BETTER NUTRITIONAL STATUS AS MEASURED BY THE MINI NUTRITIONAL ASSESSMENT TOOL IS ASSOCIATED WITH INCREASED IMMUNE RESPONSE IN ELDERLY NURSING HOME RESIDENTS WITH PRESSURE ULCERS

By

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by

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A decline in immune function occurs with age and this decline is exacerbated by malnutrition. Clinical studies demonstrate that moderate to severe malnutrition can be observed in 30 to 50% of geriatric hospitalized patients, and malnutrition is a condition associated with greater susceptibility to infection, longer hospital stay, and increased mortality. Through detection of malnutrition and impaired immune function in the elderly, early intervention may reduce the risk of these negative consequences.

Unfortunately, malnutrition and impaired immune function often go unrecognized in the elderly because of a lack of assessment methods. To partially combat this, the Mini-Nutrition Assessment (MNA) was developed as an efficient and economical assessment technique for elderly individuals. This assessment technique, which has been validated in both France and the United States for use in persons 65-90 years of age, is a comprehensive evaluation that is composed of four major parts: anthropometric measurements, dietary questionnaire, global assessment, and subjective assessment.
The purpose of this investigation was to identify a relationship between MNA score and immune response in elderly nursing home residents with pressure ulcers. The variables assessed were whole blood and lymphocyte proliferation, neutrophil respiratory burst, and delayed-type hypersensitivity (DTH). Based on MNA score, subjects were classified as malnourished (n=13, MNA score<17), at risk for malnutrition (n=7, MNA score=17-23.5), or well-nourished (n=4, MNA score>23.5); and nutrition status was compared to each immune parameter using the Kruskall-Wallis test.

Whole blood proliferation was significantly greater in the at-risk subjects compared to the malnourished subjects with both pokeweed [median (25th, 75th percentile), 1.8 (1.0, 2.2) vs. 0.6 (0.3, 1.0) dpm/cell, (p=0.017)]; and concanavalin-A [2.8 (2.3, 5.4) vs. 1.7 (0.9, 2.0) dpm/cell, (p=0.031)] mitogen, respectively. Normalized respiratory neutrophil burst was significantly greater in the well-nourished subjects than in the malnourished subjects [1.4 (1.2, 1.5) vs. 0.7 (0.5, 0.9), p=0.017].

The number of subjects who responded to at least one antigen was 3 of 4 (75%), 2 of 7 (28.6%), and 4 of 13 (30.8%), for well-nourished, at risk, and malnourished, respectively. These results were not significant. Additionally, there was no significant difference among groups in lymphocyte proliferation with concanavalin-A, pokeweed, or phytohemagglutinin mitogen. These data suggest that in elderly nursing home residents with pressure ulcers, a greater MNA score (indicative of better nutritional status) is associated with greater immune responses.
CHAPTER 1
INTRODUCTION

The prevalence of malnutrition increases with age, and is most common in the institutionalized (1,2). Malnutrition is still the most neglected problem in the elderly (3), because of inadequate nutrition training of healthcare professionals (4) and lack of validated screening methods (5), and also because of the challenge of distinguishing malnutrition from the aging process itself (6).

Malnutrition is a condition associated with greater susceptibility to infection, decreased wound healing, longer hospital stay, and increased mortality (1,2,6,7). Several studies have shown correlations between nutritional status and immune function (8,9). Other intervention trials demonstrate increased immune function with improved nutritional status, further solidifying this relationship (10,11).

The decline in immune function associated with malnutrition may further exacerbate a decline in nutritional status. It is easier to halt this downward spiral through nutritional repletion in elderly at risk of malnutrition than in those who are severely malnourished (12,13) because the immune system is not yet severely affected. Therefore, accurate nutrition-assessment tools are needed to determine the nutritional state of elderly individuals and to implement care before nutritional and immunological deteriorations occur.

The MNA was developed in the early 1990s as a quick, economical, non-invasive nutrition assessment tool (14). It was validated for use in the elderly through two successive studies in France in elderly institutionalized populations, by comparing MNA
score to biochemical indices, anthropometric measurements, and clinician assessment. A third study further validated the MNA by repeating these methods in an independently living population in New Mexico (15).

Many studies show significantly higher mortality in malnourished patients when compared to their well-nourished counterparts, regardless of nutrition assessment method used (16-18). The MNA has been shown to be predictive of mortality when the score achieved is less than 17, which categorizes the subject as malnourished (4).

Given that the MNA has demonstrated high sensitivity in detecting malnutrition in elderly institutionalized individuals (14,15,19), it is a logical assumption that MNA score will be associated with several immunological markers in a similar population. If true, this would be a significant finding because impaired immune function in the elderly often leads to a fatal outcome (9). Only a few studies to date have compared MNA with immune function (9,20), and further compared these factors in independent-living elderly with pressure ulcers (21,22), yet no studies have examined this relationship in institutionalized elderly with pressure ulcers (a more vulnerable population).

Impaired immune function has been linked to inadequate food intake in many studies (23-26), and normalization of immune parameters has been seen on refeeding (27,28). Elderly often suffer from inadequate protein and calorie intake, and this is worsened with institutionalization (1,2,29). Protein malnutrition is a contributing factor in the development of pressure ulcers (30); and undernutrition has further been linked to immunocompetence and poor wound healing (31). The MNA has been shown to anticipate nutritional decline before the physiology of immune function is distorted (9). This ability of the MNA may allow practitioners to prevent immune function decline
through identification of undernutrition and intervention through nutritional supplementation.

Therefore, the aim of this investigation was to identify a relationship between MNA score and immune status as defined by immunological assays in elderly nursing home residents with pressure ulcers. The assays performed were respiratory neutrophil burst, lymphocyte proliferation, and delayed-type hypersensitivity.
CHAPTER 2
REVIEW OF LITERATURE

Current Methods of Nutritional Assessment

Currently, valid and reliable screening tests are used in geriatric assessment of cognitive problems (Mini Mental State Exam), autonomy (KATZ ADL), gait and balance (Tinetti Performance oriented Mobility assessment), and depression (geriatric depression scale) (32,33). Yet very few reliable tools exist to assess nutritional status in seniors. Additionally, the tools that do exist are extensive, complex, and costly. No gold standard exists for determining nutritional status (34). The development of malnutrition can be described as a continuum, starting with inadequate food intake, and resulting in decreased anthropometric and biochemical values (5). Therefore, anthropometric measures and biochemical parameters have traditionally been used to assess level of nutrition (35).

One current method to determine nutritional status is the Comprehensive Geriatric Assessment (CGA), which is a multi-dimensional diagnostic process used for long-term care planning (33). The CGA format varies among institutions, but usually incorporates at least four major domains: medical status, functional status, psychological function, and socioeconomic indices.

Other tools were developed in which weighted scoring rates the individual at low, moderate, or high risk for malnutrition. Chandra and colleagues designed a 14-question screening tool to assess nutritional status in elderly Canadian patients (36).

The Determine Your Nutritional Health Checklist was developed as part of the US Nutrition Screening Initiative (NSI), a collaborative effort between the American Dietetic
Association, the American Academy of Family Physicians, and the National Council of Ageing (6). It is composed of 10 yes-or-no items that are given different weights, associated with the nutritional health of the elderly. The cumulative score can range from zero to 21, and seniors are categorized at high (score >6), moderate (score of 3-5), or low (score of 0-2) nutritional risk (6). The checklist is not a diagnostic tool, but a self-administered screening assessment, designed to identify elderly at risk of malnutrition (37). Few studies have validated the checklist (38-40).

The subjective global assessment (SGA) was originally developed for use in gastrointestinal surgery patients, but has been used in many different groups including the geriatric population. The SGA includes questions about weight loss, changes in dietary intake, gastrointestinal disturbances, and functional capacity. The physical exam section of the tool assesses subcutaneous tissue loss, muscle emaciation, and presence of edema. Results of the interview and physical exam are used to subjectively classify the senior as either well-nourished (SGA A), moderately malnourished (SGA B), or malnourished (SGA C). The SGA has a high degree of interrater agreement, and is a particularly useful method of identifying protein-energy malnutrition (18).

