INFLAMMATORY CYTOKINES, CACHEXIA AND SYMPTOMS IN PATIENTS WITH HEAD AND NECK CANCER

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Dedicated to Verna Mae Renslow
A beautiful and loving wife, mother, grandmother, sister and aunt
who faced her cancer with courage, strength and grace.
Always loved and always remembered.

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CHAPTER I

INTRODUCTION

Statement of Problem

Cancer affects all populations regardless of race, gender or age. Globally, one in four persons will die from cancer (Fearon et al., 2013). This pandemic indicates that the physical and psychological sequela of the disease is a common occurrence and affects not only the afflicted individual, but also those taking care of the patient, whether that is a doctor, nurse or family member (Hopkinson, 2010) (Huhmann & Cunningham, 2005) (McClement, 2005). One debilitating aspect of cancer that affects about half of all cancer patients is cachexia (Suzuki, Asakawa, Amitani, Nakamura, & Inui, 2013) (Tisdale, 2009).

Estimates indicate that up to 80% of all cancer patients experience cachexia (Walz, 2010). Cancer cachexia is a complex metabolic condition that is characterized by loss of skeletal muscle with or without loss of adipose tissue (Dodson et al., 2011) (Fearon et al., 2013). It is a syndrome associated with weight loss, lipolysis, muscle wasting, anorexia, chronic nausea, asthenia, with resultant changes in body image (Kim et al., 2012). Additionally, about 20%-30% of all United States cancer deaths are secondary to the debilitating effects of cachexia (Caro et al., 2007)(von Haehling & Anker, 2010)

This dissertation research focused on examining the relationship between cytokines, red blood cells (RBCs) and albumin in head and neck cancer (HNC)

patients and symptoms related to cachexia such as weight loss, fatigue, musculoskeletal dysfunction, swallowing, mucositis, anorexia, pain and depression. HNC is defined by the International Agency for Research on Cancer as cancer that arises in the head or neck region, which anatomically includes the nasal cavity, sinuses, lips, mouth, salivary glands, throat and larynx. (National Cancer Institute, 2013). More than 90% of malignant neoplasias of the head and neck area are squamous cell carcinomas (Tribius & Hoffmann, 2013). In the current Globocan statistic, which ranks the most common cancer diagnosis globally, HNC of the lip and oral cavity are in 15th (2.1%), carcinoma of the larynx in 20th (1.2%) ,and oropharyngeal cancer in 22nd place (1.1%) (International Agency for Research on Cancer, 2008). Combined, HNC is the sixth most common cancer worldwide with approximately 650,000 new cases reported annually. HNC accounts for approximately 3-5 %of all cancers diagnosed in the US. In 2013, approximately 55,000 new HNC cases were diagnosed in theUS, with a projected 30% increase to 80,000 new cases in 2015.(American Cancer Society, 2012). Despite treatment advances in multimodality therapy with surgery, radiotherapy, and chemotherapy, 5-year survival rate is still poor for patients with loco regionally advanced disease (Bonomi, Patsias, Posner, & Sikora, 2014).

Patients with HNC are particularly vulnerable to developing malnutrition and anorexia due to tumor of the upper audiodigestive tract such as the oral cavity, larynx, pharynx, paranasal sinuses, and salivary glands that makes chewing, swallowing and digestion difficult (Vissink, Jansma, Spijkervet, Burlage,

& Coppes, 2003) (Larsson, Hedelin, Johansson, & Athlin, 2005) (Jager-Wittenaar et al., 2011) (Munshi et al., 2003) (Denis et al., 2004) (Ehrsson et al., 2010). Treatment such as radiotherapy and chemotherapy results in symptoms such as mucositis, dysphagia, xerostomia, altered taste and smells. (Vissink et al., 2003) (Larsson et al., 2005). These symptoms can lead to decreased food intake of energy and nutrients can cause anorexia and wasting contributing to cachexia. The combination of location and treatment, make patients with HNC particularly vulnerable to cachexia. However, the association between symptoms induced by radiation and chemotherapy and the development and consequent progression of cachexia is not very well known in patients with HNC.

As a patient progresses through the continuum of their disease, whether or not that is met with remission, spread or recurrence of their cancer, our understanding of the many symptoms that a patient experiences and the association between those symptoms and cachexia are not fully elucidated. The hallmark phenomenon of cachexia is muscle wasting. It would be expected that as muscle wasting occurs, that a patient would experience an increased level of fatigue, as many studies have demonstrated (Dodson et al., 2011) (Fearon et al., 2013). However, within the HNC population what is not understood is how muscle wasting affects the development of other symptoms such as the ability to swallow and consume calories. Furthermore, what is less understood is the association between cytokine changes in HNC patients that both affect cachexia and symptom development. For example, does an increase in a particular cytokine promote muscle wasting that in turn promotes other muscle wasting that

increases the symptom burden that HNC patients experiences? Does this muscle wasting have a direct effect on a function such as the ability to swallow, to worsening mucositis and depression, which are not necessarily a sequela of muscle wasting? Is it the cachexia that makes symptoms worse, or is it possibly symptom burden that promotes muscle wasting, or finally, is the relationship a combination of these two paradigms? In short, our understanding of the relationship between cachexia and symptoms is not understood. This is possible because the exact physiological and biological mechanisms that promote cachexia are not understood. This makes deciphering the cause and effect of symptoms and cachexia difficult to discern.

As previously stated, the precise cause of cachexia presently remains unknown. Cachexia is a type of energy balance disorder, in which energy intake is decreased and/or energy expenditure is increased (Argiles, Busquets, Stemmler, 2014). A current accepted hypothesis is that the tumor produces cytokines, which affects metabolism and cellular respiration. Three physiological processes are affected by cytokines in the development of cachexia: 1) Cytokines cause an affect that ultimately promotes catabolism, specifically of muscle. Furthermore, the body also responds to cancer, possibly mediated by cytokines, by slowing the rate at which new muscle is produced. This leads to significant muscle wasting; 2) Adipose breakdown is promoted through various pathways. This lipolytic degradation promotes the breakdown of triacylglycerol's into glycerol and non-essential fatty acids. Glycerol stimulates liver gluconeogenesis. The tumor subsequently uses this additional fuel for growth

and production of further cytokines enhancing the effects of cytokines on further metabolic alterations. The glucose consumption additionally increases lactic acid, which inhibits and stresses other metabolic processes and this process independently requires additional energy expenditure by the body for cellular respiration; 3) Altered metabolic processes caused by muscle and adipose breakdown work in concert to continue the production of “feeding” tumors through glucose and non-essential fatty acids which continues to stress the body through increasing energy consumption. The body, therefore, responds to the tumor by increasing cytokine production, which also contributes to further altered metabolic processes experienced in cancer. The exact feedback mechanisms for the multiple processes that enhance cachexia are not entirely elucidated presently.

A key concept that appears to contribute to the severity of cachexia is cancer type. Cancer by nature is heterogeneous. There seems to be distinct differences between the cachexia experienced by different types of cancer. For example, it appears that persons with pancreatic cancer experience a greater severity of cachexia compared to those with HNC (Fearon et al., 2013). However, there are many possibilities of why the difference between different cancers occurs. Generally, pancreatic cancer is not diagnosed until the disease has progressed to stage IV and subsequently patients are considerably sicker at the time of diagnosis and treatment initiation in comparison to the HNC population (Bye et al., 2013). When the advanced disease is present, this may alter our ability to manage and diagnose the many negative impacts that cachexia

presents to the cancer patient in general. Nonetheless, no matter the type of cancer, cachexia poses a significant obstacle for the patient and clinician alike (Dalal et al., 2012). The greater the weight and muscle loss in cancer patients, the worse the outcome is for those patients (Fearon, Arends, & Baracos, 2012a) Confounding this problem, is also the variations in symptoms that patients experience in different cancers, through either disease progression or secondary to the effects of treatment. For example, the treatment modalities of HNC are different from those used in the treatment of pancreatic cancer. Therefore, symptoms are possibly affected by treatment, as well as by cytokines that are mediating the progression of cancer cachexia. Deciphering the noise between cytokines, cachexia, treatment and symptom burden is possibly impacted by the heterogeneous nature of cancer. Nonetheless, cachexia negatively affects all cancers, but what is less known are the different pathways that occur in various types of cancer.

Overall, cachexia is a devastating component of cancer illness for many patients. Unequivocally, the greater the severity of cachexia, the worse the outcome is for the patient (Steinman & Deboer, 2012). Through the study and investigation of cachexia in the HNC patient, it may be possible to find the cellular links that perpetuate this process and subsequently enable the advancement of treatments for cachexia.

Significance of the Issue and the Study

Significance of the issue to society

HNC, specifically those arising in the oropharyngeal region, are now believed to arise from two distinct pathways: one influenced by alcohol and tobacco and the other a result of genomic instability induced by human viruses such as the human papilloma virus (HPV), specifically HPV 16 (Andl et al., 1998) (Gillison et al., 2000) (Gillison et al., 2012) (Weinberger et al., 2006) (Tribius & Hoffmann, 2013) (Fakhry, Rosenthal, Clark, & Gillison, 2011) and the Epstein-Barr Virus (EBV) (Han et al., 2012) (Chai et al., 2012). Currently, alcohol and tobacco-associated HNC is decreasing globally while HPV derived HNC is increasing (Turi et al., 2013). This trend was demonstrated in a large ten-year epidemiological study in Peru where an increase in observed HNC in males aged 30-44 was 2.5%/ year, in females 15-29 4.2%/year, 30-44 3.4%/year (Walter et al., 2013). A similar increase secondary to HPV has also been reported in the United States. In 2000-2004 HPV comprised 38% of all HNC diagnosis in the United States and by 2009-2010, this increased to 59% of all HNC diagnoses (Wang, Thomas, & Zhang, 2012) (Chaturvedi et al., 2011). Other countries such as Denmark (Blomberg, Nielsen, Munk, & Kjaer, 2011) Germany (Guntinas-Lichius, Wendt, & Buentzel, 2010) Sweden (Attner et al., 2010) (Hammarstedt et al., 2007), and the United Kingdom (Conway, Stockton, Warnakulasuriya, Ogden, & Macpherson, 2006) have also reported significant increases in HPV-associated HNC diagnosis. EBV has also been associated with HNC, but this association is

seen in more patients who reside in Northern Africa and Southeast Asia. Unlike HPV, EBV-associated HNC has not significantly increased within the United States (Thompson & Kurzrock, 2004) (Lewis & Chernock, 2014). Within the scientific literature, a great deal of information has been garnered in regards to the association of HPV and cancer. However, little has changed in terms of our understanding of the association between EBV and HNC (Lewis & Chernock, 2014).

The consensus is that HNC cancers secondary to viruses have better outcomes with tumors more responsive to both surgical and non-surgical therapies and a lower risk of dying from the disease. However, the exact rationale for this difference is not understood. To confound this thought process are research studies recently published indicating that perhaps viral-mediated HNC cancers do not convey as much of a survival benefit to those induced by alcohol and smoking. Most studies indicate a propensity for increased survival in the HPV-positive groups compared to the alcohol and tobacco groups (Ihloff, Petersen, Hoffmann, Knecht, & Tribius, 2010). However, other studies challenge this notion because they found no statistically significant difference between either HNC etiologies (Cerezo et al., 2014) (Sharma et al., 2012) (Stephen et al., 2012) (Bragelmann et al., 2013). Increased survival has been attributed to the younger population of patients diagnosed with cancer and having less pro-inflammatory exposure than those with a smoking and or high alcohol intake history. Overall, a large meta-analysis indicates no survival outcome benefit to those HNC patients with a viral etiology (Isayeva, Li, Maswahu, & Brandwein-

Gensler, 2012). In terms of cachexia, the impact of the virally associated tumor on the symptomology and physiology of cachexia has yet to be determined, and studies examining this association need to be conducted. Smoking and excessive alcohol consumption independently is pro-inflammatory. This constant "assault" on the body through continual inflammation may be the reason that non-viral mediated cancers have worse outcomes. Nonetheless, whether the etiology is viral or non-viral, HNC patients experience anorexia and cachexia. The effects of cachexia negatively impact survival in the HNC population. With HNC diagnosis continuing to increase, more patients will be susceptible to the debilitating effects of cachexia.

With the combination of the number of HNC diagnosis increasing along with an estimated 15,000 deaths attributed to HNC annually within the United States, it is hypothesized that the number of HNC patients with cachexia is increasing as the population overall expands. Approximately, 80% of HNC patients experience cachexia (Gullett, Mazurak, & Hebbar, 2011)(Gullett et al., 2011) (Fearon, Glass, & Guttridge, 2012b) (Siddiqui, Pandya, Harvey, & Zaloga, 2006)(Gullett et al., 2011). Therefore, it will be significant to understand the role of cachexia in weight loss and muscle wasting to combat the profound physical and psychological burden of this disease process.

Significance of the issue to the patient and caregiver

Cachexia is a multidimensional syndrome characterized by involuntary weight loss, muscle atrophy, and physiological changes that lead to progressive functional impairment. Cachexia has a significant impact on patient morbidity and mortality, treatment tolerance, outcomes, and is an extraordinary source of psychological distress for patients and their families (Hopkinson, 2010) (Huhmann & Cunningham, 2005). Higher levels of depression, pain, decreased physical energy, performance and fatigue have been reported in patients with cachexia versus those that do not have cachexia (Giannousi, Gioulbasanis, Pallis, & Xyrafas, 2012) (Laird et al., 2011b) (Oz, Theilla, & Singer, 2008) (Wallengren, Lundholm, & Bosaeus, 2013)(Roberts, Frye, Ahn, Ferreira, & Judge, 2013). Overall, cachexia affects the cancer patient negatively and has repercussions for many aspects of daily living causing an increased need for support on the part of the patient.

The physical and psychosocial impact of cachexia on caregivers is profound (Longacre, Ridge, Burtness, Galloway, & Fang, 2012; Tamayo, Broxson, Munsell, & Cohen, 2010). HNC caregivers often report moderate levels of caregiving burden (Donnelly et al., 2008) (Chen et al., 2009; Ross, Mosher, Ronis-Tobin, Hermele, & Ostroff, 2010). Caregivers of cancer patients are often tasked with providing emotional support, taking patients to frequent clinic appointments, cleaning their homes and shopping for the patient (Precious, Haran, Lowe, & Rogers, 2012). In a recent HNC pilot study, caregivers (N=23) reported at least one neuropsychiatric symptom such as trouble with appetite and

eating, altered nighttime behaviors, depression/dysphoria, decreased alertness, inattention, apathy/indifference, anxiety, irritability/lability, agitation/aggression, and slowed behavior (Bond, Hawkins, & Murphy, 2014). Delirium is a symptom that affects both the patient and caregiver increasing the stress and anxiety of the caregiver (Bond, Dietrich, Shuster, & Murphy, 2012). The exact impact of these reported neuro-psychiatric symptoms are not fully understood and need further study, but it could be hypothesized that the impact upon the caregiver will possibly be negative by increasing the psychological distress of the caregiver. With increased wasting, specifically of the muscles, patients require support with day-to-day activities of daily living. This requires caregivers to spend increased time and effort providing care increasing their likelihood of experiencing fatigue (Clark et al., 2014). Also, caregivers experience the psychological distress associated with witnessing the wasting of their loved one. This may result in "helplessness" and "hopelessness" because of the inability to reverse the syndrome and to help their loved ones "battle" their cancer. In one study, caregivers reported cachexia, anorexia and fatigue as the three most stressful symptoms during the last week of life (Oi-Ling, Man-Wah, & Kam-Hung, 2005). Furthermore, loss of weight was associated with increased caregiver distress (Rhondali et al., 2012). The reflex reaction of caregivers is to encourage their loved one to eat and drink may result in rising interpersonal and familial stress as well as the feeling of failure among caregivers as unfortunately, increasing calories does not thwart cachexia (Tisdale, 2009). A further discussion on this occurs later in this chapter.

Due to the location of the HNC, many patients experience dysphagia and this decline in swallowing ability can increase caregiver fatigue through the necessity of planning meals and potentially isolating the caregiver from their normal social network by preventing the ability to dine at restaurants (Patterson, Rapley, Carding, Wilson, & McColl, 2012). Additionally, many HNC patients require insertion of feeding tubes to combat the inability to consume adequate calories, and this too requires caregiver management and can cause an increase in anxiety among those charged with caring for the patient at home (Penner, McClement, Lobchuk, & Daeninck, 2012).

Overall, the muscle and adipose wasting experienced by cancer patients is both distressing to themselves and to those that provide care to them. With little evidence-based medicine available to counter this weight loss, distress is elevated and therefore finding the associated physiological pathways involved in cachexia is paramount to treating this devastating aspect of cancer.

