and provide a standard comparison for post-fracture inflammatory activity.

The most consistent finding regarding the depression-inflammation association in epidemiologic studies of older adults has been an association of elevated IL-6 with depressive symptoms[91,111,112,130]. It may be of value in future studies to assess other inflammatory proteins in association with depressive symptoms in hip fracture patients, such as IL-1β, IL-1 receptor antagonist, IL-2, IL-4, IL-8, IL-10 and interferon gamma.

Examining post-fracture levels of related endogenous mediators (e.g., markers of HPA axis activity such as adrenocorticotropic hormone or cortisol) would help to uncover the biological mechanisms underlying the relationship between inflammation and depressive symptoms after hip fracture.

Depression, Inflammation and Lower Extremity Function

We expected an inverse association between depressive symptom levels at baseline and performance of lower extremity function over the year post-fracture. Our hypothesis was based on prior studies which found depression to be a risk factor for poor recovery of function following hip fracture surgery[12,13,20-22]. However, we found no evidence for any association between GDS scores at baseline and LEGS scores over the year post-fracture.

The relationship between baseline depressive symptom levels and lower extremity performance over the year after hip fracture may not have existed in this sample for various reasons. First, GDS scores at baseline were not very high, with a sample mean of 2.3 out of possible 15 points. Second, the study sample performed well on the LEGS as early as the 2-month follow-up visit, with a mean score of approximately 24 points out of possible 36 points; additionally, participants' LEGS scores improved over time. Low GDS scores and high LEGS scores are more than likely reflections of the above average health status of the women who participated in this study. Third, the prior studies on which we based our hypothesis used a variety of different instruments to assess depressive symptoms and constructs similar to lower extremity performance. Other measures of depressive symptoms have included the Center for Epidemiologic Studies Depression Scale[12,94] (CES-D) and Hamilton Rating Scale for Depression (HAM-D) [14].

Another prior study used the 15-item version of the GDS but only included hip fracture patients who scored 10/15 or greater, indicating moderate to severe depressive symptoms[105]. Differing functional measures have included walking ability measured by the Barthel Index walking sub-item[105], the Functional Independence Measure (FIM)[94], and independence in ADL/IADL[12].

Although no association between depressive symptoms and lower extremity performance was found in our study, depression is an important risk factor for poor post-fracture outcomes that should not be ignored. Depression is known to negatively impact the hip fracture patient's participation in rehabilitation programs, which can subsequently affect functional outcomes[14]. Participants' depressive symptoms may have not been severe enough to interfere with physical function recovery; and that is a potential limitation of our study.

We did find an association between the highest levels of IL-6 and sTNF-αR1 and lowest LEGS scores in the year after hip fracture. This result supports our hypothesis and confirms previous findings from our group[15]. The association was the strongest at 2 months post-fracture; study participants in the highest levels' groups of IL-6 and sTNF-αR1 had LEGS scores 4.8 and 3.0 points lower than those in the lowest levels' groups, respectively. Inflammatory cytokine levels were not associated with 2-month GDS scores (reported in Chapter 5); therefore, it was not likely that depressive symptoms mediated the cytokine-lower extremity performance relationship.

There were interesting trends in the relationships between cytokine levels and lower extremity function. The low and high IL-6 level groups eventually recovered lower extremity function to the same point (i.e., negligible difference in adjusted mean

LEGS scores between groups at 12 months). However, the high IL-6 level group started at a significantly lower level of function than the low level group and thus made greater strides in its recovery over time. Lower extremity function for the mid-level group of IL-6 reached its recovery plateau by the 6-month visit, whereas the low and high IL-6 level groups continued to show increases in LEGS scores through the 12-month visit. The middle level group had maximum functional scores at 12 months post-fracture that were lower than those of either the low or the high level group. Despite the middle group's recovery to a lesser magnitude of function, moderate inflammatory activity may be a marker of a speedier recovery of lower extremity function.

There was a trend toward a dose-dependent inverse association between sTNF-αR1 level group and LEGS scores. The low group of sTNF-αR1 had the highest adjusted mean LEGS scores at all study visits, and the high sTNF-αR1 group had the lowest adjusted mean LEGS scores at all study visits. There was no plateau of recovery, as was seen with IL-6. All three sTNF-αR1 groups' adjusted mean LEGS scores increased at each study visit.