**The Mini-Nutrition Assessment (MNA)**

Few methods of assessment existed before the introduction of the MNA. The MNA was developed through collaboration between the Toulouse University Hospital in France, the Medical School of New Mexico in the United States, and Nestle Research Centre in Switzerland (4). It was created in the early 1990s by researchers Vellas, Garry, Guigoz, and Albarede as a quick, economical, and noninvasive method of assessing nutritional status of the elderly when they enter hospitals or institutions, and monitoring any nutritional changes during their stay (5). This evaluation tool is composed of simple
measurements and a battery of questions which, when answered, provide a score out of thirty points in order to categorize the nutritional status of the person assessed (41). The MNA is administered in two parts. Part 1 is a screening questionnaire and takes only a few minutes to administer. Out of a maximum score of 14, individuals who score $\geq 12$ are considered well-nourished, and do not need further assessment. Individuals who score $\leq 11$ are considered at risk for malnutrition, and are given Part 2 of the test, which is an assessment that awards a maximum of 16 points (Appendix A). On completion of the assessment stage, the score is added back to the screening score to achieve a total MNA score. Scoring categories are as follows (42):

- **Malnourished**: Scoring less than 17 points.
- **At risk for malnourishment**: Scoring between 17 and 23.5 points.
- **Well-nourished**: Scoring greater than 23.5 points.

The MNA fulfills many criteria for both screening and diagnostic measures, meaning that it identifies those at risk for nutrition and can be used to determine outcome (42). It is composed of four major sections (43,44) that include both screening and assessment questions. Each section of the MNA is listed below, and possible scores for each section are listed in parentheses.

- **Anthropometric measurements**: BMI (0, 1, 2, 3), mid arm circumference (0.0, 0.5, 1.0), calf circumference (0, 1), and weight loss during the last three months (0, 1, 2, 3).

- **Global evaluation**: independence at home (0, 1), medications taken per day (0, 1), psychological stress or acute disease in the last three months (0, 1), mobility (0, 1, 2), neuropsychological problems (0, 1, 2), skin lesions or ulcers (0, 1).

- **Dietary assessment**: number of meals/day (0, 1, 2), consumption of dairy products (0.0, 0.5, 1.0), intake of fruits and vegetables (0, 1), recent decline in food intake (0, 1, 2), Fluid intake (0.0, 0.5, 1.0), mode of feeding (0, 1, 2).

- **Subjective self-assessment**: Nutritional problems (0, 1, 2), health status compared to people the same age (0.0, 0.5, 1.0).
Validation of the MNA

Explanation of studies

The MNA has been validated for use with the elderly by three successive studies. The first study was completed in Toulouse, France in 1991 in 155 elderly nursing home subjects whose nutritional status ranged from very healthy to malnourished. In 1993, a second study was completed in Toulouse with 120 subjects in a similar population. The Albuquerque 1993 study was based in New Mexico, and used 347 independent-living elderly (also 65 y or older), who were already participants in the New Mexico aging process study, to further validate the MNA (15). The New Mexico Aging Process study was a longitudinal study that examined nutrition and health status in the independent-living elderly over time. In all three studies, both the very frail and the very active were included. Overall, more than 600 individuals were enrolled (14).

The MNA was validated in all three studies by two principle criteria. First, a comprehensive Nutrition Assessment was performed on each participant by a researcher. Anthropometrics were taken, such as height, weight, body mass index (BMI), and skin fold measurements. An evaluation of diet was accomplished using a diet history, 3-day record, interview, and food frequency checklist. Additionally, the following biological markers were measured and used as the gold standard for nutritional health: albumin, prealbumin, transferrin, retinol-binding protein, C-reactive protein, ceruloplasmin, cholesterol, triglycerides, vitamins A, D, E, B₁, B₂, B₆, B₁₂, folate, copper, zinc, hematocrit, hemoglobin, and blood cell count.

Second, two physicians trained in nutritional assessment independently assessed each patient without knowing their MNA score. Both of the physician assessments and the detailed nutrition assessment were compared to the MNA score received by the patient.
The specificity of the MNA was determined by cross-classification of the two Toulouse studies using equations from the discriminant analysis (14). These results showed the MNA could correctly identify 70-75% of individuals as normal or malnourished without the use of biochemical indices. The rest of the population (25-30%) fell in the buffer zone between well-nourished and malnourished, and would need biochemical indices or clinical evaluation to classify further. In order to set the threshold values for the MNA, scores were cross-tabulated with albumin levels of the subjects from the Toulouse studies. All subjects with inflammation, as determined by C-reactive protein >20 mg/L were excluded. In this manner, the scores were determined for well-nourished (>24), at risk for malnutrition (17-23.5), and malnourished (<17).

**Study findings**

In both Toulouse studies, there was a strong correlation between several nutritional markers (transthyretin, serum folate, and vitamin D), energy intake, and MNA score in both males and females. Additionally, an association between a low MNA score and mortality at three months and one year was also found. Overall, the test was found to be 96% sensitive (in the ability to detect malnutrition) and 98% specific (in the ability to classify well-nourished correctly).

The New Mexico Ageing Process Study examined the nutritional status of independent-living elderly in America. Half of this study population was between the ages of 75 and 85 y(with 10% being older than 85 y). Even though this group was independent-living, and therefore, considered to be healthier than the institutionalized elderly, almost 20% were found to be at risk for malnutrition. The at risk group had a lower mean dietary intake than the well-nourished group, yet both albumin levels and BMI
were within the normal range (15). The results of the New Mexico Aging Process Study showed a correlation between a high MNA score (27-30) and successful aging (45).

Comparison of the MNA with Other Nutrition Assessment Tools

The MNA vs. the SGA

Barone and associates compared the SGA with the MNA in the detection of malnutrition over 60 days in 43 elderly hospitalized patients (age greater than 65 years) in Australia. There was no significant difference in the percentage of people identified as malnourished between SGA and MNA; however, the MNA identified a greater proportion of malnourished patients across the time intervals, suggesting a significantly more (P=0.005) sensitive degree of detection (2). The findings supported claims of other authors that the SGA cannot be used to monitor changes in nutritional status secondarily to the tool's subjective nature and nonquantitative data analysis. On the contrary, the MNA assesses patients quantitatively, which allows easier long-term monitoring of nutritional status. The MNA also has good interrater reproducibility in the institutionalized elderly (5). The SGA shows a lower interrater agreement than the MNA when used in elderly populations because it was validated for use in all hospitalized patients, whereas the MNA was specifically designed and validated for use in an elderly population (46). Overall, the MNA was considered to be a more appropriate nutritional assessment tool than the SGA in an elderly population (2).

The MNA vs. Determine Your Health Checklist (NSI)

The MNA was compared to the Determine Your Health Checklist (47) as a predictor of morbidity and mortality in an elderly independent-living Danish population. It was reported that the NSI was a poor indicator of mortality, but individuals classified as
"at-risk" by the MNA had a significantly higher mortality rate and a greater incidence of chronic disease when compared to the well-nourished group (6).

**Importance of MNA**

One advantage of the MNA is that it does not require measurements that are difficult to assess, such as blood values, but MNA score has still been shown to correlate with many aspects of health (2). Many studies show significantly higher mortality in malnourished elderly when compared to their well-nourished counterparts (16-18). Studies also show that malnutrition, as determined by the MNA score, is very predictive of mortality compared to seniors classified at risk or well-nourished by the same tool (4). This is important because the prevalence of malnutrition, relatively low in independent-living elderly (1-9%), rises considerably (20-75%) in hospitalized or institutionalized elderly (1). Moreover, undernutrition may be difficult to distinguish from the aging process itself (6,38), and physicians tend to overlook malnutrition due to their meager training in nutritional assessment (4).

Malnutrition as determined by the MNA is associated with several biological markers of malnutrition, specifically hematocrit and hemoglobin (9). Furthermore, Schiffrin and colleagues showed that by categorizing subjects by albumin level (<35 g/L, 35-40 g/L, and >40 g/L, respectively), PHA (phytohemagglutinin; a T-cell mitogen) stimulated lymphocyte proliferation was significantly less in the group with albumin <35g/L. This is a significant finding because malnutrition is still one of the most neglected problems in geriatric medicine (3) and impaired immune function in the elderly population often leads to a fatal outcome (9).