Significance of the issue to healthcare

The National Institutes of Health (NIH) estimated that in 2009 the overall annual costs of cancer were as follows: Total Cost- $216.6 billion; Direct medical costs (total of all health expenditures): $86.6 billion; Indirect mortality costs (cost of total productivity due to premature death): $130 billion (American Cancer Society, 2014). Cachexia affects patients' outcomes by decreasing the ability of cancer patients to tolerate therapy as well as decreasing quality of life. A weight loss of more than 5% at the time of diagnosis has been associated with

decreased response to medical interventions and increased mortality. The direct medical costs of cachexia are difficult to tabulate due to a lack of data and to date the NIH and the American Cancer Society do not have an estimated calculation available. A recent study estimates that in 2009 there were over 161,000 admissions for cancer cachexia (Arthur et al., 2014). These admissions cost on average $4600 more compared to cancer admission for non-cachectic reasons. A tabulation of the costs of cachexia includes therapeutic diets, nutritional supplements, medications, laboratory tests and other indirect medical costs related to the provision of care (Tan & Fearon, 2008). Furthermore, involuntary weight loss is associated with anemia, cognitive dysfunction and other sequela such as postural hypotension that can lead to an increased risk of falls and injury (Knudtson, Klein, Klein, & Shankar, 2005). According to Tan & Fearon, 2008, the economic costs of cachexia extend much further than the costs of therapeutic diets, nutritional supplements, medications, laboratory tests and supplies. The tabulated calculation must also include the provision of medical care that is provided by staff, service costs, as well as indirect medical costs. Patients with weight loss, specifically lean body mass, require increased attention by either medical professionals or by family members. Each of these directly increases the costs of cachexia. Overall, the Arthur et al., (2014) paper is the first to begin assessing the costs of cachexia, demonstrating that cachexia increases the costs of cancer treatment.

Significance to science/discipline of nursing and knowledge gaps

Significant research gaps remain in regards to the underlying biological mechanisms surrounding cachexia not only in cancer patients but also within chronic disease states such as congestive heart failure, renal failure, and chronic obstructive pulmonary disease. Currently, the underlying mechanism of cancer cachexia remains to be completely elucidated (Fearon et al., 2012a) (Vaughan, Martin, & Lewandowski, 2012) (Argiles et al., 2011) (Blum et al., 2011) (Bennani-Baiti & Walsh, 2009). Our inability to fully understand the physiological pathways of cancer translates into our inability to treat patients who experience cachexia. Additionally, cachexia is often not diagnosed until the symptoms are quite severe (Fearon et al., 2013). This is primarily in response to a disagreement among clinicians about what the diagnosis of cachexia should be currently. A further discussion of this issue occurs in chapter 2. This complexity and non-uniformity surrounding cachexia as well as our lack of physiological understanding of the process, unfortunately leaves the nurse at the forefront of delivering care to patients with cachexia with little in their armamentarium to actually treat and assuage the tedious progression of cachexia.

It is possible that our lack of understanding the pathology of cachexia has meant an inability to develop evidence-based practice guidelines to assist the nurse in their task of caring for the cachectic cancer patient. Once cachexia is established in a patient, there are limited therapies that can be initiated, with most therapies targeted at palliation of the weight loss. The only possible effective treatment has been a combination of delivering adequate dietary protein

in combination with progressive resistance exercise. However, even that intervention was targeted at sarcopenic patients and not necessarily those with confirmed cachexia (Marcell, 2003). With little to offer patients and their families, nurses must care for their patient in a holistic non-evidence based directional approach to care for the cachectic patient. Without any evidence-based practices to guide the nurse, they must provide care that is of the essence of what nurses do and that is to place the patient in the best possible position to allow nature to heal. Beyond that, the ability to specifically treat cachexia is limited.

To date, pharmacological interventions have failed to demonstrate a clinically significant impact on cachexia or disease outcomes in patients with advanced cachexia. In a recent small study, 62 patients with cancer cachexia were placed in a three arm three month study examining 1) megestrol acetate (MA) plus meloxicam (n = 23); 2) MA plus meloxicam plus oral eicosapentaenoic acid (EPA)-enriched nutritional supplement (n = 21); or 3) meloxicam plus oral EPA-enriched nutritional supplement (n = 18) (Kanat et al., 2012). No statistical significance were obtained between body weight, lean muscle mass, BMI or quality of life. A recent study examining the effects of simvastatin on cachexia has been carried out in rats due to the anti-inflammatory properties that statin drugs have with a decrease in cachexia symptoms in the treatment group (Palus, Haehling, Flach, & Tschirner, 2013). A follow-up study examining statin effects on humans with cancer has not been conducted. Again, with no actual definitive approaches to treating cachexia, the nurse must rely on fundamental nursing

principles to care for their patients. One agent that held promise for use was Ghrelin.

Ghrelin, an endogenous orexigenic hormone, does hold some promise for attenuating cachectic symptoms in humans. It is posited that Ghrelin increases appetite via melanocortin modulation in conjunction with its anti-inflammatory effects, which possibly decreases the effects of cachexia. (Steinman & Deboer, 2012). Again, Ghrelin effects on humans with cachexia is still largely unknown, and the use of Ghrelin has not been uniformly adopted (DeBoer, 2010). The short-term use of melatonin has also not demonstrated any benefit in regards to reversing cachexia symptoms (Del Fabbro, Dev, Hui, Palmer, & Bruera, 2013). Overall, there are no pharmacological interventions that can be used to combat cachexia. Rather, treatment must be aimed at reducing the different symptoms of cachexia such as fatigue, pain, muscle wasting, decreased ability to tolerate treatment, anorexia, malnutrition and an overall decline in the quality of life. Nurses, without having specific interventions available for cachexia, must rely on their nursing skills to treat the many sequelae of cachexia. One such treatment modality that many nurses strive to ensure is that their patients are consuming adequate calories. Unfortunately, the effects of cachexia are not assuaged by sufficient caloric intake.

A common hypothesis is that treating cachexia through nutritional interventions is the most prudent way to combat the debilitating effects of cachexia. However, no single intervention to improve appetite has been able to demonstrate a reversal in cachexia (Yavuzsen, Davis, Walsh, LeGrand, &

Lagman, 2005). A recent meta-analysis indicates that despite nutritional interventions to improve outcomes in patients with cancer and cachexia, reversal of cachexia effects could not be achieved and no reduction in mortality has been obtained (Baldwin, Spiro, Ahern, & Emery, 2012). Dietary supplementation and appetite stimulation alone are inadequate to reverse the underlying metabolic abnormalities of cancer cachexia and have limited the long-term impact on patient quality of life and survival (Dodson et al., 2011). This is again because cachexia is in and of itself is a condition that places the body in a negative caloric expenditure. In a study that delivered “adequate” calories through TPN, wasting still occurred in patients (Evans et al., 1985). Caloric intake presently is not the answer for treating cachexia. The nurse must understand this and must teach family members who are watching their loved ones “waste away” that increasing caloric intake will not improve cachexia.

Other factors such as nausea and vomiting decrease the ability of cancer patients to tolerate food. However, despite proper nursing and the delivery of medicines to decrease nausea and vomiting, cachexia itself will not reverse because the patient can increase their food intake. This can be distressing to all care providers. While nausea and vomiting needs to be thwarted, there are very little evidence-based guidelines to guide medical providers in their ability to combat this debilitating process (Laugsand, Kaasa, & Klepstad, 2011; Naeim et al., 2008). Prophylactic treatment of nausea and vomiting prior to chemotherapy is highly recommended, but the exact guidelines for doing so are not uniform

(Vidall et al., 2011; Schulmeister & Gobel, 2008). Uncontrolled pain can also confound nausea and vomiting.

Nurses handle adequately assessing the pain experienced by their patients and to ensure a proper intervention has been conducted to reduce a patient's pain. Ensuring that cancer patients have adequate pain control is important in aiding their ability to function and increases their quality of life. Pain control in the cachectic patient can inadvertently cause worsening cachectic symptoms by causing patients to become somnolent and not eat, as well as cause opioid-induced nausea and vomiting. Furthermore, opioids can cause gastroparesis causing bloating and often the development of ileus, which decreases the bodies' ability to consume calories due to an improperly functioning gut (Mehendale & Yuan, 2006; Kurz & Sessler, 2003). Early treatment and prevention of the ill effects of opioids on the gastrointestinal track is recommended (Holzer, 2010). With so many symptoms appearing in cancer patients such as pain, fatigue, nausea and vomiting there is a possible association between the severity of these symptoms, decreased levels of quality of life and worse outcomes. However, an exact association is still lacking in the HNC population. This lack of association, unfortunately, means that there are very few evidence-based approaches that nurses can use to treat cachexia. The inability to treat cachexia can decrease a person's quality of life.

The quality of life, as sometimes measured by levels of fatigue and pain, and its overall association with the severity of cachexia is unknown. It is posited that worse QOL indicators would correlate with worse cachexia, but this

association has not been fully elucidated though evidence does suggest that there is an association between these concepts. Early treatment and intervention of cachexia have been demonstrated to improve patient quality of life (Vigano, Del Fabbro, Bruera, & Borod, 2012) (Parmar, Swanson, & Jagoe, 2013). Whether or not this translates into increased survival and decreased recurrence rates is unknown. Nurses, through many of their interventions, can improve the quality of life. Understanding these associations is important in the treatment of cancer and cachexia.

Thus far, it has been demonstrated that there are no pharmacological interventions that are effective in combatting cachexia. Furthermore, impacts on patient's quality of life secondary to cachexia are also not fully understood. The lack of treatment modalities for cachexia comes from our lack of understanding cachexia physiology. The physiological cascade that occurs at the cellular level when cancer is present resulting in cachexia is now only starting to be illuminated. Cachexia is attributed to tumor and humoral cytokine production, which promotes muscle depletion as well as inhibition of new muscle production.

Due to the lack of effective interventions, nurses at the front line working with patients as they progress in their cancer disease burden must provide nursing care, comfort and education to patients with cancer cachexia as well as to their caregivers. Nurses handle understanding the many components that drive cachexia to help manage and treat the many symptoms of cachexia. To continue to help those nurses on the frontline deliver the best care possible

continued research into the physiology and targeted treatment modalities must continue.

Overall, there is a significant lack of understanding regarding the physiological pathways of cancer cachexia. This absence of data directly translates into fewer treatment modalities available for health care professionals to provide to their patients. Continued research into the physiology of cachexia is paramount to our ability to eventually treat and perhaps thwart this terrible sequela of cancer and thereby improve outcomes.

Summary of overall significance

Cachexia affects the majority of cancer patients, including those with HNC While it is important to study cachexia about all cancers, it should be noted that each cancer presents itself with different obstacles in terms of treatment. HNC causes unusual sequela of events for a patient compared to other cancers such as primary tumors of the breast, colon or lung. Due to that reason, it is imperative that the study of cachexia be investigated in a multitude of different cancers to assess the various manifestations of secondary symptoms that accompany the disease process. For example, is cachexia different for the lung cancer patient who cannot breathe versus the HNC patient who can no longer swallow? Do the manifestations of other symptoms, such as depression, fatigue, and social isolation affect HNC patients who have cachexia differently than those manifested in breast cancer patients? Currently, the state of science cannot answer these questions and, therefore, while it will be important to utilize the

science of cachexia in other cancers, it will be important to study HNC in its entity to define those unique aspects that affect cachexia within this patient population.

In addition to studying HNC patients singularly with or without cachexia, it should be recognized that any study of cachexia will help move the scientific communities understanding of the disease process forward as, overall, there is a lack of understanding regarding thee physiology of cachexia itself. Due to this lack of physiological understanding, our ability to not only diagnose cachexia is affected, but there are limited abilities to actually treat cachexia once it is diagnosed aside from palliation. Overall, our understanding of cachexia is limited and further studies of this devastating secondary process during cancer are necessary in order to improve our physiological underpinnings to cachexia with the ultimate goal of being able to treat and possibly thwart the process in its entirety in the future. Through this ability, we can save the lives of many cancer patients.

Purpose of the Study

The purposes of this study is to examine the relationship between cytokines, red blood cells (RBCs) and albumin in head and neck cancer (HNC) patients and symptoms related to cachexia such as weight loss, fatigue, musculoskeletal dysfunction, swallowing, mucositis, anorexia, pain and depression. By identifying potential correlatives with different biomarkers, it may become possible to develop future treatment modalities aimed at reducing the negative cascade that some biomarkers may be initiating. Furthermore,

identifying correlatives between symptoms and weight loss with specific cytokines can inform future research to both increase our ability to palliate the cachectic patients as well as identify further bench science to examine specific cytokines and the metabolic processes those identified cytokines promote. Specific aims for this study include:

1. To examine cytokines, red blood cells and albumin in HNC patients.
2. To examine the role of cytokines, red blood cells and albumin and severity of weight loss in HNC patients.
3. To examine the association between cytokines, red blood cells, and albumin and reported symptoms in HNC patients.
4. To examine the association between cytokines and changes in musculoskeletal function in HNC patients.

Research Questions

Question 1: Do baseline red blood cells (RBCs) and albumin correlate with changes in cytokine levels at 6- and 12-months?

Question 2a: Are baseline cytokines, red blood cells and albumin correlated with weight loss at 6- and 12-months?

Question 2b: Does a change in cytokine levels from baseline to 6-months correlate with weight loss at 12-months?

Question 3: Are initial cytokines, RBCs and albumin associated with changes in reported symptoms such as: depression, nutrition consumption, swallowing

difficulties, mucositis, generalized pain, mouth pain, dental discomfort, hearing, xerostomia, taste, jaw movement and voice at 6- and 12-months?

Question 4: Are baseline cytokines associated with musculoskeletal impairment (neck and shoulder movement) as measured by shoulder and neck ranges of motion, swallowing and neck disability at 12-months?

CHAPTER II

LITERATURE REVIEW

History of the Problem of Interest

In 1980, (Dewys et al., 1980), published the first paper addressing outcomes about weight loss within cancer patients. It was demonstrated that a >5% unintentional weight loss was able to predict mortality and response to treatment. However, a review of human history indicates that people have been struggling with cachexia in various forms for centuries. An article by (Ben-Noun, 2004), demonstrates the biblical description of weight loss and depression experienced by King David, who himself may have had cancer. Elizabeth I, Queen of England (1533-1603) also indicates depression, altered body image and taste changes in her final days (Bennani-Baiti & Walsh, 2009). The term cachexia is derived from the Greek word ‘kakos’ (bad) and ‘hexis’ (habit). The word anorexia is from the Greek ‘an’ and ‘orexis’, which together mean no appetite, i.e. loss of appetite (Ben-Noun, 2004). This wasting and loss of appetite is experienced in most cancers.

Analysis of Relevant Literature

Head and neck cancer is one of the several types of cancer that causes cachexia. As such, while this dissertation will study the HNC population, this evaluation of cachexia science in this chapter will analyze the science not only specific to HNC, but to other types of cancers. This broad overview of cachexia will help guide the physiological and diagnostic principles established through scientific research to date and will allow a more comprehensive presentation. Furthermore, our understanding of cachexia physiology is promoted through research no matter the type of cancer in question. It should be noted that it appears that the same mechanisms that cause cachexia in HNC are the same in other cancers.

The biology, symptoms and treatment of cancer cachexia

Different mechanisms by which cachexia occur is currently being examined. There are three main areas of research that guide cachexia research today. They are: 1) the biological processes by which adipose and muscle tissues is lost during cancer; 2) the mechanisms that drive those biological pathways and 3) the mechanisms by which inflammatory processes affect the brain, specifically the hypothalamus, and subsequent anorexia development that further potentiates the effects of cachexia. Overall, our knowledge regarding the biological process of cachexia is not completely understood.

Cachexia is a relevant comorbid condition of chronic diseases including cancer. Inflammation, oxidative stress, autophagy, the ubiquitin-proteasome system, nuclear factor (NF)-kappaB, and mitogen-activated protein kinases (MAPK) are all involved in the pathophysiology of cancer cachexia (Chacon-Cabrera et al., 2014). Presently, it is not certain whether the metabolic drivers of cachexia, specifically cytokines, are a byproduct of the tumor or a byproduct of host inflammatory cells. One hypothesis is that both tumor cell production and pro-inflammatory cytokines from the host inflammatory cells respond to tumor cells and that this response is the source of an acute phase protein increase seen in cachexia (Donohoe, Ryan, & Reynolds, 2011). However, the mechanistic physiological steps of subsequent adipose and skeletal muscle are unknown. Generally, in humans, the tumor size is less than 1% of an individual's mass when there is profound cachexia suggesting the metabolic demands of the tumor are less important than distant metabolic effects induced by the tumor upon the host (Fearon et al., 2012a) (Roshani, McCarthy, & Hagemann, 2014). Further evidence for this hypothesis is supported by the same tumor burden causing different manifestations of cachexia in different persons (Tan & Fearon, 2008). These differences are probably based in genetics (Derynck, Akhurst, & Balmain, 2001) (Saini, Al-Shanti, & Stewart, 2006).