This is the first study to report an association between elevated sTNF-αR1 levels and poor lower extremity performance after hip fracture, as measured by the LEGS. Results from this study confirm the inverse association between IL-6 and lower extremity performance found by Miller and colleagues[15]. Together, these results further highlight the importance of regulated pro-inflammatory cytokine activity in the post-fracture recovery period. Inflammation plays a critical role in the regeneration of bone after fracture or injury. If the acute inflammatory response does not resolve within the first week of the injury or is abnormally elevated, the injured bone may not heal properly.

This becomes evident as decreased load-bearing capability, which could ultimately impact the patient's ability to recover the function of their lower extremity.[15,129]

7.2 Limitations

This study has several limitations. The GDS is not a diagnostic instrument, though it is appropriate for screening and identification of older patients who may be at risk for developing depressive disorders. Regarding the study sample, only 19% of hip fracture patients identified for the study were eligible[3]. Hip fracture patients enrolled in this study were quite robust compared to the general hip fracture population, in that they were more cognitively intact, suffered from fewer comorbid conditions, and they had been able to perform the study's exercise intervention, thus making the findings from this sample less generalizable to the hip fracture population. Also, the majority of patients included in these analyses were white women; thus, we are unable to assess whether similar associations exist in men or in women of other ethnicities. A final limitation was the inclusion of hip fracture patients with MMSE scores as low as 20. Due to the lack of validity of the MMSE, participants with a score of 20 may have significant cognitive decline if tested with more sensitive tools. Because MMSE is an important confounder, we included it in the analysis. Even with MMSE scores as low as 20, the study patients had cognitive function that was good enough to allow them to participate in the year-long exercise trial. To generalize to a population of female hip fracture patients with cognitive decline, inclusion of patients with some cognitive impairment may be deemed appropriate for future studies. Indeed, chronic inflammation post hip fracture might predict cognitive impairment as well as depressive symptoms.

7.3 Strengths

This project is novel in its objective to determine the association of depressive symptoms and inflammatory cytokines following a hip fracture. The relationship between depression and inflammation has not previously been assessed in the hip fracture population. Whereas the majority of the prior research has been cross-sectional, this study presents a longitudinal analysis of the bi-directional associations between depressive symptoms and inflammatory cytokine levels. The longitudinal study design and analysis allow for the assessment of relationships between variables over time. The GEE analytic method uses data from all visits with both an independent variable and dependent variable and enables performing longitudinal analyses when some study visits are missing.

Despite the lack of generalizability to the hip fracture population, the highly functioning capacity of the hip fracture patients included in this study may allow for these results to be generalized to a healthy population of older adults who do not suffer from many comorbidities or cognitive impairment.

Finally, this research was supported by the staff of the Baltimore Hip Studies (BHS), which has more than 25 years of experience designing and conducting population-based studies of the hip fracture recovery process. The infrastructure of BHS aided in implementation of protocol, recruitment and retention of subjects, and management of data.

7.4 Implications and Future Work

Not only do the presence and persistence of depressive symptoms have implications for recovery of physical function, but based on these findings, physiologic

function may be affected by depressive symptoms as well. Results from this study contribute to the growing literature regarding the roles of inflammatory cytokines in the post-fracture recovery. Prior studies of post-fracture IL-6 and sTNF-αR1 levels have assessed levels of both cytokines in association with markers of bone[137] turnover and dietary antioxidants[138]. Results from our study contribute an additional role of inflammation in the recovery of both physical and psychological function after hip fracture.

Findings such as these underscore the importance of routine screening, evaluation and treatment of depression and depressed mood in rehabilitation settings during the post-fracture recovery period. Identifying subgroups of hip fracture patients with higher levels of depressive symptoms is important because of the impact of depression on level of recovery and length of rehabilitation. Patients with depression due to disability require longer rehabilitation, and prevalence and persistence of depressive symptoms are risk factors for poor functional recovery post-fracture[13,92,95]. Finally, the relationship between depression and inflammation also has important implications for treatment, as depressed patients with increased levels of inflammatory biomarkers have been found to be more likely to exhibit treatment resistance[25]. Anti-inflammatory agents have been shown to reduce depressive symptoms in patients with treatment resistant depression[133], which could be extended to the hip fracture population experiencing persistently high depressive symptoms. A better understanding of this chronic immune dysregulation after hip fracture is needed, in order to develop new treatment targets for both depressive symptoms and functional recovery. Further research should establish the cause and effect of this relationship as well as long-term correlates.