Based on these research studies, elderly nursing home residents are at a much greater risk for malnutrition and impaired immune function than the general population. More
importantly, the term "at-risk" implies that without intervention, the population so noted will progress to a more severely malnourished or immune compromised state (38).

Therefore, reliable assessment tools are needed to identify elderly at-risk of malnutrition. The MNA has been validated through successive studies to assess nutritional status in elderly persons (15). A recent study completed in two long-term geriatric units in Spain, found the MNA to be reliable in terms of internal consistency and in terms of interobserver test-retest reliability (48).

Identifying comorbidities of malnutrition

Malnutrition has been associated with many comorbidities of malnutrition. A study conducted in independent-living elderly showed a direct association between moderate score (defined at risk) on the MNA with lower BMI, energy intake, functional, and mental abilities compared to seniors who were categorized as well-nourished (41). More specifically, Havlik and associates demonstrated that the increase in functional limitations associated with aging often impairs ability to grocery shop and prepare food (49).

Covinsky and colleagues discovered malnourished elderly were more likely to be dependent in at least one activity of daily living (ADL) after hospital discharge, resulting in a higher probability of nursing home use in the year following hospitalization (18).

Griep and colleagues discovered a relationship between poorer health, poorer odor perception, fewer natural teeth and being at risk for malnutrition based on an MNA score of less than 24. Many studies show that odor perception decline can cause changes in food consumption and decreased food appreciation (50). Poor oral health is often seen in the elderly and plays a large role in food intake (Figure 1). For example, dental caries, ill-fitting dentures and missing teeth often hinder mastication, furthering risk of malnutrition (51,52).
**Pressure ulcers and nutrition**

A pressure ulcer is a wound that often arises from too much pressure on the skin; however, it can also occur from friction by rubbing against an object (e.g. bed sheet). In long-term care facilities, pressure ulcers often arise as a result of the resident not being turned, cleaned, or fed as often as the ideal standard of nursing would dictate.

Nutritional status also plays a large role in skin integrity. Protein malnutrition can alter dermal extracellular matrix turnover, increasing risk for pressure ulcer development (53). Protein requirements are higher in stressed populations secondary to hypermetabolic state, and are necessary for wound healing (53,54). Additionally, many nutrients play a role in wound healing; vitamin C is necessary for collagen synthesis, vitamin A enhances epithelialization, and zinc is vital for cell mitosis and proliferation (7). Wissing and colleagues demonstrated the relationship between ulcers and nutrition by correlating reduced MNA score in seniors with open ulcers, and an increase in MNA score upon healing (21). Severity of the pressure ulcer is determined by “stage”, or how deeply the wound penetrates the tissue (see Appendix A).

In addition to diminished quality of life, pressure ulcers are a financial burden to health institutions. In 1993, one study concluded physician and hospital cost for treating a pressure ulcer patient was $230,575 (55). The same study estimated that for the approximately 34,000 seniors with pressure ulcers in the United States, that total charges run over 800 million dollars annually, and expense increases with pressure ulcer stage (56). Identifying seniors at risk of malnutrition and intervening prior to ulcer formation would not only reduce costs, but could also improve quality of life.
Improving prognosis through intervention

One prospective, randomized controlled study demonstrated the effectiveness of nutrition intervention in 88 elderly nursing home residents at risk for malnutrition (57). Nutritional status was determined at Day 0, 30, and 60 by a 3-day diet record and simultaneous evaluation by the MNA. Subjects were then divided into 4 groups according to their MNA score. Those with a score >24 (well-nourished) did not receive supplement. Those with a score between 17 and 23.5 (at risk) were randomized into one of two groups. One group received supplementation, and the other did not. The group with MNA scores < 17 (malnourished) also received oral supplements. Resident compliance with oral supplements was good.

The majority of the residents receiving oral supplements improved their MNA score and increased their weight significantly by 1.4±0.5kg. The well-nourished and at risk group that did not receive supplementation did not change in MNA score or weight. Intervention supplementation was shown to be successful in improving weight and increasing MNA score in malnourished and at-risk elderly nursing home resident (57).

Some studies suggest the need for intervention. Several clinical studies have shown increased post-operative morbidity and mortality in seniors over 60 years of age (58,59). This risk was found to be exacerbated by malnutrition (60). When the MNA was used in a preoperative setting, 9% of the subjects were found to be malnourished, and 5% were at risk of malnutrition. These results suggest a need for pre-operative nutrition that might reduce morbidity and mortality risk (61).

Intervention could also reduce weight loss, which is associated with increased risk for morbidity and mortality compared to weight gain or maintenance (62). Additionally,
weight loss often reduces functional activity, and has been associated with depressed immunity, which also increases risk of morbidity and mortality (63).

In a study of 120 hematology patients, malnutrition did not appear to be more prevalent than in the normal elderly population when assessed using the MNA; however, the authors agreed that the MNA was an important tool for use in this population, because nutritional influence could influence the outcome of the disease (64).

In conclusion, many studies have shown the relationship of impaired nutritional status with increased risk for morbidities and mortality, suggesting an opportunity for intervention to reduce these risks. Overall, prevention of malnutrition is more successful than treatment (65).

**Immune Function**

**Malnutrition and Immune Function**

Malnutrition can impair the immune system through a number a mechanisms, including impairment of antigen presenting cells and proliferative responses and reduction of phagocytic and cytolytic ability (66). In fact, suboptimal nutrition is believed to contribute to immune senescence (11).

Elderly are at risk for protein energy malnutrition (29), which has been associated with impairment of cell-mediated immunity, phagocyte function, complement system, and cytokine production (27). Chandra stated immunocompetence results from deficiencies in vitamin A, beta-carotene, folic acid, vitamin B\(_6\), vitamin B\(_{12}\), vitamin C, and vitamin E. Research has shown that these immune function impairments can be reversed with feeding. Allende and associates noted a normalization of total lymphocytes and lymphocyte proliferation upon refeeding in anorexic patients (28).
Malnutrition as defined by the MNA seems to anticipate change in nutritional markers before the physiology of immune response is overwhelmingly altered (9), presenting an opportunity for intervention if the malnutrition is identified. Schiffren and associates found significantly lower hemoglobin and hematocrit levels in malnourished vs. well-nourished subjects as determined by the MNA even though total lymphocyte count (TLC) and lymphocyte proliferative response were not significantly different between groups (9). These researchers concluded that although the malnourished groups showed markers of malnutrition, these were not severe enough to alter immune status.

**Total lymphocyte count (TLC) and lymphocyte proliferation**

TLC is a sensitive, but not specific, indicator of nutritional status that correlates with morbidity and mortality (67); however, it can be affected by several other factors, such as aging and infection (11,66,68). TLC is calculated by multiplying the white blood cell count by percentage of lymphocytes. Normal, mild, moderate, and severe nutritional depletion are associated with lymphocyte counts of >1800 mm$^3$, 1500-1800 mm$^3$, 900-1500 mm$^3$, and <900 mm$^3$, respectively (69).

The ability to mount a proliferative response has also been associated with nutritional state. Impairment of T-lymphocyte proliferation has been seen in nursing home residents as defined by MNA score (11,70), and albumin levels (9), yet few studies examine T-lymphocyte proliferative response in a pressure ulcer population (71). It is important for clinicians to understand the impact a pressure ulcer has on the immune system, so they can provide patient better care.

Immune senescence also may play a role in lymphocyte proliferation. T-lymphocyte proliferation is one immune response that has been shown to decrease with age from 20-70 years of age, and plateau after 70 (72,73). A study involving 403 independent-living
elderly (70-106 years) revealed 18% were incapable of mounting a proliferative response to PHA, ConA (a T-cell mitogen), and PW (a T and B-cell mitogen), and this decreased response was associated with a mortality risk two times greater than in positive responders (74). Individuals who demonstrated <10% of the mean response of the young control to PHA, PW and ConA were classified as negative responders. The authors concluded that decreased proliferative response may have been due to an underlying disease; however, when this variable was excluded, the negative responders still had a higher mortality rate when compared to the positive responders. This study suggested that decreased lymphocyte proliferation to mitogens is a “marker of physiologic aging” and might be a predictor of death in the elderly (74).