Different metabolic processes affect the loss of muscle and adipose tissue as well as the process by which the human body regulates its diet. These metabolic processes are not fully understood negatively impacting the ability to develop therapies to counter the devastating effects of cachexia. Thus, our

limited understanding of these pathways directly affects our ability to treat cachexia. It is imperative that studying of these pathways not only to understand cachexia physiology, but also also to develop novel therapies to abate potentially the effects of cachexia. Furthermore, cachexia is often not identified and treated in late stages of the process are occurring making reversal of cachexia very difficult to obtain (Tan, Deans, Skipworth, Ross, & Fearon, 2008).

Cytokines are protein molecules released by lymphocytes or monocyte macrophages, and in some instances the same cytokine can be released by both organelles (Germolec, Frawley, & Evans, 2010). The production of chemical-mediators associated with cachexia is derived from either the tumor or from humoral factors, which are mainly exhibited in the form of cytokines that are derived from cellular responses in the tumor microenvironment (Bing & Trayhurn, 2009) (Bing, 2011). Several cytokines have been linked with cachexia. However, one difficulty in the identification of cachexia is that consistent results between different cytokines and cachexia development have not been elucidated.

Macrophages, dendritic cells, fibroblasts, NK cells, T and B cells, keratinocytes and tumor cells produce Tumor Necrosis Factor Alpha (TNF-α). It was originally named “cachectin” due to its catabolic nature. At the time of its discovery, it was shown to induce cachexia in mice after ovarian cancer was transfected with the human gene for TNF-α (Oliff et al., 1987) as well as to slow the insulin signaling pathway (Hotamisligil, 1999). However, TNF- alpha has not consistently demonstrated a cause and effect of the development of cachexia (Maltoni et al., 2001). In a recent study of 32 HNC patients, no association was

found between TNF-α levels and weight loss (de Carvalho et al., 2013). However, within HNC, a majority of cancer is correlated with alcohol consumption, and a significant difference in TNF-α levels between these groups has been seen, but these levels did not correlate with weight loss (Heimdal, Aarstad, Klementsen, & Olofsson, 1999).

Multiple animal studies indicate that TNF-α is a cytokine that frequently induces cachexia (Tisdale, 2005). In one study of 63 persons with pancreatic cancer, only 36.5% of them had detectable levels of TNF-α, with higher levels in those with metastatic disease (Karayiannakis et al., 2001). An examination of tumor TNF-α production does not demonstrate that tumors are independently the source of increased TNF-α in all cases of cachexia (Fearon et al., 2013). Unfortunately, while it is known that TNF-α directs catabolism, the exact mechanism in vivo remains elusive (Laine, Iyengar, & Pandita, 2013). TNF-α appears to be a stimulus for the production of other catabolic cytokines that subsequently cause the development of cachexia (Argiles, Busquets, Stemmler, & Lopez-Soriano, 2014). This conclusion is further supported by evidence that intraperitoneal injection of soluble recombinant human TNF-receptor antagonist improved anorexia in tumor-bearing animals (Torelli et al., 1999).

A recent study also showed a reversal in cachexia in humans with TNF-α antibody treatment, but the exact mechanism is not understood (Jatoi et al., 2010). Further complicating the picture of TNF-α in cancer is the fact that it appears to be able to act as both a tumor promoter as well as a tumor inhibitor (Balkwill, 2002). Additionally, TNF-α appears to affect the hypothalamus through

pro-inflammatory signals that affect neurotransmitters and subsequent hunger signaling to patients perpetuating the cycle of cachexia by inducing anorexia (Amaral et al., 2006). TNF-α also promotes fat lipolysis and fat depletion (Tsoli & Robertson, 2013). TNF-α has also been recently identified to regulate lipolysis through the GO/G1 switch gene 2 (GOS2). By inhibiting the expression of GOS2, an increased level of lipolysis occurs (Yang, Zhang, Heckmann, Lu, & Liu, 2011). Presently, it appears that TNF-α directly relates to adipose tissue loss, but not directly to the loss of muscle protein (van Hall, 2012).

A closely related member of TNF-α is the TNF-like weak inducer of apoptosis (TWEAK). It is a regulator of inflammation and skeletal muscle mass and works through binding to Fn14 (fibroblast growth factor-inducible receptor 14), which is a cell-surface receptor that is linked to various intracellular signaling pathways. The TWEAK-Fn14 axis handles the promotion of tissue repair following an acute injury. It is now recognized as a novel inducer of skeletal muscle wasting (Kumar, Bhatnagar, & Paul, 2012). The TWEAK-Fn14 can contribute to starvation-induced muscle atrophy (Penna et al., 2013). Genetic ablation of TWEAK in a murine model has demonstrated a decrease in the loss of muscle mass (Mittal et al., 2010).

TWEAK suppresses satellite cell self-renewal reciprocally regulating NOTCH and NF-KB and P38 mitogen-activated protein kinase (MAPK) pathways. In short, TWEAK up-regulates NF-KB levels, which have been linked to the progression of cancer-related symptoms including cachexia (Gupta et al., 2011) (Cleeland et al., 2003; Myers, 2008) (Rhoads, Kandarian, Pacelli,

Doglietto, & Bossola, 2010). Interleukin-1 can also activate the NF-KB pathway (Johns, Stephens, & Fearon, 2013). NF-KB is a protein complex that controls transcription of DNA and is found in almost all animal cell types. In regards to cachexia, NF-KB has been linked to muscle-protein breakdown and wasting (Op den Kamp et al., 2013). This muscle wasting appears to be regulated by the ability of NF-KB to increase Pax7 expression, which inhibits myofiber regeneration leading to muscle wasting (He et al., 2013). Additionally, TWEAK handle up-regulation of the E3 ubiquitin ligases MuRF1 (muscle ring finger 1) through NF-KB signaling. The ubiquitin-proteasome pathway/ubiquitin-mediated proteolytic (UPP) system is involved in the disease-related hyper catabolism (Mitch & Goldberg, 1996). The UPP handles protein catabolism at the mammalian cytosol and nucleus. MuRF1 participates in the regulation of amino acid metabolism, including the control of free amino acids and their supply to other organs under catabolic conditions and in the regulation of ATP synthesis under metabolic stress conditions where MuRF1 expression is induced (Koyama et al., 2008). This up-regulation results in the loss of myosin heavy chain (MyHC) and other thick filament components (Cohen et al., 2009). The inhibition of the NF-KB induced MuRF1 pathway has demonstrated the decrease of muscle wasting in mice (Moore-Carrasco et al., 2007). Further studies surrounding the regulation of P38 MAPK needs be conducted to identify the exact mechanism through which protein synthesis is decreased (Wing, 2013). Presently, strong evidence exists that tumors directly induce signaling pathways that upregulate enzymes that induce skeletal muscle breakdown. However, in the absence of

these pathways, such as MuRF1, muscle breakdown does not occur despite tumor burden, further indicating that genetics possibly dictate the microenvironment response to the tumor..

However, conflicting evidence has recently been reported in regards to both serum TWEAK levels and tumor TWEAK levels in HNC patients (Aviles-Jurado et al., 2014). Study participants who had lower TWEAK serum levels had significantly increased recurrence of disease (N=37). Furthermore, higher TWEAK levels within the tumor were correlated with worse prognosis. However, an examination of these levels and weight loss was not conducted in this study. It can be speculated that elevated TWEAK levels hinder tissue repair through the promotion of catabolism, which enhances cachexia. Further studies examining this association will need to be conducted in a large RCT.

TNF-α and TWEAK appear to work in concert with the cytokine interleukin 6 (IL-6) in the progression of cachexia. IL-6 stimulates the liver to induce an acute phase response. However, unlike TNF-α, circulating levels of IL-6 have been shown to correlate with weight loss and survival in cancer patients, (Scott, McMillan, Crilly, McArdle, & Milroy, 1996) (Moses, Maingay, Sangster, Fearon, & Ross, 2009) as well as a predictor of survival in patients with advanced cancer (Suh et al., 2013). Macrophages, dendritic cells and T and B cells produce IL-6. IL-6 levels have been shown to increase significantly in head and neck cancer patients (Pries & Wollenberg, 2006) (Richey et al., 2007). In a large prospective cohort study of HNC patients, higher IL-6 levels were significantly associated with worse overall prognosis (n-444) (Duffy et al., 2008). However, in a recent study

of lung and colorectal cancer patients, elevated IL-6 levels did not significantly correlate with weight loss until after 6-months duration (Kim et al., 2012). However, in an attempt to understand the association between levels of IL-6 and muscle wasting, only supra-physiological levels of IL-6 when administered to healthy animals, in the absence of disease, was sufficient to initiate muscle wasting and subsequent loss (Baltgalvis et al., 2008). Additionally, a recent trial of IL-6 monoclonal antibody in weight losing lung cancer patients has shown reversal of anorexia, fatigue and anemia, but not of muscle atrophy (Bayliss, Smith, Schuster, Dragnev, & Rigas, 2011). IL-6 has also been shown to stop muscle catabolism but was unable to reverse the depression of muscle synthesis (White et al., 2012). From a prognosis perspective, it appears that IL-6 rises in the final week before death (Martin et al., 1999). IL-6 seems to elevate slowly at the initial onset of cachexia and increase exponentially at the end of life (Tisdale, 2009).

IL-6 has been additionally linked to cachexia within the concept of fat-muscle crosstalk, specifically in the setting of the C26 tumor (Das et al., 2011). Physiological important crosstalk between adipose tissue and skeletal muscle exists in the context of cancer cachexia (Zechner et al., 2012). In a recent study, autophagy was shown to increase in mice implanted with C26 tumor (Penna et al., 2013). Ablation of the adipose triglyceride lipase, also known as hormone-sensitive lipase, (Das et al., 2011), was able to preserve muscle mass. This data suggests that the tissue cross-talk between tumor, fat and muscle is important to our understanding of the cachectic process.

C26 abundantly secretes myostatin, which is a member of the transforming growth factor-beta family. Myostatin regulates the glucose metabolism of muscle cells while deregulated myostatin activity is associated with some metabolic disorders, including muscle cachexia, obesity and type II diabetes (Liu et al., 2013). Through the activation of the UPP, myostatin has been shown to upregulate MuRF1 and NF-κB . Myostatin also inhibits the activity of IGF-1. The skeletal muscle cells further secrete myostatin and first signals through the activin type II receptor, which then recruits Akt family kinase resulting in the activation of SMAD2 and SMAD3 transcription factor complexes. Akt, in conjunction with TORC1, is down-regulated resulting in a decrease in muscle protein production (Thomas & Mitch, 2013). By inhibiting myostatin, a reduction in muscle wasting has been accomplished. Within a mouse model, myostatin antagonism demonstrated the ability to prevent cachexia (Benny Klimek et al., 2010). Furthermore, in a separate mouse model, the utilization of actRIIB antagonism inhibited myostatin thus blocking MuRF1 and improving muscle mass and weight (Busquets et al., 2012). Furthermore, inhibition of the FoxO transcriptional factor also blocked MuRF1 and prevented muscle atrophy (Reed, Sandesara, Senf, & Judge, 2012). The recent successes in mouse models regarding myostatin holds promising leads into the quest to reverse and prevent cachexia. The muscle weakness produced by myostatin has also been linked to increased diaphragmatic weakness in patients leading to profound respiratory failure and intubation (Roberts et al., 2013).

Three major proteolytic pathways responsible for the degradation of proteins in skeletal muscle exist. These are 1) the lysosomal system including the cysteine proteases cathepsins B, H and L as well as the aspartate protease cathepsin D (Tisdale, 2009). When myostatin was inhibited causing a decrease in MuRF1 up-regulation, there was also a subsequent decrease in cathepsins L (Busquets et al., 2012). 2) Protein degradation through the UPP. This pathway is the main driver behind the degradation of myofibrillar proteins, especially in patients with a weight loss of greater than 10% (Khal, Hine, Fearon, Dejong, & Tisdale, 2005) (Smith, Khal, & Tisdale, 2005). The exact process by which the UPP promotes protein degradation is not known but is possibly associated with a genetic component that takes its initiation from cytokine mediators (Bhattacharya et al., 2014) 3) The calcium-activated system. Research suggests that myofilaments are released from the sarcomere by the action of calcium-activated calpains followed by the degradation of the myofilaments by the ubiquitin-proteasome system (Adams, Anker, & Schuler, 2011). This process in conjunction with the UPP drives muscle degradation further.

Further muscle breakdown can be initiated by the tumor itself through the production of proteolysis-inducing factor (PIF). The action of PIF is mediated through a high-affinity membrane receptor in muscle (Mirza, Wyke, & Tisdale, 2011). PIF has been shown to increase directly the activity of the UPP (Russell, Siren, Siren, & Tisdale, 2010). The exact role of PIF is not entirely understood. Several studies have correlated a presence of PIF in the urine of cachectic patients that was related to weight loss (Wang et al., 2003) (Williams et al.,

2004), while other studies have questioned this association (Jatoi et al., 2010) (Whitehouse, Smith, Drake, & Tisdale, 2001).

The human stress response to disease is often linked to an increase in the readily available release of glucose into the blood stream. Furthermore, many patients today have increased insulin resistance either from metabolic syndrome or diabetes. Insulin resistance has been linked to cancer progression in several cancers (Hursting & Berger, 2010). However, in relation to cachexia, the increased insulin resistance is problematic in that it potentiates several pathways that have been linked to adipose and muscle mass loss. The binding of insulin, or insulin growth factor-1 (IGF-1) to cell surface receptors on tumors activates the PI3K/Akt (phosphoinositide-3-kinase/Akt) and glycogen synthase kinase (GSK-3beta) pathways leading to the activation of the mammalian target of rapamycin (mTOR) (Pollak, 2008). This activation triggers muscle growth. However, under catabolism, IGF-1 ability to function appropriately is altered. Increased metabolically altered IGF-1 levels directly enhance the role of myostatin potentiating cachexia (Bonetto et al., 2013). Evidence suggests that IGF-1 and insulin are impaired under catabolic stress conditions, potentiating the production of these factors and driving cachexia. Perhaps what is altered at the cellular level is the IGF-1 and insulin receptors. The IGF-1 administration failed to reverse the muscle atrophy in experimental mouse models (Costelli et al., 2006). Remodeled post-receptor insulin, GH, and IGF-1 pathways constitute a potential target for pharmacological treatment in the setting of body wasting and cachexia (Trobec,

von Haehling, Anker, & Lainscak, 2011). It should also be noted that IGF-1 studies in HNC patients in regards to cachexia have not occurred.

Standard treatments in the cachectic patients such as glucocorticoids to stimulate appetite and food intake may be promoting further muscle loss. Glucocorticoids (GC) promote muscle atrophy through enhancement of the UPP (Schakman, Kalista, Barbe, Loumaye, & Thissen, 2013), specifically through the FOX-O transcription factor (Sandri et al., 2004). FOX-O3 deletion results in protection against muscle wasting (Zhao et al., 2007). The inhibitory effect of GC on muscle protein synthesis is thought to result mainly from the inhibition of the mTOR/S6 kinase-1 pathway (Schakman et al., 2013). These changes in muscle protein turnover could be explained by changes in the muscle production of two growth factors, namely IGF-1, a muscle anabolic growth factor and myostatin, a muscle catabolic growth factor (Schakman et al., 2013). Research has indicated that by suppressing myostatin, this also reduces the metabolic derangements IGF-1 and insulin receptors experience under stress, thus enhancing muscle growth (Han & Mitch, 2011).

Conversely, data indicates that IGF-1 is not necessarily negative in the promotion of cachexia. Under normal physiological conditions, IGF-1 promotes muscle growth. High levels of Angiotensin II can affect IGF-1. Angiotensin II is produced through the renin-angiotensin system and has been associated with weight loss (Tisdale, 2009). The idea that angiotensin II could promote weight loss was first discovered in heart failure patients who began taking angiotensin converting enzyme inhibitors. Subsequent mouse studies have shown that

injection of angiotensin II promoted muscle and adipose tissue loss (Brink et al., 2001). Angiotensin II infusion induces skeletal muscle atrophy in rodents and mechanisms include increased expression of the E3 ligases atrogin-1/MuRF-1, an elevated rate of ubiquitin-proteasome mediated proteolysis and increased reactive oxygen species (ROS) levels, closely mimicking conditions of human cachexia (Sukhanov et al., 2011).

Single polymorphisms of IL-1, IL-6 and IL-10 have also been implicated in the development of cachexia (Fearon et al., 2012a) (Tan & Fearon, 2008). In a large study of 203 patients examining multiple cytokine polymorphisms, only IL-10-1082 polymorphism was linked to the development of cachexia in gastric cancer (Deans et al., 2009). Increased IL-10 serum expression was significantly associated with worse survival in early stage oral squamous cell carcinoma (Chen et al., 2013). However, this same association was not witnessed in myelofibrosis (Tefferi et al., 2011). An affirmative association with circulating IL-10 levels and cachexia is currently lacking. Nonetheless, various cytokines have been linked to the process of cachexia through both down-regulation and up-regulation of metabolic processes. Furthermore, cytokines are also responsible for metabolic processes within the brain.