Findings from these studies can be applied to methodology of future studies of depressive symptomatology and inflammatory cytokines in samples of hip fracture patients. In particular, this research may be continued to determine if depressive symptom levels and/or cytokine levels have a predictive value in mortality and morbidity in a hip fracture population. Sex differences in the associations between depressive symptoms, inflammatory cytokine levels, and lower extremity performance can also be examined, because men are at risk for worse post-fracture outcomes (specifically, death) in the first year after the event. Further work in this area may also lead to identification of elderly individuals at high risk for developing depressive symptoms after hip fracture based on the severity of their inflammatory response. Determining a clinically relevant cutoff point for "high" inflammatory cytokine levels would be the next logical step in this line of work.

7.5 Conclusion

Elevated pro-inflammatory cytokine levels may play an important role in the presence and persistence of depressive symptoms and impaired recovery of physical function in the year following hip fracture. Though the directionality of the relationship between depressive symptomatology and cytokine biomarkers is not completely clear, findings from this research support the hypothesis of bi-directionality. It may be of clinical value to focus on subgroups of patients within the hip fracture population who have the highest levels of inflammatory cytokines, as they may be at risk for poorer post-fracture affective and physical outcomes than hip fracture patients with low cytokine levels. However, a reliable and clinically relevant cutoff for high versus low inflammatory cytokine levels would need to be determined. It is also apparent from this

research that interventions targeting physical, psychological and physiologic rehabilitation within the first two months post-fracture will likely positively impact the trajectory of the hip fracture patient's recovery over the entire first year after fracture.

Appendix

Geriatric Depression Scale (short form)[101]

Instructions:	Circle the answer that best describes how you felt over the <u>past week</u>.		
	1. Are you basically satisfied with your life?	yes	no
	2. Have you dropped many of your activities and interests?	yes	no
	3. Do you feel that your life is empty?	yes	no
	4. Do you often get bored?	yes	no
	5. Are you in good spirits most of the time?	yes	no
	6. Are you afraid that something bad is going to happen to you?	yes	no
	7. Do you feel happy most of the time?	yes	no
	8. Do you often feel helpless?	yes	no
	9. Do you prefer to stay at home, rather than going out and doing things?	yes	no
	10. Do you feel that you have more problems with memory than most?	yes	no
	11. Do you think it is wonderful to be alive now?	yes	no
	12. Do you feel worthless the way you are now?	yes	no
	13. Do you feel full of energy?	yes	no
	14. Do you feel that your situation is hopeless?	yes	no
	15. Do you think that most people are better off than you are?	yes	no

Geriatric Depression Scale (short form)
Scoring Instructions

Instructions:	Score 1 point for each bolded answer. A score of 5 or more suggests depression.		
	1. Are you basically satisfied with your life?	yes	**no**
	2. Have you dropped many of your activities and interests?	**yes**	no
	3. Do you feel that your life is empty?	**yes**	no
	4. Do you often get bored?	**yes**	no
	5. Are you in good spirits most of the time?	yes	**no**
	6. Are you afraid that something bad is going to happen to you?	**yes**	no
	7. Do you feel happy most of the time?	yes	**no**
	8. Do you often feel helpless?	**yes**	no
	9. Do you prefer to stay at home, rather than going out and doing things?	**yes**	no
	10. Do you feel that you have more problems with memory than most?	**yes**	no
	11. Do you think it is wonderful to be alive now?	yes	**no**
	12. Do you feel worthless the way you are now?	**yes**	no
	13. Do you feel full of energy?	yes	**no**
	14. Do you feel that your situation is hopeless?	**yes**	no
	15. Do you think that most people are better off than you are?	**yes**	no

Total Score _____

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