**Neutrophils**

Neutrophils are the most abundant white blood cell (50-70%) and are primarily responsible for destroying foreign microbes through phagocytic processes (66). When neutrophils are stimulated by cytokines, they migrate to the infected area and engulf foreign microbes. Once the microbe is inside the neutrophil, it is dissolved by the discharge (degranulation) of highly reactive oxides (including superoxide anions, hydrogen peroxide, hydroxyl radicals, and hypochlorous acid) into the phagocytic vacuole (66).

Neutrophil activity can be measured many ways. One method is called a neutrophil respiratory burst test (NBT). NBT is accomplished by stimulating neutrophils in the presence of a reducible compound (often ferricytochrome c) that changes color upon reduction. This color change is then detected spectrophotometrically (75). It has been suggested that aging may negatively effect neutrophil function (76). Conversely, one recent study found no significant difference in neutrophil function in elderly without infection compared to their infected counterparts and the young control (77). Although it is
still controversial whether age plays a role in neutrophil dysfunction, it is generally accepted that nutritional status has a great impact on neutrophil function in both animals (23) and humans (25).

Delayed-type hypersensitivity

Delayed-type hypersensitivity (DTH) is a measure of in vivo immune function and is often administered to nursing home residents in the form of a tuberculosis test. A positive response to DTH testing indicates previous exposure to the antigen and the production of memory cells. Inability to produce a DTH reaction has been associated with increased mortality in consecutive studies (78,79).

One limitation of DTH is that malnutrition may result in anergy regardless of previous exposure (80). The antigens used in this study are Candida albicans, mumps antigen, and tetanus toxoid. These antigens were chosen because of their common exposure in this population, from either the environment or vaccination. Since it was known that most of the observed population had been previously exposed to these antigens, it was expected that most of the subjects would show a positive response, and a negative response may possibly be attributed to malnutrition.

Aging and Immune Function

Immunosenescence can be defined as “a state of dysregulated immune function that contributes to increased susceptibility of the elderly to infection (81).” By the age of 40 years, there is a 90% complete involution of the thymus.

Decreased immune function in the elderly has been studied in an effort to determine its etiology. While the exact etiology remains unclear, some mechanisms are understood, as explained by Schiffin and colleagues (9):
An altered (impaired) immune response has previously been detected in elderly individuals. Several investigators have suggested that this impaired immune function is caused by a basic defect in receptor signaling of immune cells and an altered capacity to respond to antigen-dependent activation signals. In fact, any immune response implies the activation and expansion (proliferation) of antigen-specific cells, and it has been reported that with aging a smaller number of immune cells enters the cell cycle upon antigen or mitogen stimulation. Two major mechanisms could be responsible for this observation: (i) a different distribution of lymphocyte subsets with the aging process owing to an abnormal lymphopoietic function, including cellular maturation, and (ii) an intrinsic cellular deficiency resulting in an abnormal response to activation signals.

Although the etiology of immunosenescence may still be unclear, it is known that responses to vaccination are diminished in the elderly. Burns and Goodwin found a 40-150-fold increase in the rate of influenza infection in elderly individuals with chronic illnesses such as diabetes, emphysema, or chronic renal insufficiency (82). Chronic illnesses may go undiagnosed in the elderly, which presents the complication of enrolling “apparently healthy” seniors into research studies. The SENIEUR protocol was developed by a the EURAGE Concerted Action Programme on Ageing of the European Community. This protocol describes exclusion criteria in effort to observe only the “truly healthy” elderly population, and standardize subject selection between studies investigating the interaction between aging and immune dysregulation (83).

The SENIEUR protocol elucidated one very important fact: Immune senescence does not happen to everyone, and often it exists concurrently with other comorbidities (83). Given that many studies have demonstrated the relationship between poor nutritional status and declined immune function (11,66,68,70,74) one could argue that poor nutrition is a preventable comorbidity to immune senescence.

**Purpose for Study**

In general, there is a greater occurrence of malnutrition in the elderly population, but most predominately in elderly who have been hospitalized or institutionalized (13,84). If
we define nutrition as "decreased nutrient reserves," malnutrition is found in up to 15% of the independent-living elderly, whereas the incidence of malnutrition rises to 25-60% in the institutionalized elderly (44). Malnutrition has been shown to develop as a result of diminished odor perception associated with age (50), which can then effect nutrient intake from diminished food appreciation (Figure 1). A decline in nutritional parameters (e.g. hemoglobin, hematocrit) has been associated with the presence of pressure ulcers. Furthermore, a distinct relationship has been identified between nutritional status, immune response, and pressure ulcers, as shown in Figure 2.

Due to the relationship between malnutrition, pressure ulcers, and immune function decline, we would anticipate impaired nutritional and immunological status in a pressure ulcer population, which would be further compounded by institutionalization. Therefore, the purpose of this study is to identify a relationship between parameters of immune function and score achieved on the MNA. If a relationship does exist, the MNA could be used clinically as a quick, economical, non-invasive method of predicting immune function decline. By repleting nutritional status in subjects identified at risk of malnutrition by the MNA, a decline in immune function can be avoided, and risk of mortality decreased.

Figure 2-2. Relationships among nutrition, pressure ulcers, and immunity
CHAPTER 3
STUDY DESIGN AND METHODS

Data for this cross-sectional study were collected prospectively and represent the baseline data from an interventional trial. MNA score was assessed for each enrolled subject. Venous blood was collected to determine total lymphocyte count (TLC), serum albumin, and immune function studies. Bioelectrical impedance analysis (BIA) was performed on each patient to assess body composition.

Inclusion/Exclusion Criteria

Thirty nursing home residents from the Lake City Veterans Administration (VA) Nursing Home, Lake City, Florida were recruited for the study. Eligible subjects were 65 years of age or older, and had a stage II or more severe pressure ulcer. Residents were excluded if they were receiving immunosuppressive medications, suffered from egg or thimersal allergies, had diabetes treated by insulin, or an immune deficiency disease. Additionally, residents with renal or hepatic failure were excluded due to the protein load of the intervention product for the larger study.

Sample Collection

A 30 mL blood sample was drawn at 9 AM and collected in three 10 mL sodium heparin Vacutainers (Becton Dickenson, Franklin Lakes, NJ). The Lake City VA laboratory completed the CBC with differential and serum albumin on one of the 10 mL tubes. The remaining two 10 mL tubes were transported to Gainesville, Florida where they were processed for neutrophil burst and lymphocyte proliferation assays.
Immune Function Tests

Isolation of neutrophils and lymphocytes from whole blood

Two 10 mL sodium heparin Vacutainers of whole blood were received per patient. To isolate the leukocyte-rich plasma (LRP) layer, whole blood was mixed with dextran (Accurate Chemical and Scientific Corp., Westbury, NY) solution (6% w/v dextran 500 + 9% w/v NaCl) in a 9:1 ratio and allowed to stand at room temperature for 40 minutes. During this period, prepare a final concentration of 1.077 g/mL of Optiprep (Accurate Chemical and Scientific Corp., Westbury, NY) in 0.85% sodium chloride (NaCl), containing 1 mM ethylenediamine tetra acetic acid (EDTA), 20 mM HEPES, pH 7.4: a 1.095 g/mL Optiprep in the same buffer. A density gradient was then prepared for each sample consisting of 4 mL of the 1.077 g/mL Optiprep prepared above layered on top of 4 mL of the 1.095 g/mL Optiprep solution.

After 40 minutes, the dextran caused red blood cells (RBC) to settle to the bottom of the tube and the top LRP fraction was layered on the prepared density gradient. The layered sample was then centrifuged in a 15 mL conical tube at 800 x g for 30 minutes in a Jouan C312 Centrifuge without brake (Jouan, Inc., Winchester, VA). Centrifugation results in the formation of 2 distinct bands: the upper band contains lymphocytes and the lower band contains neutrophils. The lymphocyte layer was removed using a sterile fine-tip transfer pipette and placed in a 50 mL conical tube. The neutrophil layer was also removed using a sterile fine-tip transfer pipette and placed in a separate 50 mL conical tube.