Various circulating levels of cytokines affect brain neuropeptides. Many cytokines have an impact on appetite such as IL-1α, IL-1β, IL-6, TNF-α and interferon-gamma (Patra & Arora, 2012). These cytokines transport across the blood-brain barrier and interact on the luminal surface of brain endothelial cells and release substances driving or hindering appetite (Banks, 2001). Cytokines

modulate the activity of neuropeptides and hormones that control energy homeostasis and/or illness behaviors (Krasnow & Marks, 2010). Cytokines seem to play a fundamental role in energy balance through persistent activation of the melanocortin system and inhibition of the central neuropeptide Y and agouti-related protein (AgRP) pathways (Patra & Arora, 2012). Under normal physiological conditions, activation of neuronal nitric oxide synthase results in increased adenosine monophosphate kinase and a decrease in malonyl-coenzyme-A, leading to increased food intake (Morley & Farr, 2008). However, under various disease states, these processes can be altered leading to an imbalance in anorexigenic and orexigenic peptides which can result in suppression of appetite and increased satiety ultimately leading to weight loss (Perboni & Inui, 2006). The pathophysiological role of cytokines in the hypothalamic accurate nucleus is not yet fully understood, but the evidence suggests a strong association between cytokines and the brain, which in turn can promote cachexia, and symptoms, such as depression and anorexia during cancer. Complicating this notion are two studies. In a mouse model, intracerebroventricular injection of inflammatory cytokines reduced food intake indicating that the brain can directly respond to inflammatory messages (Braun & Marks, 2010). However, in a separate study a negative association was reported between food intake and IL-1 (Braun & Marks, 2010). A recent study also indicated that association between glucocorticoid levels and CNS activation of muscle catabolism (Braun et al., 2011). While it is agreed that the hypothalamus

responds to cytokines, the exact pathological path is not clearly identified further complicating the treatment of cachexia.

One such cytokine recently implicated in the regulation of appetite on the hypothalamus was the TGF-beta cytokine MIC-1/GDF 15 (Tsai, 2012). Evidence for this has been demonstrated in a mouse model where tumors overexpressing MIC-1/GDF15 are present, a decrease in food intake and loss of lean body mass occurs (Johnen et al., 2007). Additionally, in humans with heart failure, those with the highest MIC-1/GDF levels had the lowest BMI (Kempf et al., 2007). This MIC-1/GDF15 association is possibly a factor in HNC cachexia, but presently no such examination to date has been conducted. In regards to symptoms such as anorexia, there appears to be an association with cytokine levels and symptoms. However, the association with cytokines and other symptoms is not fully understood.

Recent scientific research suggests that some of the most common symptoms reported by oncology patients are associated with changes in the levels of pro and anti-inflammatory cytokines (Miaskowski & Aouizerat, 2012) (Serugua, et al., 2008). Despite this increased association, there is mixed data regarding the development of symptoms, such as pain, anorexia and lethargy that occur in cancer patients and the association between these symptoms and cachexia. This is partially because there appears to be a lag between an elevation of cytokines and subsequent symptom development (Lucia, Esposito, Fanelli, & Muscaritoli, 2012). Also, there is an indication that symptoms can be present, such as pain, lethargy, depression, prior to muscle and adipose wasting

(Laird et al., 2011b). This is a classic conundrum of trying to decipher what occurs first in patients. Do symptoms predicate the development of cachexia, or is it cachexia that predicates the development of symptoms? Alternatively, is it a combination of both? What is hypothesized is that cytokine dysregulation caused by tumor burden begins to have a cascade effect on the physiological processes of the body. This cascade is possibly regulated by tumor burden and location. In one study of breast cancer patients, it was found that increased cytokine dysregulation with IL-6 correlated to worse symptoms of pain, fatigue, sleep disturbance and depression (Doong, et al., 2014). Further complicating the dysregulation of the cytokine is the additional dysregulation that can be caused by treatment modalities such as chemotherapy, radiation and medications (Cheung, et al., 2013). Many studies do not follow patients over a long enough period to demonstrate how inflammatory cytokines correlate with future developments of symptoms and wasting associated with cachexia (Fearon et al., 2012a). Further explorations between symptoms and cachexia must occur in order to provide better treatment options for patients with cachexia and other symptom burdens.

Summary of the biology, symptoms and treatment of cancer cachexia

Overall, our knowledge in regards to cancer cachexia continues to accelerate. In regards to HNC, fewer studies have been conducted in regards to specific pathways, such as IGF-1, MIC-1/GDF15 and E3 ligases atrogin-1/MuRF-1. Furthermore, contradictory evidence has been shown in HNC patients with

more established cytokines such as IL-6. To improve our understanding of HNC cachexia, further large prospective studies will need to be conducted to improve our understanding of the many complex mechanisms involved in cachexia. The effects of cytokines and associations with symptoms such as depression, appetite suppression and anorexia development have only recently begun to be understood.

The association between cytokines and other symptom development such as mucositis and fatigue are less known. In regards to fatigue, the association between cytokines and development of lethargy has not been fully elucidated. Fatigue is possibly due to a conglomeration of processes from the disease process itself and treatment. Additionally, the role of cytokines in cancer and the associations with musculoskeletal dysfunction and cytokines is also not understood. However, muscle dysfunction is possibly related to the progression of tumor burden and the subsequent inhibition of new muscle production as well as degradation of muscle previously discussed in this chapter. Overall, the association between cytokines, symptoms and cachexia experienced by cancer patients is not understood, but it is agreed that these three principles make the experience of cancer patients difficult.

Definition of Terms

A major impediment to research in cachexia has been the lack of a consensus clinical definition and diagnostic criteria to help design and interpret clinical studies, and appraise the relevance of findings from animal tumor models

of cachexia (Tsoli & Robertson, 2013). Historically, cachexia has generally been defined as involuntary weight loss of >5% from baseline, a body mass index (BMI) <20kg/m2 with any degree of weight loss (Vaughan et al., 2012). However, this vague definition does not take into account severity of illness, and other measurable factors such as cytokine levels, inflammation, treatment toxicity and impact on quality of life (von Haehling & Anker, 2010).

In 2010, key individuals identified as experts in cancer cachexia and members from the European Palliative Care Research Collaborative, the Society on Cachexia and Wasting Disorders, the National Cancer Research Institute, Palliative Care Clinical Studies Group and the European Society for Clinical Nutrition Metabolism Special Interest Group on Cachexia participated in focus groups and two Delphi rounds to identify a working definition of cancer cachexia that could be applied to future research. The working group published the following consensus:

> *Cancer cachexia was defined as a multifactorial syndrome defined by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment. Its pathophysiology is characterized by a negative protein and energy balance driven by a variable combination of reduced food intake and abnormal metabolism. The agreed diagnostic criterion for cachexia was weight loss greater than 5%, or weight loss greater than 2% in individuals already showing depletion according to current bodyweight and height (body-mass index [BMI] <20 kg/m(2)) or skeletal muscle mass (sarcopenia). An agreement was made that the cachexia syndrome can develop progressively through various stages--precachexia to cachexia to refractory cachexia. Severity can be classified according to degree of depletion of energy stores and body protein (BMI) in combination with degree of ongoing weight loss. Assessment for classification and clinical management should include the following domains: anorexia or reduced food intake, catabolic drive, muscle mass and strength, functional and psychosocial impairment. Consensus exists on a framework for the definition and classification of cancer cachexia.*

> *After validation, this should aid clinical trial design, development of practice guidelines, and, eventually, routine clinical management.*
> (Fearon, Strasser, Anker, Bosaeus, & Bruera, 2011)

Cachexia is a multi-organ syndrome, characterized by weight loss (at least 5%), muscle and adipose tissue wasting and inflammation. Metabolic abnormalities, carbohydrate levels, lipid and protein metabolism are thought to be caused by the tumor-induced production of humoral inflammatory mediators or other mediators produced directly by the tumor (Fearon et al., 2011). In 2013, cachexia was classified into three distinct areas:

1. Pre-cachexia: when a patient has weight loss <5%, but has not yet developed serious complications.
2. Cachexia: where the syndrome is progressing, with weight loss exceeding the parameters mentioned above, but still potentially able to be treated.
3. Refractory cachexia: the point at which the disease is no longer responsive to treatment or when treatment benefits are outweighed by burden and risk.

(Vaughan et al., 2012)

However, while an acceptable definition of cancer cachexia can be agreed upon, translating this definition into the clinical diagnosis of cachexia will still be difficult despite the identification of the five domains of cachexia. The key concept from the group's consensus is identification that lean muscle mass loss is the greatest link with poor outcomes in cancer patients. However cachexia

itself appears to be affected by a host of mediators that affect both the adipose and skeletal muscle simultaneously, suggesting that multiple pathways are occurring that can lead to cachexia (Dalamaga, 2013).

Key concepts in head and neck cancer cachexia

Within the following section, there are three separate tables for identification of key concepts relating to cachexia. They are general definitions, cytokines/chemokine's, and symptoms. Cytokines are important physiological agents that handle the regulation of cellular functioning and human stress response. They are molecular messengers that assist in coordinating the interplay of the human immune system. The half-life of cytokines are relatively short and therefore have limited biological importance under normal conditions. However, during acute and chronic illnesses, the production of cytokines increases and often remains elevated. Cytokine elevation leads to inflammation and disease. The cytokines IL-1,2,6, TNF-a, INF-y have consistently been shown to correlate with cancer cachexia (Tisdale, 2009). (Antoun et al., 2010) (Bye et al., 2013).

Table 1: Cancer Cachexia General Definitions

Acute phase reactants	APR- during any inflammatory response, production of these reactants by the liver ensues. These reactants consume albumin. The body does not increase its production of albumin. Hypoalbuminanemia is due to increased trans capillary escape and increased albumin degradation by the APR (Fearon et al., 2012a)
Body Mass Index	Body Mass Index (BMI): the current standard for measuring weight and obesity and is a number calculated from a person's weight and height. BMI is a fairly reliable indicator of body fatness for most people. BMI does not measure body fat directly, but research has shown that BMI correlates to direct measures of body fat, such as underwater weighing and dual energy x-ray absorptiometry (DXA) BMI = weight (lb.) / [height (in)] ^{2} x 703 (English measurements) BMI = weight (kg) / [height (m)]^2 (metric measurements) • Less than 18.5 = underweight • 18.5 to 24.9 = normal weight • 25 to 29.9 = overweight • 30 and above = obesity o 30-34.9 (class 1 obesity) o 35-39.9 (class 2 obesity) o 40 and above = morbid obesity (Martin et al., 2013)
Cachexia Diagnosis	Cachexia can be diagnosed in the following way: a weight loss of at least 5% or more in 12-months or less in the presence of underlying illness, plus three of the following criteria: decreased muscle strength, fatigue, anorexia, low fat-free mass index, abnormal biochemistry (increased inflammatory markers (C-reactive protein >5.0 mg/l), IL-6 >4.0 pg/ml), However, other diagnostic tools should not be discarded, such as decreased physical performance (total activity, handgrip strength, stairs climb, or 6-min walk distance) or biochemical tissue analysis (activation of proteolysis or apoptosis in skeletal muscle biopsies (Couch et al., 2014)
Cachectic Syndrome	A complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass. The prominent clinical feature of cachexia is weight loss in adults (*corrected for*

	fluid retention) or growth failure in children (*excluding endocrine disorders*) (Fearon et al., 2011)
CASCO	**Ca**chexia **Sco**re: a numerical scale, classifies cachexia into mild (0–25), moderate (26–50), severe (51–75), and terminal (76–100). The higher the score, the worst the syndrome (Argiles et al., 2011).
C-reactive protein (CRP)	C-reactive protein (CRP) is a nonspecific but sensitive marker of inflammation. Interleukin-6 (IL-6), IL-1, and tumor necrosis factor alpha induce the synthesis of CRP in hepatocytes. It is positively correlated with weight loss, anorexia-cachexia syndrome, the extent of disease, and recurrence in advanced cancer. (Mahmoud & Rivera, 2002). However, its uses as a prognostic indicator in cancer are controversial (Chung & Chang, 2003). Decreased quality of life scores have been associated with increased CRP (Imayama et al., 2013) (Wallengren et al., 2013). Reducing CRP levels has helped patients increase their energy levels and improve their quality of life (Kamath, 2012). Elevated levels have been associated with increased depression levels in cancer patients (Archer, Hutchison, Dorudi, Stansfeld, & Korszun, 2012). CRP levels have also been correlated with cancer pain (Laird et al., 2011a).
Cytokine	Any of a class of immunoregulatory proteins (as interleukin, tumor necrosis factor, and interferon) that are secreted by cells especially of the immune system (Bennani-Baiti & Walsh, 2009).
Muscle Atrophy	The involuntary wasting or loss of muscle tissue (Whitehouse et al., 2001).
Obesity Paradox	The concept that obese persons have a decreased mortality rate from certain cancers compared to normal and underweight persons (Gonzalez, Pastore, Orlandi, & Heymsfield, 2014)
Pro-Inflammatory	Any substance that causes inflammation. Chronic inflammation plays a major role in carcinogenesis, and cancer cells potentially rely on production of proinflammatory mediators for growth, protection from apoptosis, and promotion of angiogenesis/metastasis (Donohoe et al., 2011)
Total Energy Expenditure	TEE is divided into resting energy expenditure (REE), diet-induced energy expenditure (DEE) and the energy cost of physical activity (AEE). A majority of cancer patients are hyper metabolic (Solheim et al., 2014).
Tumorkine	Tumor derived factors and cytokines that directly affect the bodies' metabolism, lipogenesis, lipolysis, myogenesis, myolysis, and neuroendocrine control of

	appetite and production of acute phase proteins. Cytokines derived from tumors appear to be the key agent in causing cachexia, rather than those from human innate immunity (Cahlin et al., 2000).

Table 2: Cytokines Relating to Cachexia

Cytokine	Definition
Tumor Necrosis Factor- alpha (TNF-α)	Synthesized by activated macrophages. Originally called cachectin due to its relation to weight loss in rabbits (Beutler & Cerami, 1986). Later demonstrated to correlate with weight loss in (Oliff et al., 1987). Contributes to insulin resistance by degrading the insulin-signaling pathway (Hotamisligil, 2000). Promotes atrophy through the induction of E3 ligase genes that mediate the breakdown of myofibrillar proteins by the ubiquitin-proteasome pathway (Sishi & Engelbrecht, 2011). However, systemically it is unclear as to its relevance in the development of human cachexia (Fearon et al., 2013)
Interleukin-6	Appears to works in synergy with TNF-a and other cytokines. Circulating levels of IL-6 have been shown to correlate with weight loss and increased mortality in cancer patients (Scott et al., 2002). IL-6 receptor antibody blocked cachexia progression via suppression of muscle protein degradation, while not rescuing the suppression of synthesis (White et al., 2011). IL-6 is an acute phase reactant, but will remain at elevated levels during a chronic illness. Causes a reduction in erythropoietin production by the kidneys and can contribute to the anemia of chronic illness. Also promotes erythrophagocytosis by macrophages and in conjunction with IL- **β**1 and IFN-y decreases erythroid progenitor proliferation.
Interleukin-1	Responsible for activating T and B-lymphocytes. Is an acute phase responder and is responsible for the production of other cytokines. IL-1 levels have been attributed to muscle wasting and the development of cachexia in cancer patients (Patra & Arora, 2012).
Interleukin-2	Are produced by T and B-lymphocytes. Augments NK cell activity (Tefferi et al., 2011).
Interleukin-8	IL-8 is a designated chemokine. Its role in cachexia is unclear presently; however, levels have been correlated with the nutritional status of cancer patients suggesting a role in driving muscle wasting (Gioulbasanis, Patrikidou, & Kitikidou, 2012).

Interleukin-10	Considered an anti-inflammatory cytokine the can down-regulate TNF-α. (Mydlarz et al., 2014).
Interferon-y	A type II interferon that is part of human adaptive immunity against viral, bacterial and tumor regulation. Activates macrophages and stimulates the production of IL-1 and TNF-α. The essential role of IFN-γ in the pathogenesis of cachexia was confirmed by the demonstration that monoclonal antibodies (MAbs) against IFN-γ, given prior to injection of the tumor cells in mice, prevented cachexia (Matthys et al., 1991).
MIC-1/GDF-15	MIC-1/GDF15 is a transforming growth factor beta (TGF-β) family cytokine. In experimental animals, serum MIC-1/GDF15 levels at the lower end of this range induce anorexia by direct actions of the circulating cytokine on feeding centers in the brain (Tsai, et al., 2012).
Transforming Growth Factor B	TGF-β assists in the regulation of tissue repair and regeneration following an injury. Released from platelets at the time of tissue injury, it handles regulating TNF-α, IL-2. MIC-1/GDF15 is a TGF-β family cytokine that is found in the serum of all normal individuals at an average concentration of about 0.6 ng/ml. Its increased expression in both cancers and other diseases can result in 10-100-fold or more elevation of its serum levels. In experimental animals, serum MIC-1/GDF15 levels at the lower end of this range induce anorexia by direct actions of the circulating cytokine on feeding centers in the brain (Tsai et al., 2012).
Nuclear Factor-kB	NF-κB has been called a master transcriptional switch in inflammation that regulates dozens of targets involved in inflammation and other biochemical processes. IL-1, 6 and TNF-a enhance NF-κB and mediates muscle loss and dysfunction (Wysong et al., 2011).
MMP2A/9A	Biological important in terms of tissue remodeling. Possibly contributes to tumorgenesis (Nagase & Woessner, 1999).
TWEAK	TWEAK has multiple biological activities, including stimulation of cell growth and angiogenesis, induction of inflammatory cytokines, and under some experimental conditions, stimulation of apoptosis (Wiley & Winkles, 2003).

Table 3: Symptoms affecting Quality of Life that may or may not impact Cachexia

Symptom	Definition
Anorexia	Unintentional weight loss and non-reversible despite adequate nutritional support (Fearon et al., 2011)
Body Image Disturbance	Alteration in body image perception secondary to highly visible disfigurement resulting from primary cancer and its treatment (Rhoten, Murphy, & Ridner, 2013). Can also be from resultant changes from weight loss.
Depression	Major depressive disorder, or major depression, is characterized by a combination of symptoms that interfere with a person's ability to work, sleep, study, eat, and enjoy once pleasurable activities. Major depression is disabling and prevents a person from functioning normally. Minor depression is characterized by having symptoms for 2 weeks or longer that do not meet full criteria for major depression. Without treatment, people with minor depression are at high risk of developing the major depressive disorder (Health, 2013).
Dyspnea	Shortness of breath, the inability to catch one's breath (Landis et al., 2014).
Dysphagia	Difficulty or the inability to swallow content into the esophagus (Raber-Durlacher et al., 2012).
Dysphonia	Hoarse voice, inability to phonate
Fatigue	Cancer-related fatigue (CRF - sometimes simply called "cancer fatigue") is one of the most common side effects of cancer and its treatments. It is often described as "paralyzing." Usually, it comes on suddenly, does not result from activity or exertion, and is not relieved by rest or sleep. It may not end - even when treatment is complete. The exact reason for cancer fatigue is unknown. CRF may be related to both the disease process and treatments, including surgery, chemotherapy, and radiation therapy (Moubayed et al., 2014).
Insomnia	Trouble falling asleep or staying asleep through the night(Oi-Ling et al., 2005)
Malnutrition	Occurs when the body does not get enough nutrients for proper metabolic functioning (O'Neill & Shaha, 2011).
Mucositis	Cytokine-induced inflammation of the mucosal lining of the mouth and esophagus usually caused by treatment modalities (Nicolatou-Galitis et al., 2013).
Otalgia	Pain in the ear
Pain	The International Association for the Study of Pain says it is "an unpleasant sensory and emotional experience in

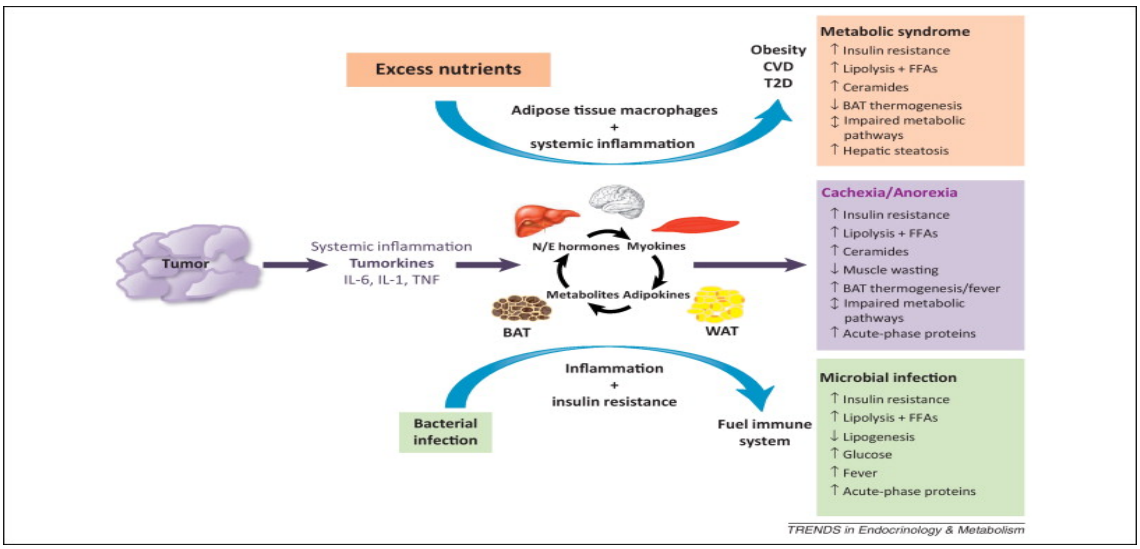
	association with actual or potential tissue damage, or described in terms of such damage." (Cancer-Pain.Org, 2014, #25133)
Rhinorrhea	Mucous discharge from the nose that has increased secondary to tumor burden and treatment (Dallapiazza et al., 2014)
Shoulder and Neck Dysfunction	Alterations in the ability to use either or both the neck and shoulder following surgical resection of HNC and or secondary to tumor or lymphadenopathy (Patterson et al., 2012).
Social Isolation	Refers to a complete or near-complete lack of contact with people and society for members of a social species (Rhoten et al., 2013)

Theoretical Framework

As presented previously, cachexia is a conglomeration of metabolic derangements that occur as a result of tumor-derived signals as well as altered metabolism occurring in the host microenvironment. There are several conceptual models that have been proposed regarding the development of cachexia. Due to the heterogeneity of cancer, the heterogeneity of humans, and our limited knowledge of the physiology that is occurring during cancer and the subsequent development of cachexia, a precise model does not exist. However, several models help to inform the current state of cachexia science. Many are overlapping in their context, but each has its variations.

Figure 1: The Tsoli and Robertson Model of Cachexia Physiology

This model highlights the involvement of tumor and the production of tumor-derived cytokines. These cytokines have an impact on the brain, muscle, liver and adipose tissue. In regards to the brain, the tumor itself in conjunction with tumor produced cytokines causes anorexia due to decreased caloric intake because the neuroendocrine system is negatively altered by the cytokines causing a reduction in caloric intake (Laviano, Seelaender, Rianda, Silverio, & Rossi Fanelli, 2012). The model indicates that decreased muscle mass secondary to muscle wasting results in increased fatigue. In regards to impairment in hepatic function, a decrease in drug clearance is experienced secondary to the tumor kind increasing the likelihood of drug toxicity (Prado, Antoun, Sawyer, & Baracos, 2011). The model also demonstrates that lipolysis is increased in both brown and white adipose tissue. While the model identifies a very good overall synopsis of cachexia, it does not fully highlight the many different pathophysiology's that are potentially occurring at the cellular level in the brain, liver, fat and muscle tissue. However, this is not necessarily a negative as the process of cachexia is very complex, and this model is very basic allowing for further in-detail analysis to occur. Overall, the model is very accurate in its current format.

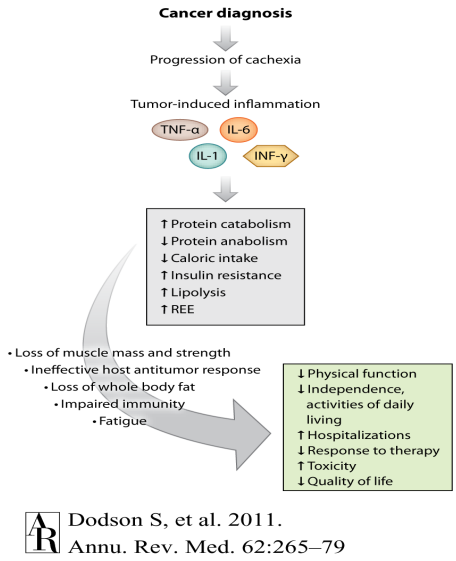


(Tsoli & Robertson, 2013)

Figure 2: The Dodson Model of Cancer Cachexia Physiology

This model is more detailed than the Tsoli and Robertson model in terms of cachectic effects on the patient and the symptoms that they experience. The premise of this model is that there is a definitive cancer diagnosis, which then leads to a progression in cancer cachexia. This progression of cancer cachexia leads to inflammatory cytokines to cause an increase in protein catabolism, insulin resistance, lipolysis and resting energy expenditure as well as a decrease in protein anabolism and caloric intake (Evans et al., 2008)These changes lead to a loss of muscle mass and strength, loss of whole body fat, increased fatigue and a decline in a patient's immune system (Dillon et al., 2012). From these

changes, the patient experiences a decrease in physical function and the ability to perform activities of daily living, a decreased response to therapy and quality of life (Barrera & Norton, 2009). The patient furthermore experiences an increase in hospitalizations and drug toxicity. The positives, in regards to this model, are the association between a progression of cachexia and changes in a patient's symptom presentation. Additionally, the model is not very detailed in the exact process by which these symptoms develop, but this is not necessarily a negative in that it allows for the recognition that we do not fully understood the exact pathways yet between cachexia and symptoms. Furthermore, the model begins with a cancer diagnosis and a secondary progression of cachexia. Research clearly indicates that a patient is possibly experiencing cachexia even before a diagnosis is made (Citrin et al., 2012). However, without a diagnosis of cancer, there would be no diagnosis of cancer cachexia. Additionally, the model states that a progression of cachexia occurs, and then inflammatory cytokines begin to increase. Most likely, both are occurring simultaneously because cytokines increase systemically at the very start of cancer (Lucia et al., 2012). The models insinuation that the progression of cachexia is occurring before the inflammatory cytokines begin to increase, or perhaps even circulate, is very misleading and not an accurate representation of the progression of cachexia. The model would have been better had arrows been included highlighting the feedback loop of cachexia progression, cytokines, and symptoms.

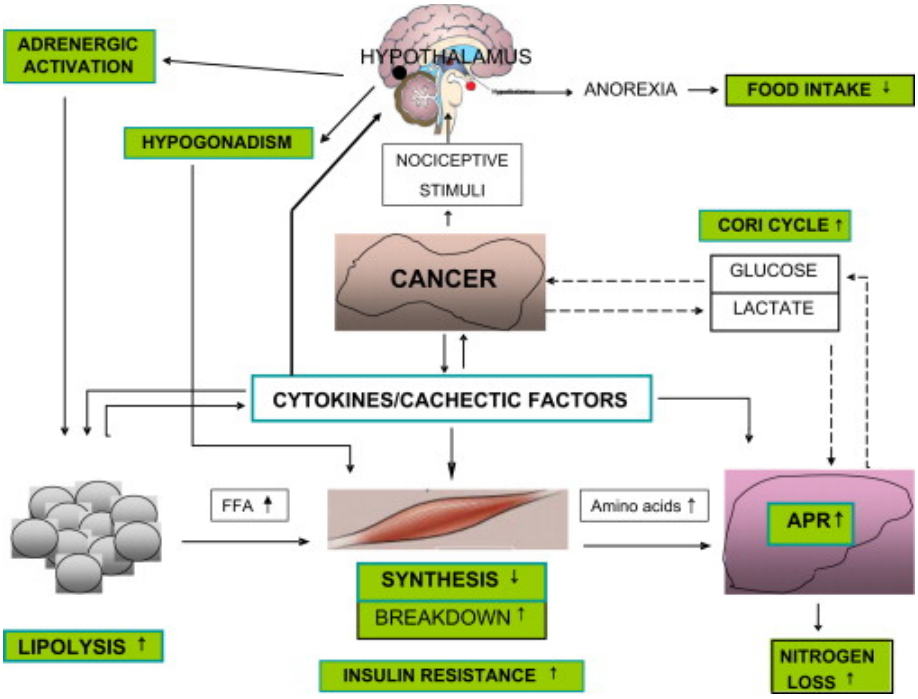


(Dodson et al., 2011)

Figure 3: The Fearon Model of Cancer Cachexia Physiology

This model highlights the involvement of tumor and the production of cytokines. However, this model is unique In that it demonstrates a feedback mechanism from the cytokines produced by the tumor itself and the cytokines produced in the microenvironment secondary to the tumor. This model also takes into consideration the effects of hypogonadism, which is a very common occurrence in persons with cachexia as well as the Cori cycle which is affected by the development of acute phase reactants (APRs) secondary to cytokines. The alteration in the CORI cycle increases lactate as well as increases the production of glucose by the Liver (Trendelenburg, Meyer, Jacobi, Feige, & Glass, 2012).This in turn helps to feed the tumor itself with sustenance for its

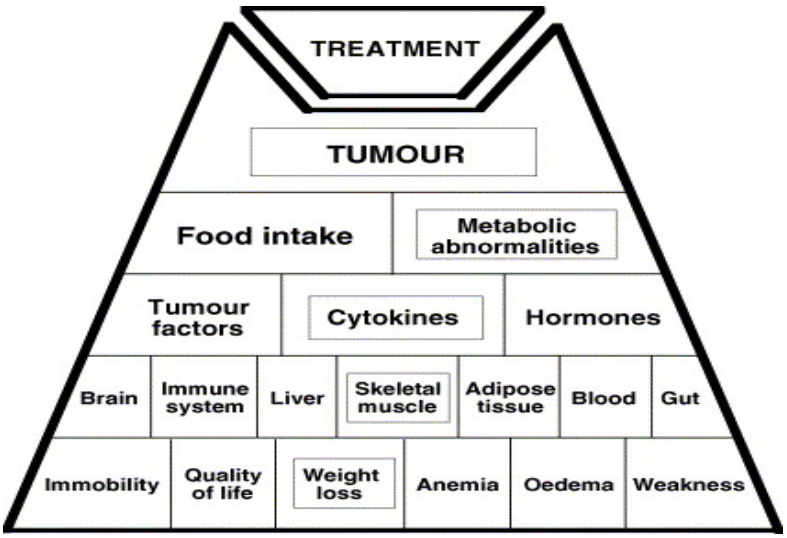
growth potential. The model also takes into consideration both the effects of insulin resistance and the increasing occurrence of nitrogen loss. Insulin resistance is an important component of increased muscle catabolism. This increased catabolism increases the amount of circulating amino acids, which increases the APRs from the liver (Trendelenburg et al., 2012). The effects of increased free fatty acids are also linked to muscle breakdown. Overall, the model is one of the better models demonstrating the physiological loop of cachexia. It demonstrates the plethora of biological processes that are altered in cachexia.



(Fearon et al., 2012)

Figure 4: The Argiles, Busquents, Felipe and Lopez-Soriano Model of Cancer Cachexia

This model of cachexia is unique in that it presents cachexia in a pyramid rather than a loop mechanism like the previous three models. The model is a progression of processes that occur from top to bottom with the tumor at the top. The tumor causes a change in food intake and metabolic abnormalities (Siddiqui et al., 2006). These changes interplay with tumor factors, cytokines and hormones (Bosaeus, Daneryd, Svanberg, & Lundholm, 2001). As these biological factors increase, the brain, immune system, Liver, skeletal muscle, adipose tissue, blood and gastrointestinal system are altered which leads to immobility, quality of life issues, weight loss, anemia, edema and weakness (Laviano et al., 2012). The pyramid attempts to capture the various sequela of cancer as it causes cachexia. What is excellent about this model is the fact that it attempts to have a foundational effect of the tumor on the many processes that promote cachexia. As cachexia progresses, a change and increase in symptom burden is finally achieved. The model captures the areas within the body that are affected by metabolic abnormalities, the increase in cytokines and the progression of cachexia. The cytokines affect multiple organs, and the penultimate development of symptoms.



(Argiles et al., 2005)

Conceptual and Theoretical Limitations

One of the limiting factors in the development of a precise cachexia model is our limited knowledge regarding the role of neuroinflammation in muscle and adipose tissue wasting. What is known is that the brain responds to tumor-generated cytokines negatively in that it causes anorexia. The precise biological pathway(s) involved in this process is still limited, but overall the role of the hypothalamic arcuate nucleus in food intake appears to be a central component that can impact cachexia (Laviano et al., 2012). None of the models incorporate the role of neuropeptide Y (NPY) and agouti-related protein (AgRP), which are both potent stimulators of food intake as well as proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) which are both appetite suppressants (Trivedi, 2014). While it is assumed that the tumor impacts

these biochemical agents, the precise role is not fully understood. Hence, most models in regards to cachexia are at this point vague in their association between neuroinflammation and the perpetuation of cachexia. Exacerbating this lack of understanding is also the unknown role of different tumors and the enhancement of appetite suppressant within the brain (Fearon et al., 2012b). The role of different cytokines and their levels in this process is also not understood. Therefore, cachexia models that are incorporating the role of neuroinflammation are at this point limited by the current knowledge.