Lymphocyte proliferation assay

Isolated lymphocytes were re-suspended in 20 mL of RPMI-wash (RPMI-1640 containing 50 µM 2-mercaptoethanol, 2 mM L-glutamine, 50 units/mL penicillin, 50
µg/mL streptomycin, and 25 mM HEPES buffer, Cellgro, Herndon, VA) and centrifuged in the Jouan C312 centrifuge for 15 min at 400 x g. The supernatant was removed and the pellet was washed twice with RPMI-wash using the same procedure as above. After the final wash, the supernatant was removed and the pellet was re-suspended in 1 mL of RPMI-complete [RPMI wash (as described above) supplemented with 10% VSP (Human serum gamma depleted/heat inactivated); Biocell Laboratories, Inc., Ranch Dominguez, CA]. Cells were quantified using a hemocytometer, and then adjusted to a stock concentration of 4 x 10^6 cells/mL in RPMI-complete.

In a 96-well round bottom culture plate, cells were plated at a concentration of 2.0 x 10^5 cells/well and a final volume of 200 µl/well. Cells were stimulated with a final concentration of 10 µg/mL phytohemagglutinin (PHA, a T-cell mitogen), 1 µg/mL pokeweed (PW, a T and B-cell mitogen), and 12.5 µg/mL concanavalinA (ConA, a T-Cell mitogen) and the remaining wells served as controls with RPMI complete and cells only. The final concentrations for PHA, ConA, and PW were used because they were found through experimentation in our lab to evoke the greatest response in our elderly lymphocytes.

Cells were then incubated for 66 hours in 5% CO₂ at 37°C. After a 66-hour incubation, 1 µCi of H³-thymidine in RPMI-wash (specific activity 20 Ci/mmol: NEN Life Science Products, Inc., Boston, MA) was added to each well and then each well was harvested 6 hours later. Cells were harvested onto glass filter paper using a Skatron Cell Harvester (Denton Harbor, MI) and filter paper was placed into scintillation tubes containing 5 mL of Scinti-Safe scintillation fluid (Fisher Scientific, Suwannee, GA). Samples were then counted using a Beckman Scintillation counter LS6000SC (Beckman,
The average counts per minute (cpm) minus the average unstimulated counts were divided by 0.6 to compensate for the 60% efficiency of our liquid scintillation counter for $^3$H. Data are reported in disintegrations per minute (dpm).

**Whole blood proliferation assay**

In a 15 mL conical tube, 1 mL of patient whole blood was mixed with 3 mL RPMI-wash (see above description) resulting in a 1:4 dilution. In a 96-well plate, cells were stimulated with a final concentration of 10 µg/mL PHA, 1 µg/mL PW, and 12.5 µg/mL ConA and the remaining wells served as controls with RPMI complete and cells only. Each well was adjusted to a final volume of 200 µl/well with RPMI complete, and 200 µl of sterilized Milli-Q water was added to each well that was empty after all other wells had been plated. Cells were then incubated for 72 hours in 5% CO$_2$ at 37°C. After a 72-hour incubation, 1 µCi of H3-thymidine in RPMI-wash (specific activity 20 Ci/mmol: NEN Life Science Products, Inc.) was added to each well and then each well was harvested 24 hours later. Cells were harvested onto glass filter paper using a Skatron Cell Harvester (Denton Harbor, MI) and filter paper was placed into scintillation tubes containing 5 mL of Scinti-Safe scintillation fluid (Fisher Scientific). Samples were then counted using a Beckman scintillation counter. The average counts per minute (cpm) minus the average unstimulated counts divided by 0.6 to compensate for the 60% efficiency of our liquid scintillation counter for 3H. The data are reported as dpm.

**Neutrophil oxidative burst assay**

The neutrophils isolated from the whole blood by density gradient centrifugation were washed with 0.85% NaCl containing 1mM EDTA and 20 mM HEPES, pH 7.4. This was centrifuged for 10 minutes at 260 x g in the Joan C312 centrifuge. If RBC
contamination was noted in the pellet, 3 mL lysing buffer (0.83% NaCl with 10mM HEPES, pH 7.0) was added and the sample was incubated at 37°C for 7 minutes. After lysing, the sample was centrifuged at 260 x g for 10 minutes and the resulting pellet was washed in 10 mL Hank’s Balanced Salt Solution without Mg$^{2+}$ or Ca$^{2+}$ (Mediatech, Inc., Herndon, VA) and centrifuged at 350 x g for 10 minutes. The supernatant was removed and 1 mL of glucose (Sigma, St. Louis, MO) in Dulbecco’s Phosphate-Buffered Saline (DPBS) (1g/L) was added to the pellet and the neutrophils were counted in a Beckman Coulter Instrument (Coulter Corporation, Miami, FL). Neutrophil concentration was adjusted to 5.0 x 10$^6$ cells/mL. Fifty microliters of neutrophils were plated for a final concentration of 2.5 x 10$^5$ cells/well in a 96-well flat bottom plate. To each well was added 100 µl DPBS with glucose (as prepared above); 10 µl of 1µg/mL phorbol 12-myristae 13 acid (PMA; Sigma, St. Louis, MO) in DPBS with glucose, and 10 µl of 18.6 mg/mL ferricytochrome c, horse heart muscle (Sigma, St. Louis, MO).

The neutrophil oxidative burst was measured by a colorimetric kinetic assay using UV Max (Molecular Devices, Sunnyvale, CA). Each well, therefore, contains cell suspension from a subject, media and substrate for those cells (DPBS + glucose solution), a stimulator of T and B lymphocytes (PMA), and an iron-containing molecule that changes color upon oxidation. When the neutrophils are stimulated in vitro they produce highly reactive oxides that are used to kill bacteria engulfed by the neutrophil or are secreted to clear microbes that are too large for the neutrophil to engulf. These oxidative agents made by the neutrophil include superoxide anions, hydrogen peroxide, hydroxyl radicals, and hypochlorous acid. These agents reduce the iron molecule in
ferricytochrome, causing a color change that is measured as a change in absorbance by the UV Max instrument.

In theory, healthier neutrophils will react more readily to an outside stimulant (PMA, in this case), and therefore cause a greater color change over a shorter period of time. The plates were read for thirty minutes total; however, the reaction occurred at the highest linear change in Vmax from 3 to 10 minutes; for this reason, the 3 to 10 minute data are reported and compared between subjects. Results were normalized with a young control to account for any variation in assay technique. A young control (25-40 y) was an individual without any overt medical complication. Young control blood was drawn the same day and time as subjects, and processed concurrently with subject blood.

Delayed-type hypersensitivity (DTH) test

DTH testing was completed with the use of three antigens and a saline control purchased from ALK-Abello (Round Rock, Texas); these antigens were Candin (Candida albicans), MSTA (mumps antigen), and tetanus toxoid. These antigens were chosen because of their common exposure in this population. All three antigens and the control were injected intradermally. Candin and MSTA were purchased ready to use, but the tetanus toxoid was mixed in a 1:10 dilution with sterile 0.9% normal saline and 0.4% phenol mixture (ALK-Abello). Induration (swelling) was measured at 48 and 72 hours post administration. Subjects were classified as responding if they had a minimum induration of 5 mm to any of the 3 antigens at either time point, or anergic if they did not respond to any of the antigens. Measurements were taken by averaging the two widest points of the induration. Erythema (redness) without induration was considered not significant. Total induration was calculated by adding the diameter of all positive responses.
The MNA Administration

The MNA, as developed by Guigoz and associates, leaves much room for professional judgment (Appendix B). To maintain consistency between interviewers, interpretation and clarification of several questions of the MNA were developed, and patients were assessed according to these definitions. It is important to note that the decisions made to clarify these MNA questions may not have been the same decisions made by the researchers who validated the MNA; however, all researchers in our study wanted continuity of assessment, and we felt question clarification would help achieve this. Either a researcher or a research assistant who had been trained to use the tool administered the MNA. The question clarifications are listed below and justifications for these adjustments are:

Question 1 asks if food intake has declined over the past three months due to loss of appetite, digestive problems, chewing or swallowing difficulties. Subjects received full points if there was no decline in food intake over the past three months, and zero points if food intake had severely declined. All researchers involved agreed that it would be more pertinent to determine caloric intake rather than appetite to assess nutritional status. Appetite may not necessarily affect intake, especially in an institutionalized setting where residents receive assistance with meals and are constantly monitored by a registered dietitian.

Question 3 assesses mobility, but level of mobility is poorly defined. Clarification of this question was as follows: Patients received no points if they were in contracture or could not hold themselves up if placed in a chair. Subjects received a score of 1 if they were mobile but chose not to leave their room or the subject was able to get out of bed with assistance or independently but did not leave the nursing home. Subjects received a
full score if they were able to leave the nursing home and were mobile once in their wheelchair or walker (either with assistance or independently). Some studies have shown that persons with decreased mobility are at risk for poor diets (22). This is unlikely in an institutional setting where food is prepared for and delivered to the residents.

Question 6 pertained to body mass index (BMI). Weights were obtained from the patient’s chart. For amputees, BMI’s were calculated using an adjusted body weight through a method by Lee and Nieman (1996):

\[ \text{Adjusted body weight} = \frac{\text{measured weight}}{(100\%-\% \text{ of amputation})} \]  \hspace{1cm} (3-1)

Table 1 represents percentage of total body weight values for weight adjustment of amputees.

All heights were calculated through knee height measurement using a knee height caliper (Ross, Columbus, OH). Subjects were asked to lie in a supine position and bend both the left knee and left ankle at 90°. The fixed blade was placed under the heel of the left foot while the sliding blade was pressed down against the thigh distal to the patella. The shaft of the caliper was in line with long bone in the lower leg (tibia) and crossed over the lateral malleolus. The locking lever was pushed away from the blades to hold the measurement and was read through the viewing window to the nearest 0.1 cm. Two measurements were made in immediate succession, and the average measurement was recorded.

Actual stature was estimated by inserting the knee height into the following equations 3-2 by Chumlea (Chumlea, W. C. et al in Nutritional assessment of the elderly through anthropology, 1987), and patients older than the equation’s defined age range were still assess with the same equation:
White male (age 60-90 yr.): [KH (cm) x 2.08] + 59.01
Black male (age 60-80 yr.): [KH (cm) x 1.37] + 95.79
White female (age 60-80 yr.): [KH (cm) x 1.91] – [Age (yr.) x 0.17] + 75
Black female (age 60-80 yr.): [KH (cm) x 1.96] + 58.72

Question 8 asks if the subject takes more than 3 prescription drugs per day. In many nursing home settings, any medication is considered a prescription drug because it must be ordered by a physician, including over the counter medications like aspirin. For clarification, prescription drugs were defined as medications not available over the counter to the general public.

Question 10 asks how many full meals a subject eats per day. A full meal was considered to be 75% or more of a meal. Subjects received full points if they were eating three meals per day or if they were receiving 100% of their energy need though a feeding tube alone, through a tube feeding and oral diet, or through meals and between meal supplements.

Questions 11, 12, and 13 are designed to assess intake of protein, fruits and vegetable, and fluids, respectively. Patients received full points for each question if they were meeting nutrient needs via tube feeding.

Question 14 was designed to assess the subject's mode of feeding. The patient receives no points if unable to self-feed without assistance. Previous research has shown a correlation between MNA score and need for public help with activities of daily living (41); however, in an institutionalized setting, subjects receive additional help with all meals, and often receive supplements between meals. For these reasons, the researchers agreed to assign no points only if the patient completely refuses to eat.
Questions 15 and 16 are self-perception questions regarding health, and the subject answers them. If subjects could not answer questions independently, due to either cognitive impairment or physical impediment, the primary caregiver for that resident was consulted.

Question 17 requires the researcher to measure the subject's mid-arm circumference. To maintain consistency between researchers, the following protocol was followed: Patients were asked to lie in a supine position with their arms at rest by their sides. A tape measure was used to determine the mid-point of the arm by measuring the distance between the acromial surface of the scapula (bony protrusion surface of the upper shoulder) and the olecranon process of the elbow (bony point of the elbow on the back of the arm). The mid-point was marked with a black marker. The tape measure was then positioned at the previously marked mid-point on the upper arm and tightened snugly, but not tight enough to cause indentation of the skin. Measurements were repeated twice and average was recorded.

Question 18 requires the researcher to measure mid calf circumference. Again, to maintain consistency between researchers, the following protocol was followed: The subject was asked to lie in a supine position, with one knee bent at a 90° angle while their foot rested on the bed. The assessor visually verified the 90° angle with a cardboard cutout of a right triangle by holding the triangle up to the subject's knee. The assessor then subjectively chose the largest circumference of the calf and placed a measuring tape around this area to determine the calf circumference in centimeters. Measurements were repeated twice, and the average of the two measurements was recorded.
BIA was measured using the RJL Fluid and Nutrition System developed by RJL Systems, Inc (RJL Systems, Clinton, MI). Subjects were excluded from the procedure if they had an amputation. Hydration status was screened before enrollment by completing a comprehensive metabolic panel on each subject, followed by fluid or electrolyte replacement if necessary. Hydration status was not assessed the day BIA was completed, which may be another limitation to the study.

All jewelry was removed from the subject. Subjects were placed in a supine position on a non-conductive surface with arms 30° from the body and legs slightly apart. The subject was instructed to lie quietly during the procedure, and the tester explained the testing procedure to the patient, as it was completed.

The subject’s right sock was removed and the electrode sites were swabbed with alcohol. Electrodes and cables were attached according to the illustration (Appendix C). Once the subject was relatively still, the current was turned on, and the resistance and reactance measurements were recorded. The recorded values for resistance and reactance were entered into the Cyprus software program (RJL Systems, Clinton, MI) along with age, weight obtained from chart, and height calculated from knee height measurement. The Cyprus program then displayed body composition results.

Statistics

Statistical analysis for neutrophil respiratory burst, lymphocyte and whole blood proliferation was performed using the Kruskall-Wallis test with significance taken at p<0.05. Post-hoc analyses were accomplished using Dunn's Multiple Comparison Test. Number of responders to DTH was analyzed using a chi-squared test for independence.
Box Plots are given for all data except BIA measures (total body water, fat pounds, fat percentage, and phase angle). BIA measures are reported as scatter plots because “n” was too small in the well-nourished group to report in a box plot. Each box is set up so that the bottom of the box represents the 25th percentile and the top of the box represents the 75th percentile. The horizontal line inside the box is the mean. The median can be estimated by finding the point equidistant from the bottom (25th percentile) and the top (75th percentile) of the box and drawing a horizontal line through it so that it bisects the box. The whisker extending from the top of the box represents the top quartile (75-100%), and the highest horizontal line on the top whisker represents the highest data point. The whisker below the box represents the lowest quartile (0-25th%) and the lowest horizontal line on the whisker represents the smallest data point.

Table 3-1. Percentage of total body weight values for weight adjustment of amputees

<table>
<thead>
<tr>
<th>Body part</th>
<th>Percent (%) of total body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot</td>
<td>1.8</td>
</tr>
<tr>
<td>Below knee</td>
<td>7.1</td>
</tr>
<tr>
<td>Entire leg</td>
<td>18.5</td>
</tr>
<tr>
<td>Hand</td>
<td>0.8</td>
</tr>
<tr>
<td>Below elbow</td>
<td>3.1</td>
</tr>
<tr>
<td>Entire arm</td>
<td>6.5</td>
</tr>
<tr>
<td>Head</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Thirty nursing home residents were recruited for this study. Of the thirty residents recruited, five dropped out prior to initiating the study protocol. Reasons for drop out included discharge to home (n=1), MD request (n=1), blood draw refusal (n=2), and two patients did not meet inclusion criteria. The final number of residents included in the study was 24. Subjects were categorized by MNA score as malnourished (score <17), at risk for malnutrition (score =17-23.5), and well-nourished (score >23.5). Demographics for the 24 residents who met inclusion criteria are listed by MNA category in Table 2.