In regards to the brain during cachexia, what is also lacking in terms of our understanding is the role in which other attributed sequela of cachexia affects the brain and appetite regulation (Perboni & Inui, 2006). Symptoms such as mucositis, nausea, lethargy, insomnia, depression, pancytopenia and leukocytosis impact the overall state of health in cancer patients (Laird et al., 2011b) (Oi-Ling et al., 2005). What are not understood is how these symptoms affect the brain and how those impacts are manifested in terms of cachexia (Grossberg, Scarlett, & Marks, 2010). Presently, the role of the brain in relationship to tumor-induced regulation of neuropeptides as well as the brain's response to various symptoms commonly associated with cancer is not fully understood (Johnen et al., 2007). This lack of understanding presently limits the ability to capture fully the role of neuroinflammation precisely in a cachexia model unfortunately leaving many models significantly shallow in their ability to capture this process.

In addition to the many components of cachexia, that may or may not be impacted by the brain, is the lack of understanding of cytokine levels and cachexia. For example, in some studies, cachexia appears to be strongly correlated with certain cytokines, but in other studies this association is not statistically significant (Bennani-Baiti & Walsh, 2009) (Dodson et al., 2011). Therefore, the different models that utilize various cytokines do so with the understanding that different levels of individual cytokines may or may not be involved in the cachectic process. This variation in the association between cytokines and cachexia is possibly rooted in the overall heterogeneity of cancer (Fearon et al., 2012b). Every type of cancer appears to cause different outcomes and processes in humans. Why this is the case is possibly attributed to the location of the tumor and the impairment that various organ dysfunction can also cause upon the body. Even within the HNC population different studies have demonstrated mixed results in terms of correlating cytokines with outcomes (Duffy et al., 2008) (Heimdal, Kross, Klementsen, Olofsson, & Aarstad, 2008).The various cachexia models do not adequately depict this variation. However, the models do serve as a starting point for developing an understanding of cachexia as well as formulating studies that can help investigate these associations.

The heterogeneity of cancer is also possibly confounded by the lack of understanding regarding the role of genetics within the development of cachexia (Fearon, 2012) The process by which myosin and adipose degradation occurs is in response to tumor mediated inflammatory markers is possibly affected by the

individual's genetic make-up (Whitehouse et al., 2001). Additionally, the relationship is further confounded by our lack of understanding regarding the role of demographics and other factors such as pre-diagnosis state of health. For example, men overall have worse cachexia as determined by muscle wasting (Marcell, 2003). This is likely due to the fact that men typically have larger muscle stores compared to women. Presently, the models do not take into consideration these other factors that possibly affect cachexia.

Summary of conceptual and theoretical limitations

There are significant factors that appear to affect the brain during cancer and the subsequent development of cachexia. However, to date the precise sequence of these mechanisms have not been fully elucidated. In regards to HNC patients, the disease process itself may affect the brain and thus alter the neurochemical balance of the brain negatively. Symptoms such as lethargy, insomnia, mucositis, xerostomia and dysphagia possibly affect the ability to eat, but the exact associational effects of these symptoms on the brain are unknown. The probable hypothesis is that these symptoms negatively influence the brain through depression and likely cause a decline in appetite. However, the role of these symptoms in the brain and secondary cachexia development and promotion remains unclear. Further studies of these symptoms and their effects on the brain and cachexia in the HNC population are needed.

Discussion of Theoretical Knowledge

Despite the discussed shortcomings of the current state of cachexia models, the models that have been presented are robust in the synthesis of the present state of cachexia research. Due to the heterogeneity of cancer, the models represent all cancers and cachexia. There are no specific cachexia models for a single type of cancer such as HNC.

Presently, all models indicate a role of the cytokine. While the precise function of a single or group of cytokines has not been elucidated presently, what is known is that the cytokines participate in the ignition switch that starts and then perpetuates muscle and adipose tissue wasting. The models are adequate for their presentation for why cachexia occurs. As cytokines increase, whether from the tumor itself, or cell mediates pathways distal to the actual tumor, a progression of events occur in various organs, such as the liver and brain that potentiates cachexia through muscle and adipose breakdown. Furthermore, the regeneration of muscle and adipose tissue is severely limited placing the person in a negative net balance, which ultimately leads to weight loss. The models present this progression of events very well. What is limited in our understanding is why in certain cases this does not occur.

Not all cancer patients experience cachexia. The models do not necessarily account for this discrepancy. Presently it is unknown why some persons with cancer do not develop cachexia despite having identical cancers. However, even persons without a profound experience of weight loss, muscle wasting appears to occur in every patient (Argiles et al., 2014). The difference

between severe muscle and mild muscle wasting has not been elucidated presently. However, the models do indicate the various molecular pathways for this process, even though a person may not experience a significant loss of muscle. Therefore, our models capture the overall process of muscle wasting. What they do not capture presently are the inhibitory factors that prevent an extraordinary loss of muscle. This is significantly in part secondary to the lack of understanding of what these inhibitory processes are and the fact that the molecular pathway for cachexia is only slowly being pieced together. Thus, the development of the many pathways secondary to cytokine production that induces cachexia are still be identified and until this process can be elucidated in may be difficult to determine the mechanisms that actually inhibit muscle wasting in general. The processes by which cachexia is occurring is not entirely identified.

A question that cannot be answered presently is, do all cancers produce cachexia through the same pathways? In regards to HNC, do the symptoms experiences of HNC patients differ compared to those of lung or breast cancer? The answer is yes. Therefore, is it possible that the specific symptoms that are associated with HNC negatively or positively affect cachexia? Is there a difference between patients who cannot swallow from HNC tumor burden verses that patient who cannot swallow due to shortness of breath from lung cancer? In a recent study examining symptom clusters and various cancers such as lung, gastrointestinal, genitourinary, breast and HNC, it was found that now significant difference in symptoms were reported between the lung and gastrointestinal

cancers (Jimenez et al., 2011). An analysis of HNC in this study was not conducted due to sample size. Nonetheless, the study indicates that various symptoms are universal to all cancers. Currently what is unknown is how these symptoms potentially affect cachexia. However, if symptoms are equally shared between cancers, the symptoms affecting cachexia in HNC patients may be similar to those of other cancers. It will be important for future research to examine these symptoms and their neurological impacts and subsequent cachexia development.

Theoretical Framework for Study

Currently, there is not one universal model that precisely describes the physiology of cancer cachexia. However, given the strengths and limitations of the available models, the best framework to guide this dissertation research is the Model of Cancer Cachexia proposed by Argiles, Busquents, Felipe and Lopez-Soriano. In this model, the tumor produces metabolic abnormalities that promote cytokine production, causing muscle wasting, and other symptoms ultimately leading to cachexia. As this dissertation research is focused on examining the association between cachexia, symptoms and cytokines, this model best fits the research questions proposed.

CHAPTER III

METHODOLOGY

Research Design

This dissertation is a secondary analysis of data from a four-year prospective longitudinal, descriptive study that was recently completed. The following assumptions guided this study: 1) many patients with HNC develop cachexia; 2) the physiological underpinnings of cachexia in this population are not well articulated; 3) inflammatory processes contribute to cachexia; 4) other symptoms besides weight loss can accompany cachexia such as depression, fatigue and pain; and 5) long-term associations between baseline cytokines and symptoms progression are not well understood. To examine these assumptions, this secondary analysis examined cytokines, red blood cells (RBCs), albumin and changes in symptoms and weight over a 12-month period post treatment. It assessed how changes in cytokines over time correlated with variables of cachexia and cancer symptoms as well as changes in musculoskeletal functioning over the 12-month period. Data values were collected on the patients at the initiation and conclusion of treatment as well as at 6, 12, 24, 30, 36, 42 and 48 weeks. This data analysis only included individuals who completed their 48-week assessment.

Description of Research Setting

Recruitment, as well as all study visits, took place in private clinic rooms at Vanderbilt-Ingram Cancer Center (VICC) in Nashville, TN. The VICC was established in 1993 and integrates the cancer-related expertise and resources of the School of Medicine, School of Nursing, School of Arts and Sciences, School of Engineering and the Peabody School of Education as well as the fully integrated Veterans Administration Medical Center. The VICC is a National Cancer Institute designated Comprehensive Cancer Center.

Sample and Sampling Plan

Inclusion and exclusion criteria

The targeted population for the parent study consisted of patients with carcinoma of the head and neck. Eligibility criteria included: a) newly diagnosed, histologically proven carcinoma involving the head and neck; b) Stage II or greater; c) age of 21 or over; d) willing and able to undergo baseline and follow-up assessment at the VICC; and e) the ability to speak English. Patients were excluded if a) they had medical record documentation of cognitive impairment that would preclude the capacity to provide informed consent; b) were unwilling to undergo routine follow-up at the VICC; or c) had recurrent cancer. No restriction was placed on the type of treatment; however, treatment parameters were meticulously documented. To accomplish the aims of this study, which was to examine the association between baseline inflammatory markers and

symptoms one year later, only persons completing the 48-week assessment were included in this analysis. Some patients had their 48-week assessment prior to and after the precise 48-week mark. Therefore, persons completing their 48-week assessment 6 weeks prior to and after 48 weeks, giving a range of 42-54 weeks, were included in this analysis.

Recruitment

For the primary study, one hundred subjects were recruited over 25 months from newly diagnosed patients with carcinoma of the head and neck undergoing treatment at the Vanderbilt-Ingram Cancer Center. Study staff met with individuals who were interested in participating in the study and reviewed the informed consent document with potential participants. Individuals who selected to participate in the study signed an informed consent document. For this secondary analysis, only those individuals who completed their week 48 assessment were included.

Retention

The study was designed to place as minimal amount of burden on study participants as possible. Study visits were scheduled to take placed shortly before or after a participant's regularly scheduled visit. The study sessions took more than 60 minutes. Participants received U.S. postal reminders of their study visits and were additionally thanked during that time. Patients were incentivized with increasing amounts of money for each assessment that they completed

ranging from $15 to $25. Of the 96 individuals who enrolled in the study, 56 persons finished the week 48 assessment.

Protection of Human Subjects

The data from the parent study was obtained through IRB approved procedures at Vanderbilt University Medical Center and the VICC Scientific Review Committee. All participants gave informed consent. The data analysis conducted for this research was void of name and other identifiable characteristics.

Data Collection Methods

The timeline for data that was collected for this secondary analysis is included in Table 4. Trained research individuals collected data.

Table 4: Data Collection Timeline

Task	BL	EoT	Wk 6	Wk 12	Wk 18	Wk 24	Wk 30	Wk 36	Wk 42	Wk 48
Med. Hx[1]	X									
Weight & Height[3]	X	X	X	X	X	X	X	X	X	X
Red Blood Cell Count[3]	X									
Albumin[3]	X									
Demographics[2,3]	X									
VHNSS[2]	X	X	X	X	X	X	X	X	X	X
Cytokines[3]	X	X	X	X	X	X	X	X	X	X
CROM[3]	X	X	X	X	X	X	X	X	X	X
NDI[2]	X	X	X	X	X	X	X	X	X	X
CESD[2]	X	X	X	X	X	X	X	X	X	X

BL= Baseline, EoT= End of Treatment, VHNSS= Vanderbilt Head and Neck Symptom Survey, CROM= Cervical Range of Motion, NDI= Neck Disability Index, CESD= Center for Epidemiological Studies Depression
1= interview, 2= patient completed, 3= physicians or study staff/completed task

Instruments

The following data collection instruments were used for each aim:

AIM 1: To examine cytokines, RBCs and albumin in HNC patients. The Vanderbilt Flow Cytometry and Human Immunology Core handled all storage, processing and analyzes of the cytokines, red blood cells and albumin used in this study. IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, IFN-γ**,** MMP2A, and MMP9A were measured using a sensitive flow cytometric bead array (CBA; Becton Dickinson) assay that allows multiple cytokine analyses of a single sample (American Cancer Society, 2006). Two-color flow cytometric analysis was performed using an LSRII flow cytometer (Becton Dickinson). Data was acquired and analyzed using Becton Dickinson CBA software. Forward-scatter versus side

scatter gating was used, to exclude any sample particles other than the 7.5-mm polystyrene beads. CRP and TGF-β1, TGF-β2, TGF-β3 were measured using electro- chemiluminescence multiplex array technology from Meso-Scale Discovery (Gaithersburg, MD) according to manufacturer's instructions. Data were acquired using Sector 2400 imager. Red blood cells were measured using a hemacytometer and reported in million cells per microliter (cells/mcl). Albumin was measured using the Beckman UniCell DxC800 Synchron method. Results were reported in grams per deciliter (g/dL).

AIM 2: To examine the role of cytokines, RBCs, albumin and severity of weight loss in HNC patients. The cytokines, red blood cells and albumin analyzed for AIM 1 were used for this aim. Weights, measured in kilograms that were collected/obtained at the time of visit, were used for this analysis. Heights were also collected on all patients at the start of the study. BMIs were calculated using the original weight and height with the following formula (weight (kg) / [height (m)]^2).

AIM 3: To examine the association between cytokines, RBCs, albumin and reported symptoms in HNC patients. In regards to cytokines, red blood cells, and albumin, using the specimens collected as described in Aim 1 completed this objective. Additionally, the following tools were utilized for this study: Vanderbilt Head and Neck Symptom Survey (VHNSS) version 2.0. The Vanderbilt Head and Neck Symptom Survey (VHNSS) 2.0 is a 50-item patient reported outcome measure that identifies and quantitates acute and late symptom burden and functional deficits experienced by HNC patients. The

VHNSS has 13 domains including nutrition, swallowing, xerostomia, mucositis, excess mucous, speech, hearing, taste change, smell, dental health, Furl sensitivity, a range of motion and pain. Items are scored on a Likert scale rating the severity of the symptom from 0 (none) to 10 (severe). The Vanderbilt Head and Neck Symptom Survey version 2.0 was designed to provide an expanded inventory of symptoms that an included an emphasis on oral health outcomes and was an expansion of version 1.0 (Cooperstein, et al., 2012) (Murphy, et al, 2010). Within version 2.0 the internal consistency of the ten clusters was good to excellent, with Cronbach's alpha coefficient above 0.90 in 6 symptom clusters and above 0.70 in four remaining clusters (ranges 0.74 to 0.95). The Cronbach's alpha for each domain are as follows: nutrition (0.83), swallowing solids (0.92), swallowing liquids (0.74), xerostomia (0.92), mucous (0.95), voice (0.89), taste/smell (0.91), dental health (0.75), mouth pain (0.89), general pain (0.94). Within this study, the internal reliability of the VHNSS version 2.0 ranged from 0.82-0.86 for Cronbach's alpha. The Cronbach's alpha for each domain in this study are as follows: nutrition (0.82), swallowing solids (0.82), swallowing liquids (0.84), xerostomia (0.83), mucous (0.84), voice (0.83), taste/smell (0.85), dental health (0.85), mouth pain (0.82), general pain (0.84).

Center for Epidemiological Studies Depression Scale (CESD; Depressive symptomatology). The CESD is a 20 item self-report measure that assesses the presence and severity of depressive symptoms occurring over the past week from the patient's perspective (Ridner & Dietrich, 2008). All items are rated on a 4-point scale. Psychometric properties of the CESD have been extensively

examined and the scale has been used widely in cancer research. In breast cancer survivors, it has reported Cronbach's alpha of .89 (Ridner & Dietrich, 2008). Within this study, the internal reliability ranged from 0.81-0.86 for Cronbach's alpha.

AIM 4. To examine the association between cytokines and changes in musculoskeletal function in HNC patients.

Cervical Range of Motion. Cervical Range of Motion Kit. The Dynatronics CROM-D (Litchfield, Minnesota) was used to measure CROM in all planes of movement. The CROM-D measures cervical flexion and extension, lateral extension, and cervical motion. (Youdas, Carey, Garrett, 1991) (Jordan, 2000) (Dynatronics, 2014). This instrument was utilized to determine if there was any association with increasing symptoms of cachexia and a decreased ability for the patient to move their neck. It has demonstrated the clinical validity and reliability (ICC= .89-.98) when compared with other measures of cervical range of motion (r=.93-98) (Audette, Dumas, Cote, De Serres, 2010). The CROM measures 6 movements: 1) head and forward neck flexion, 2) head and neck extension, 3) left lateral flexion, 4) right lateral flexion, 5) left lateral rotation and 6) right lateral rotation. Within this study, the internal reliability of the CROM ranged from 0.72 to 0.80 for Cronbach's alpha.

Neck Disability Index (NDI). This 10-item instrument taps components of daily life that may be affected by neck pain and dysfunction. Items include pain, personal care, lifting, reaching, headache, concentration, work, driving, sleeping, and recreation. A Gutman-style response format is used, with 6 possible responses

reflecting increasing degrees of disability. Items are summed to provide a total score. The NDI categorizes levels of disability through percentage. The questionnaire consists of 10 items, each scored from 0-5. The maximum score that can be obtained is fifty. The score is multiplied by 2 to and divided by 100 to compute the percentage. A raw score of 0-4 indicates no disability, 5-14 mild disability, 15-24 moderate disability, 25-34 severe disability, and 35 or greater complete disability. Internal consistency of the 10-item scale has been adequate in previous research (α= .89 - .92) (Vernon & Mior, 1991) (Hains, Waalen, & Mior, 1998). Factor analysis identified 1 dimension. Stability of the instrument also was adequate (r = .89). For this study, internal reliability for the NDI was 0.89 for Cronbach's alpha.

Other data collection instruments

Demographic variables (age, race/ethnicity, marital status, employment, insurance, and education) were collected through self-report in the primary study. Clinical variables (date of cancer diagnosis, treatment received/planned, radiation dose, radiation schedule, stage, and medications taken) were obtained from participant medical records and collected at the start of the primary study.

General Data Analysis and Sample

Ninety-six individuals originally enrolled in the parent study. Cases with data assessments within plus or minus 6 weeks of 12-months post-treatment were used in this study. The inclusion criteria resulted in a sample size of 56

persons. Randomly missing responses to items within the self-report assessment tools (e.g., CESD, VHNSS, NDI) were handled via protocols specified by the instrument developers. Within this sample, randomly missing values occurred for baseline, 6-months and 12-months post-treatment lab and self-report measures resulting in minimally differing samples size for specific analyses.

Data were analyzed using SPSS Version 22 (Chicago, IL). Descriptive statistics were used to summarize all of the variables used in this study. Frequency distributions were used to summarize and evaluate nominal and ordinal data values. Information from these summaries was also used to inform whether specific categories contained sufficient cases to conduct analysis or if categories needed to be combined into clinically appropriate larger categories. Due to extreme skewness, common to symptom data, cytokine distributions and outlying values, all of the continuous data were summarized using median, 25^{th}-75^{th} inter-quartile range (IQR, minimum, and maximum values). Distributions were rank transformed for use in parametric statistical methods.

Chi-Square Tests of Independence (nominal, ordinal) and Mann-Whitney tests were used to test for differences between the samples of 56 individuals from the parent study that were included in this study to the remaining 40 individuals from the parent study that was not included. There was no correction to the Type I error rate for any of the statistical tests conducted in this study. An alpha of 0.05 was used for each test. Many statistical tests were conducted in this study therefore results were interpreted with extreme caution. Accordingly, effect sizes were the primary focus.

Analysis of Specific Aims

AIM 1: To examine concentration of red blood cells and serum cytokines and albumin in HNC patients.

Question 1: Do baseline red blood cells and baseline albumin levels correlate with changes in cytokine levels at 6- and 12-months?

Method of analysis: The operationalization of "change" in cytokines was accomplished by subtracting baseline cytokines values from respective values at 6-months and 12-months post-treatment. A multiple linear regression of baseline red blood cell count on each of the resulting change values was conducted that included the respective cytokine's baseline value. Another set of such regressions was conducted for the baseline albumin levels.

AIM 2: To examine the role of cytokines, red blood cells, albumin and severity of weight loss in HNC patients.

Question 2a: Are baseline cytokines, baseline red blood cells and baseline albumin levels correlated with weight loss at 6- and 12-months?

Question 2b: Does a change in cytokine levels from baseline to 6-months correlate with weight loss at 12-months?

Method of analysis: For question 2a, the operationalization of "change" in weight was accomplished by subtracting baseline weight values from respective values at 6-months and 12-months post-treatment. A multiple linear regression of baseline cytokines, red blood cells and albumin levels on each of the resulting weight change values was conducted. For question 2b, the operationalization of "change" in cytokines values was accomplished by subtracting baseline

cytokines values from respective values at 6-months. A multiple linear regression of 6-month cytokine values was conducted on 12-month weight changes calculated for question 2a.

AIM 3: To examine the association between cytokines, red blood cells, and albumin and reported symptoms in HNC patients.

Question3: Are initial cytokines, RBCs and albumin associated with changes in reported symptoms such as: depression, swallowing difficulties, mucositis, generalized pain, mouth pain, nutrition consumption, dental discomfort, hearing, xerostomia, taste, jaw movement and voice at 6- and 12-months?

Instrument: Vanderbilt Head and Neck Symptom Survey (VHNSS).

Method of analysis: The operationalization of "change" in symptoms was accomplished by subtracting baseline symptoms scores from respective values at 6-months and 12-months post-treatment. A multiple linear regression of baseline cytokines, red blood cells and albumin on each of the resulting symptom change values was conducted.

Descriptive statistical summaries of cytokines and nutritional biomarkers prior to treatment and summaries of symptoms 12-months after enrollment were obtained and analyzed. To ensure that parametric statistical methods were used appropriately, the distributions and shapes of the continuous measures were evaluated to determine if any violations of parametric statistical assumptions such as normality exist. Multiple linear regressions were performed on the pretreatment cytokine values, and one-year symptom measurements obtained from the VHNSS. Each variable prior to analysis was ranked and transformed

AIM 4: To examine the association between cytokines and changes in musculoskeletal function in HNC patients.

Question 4: Are baseline cytokines associated with musculoskeletal impairment (neck and shoulder movement) as measured by shoulder and neck ranges of motion and neck disability at 12-months?

Instrument: Neck Disability Index (NDI), Cervical Range of Motion (CROM),

CHAPTER IV

RESULTS

Sample

The demographic characteristics of all participants in the parent study (N=96), the subsample included in this study (N=56) and those not included (N=40) are summarized in Table 5. There were no statistically significant differences in demographic characteristics between those included in this study and those not included. The majority of participants in this secondary analysis were male (N=40, 71.4%). Most persons in the analysis were White (N=51, 91.1%). A majority of patients were married (N=42, 75.0%), resided in the country (N=31, 55.4%) and had at least finished high school (N=52, 92.9%). The group was evenly distributed between currently employed (N=27, 48.2%) and not employed (N=29, 51.8%) as well as between those who currently consume alcohol (N=15, 48.4%) and those who do not (N=16, 51.6%). The average age of those in this study was 57.7 years. The minimum age was 29 and the maximum age was 80. Most persons indicated that they resided in homes where the income level was less than $50,000 annually (N=37, 48.2%).

Table 5: Demographic Characteristics

	Parent Study	Not in Current Study	In Current Study	p-value
	Total (%) N=96	**Total (%) N=40**	**Total (%) N=56**	
Gender				.698
Male	70 (72.9)	30 (75.0)	40 (71.4)	
Female	26 (27.1)	10 (25.0)	16 (28.6)	
Race				.314
White	86 (89.6)	35 (87.5)	51 (91.1)	
Black or African American	7 (7.3)	4 (10.0)	3 (5.4)	
Other	3 (3.1)	1(2.5)	2 (3.6)	
Marital Status				.952
Single/Widowed	21 (21.9)	9 (22.5)	12 (21.4)	
Married/Partnered	72 (75.0)	30 (75.0)	42 (75.0)	
Other	3 (3.1)	1 (3.6)	2 (3.6)	
Employment				.492
Full & Part Time	42 (43.8)	15 (37.5)	27 (48.2)	
Unemployed/Retired/Home-maker/Other	54 (56.2)	25 (62.5)	29 (51.8)	
Location of Residence				.130
City	42 (43.8)	22 (55.0)	20 (35.7)	
Country	45 (46.9)	14 (35.0)	31 (55.4)	
Other	9 (9.4)	4 (10.0)	5 (8.9)	
Smoking (now or past use)				.448
Yes	68 (70.8)	30 (75.0)	38 (67.9)	
No	28 (29.2)	10 (25.0)	18 (32.1)	
Alcohol History (now or past use)				.972
Yes	55 (57.3)	23 (57.5)	32 (57.1)	
No	41 (42.7)	17 (42.5)	24 (42.9)	
Current Alcohol intake				.309
Yes	27 (54.0)	12 (63.3)	15 (48.4)	
No	23 (46.0)	7 (36.8)	16 (51.6)	
Income per year				.496
<$50,000	51 (53.1)	24 (60.0)	27 (48.2)	
>50,000	28 (29.2)	10 (25.0)	18 (32.1)	
Did not care to respond	17 (17.7)	6 (15.0)	11 (19.6)	
Education				.258
< High School	9 (9.4)	4 (10.0)	4 (7.1)	
> High School	85 (88.5)	30 (90.0)	52 (92.9)	

The disease and treatment characteristics of all participants in the parent study (N=96), the subsample included in this study (N=56) and those not included (N=40) are summarized in Table 6. The most common type of cancer diagnosis in the current study sample was squamous cell carcinoma (N=48, 84.9%). The predominant location of the cancer was in the oropharynx (N=28, 50.0%). Additionally, most patients underwent chemotherapy (N= 54, 96.4%) There was a statistically significant difference in the rates of viral etiology within the set of patients in the current study and those not in this study (p=0.038). This rate was higher in the study sample (N=30 of 56, 53.8%) than it was in the sample not included (N=15 of 40, 37.5%). There were no other statistically significant differences in disease and treatment characteristics between the groups.

Table 6: Description of Disease and Treatment

	Parent Study	Not in Current Study	In Current Study	p-value
	Total (%) N=96	**Total (%) N=40**	**Total (%) N=56**	
Type of Cancer				.517
Squamous Cell Carcinoma	81 (84.4)	33 (82.5)	48 (84.9)	
Other	15 (15.6)	7 (17.5)	8(14.3)	
Location of Cancer				.427
Oral Cavity	20 (20.8)	9 (22.5)	11 (19.6)	
Oropharynx	41 (42.7)	13 (32.5)	28 (50.0)	
Larynx	13 (13.5)	8 (20.0)	5 (8.9)	
Other	22 (22.9)	10 (25.0)	9 (16.1)	
Known Viral Etiology				.039
None	51 (53.1)	25 (62.5)	26 (46.4)	
HPV	28 (29.2)	5 (12.5)	23 (41.1)	
Other	17 (17.7)	10 (25.0)	7 (12.5)	
Surgery				.422
Yes	41 (42.7)	19 (47.5)	22 (39.4)	
No	55 (57.3)	21 (52.5)	34 (60.7)	
Chemotherapy				.227
Yes	94 (97.9)	40 (100)	54 (96.4)	
No	2 (2.1)	0 (0)	2 (3.6)	
Type of Surgery	*(N=41)*	*(N=19)*	*(N=22)*	.764
RND	2 (4.9)	1 (5.3)	1 (4.6)	
Modified ND	25 (61.0)	10 (52.6)	15 (68.2)	
Total/Partial Laryngectomy	4 (9.8)	3 (15.8)	1 (4.6)	
Other	10 (24.4)	5 (26.3)	5 (22.7)	
Concurrent Radiation				.239
Yes	(97.9)	38 (100)	54 (96.4)	
No	2 (2.1)	0 (0)	2 (3.6)	
PEG				.078
Yes	49 (52.1)	24 (63.2)	25 (44.6)	
No	45 (47.9)	14 (36.8)	31 (55.4)	
Type of Treatment				.380
Chemo XRT	15 (16.0)	7 (18.4)	8 (14.3)	
Induction + ChemoXRT	42 (44.7)	14 (36.8)	28 (50.0)	
Surgery + ChemoXRT	25 (26.6)	11 (28.9)	14 (25.0)	
Surgery + Radiotherapy	2 (2.1)	0 (0)	2 (2.1)	
Induction + Chemo XRT+Surgery	10 (10.6)	6 (15.8)	4 (7.1)	
Distant Metastasis				.445
Yes	5 (5.2)	3 (7.5)	2 (3.6)	
No	91 (94.8)	37 (92.5)	54 (96.4)	
Tumor TNM Stage				.317
Stage 0-3	45 (46.9)	20 (50.0)	25 (44.6)	
Stage 2a,2b,2c, 3b	51 (53.1)	20 (50.0)	31 (55.4)	
Stage of Tumor				.849
Stage 1-3	26 (27.1)	11 (27.5)	15 (26.8)	
Stage 4a and 4b	70 (72.9)	29 (72.5)	41 (73.2)	
Size of the Tumor				.259
3 or less	67 (68.8)	23 (57.5)	44 (76.8)	
4	12 (12.5)	9 (22.5)	3 (5.4)	
4a/b	11 (11.4)	5 (12.5)	6 (10.7)	
X	6 (6.3)	3 (7.5)	3 (5.4)	

Results for each AIM

AIM 1: To examine cytokines, RBCS and albumin in HNC patients.

Question: *Do baseline red blood cells and baseline albumin levels correlate with changes in cytokines at 6- and 12-months?*

RBCs in this study were measured in million cells per microliter (cells/mcL). The normal range for an adult male is 4.7 to 6.1 cells/mcL and for an adult female is 4.2 to 5.4cells/mcl. However, variations within these ranges can occur for several reasons (NIH, 2015). The median for males and females in this study (4.51) were within their respective normal ranges (Table 7). The median for females (4.28) and males (4.73) are within the normal range for both sexes. Normal albumin level is approximately 4m/dL. The median and IQR albumin values for the sample in this study were slightly higher than that norm.

Table 7: Description of Red Blood Cells and Albumin at Baseline

	Red Blood Cells Entire Study Group	Red Blood Cells Male	Red Blood Cells Female	Albumin
N	55	39	16	53
Median	4.51	4.73	4.28	4.20
IQR	4.21, 4.87	4.37, 4.96	3.63, 4.46	4.05, 4.40
Min, Max	3.08, 5.56	3.62, 5.56	3.08, 5.44	3.10, 4.70

Descriptive summaries of the cytokines assessed in this study at baseline and the changes in those cytokines from baseline at 6-months and 12-months post treatment are summarized in Tables 8-11. The interleukins assessed included IL-1β, IL-6, IL-8, IL-10, and IL-12p70. As shown in Table 8, there was a high degree of variability among the interleukins at baseline and changes in

those interleukins throughout the first year. Furthermore, it was not uncommon to see many patients with zero detectable IL-1β and IL-12p70 at baseline and little change in values throughout the study period. In contrast to, IL-6, IL-8 and IL-10, a majority of patients had detectable levels of those cytokines at baseline and varying levels of change during the study period.

Table 8: Interleukins at Baseline and Changes from Baseline at 6- and 12-months

IL-1β			
	BL	6 Month Change	12 Month Change
N	56	54	56
Median	0.03	-0.02	0.00
IQR	0.00, 0.18	-0.10, 0.00	-0.14, 0.01
Min, Max	0.00, 6.51	-6.48, 1.09	-6.51, 0.14
IL-6			
	BL	6 Month Change	12 Month Change
N	56	54	56
Median	1.37	-0.03	-0.08
IQR	0.65, 3.01	-1.04, 0.43	-0.89, 0.44
Min, Max	0.10, 28.30	-27.63, 9.37	-9.65, 463.70
IL-8			
	BL	6 Month Change	12 Month Change
N	56	54	56
Median	12.35	-0.84	-2.27
IQR	8.64, 17.18	-6.12, 2.89	-7.13, 1.44
Min, Max	2.27, 6845.00	-6833.70, 671.30	-6836.89 32,140.50
IL-10			
	BL	6 Month Change	12 Month Change
N	56	54	56
Median	0.44	-0.06	-0.07
IQR	0.26, 2.06	-0.61, 0.16	-0.44, 0.54
Min, Max	0.07, 852.00	-851.87, 2.92	-851.80, 4.07
IL-12p70			
	BL	6 Month Change	12 Month Change
N	56	54	56
Median	0.07	-0.002	0.00
IQR	0, 0.18	-0.11, 0.4	-0.08, 0.05
Min, Max	0, 0.57	-0.48, 1.26	-0.57, 1.14

The matrix metalloproteinases assessed in this study included MMP2A and MMP9A in this study. There was high variability amongst MMP2A and MMP9A (see Table 9). Both cytokines trended downward over the one-year of the study.

Table 9: Matrix Metalloproteinases at Baseline and Changes from Baseline at 6- and 12-months.

MMP2A			
	BL	6 Month Change	12 Month Change
N	56	54	56
Median	1.1×10^4	8187.50	5820.00
IQR	9.2×10^5 3.4×10^5	-5477.00, 2.8×10^4	-3898.50, 1.8×10^4
Min, Max	5.5×10^4 4.5×10^5	-3.2×10^5, 1.2×10^4	-4.7×10^4, 1.8×10^4
MMP9A			
	BL	6 Month Change	12 Month Change
N	56	54	56
Median	1.5×10^5	-5.3×10^4	-4.5×10^4
IQR	1.1×10^5, 1.5×10^5	-1.6×10^5, 8030.50	-1.4×10^5, -1704.00
Min, Max	5.3×10^5, 6.0×10^5	$-4.2 \times 10 \times 10^5$, 8030.50	-1.4×10^5, -1704.00

The tumor growth factor families measured in this study included TGF-β1, TGF-β2 and TGF-β3. As shown in Table 11, a general decrease in these cytokines occurred over the one-year study period. It should be noted that lab analyses of TGF-β3 at the three times of assessment was available for less than half of the participants in the study (25 of 56).