The MNA as a Measure of Nutritional Status

Figure 4-1 shows the nutritional parameters of the MNA. There was no difference in height among the three groups (data not shown). In Figure 4-1A, BMI was significantly (p=0.0044) lower in malnourished subjects compared to well-nourished subjects. Figure 4-1B shows no significance among groups in mid-arm circumference. Body weight was significantly (p=0.0164) greater in well-nourished residents when compared to their malnourished counterparts (Figure 4-1C). Mid-calf circumference was also significantly (p=0.0283) greater in well-nourished versus malnourished residents (Figure 4-1D).

Nutritional parameters that are not components of the MNA include BIA, albumin, hematocrit, and hemoglobin. Figure 4-2 compares measures of the BIA with score achieved on the MNA. The measures shown versus MNA score are total body water (Figure 4-2A), phase angle (Figure 4-2B), fat percentage of body weight (Figure 4-2C),
and pounds of body fat (Figure 4-2D). The only significant component of the BIA was total body water, which was significantly (p=0.0221) lower in the malnourished compared to both the well-nourished and the at-risk group. There also appeared to be a trend toward greater pounds of body fat in residents with increased MNA score (p=0.1049); however, this trend did not reach significance. There was no significant difference among groups for fat percentage or phase angle.

Figure 4-3 compares serum albumin level and MNA category. There was no significant difference (p=0.2837) between the groups; however, one of the residents in the well-nourished category had an unusually low albumin number. One week after completing the MNA, he was diagnosed with renal insufficiency. This resident refused nephrology consultation to determine the exact etiology of his renal insufficiency, therefore his condition during enrollment remains unknown; however, pre-existing nephrotic syndrome would explain the unexpectedly low albumin level for a patient classified as otherwise well-nourished. If this resident's data had been excluded, the results would have almost been significant (p=0.0563). Hemoglobin and hematocrit levels were not significant among groups in our study (data not shown).

Comparison of the MNA with Immune Parameters

The immune parameters observed in this study were TLC, DTH, lymphocyte and whole blood proliferation, and neutrophil respiratory burst. Figure 4-4 compares TLC with MNA. There was no significant difference among the groups.

The number of DTH responders for the well-nourished, at risk, and malnourished groups were 3 of 4 (75%), 2 of 7 (28.6%), 4 of 13 (30.8%). These results were not significantly different among groups.
Figure 4-5 compares lymphocyte proliferation to three different mitogens, and the level of responders for each MNA group. Of the 24 residents included in this study, four residents are not included in any of the lymphocyte proliferation or responder data. One resident withdrew from the study prior to the blood draw. Two residents did complete a blood draw, however, they were excluded due to a different cell separation technique in the protocol, which we used for these first two residents only. We were unable to collect lymphocyte proliferation data for another resident because the young control had an insufficiently low lymphocyte yield for that particular day. Finally, a fifth resident only had data for PHA mitogen because of complications isolating the lymphocytes, which resulted in insufficient cells to plate with all three mitogens. Lymphocyte proliferation data is shown with stimulation with PHA (Figure 4-5A), PW (Figure 4-5B), and ConA (Figure 4-5C). Figure 4-5D shows the level of response to stimulation compared to the young control.

Lymphocyte proliferation to the three mitogens and responder data were not significant; however, with PHA stimulation, a major outlier in the at risk group may have been responsible, as removing this outlier would result in a significant difference between malnourished and at risk groups as well as at risk and well-nourished groups. If this subject were omitted the p value would become (p=0.0052). Additionally, the exclusion of this outlier would result in a significant (p=0.0387) difference between malnourished and at-risk groups for PW.

Figure 4-6 compares whole blood stimulated with three separate mitogens with score achieved on the MNA. Figure 4-6A represents whole blood proliferation with PHA, which was not significant among groups. Whole blood proliferation was found to
be significantly lower in the malnourished subjects with both PW (p=0.031) and Con A (P=0.017) mitogen compared to the at-risk groups (Figures 4-6B and 4-6C).

Figure 4-7 compares normalized respiratory neutrophil burst with MNA score. Malnourished subjects were significantly (p=0.017) lower than their well-nourished counterparts.

Table 4-2: Characteristics of 24 subjects included in the study

<table>
<thead>
<tr>
<th></th>
<th>Mini Nutrition Assessment Score (median)</th>
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<tr>
<td></td>
<td>&lt;17 (13)</td>
</tr>
<tr>
<td>n (%)</td>
<td>13 (54%)</td>
</tr>
<tr>
<td>Median MNA score</td>
<td>24 (24, 25)</td>
</tr>
<tr>
<td>(25th, 75th percentile)</td>
<td></td>
</tr>
<tr>
<td>Age+SEM</td>
<td>79.2±1.8</td>
</tr>
<tr>
<td>Race</td>
<td>-</td>
</tr>
<tr>
<td>Caucasian</td>
<td>5</td>
</tr>
<tr>
<td>African American</td>
<td>8</td>
</tr>
<tr>
<td>Gender</td>
<td>-</td>
</tr>
<tr>
<td>M</td>
<td>13</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
</tr>
<tr>
<td>Ulcer Stage</td>
<td>4 (1.5-3)</td>
</tr>
<tr>
<td>(Median+25, 75 percentile)</td>
<td></td>
</tr>
<tr>
<td>Number of Ulcers+SEM</td>
<td>3.3±0.5</td>
</tr>
</tbody>
</table>
Figure 4-1. Nutritional parameters contained within the MNA vs. MNA score. A) BMI vs. MNA score. B) Mid-arm circumference vs. MNA score. C) Body weight vs. MNA score. D) Calf circumference vs. MNA score. * p<0.05, **p<0.01 vs. well-nourished (>23.5 MNA score)
Figure 4-2. Measures of BIA vs. MNA score. A) Total body water vs. MNA score. B) Phase angle vs. MNA score. C) Percentage of body fat vs. MNA score. D) Pounds of body fat vs. MNA score. * p<0.05 vs. well-nourished (>23.5 MNA score), #p<0.05 vs. at risk (17-23.5 score)
Figure 4-3. Albumin vs. MNA score

Figure 4-4. Total lymphocyte count vs. MNA score
Figure 4-5. Lymphocyte proliferation vs. MNA score. A) Lymphocyte proliferation with PHA vs. MNA score. B) Lymphocyte proliferation with PW vs. MNA score. C) Lymphocyte proliferation with ConA vs. MNA score. D) Level of responders vs. MNA score.
Figure 4-6. Whole blood proliferation vs. MNA score. A) Whole blood proliferation with PHA vs. MNA score. B) Whole blood proliferation with PW vs. MNA score. C) Whole blood proliferation with ConA vs. MNA score. # p<0.05 vs. at risk (17-23.5 MNA score)
Figure 4-7. Neutrophil burst (normalized) vs. MNA score. * p<0.05 vs. well-nourished (>23.5 MNA score)
CHAPTER 5  
DISCUSSION

The hypothesis of this study was that there would be a relationship between immune parameters and score achieved on the MNA. Our results did show a significant decline in some immune parameters with malnutrition, as defined by the MNA. For instance, malnourished subjects had significantly decreased whole blood proliferation with Con A (p=0.017) and PW (p=0.031) mitogens compared to subjects who were only at-risk for malnutrition. Additionally, respiratory neutrophil burst was significantly (p=0.017) impaired in malnourished subjects when compared to their well-nourished counterparts.

On the contrary, lymphocyte proliferation data showed no significant difference between MNA groups, even though we found a significant difference in whole blood proliferation with MNA score. It is important to note that whole blood proliferation is a stimulation of lymphocytes within whole blood, which provides an environment that may more closely mimic in vivo conditions. However, under these conditions, change in whole blood lymphocyte proliferation with malnutrition cannot be directly attributed to a change in lymphocyte function. In fact, because there was no effect of malnutrition on lymphocyte proliferation when lymphocytes were separated from other cell populations and the subject’s serum, it is possible that non-lymphocyte factors were responsible for a decreased proliferative response in the whole blood sample.

The number of DTH responders for the well-nourished, at risk, and malnourished groups were 3 of 4 (75%), 2 of 7 (28.6%), and 4 of 13 (30.8%), respectively. These
results were not significantly different between groups, most likely due to the small sample size. However, even though these results were not significant, it is still interesting to note that the malnourished and at risk subjects were only half as likely to respond to DTH testing than the well-nourished subjects.

The only significant component of the BIA was total body water, which was (p=0.0221) lower in malnourished subjects compared to both the well-nourished and the at-risk groups. This may suggest that malnourished patients are slightly dehydrated. Furthermore, if malnourished persons are dehydrated, this would falsely elevate albumin, hemoglobin, and hematocrit levels, making them not significantly different from the well-nourished or at risk patients, as was observed. Of course, another limitation to this study is that we did not reassess patient hydration status prior to completing BIA.

An assumption of this study was that the MNA is a sensitive indicator of nutritional status in the elderly as demonstrated through previous trials (14,15). To check that this assumption was correct, hematocrit, hemoglobin, and albumin were measured in each subject. In addition, MNA score was compared to nutritional parameters contained within the MNA. Our results were consistent with previous findings that MNA score is associated with nutritional parameters contained within the MNA (e.g. BMI, body weight, and calf circumference). Furthermore, our results confirmed this association in a pressure ulcer population. This is significant because body weight is a predictor of mortality in this population (85). However, our results showed there were no significant differences among MNA groups in hematocrit or hemoglobin even though previous findings have shown significant associations between these values and MNA score (9). Furthermore, there were no differences among groups for albumin, which may have been
due in part to the suggested dehydration in malnourished subjects as suggested by total body water results. Dehydration in the malnourished group may have falsely elevated albumin levels and erased any significant difference from albumin levels in well-nourished subjects.

More than likely, the observed population was in a state of chronic inflammation from the presence of pressure ulcers. Previous studies show depressed levels of both hematocrit and hemoglobin in patients with chronic inflammation (54,86,87). Anemia from chronic inflammation is characterized by normal iron stores in the reticuloendothelial system, and is believed to result from the inability to access these stores (88,89). The overall average hematocrit and hemoglobin levels in our studied population demonstrate this inflammatory state. The overall average hematocrit value was 32.1%, which is significantly below the deficient levels of <36% for females and <41% for males (90). To further demonstrate the level of deficiency in this group, it is important to note that there was only one female in the observed population. Hemoglobin was also deficient in this group (µ=10.6 g/dL) compared to acceptable levels in males (>13.5 g/dL) and females (>12 g/dL). It is important to note that hemoglobin and hematocrit are not measures of nutrition alone. In fact, hemoglobin and hematocrit levels can be affected by age, exercise, and disease.

In addition to low hematocrit and hemoglobin, the observed population displayed hypoalbuminemia. This finding was consistent with other studies that observed depressed albumin levels in seniors with pressure ulcers (53,54). The average albumin for the entire group was 3.1 g/dL, which is considered mild depletion (90), although research shows this is a result of decreased hepatic albumin synthesis, and not serum
protein leakage from the wound site (54). Furthermore, C-reactive protein (CRP), a marker of inflammation, has been indirectly associated with albumin levels (54). Although we were unable to measure CRP in our study, it may be that CRP levels were elevated, and the low albumin in our residents may partially be a result of inflammation from a chronic disease, or, the pressure ulcer itself (18,91). Thus, if the entire observed population is in an inflammatory state, then albumin, hemoglobin, and hematocrit may not be good measures of nutritional status.

Nutritional status for all residents was measured in this study using prospective clarifications of MNA questions. The MNA assesses nutritional status within the past three months. However, not all residents enrolled in this study had been at the Lake City VA medical center for this amount of time. If the resident’s home care had been assessed, it is likely their MNA score would have been lower because they would not have been receiving specialized care. Therefore, this was a limitation to the study.

Yet, another limitation of the study was the size of the studied population. Our sample size was too small to assume Gaussian distribution, and more than likely, a larger “n” would have shown significance where our study only found trends. Furthermore, out of 25 seniors examined, only four were considered to be well-nourished, thus creating unequally sized groups for comparison. In retrospect, the small number of well-nourished subjects with pressure ulcers should have been expected because poor nutritional status is a risk factor for pressure ulcer development. Future studies may want to consider a larger study size. This would more than likely increase significance among MNA groups, as well as correct for unequally sized groups for comparison.
CHAPTER 6

CONCLUSION

In conclusion, the results of this study suggest the MNA can be used to determine level of immune function parameters in elderly nursing home residents with pressure ulcers. This may be due in part to the interrelationship between nutrition and immunity.

Malnutrition and declining immune function are intertwined, accelerating each other to a vicious downward spiral that can only be stopped through nutritional intervention. MNA score is a predictor of nutritional decline in pressure ulcer patients, and decreased MNA score is associated with increased morbidity and mortality. Since MNA score is related to decreased immune function, this may partially help explain the reason decreased MNA score is related to increased morbidity. Because elderly with pressure ulcers are more likely to be nutritionally deficient, nutritional intervention is imperative.
APPENDIX A
PRESSURE ULCER STAGES

Figure A-1. Pressure ulcer stages. Reprinted with permission from "Pressure Ulcers in Adults: Prediction and Prevention" Clinical Practice Guideline Number 3, May 1992.

Description of Stages

- **Stage 1:** The ulcer appears as a defined area of persistent redness in lightly pigmented skin, whereas in darker skin tones, the ulcer may appear with persistent red, blue, or purple hues. Warmth, edema, induration, or hardness may also be indicators.

- **Stage 2:** Partial thickness skin loss involving epidermis, dermis, or both. The ulcer is superficial and presents clinically as an abrasion, blister, or shallow crater.

- **Stage 3:** Full thickness skin loss involving damage to or necrosis of subcutaneous tissue that may extend down to, but not through, underlying fascia. The ulcer presents clinically as a deep crater with or without undermining of adjacent tissue.

- **Stage 4:** Full thickness skin loss with extensive destruction, tissue necrosis, or damage to muscle, bone, or supporting structures (e.g., tendon, joint capsule). Undermining and sinus tracts also may be associated with Stage 4 pressure ulcer.
## APPENDIX B

**MINI NUTRITIONAL ASSESSMENT**

<table>
<thead>
<tr>
<th>Last name:</th>
<th>First name:</th>
<th>Sex:</th>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Age:**  
**Weight, kg:**  
**Height, cm:**  
**I.D. Number:**

Complete the screen by filling in the boxes with the appropriate numbers. Add the numbers for the screen. If score is 11 or less, continue with the assessment to gain a Malnutrition Indicator Score.

### Screening

<table>
<thead>
<tr>
<th>Question</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Has food intake declined over the past 3 months due to loss of appetite, digestive problems, chewing or swallowing difficulties?</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>B Weight loss during last months</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>C Mobility</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>D Has suffered psychological stress or acute disease in the past 3 months</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>E Neuropsychological problems</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>F Body Mass Index (BMI) (weight in kg) / (height in m)²</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**Screening score** (subtotal max. 14 points)

12 points or greater Normal — not at risk — no need to complete assessment

11 points or below Possible malnutrition — continue assessment

### Assessment

<table>
<thead>
<tr>
<th>Question</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>G Lives independently not in a nursing home or hospital</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>H Takes more than 3 prescription drugs per day</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>I Pressure sores or skin ulcers</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**Assessment** (max. 16 points)

**Screening score**

**Total Assessment** (max. 30 points)

### Malnutrition Indicator Score

17 to 23.5 points at risk of malnutrition

Less than 17 points malnourished
Figure C-1. The R JL systems electrode placement.
http://www.rjlsystems.com/research/electrodes.htm
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Jan Hudgens is a native Floridian, and has resided in Gainesville, Florida for most of her life. Her early interest in science led her to volunteering opportunities in multiple organizations, including North Central Florida AIDS Network, Shands Teaching hospital, and numerous nursing homes throughout Gainesville, Florida. At 16 years of age, she began working with the Florida Institutional Review Board of Gainesville, Florida, until her admission to college in 1997. Jan attended the University of Florida where she graduated cum laude in May of 2001 with a B.S. in Food Science and Human Nutrition and a specialization in Dietetics. She was accepted into the combined Master of Science/Dietetic Internship program later that year, and was awarded a departmental teaching assistantship based on her previous academic achievements. While in graduate school, Jan worked under the supervision of Dr. Bobbi Langkamp-Henken, and assisted in the orchestration of several human studies. In the future, Jan plans to obtain a doctorate in Human Anatomy, and teach at the graduate level.