Table 10: Transforming Growth Factors (TGF-β1, TGF-β2 and TGF-β3) at Baseline and Changes from Baseline at 6- and 12-months.

TGF-β1			
	BL	6 Month Change	12 Month Change
N	56	54	56
Median	3.3 $X10^4$	-6500.00	-5127.00
IQR	2.4 X $10^{4,}$ 4.1 X 10^4	-1.6 $x10^4$, 530.25	-1.0 X 10^4, 437.50
Min, Max	2399, 4.1 $X10^4$	-2.8 x 10^4, 1.6 X 10^4	-4.3 x 10^4, 3.1 $x10^4$
TGF-β2			
	BL	6 Month Change	12 Month Change
N	54	53	54
Median	1637.50	-280.00	-283.00
IQR	1080.00 2164.25	-692.25, 191.75	-592.25, 130.50
Min, Max	560.00, 4842.00	-2587.00, 1511.00	-2556.00, 1272.00
TGF-β3			
	BL	6 Month Change	12 Month Change
N	24	25	26
Median	85.00	6.50	-37.05
IQR	30.32, 142.75	-17.38, 31.93	-81.78, 31.58
Min, Max	9.00, 416.00	-69.30, 133.90	-347.70, 85.00

There was very little variability in IFN-γ during the study, but overall the median level trended downward over one-year (see Table 11). TNF-α also had very little variability over one year and overall had a slight decrease over the one-year study period. CRP levels had great variability over the one-year period. CRP also decreased over the one-year study period.

Table 11: Description of TNF-α, IFN-γ & CRP at Baseline and Changes from Baseline at 6- and 12-months.

TNF-α			
	BL	6 Month Change	12 Month Change
N	56	54	56
Median	2.59	-0.31	-0.25
IQR	1.82, 3.72	-0.93, 0.13	-1.07, 0.08
Min, Max	0.36, 69.8	-65.23, 0.97	-52.00, 2.30
IFN-γ			
	BL	6 Month Change	12 Month Change
N	56	54	56
Median	3.69	-0.09	-0.19
IQR	0.80, 7.79	-2.12, 1.43	-2.36, 1.00
Min, Max	0.00, 32.90	-26.70, 19.00	-23.51, 24.71
CRP			
	BL	6 Month Change	12 Month Change
N	56	54	56
Median	1.60×10^6	-7815.00	-2.2×10^5
IQR	$0.2x10^5$ $6.5x10^6$	-1.1×10^6, 5.8×10^4	$-2.3 x10^6$, 1584.50
Min, Max	817, $1.1 x10^6$	-1.8×10^6, 2.1×10^7	-1.1×10^8, $1,8 \times 10^7$

Associations of baseline red blood cells (RBCs) with changes in cytokines from baseline to 6- and 12-months are shown in Table 12. Associations of pre-treatment albumin levels with the same cytokine changes are shown in Table 13. After controlling for respective pre-treatment cytokine levels, there were statistically significant inverse associations of pretreatment RBCs levels changes from pre-treatment in MMP9-A and TNF-α (MMP9-A: *beta*= -0.22, p=0.018; TNF-α: *beta*= -0.24, p=0.045). Patients with higher RBCs at baseline had less increase or greater decrease in both MMP9-A and TNF-α through the study period. A similar pattern of association was found between pre-treatment levels of albumin and changes in IL-1β from pre-treatment to 12-months post-treatment (*beta*= -0.22, p=0.027, see Table 13).

Table 12: Association with Red Blood Cells at Baseline and 6- and 12-Month Changes in Cytokines from Baseline

Cytokines	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	-0.12	0.120	-0.14	0.169
IL-6	0.03	0.835	-0.07	0.574
IL-8	0.05	0.675	-0.03	0.755
IL-10	-0.05	0.690	-.15	0.183
IL-12p70	<0.01	0.987	-0.06	0.616
MMP2A	0.20	0.157	<0.01	0.993
MMP9A	-0.16	0.065	-0.22	0.018
TGF-β1	-0.02	0.813	0.06	0.592
TGF-β2	-0.12	0.310	-0.07	0.546
TGF-β3*	0.15	0.576	-0.41	0.055
TNF-α	-0.09	0.501	-0.24	0.046
IFN-y	0.23	0.090	0.05	0.675
CRP	0.10	0.419	0.17	0.126

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Table 13: Association with Albumin at Baseline and 6- and 12-months Changes in Cytokines from Baseline

Cytokines	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	-0.15	0.055	-0.22	0.027
IL-6	0.03	0.852	-0.11	0.439
IL-8	0.07	0.524	-0.08	0.374
IL-10	-0.10	0.424	-0.22	0.073
IL-12p70	-0.07	0.550	-0.15	0.226
MMP2A	0.19	0.190	-0.05	0.751
MMP9A	<0.01	0.993	-0.06	0.570
TGF-β1	0.11	0.260	-0.06	0.637
TGF-β2	0.15	0.177	0.04	0.757
TGF-β3*	-0.11	0.691	-0.04	0.861
TNF-α	-0.05	0.685	-0.06	0.647
IFN-y	-0.03	0.839	-0.03	0.820
CRP	0.13	0.348	0.15	0.203

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

AIM 2: To examine the role of cytokines, RBCs, albumin and severity of weight loss in HNC patients.

Question 2a: *Are baseline cytokines, RBCs and Albumin correlated with weight loss at 6- and 12-months?*

Weight (kg) and BMI measured at baseline, and changes from baseline BMI and weight at 6-months and 12-months post treatment are shown in Table 14. The median baseline BMI of 28.82 is above 25. A BMI > 25 is considered overweight. The 25% IQR at baseline is also above 25 indicating that a majority of patients were overweight at the time of enrollment. While the median BMI does decrease from baseline at both 6- and 12-months, a majority of patients remained overweight. At 12-months, the median change in BMI was slightly less than 6-months. This indicates that the group as a whole remained stable with their weight after approximately 6-months.

Table 14: Description of Weight and BMI at Baseline and Changes from Baseline at 6- and 12-months

Weight (kg)			
	BL	6 Month Change	12 Month Change
N	56	53	53
Median	86.00	-9.10	-6.84
IQR	78.12, 99.55	-14.26, -2.20	-12.22, 3.00
Min, Max	48.10, 151.40	-32.41, 10.54	-31.69, 12.62
BMI			
	BL	6 Month Change	12 Month Change
N	56	53	53
Median	28.82	-2.86	-2.20
IQR	25.81, 32.15	-4.67, -0.67	-4.23, 1.00
Min, Max	18.20, 41.72	-11.53, 3.15	-8.50, 5.62

Associations of pre-treatment cytokine levels with changes in weight and BMI from pre-treatment to 6- and 12-months post-treatment are shown in Tables 15 (weight) and 16 (BMI). Pre-treatment levels of IL-6 provided the single

statistically significant pattern of associations with changes in weight at 6-months post-treatment (*beta*= -0.23, p=0.045). Patients with higher IL-6 levels at baseline had greater decrease in weight through the study period than patients with lower IL-6 pre-treatment values. By 12-months post-treatment a similar direction yet slightly smaller coefficient and no longer statistically significant coefficient was demonstrated (*beta*= -0.23, p=0.045).

Table 15: Association of Pre-Treatment Cytokine Levels, RBC, and Albumin with Change in Weight from Pre-Treatment to 6- and 12-months Post-Treatment

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	-0.12	0.324	-0.03	0.826
IL-6	-0.23	0.045	-0.21	0.082
IL-8	0.06	0.606	0.03	0.820
IL-10	-0.04	0.760	0.03	0.811
IL-12p70	0.03	0.784	0.01	0.944
MMP2A	0.05	0.655	<0.01	0.974
MMP9A	<0.01	0.983	0.14	0.264
TGF-β1	0.13	0.275	0.20	0.096
TGF-β2	-0.12	0.321	-0.14	0.285
TGF-β3*	-0.12	0.559	-0.09	0.680
TNF-α	0.01	0.914	-0.01	0.970
IFN-y	-0.10	0.427	-0.06	0.617
CRP	-0.03	0.817	-0.02	0.863
RBC	0.16	0.230	0.17	0.267
Albumin	0.22	0.082	0.21	0.119

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Table 16: Associations of Pre-Treatment Cytokines Levels, RBC, and Albumin with Change in BMI from Pre-Treatment to 6- and 12-months Post-Treatment

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	-0.07	0.522	0.02	0.861
IL-6	-0.18	0.101	-0.16	0.199
IL-8	0.07	0.516	0.02	0.849
IL-10	-0.01	0.933	0.06	0.631
IL-12p70	-0.03	0.785	-0.03	0.819
MMP2A	0.03	0.768	<0.01	0.972
MMP9A	0.01	0.915	0.15	0.230
TGF-β1	0.10	0.369	0.19	0.110
TGF-β2	-0.09	0.438	-0.08	0.509
TGF-β3*	-0.19	0.908	-0.04	0.851
TNF-α	0.03	0.758	0.03	0.806
IFN-y	-0.11	0.337	-0.09	0.482
CRP	<0.01	0.990	-0.01	0.955
RBC	0.09	0.434	0.10	0.431
Albumin	0.21	0.078	0.19	0.128

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Question 2b: *Does a change in cytokine levels from baseline to 6-months correlate with weight loss at 6- and 12-months?*

Correlations of changes in cytokine levels from pre-treatment to 6-months and 12-months post-treatment with respective changes in weight and in BMI are summarized in Tables 17 and 18. Similar patterns of associations resulted. During the early post-treatment period the strongest and only statistically significant association was an inverse association of changes in IL-10 with weight and BMI changes over the same period (weight: *beta*= -0.36, p=0.012; BMI: *beta*=-0.31, p=0.025). In other words increasing IL-10 levels were associated with decreasing weight and/or BMI over the same time period. For the longer post-treatment period (12-months), the strongest associations and statistically

significant associations with changes in weight/BMI from pre-treatment were with changes in IFN-y and TGF-β1.Contrary to the pattern seen with IL-10 during the short-term, decreasing levels of both of these cytokines were associated with decreasing weight and/or BMI (see Tables 17 and 18).

Table 17: 6-Month Change in Cytokines, RBC, and Albumin with Change in Weight from Baseline at 6- and 12-months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	-0.31	0.157	-0.14	0.435
IL-6	0.10	0.484	0.25	0.112
IL-8	0.21	0.190	0.20	0.309
IL-10	-0.36	0.012	-0.18	0.241
IL-12p70	<0.01	0.983	-0.22	0.141
MMP2A	0.19	0.114	-0.05	0.734
MMP9A	0.15	0.441	0.09	0.653
TGF-β1	0.20	0.256	0.32	0.033
TGF-β2	0.21	0.200	-0.05	0.743
TGF-β3*	0.30	0.351	-0.35	0.361
TNF-α	0.10	0.474	0.18	0.220
IFN-y	-0.01	0.971	0.32	0.019
CRP	-0.03	0.797	0.10	0.562
RBC	0.47	0.088	0.71	0.356
Albumin	-0.51	0.117	-0.10	0.753

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Table 18: 6-Month Change in Cytokines, RBC, and Albumin with Change in BMI from Baseline at 6- and 12-months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	-0.19	0.360	-0.10	0.581
IL-6	0.11	0.409	0.26	0.087
IL-8	0.19	0.200	0.11	0.578
IL-10	-0.31	0.025	-0.21	0.158
IL-12p70	<0.01	0.985	-0.20	0.162
MMP2A	0.17	0.118	-0.07	0.549
MMP9A	0.26	0.149	0.14	0.451
TGF-β1	0.10	0.559	0.29	0.047
TGF-β2	0.09	0.596	-0.06	0.729
TGF-β3*	0.29	0.331	-0.33	0.287
TNF-α	0.10	0.435	0.19	0.193
IFN-y	0.03	0.785	0.31	0.020
CRP	-0.08	0.549	0.05	0.768
RBC	0.28	0.070	0.31	0.666
Albumin	-0.36	0.268	0.02	0.949

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

AIM 3: To examine the association between cytokines, RBCs, albumin and reported symptoms in HNC patients?

Question 3: *Are initial cytokines, RBCs and albumin associated with changes in reported symptoms such as: depression, nutrition consumption, swallowing difficulties, mucositis, generalized pain, mouth pain, dental discomfort, hearing, xerostomia, taste, jaw movement and voice at 6- and 12-months.*

Depressive Symptoms

Descriptions of the CESD measured at baseline, and changes in that measure at 6-months and 12-months is provided in Table 19. There was considerable variability in self-reported CESD scores over the duration of this study. This variability likely made it possible to identify associations with other variables such as cytokines.

Table 19: Description of Depressive Symptoms as Measured by CESD at Baseline and Changes from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	12.00	-3.50	-4.00
IQR	7.00, 21.75	-10.00, 1.00	-10.00, 1.00
Min, Max	0.0, 39.00	-34.00, 16.00	-34.00, 15.00

Associations of initial levels of cytokines with changes in levels of depression over 6- and 12-months post treatment are presented in Table 20. Lower levels (relative to higher levels) of TGF-β2 prior to treatment were statistically significantly associated with greater increases in CESD scores from prior to treatment to 12-months post treatment (*beta*= -0.41, p=0.002). In addition, a positive association with pre-treatment CRP levels was observed (*beta*= 0.28, p=0.035) indicating the patients with higher CRP levels pre-treatment tended to have greater increases in depressive symptoms in the 12-months post-treatment (relative to their symptoms prior to treatment) than did patients with lower pre-treatment CRP levels.

Table 20: Initial Biomarker Levels and Changes in Depression from Baseline at 6- and 12-months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	<0.01	0.987	-0.01	0.957
IL-6	0.22	0.079	0.04	0.750
IL-8	0.17	0.184	0.20	0.139
IL-10	0.08	0.543	-0.13	0.347
IL-12p70	-0.08	0.511	0.12	0.362
MMP2A	-0.08	0.545	-0.15	0.255
MMP9A	-0.19	0.129	-0.10	0.445
TGF-β1	-0.21	0.099	0.07	0.584
TGF-β2	-0.21	0.119	-0.41	0.002
TGF-β3*	0.12	0.443	-0.02	0.902
TNF-α	0.16	0.207	-0.07	0.599
IFN-y	0.14	0.270	0.20	0.125
CRP	0.17	0.168	0.28	0.035
RBC	-0.20	0.112	0.01	0.966
Albumin	0.01	0.941	-0.03	0.857

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Nutrition-Related Symptoms

Descriptions of the VHNSS Nutrition cluster scores at baseline and changes from baseline at 6-months and 12-months post treatment are presented in Table 21. The median baseline score was <0.3 on a 0-10 scale. Fifty percent of patients demonstrated no change from baseline to 6- or 12-months; 75% did not increase more than 0.75 over the course of the study period.

Table 21: Reported Difficulties with Consuming Nutrition at Baseline and Changes from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	0.25	0.00	0.00
IQR	0.00, 1.50	-0.50, 0.75	-1.00, 0.25
Min, Max	0.00, 7.00	-5.25, 7.75	-7.00, 3.75

Associations of initial levels of cytokines and changes in the VHNSS Nutrition cluster scores from baseline at 6- and 12-months post treatment are presented in Table 22. Higher levels of IL-6, IL-10 and MMP2a at baseline were associated with worsening nutrition consumption at 6-months (IL-6: *beta*= 0.35, p=0.006; IL-10: *beta*= 0.33, p=0.008; MMP2A: *beta*= 0.27, p=0.035). Lower levels of TGF-β1 and IFN-y at baseline were associated with worsening nutrition symptoms at 6-months: (TGF-β1: *beta*= -0.31, p= 0.014; IFN-y: *beta*= -0.25, p= 0.048). IL-6 was the only biomarker with short-term effects that remained at a statistically significant level over the longer 12-month post-treatment period (*beta*= 0.21, p=0.041).

Table 22: Initial Biomarker Levels and Changes in Nutrition-Related Symptoms from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.06	0.660	0.06	0.592
IL-6	0.35	0.006	0.21	0.041
IL-8	-0.06	0.641	0.06	0.568
IL-10	0.33	0.008	0.12	0.237
IL-12p70	-0.06	0.631	-0.03	0.764
MMP2A	0.27	0.035	0.11	0.297
MMP9A	-0.17	0.202	0.03	0.765
TGF-β1	-0.31	0.014	-0.06	0.600
TGF-β2	0.12	0.359	-0.14	0.201
TGF-β3*	-0.04	0.854	-0.36	0.096
TNF-α	0.18	0.155	0.17	0.112
IFN-y	-0.25	0.048	-0.03	0.807
CRP	-0.24	0.064	-0.03	0.789
RBC	-0.13	0.321	-0.04	0.727
Albumin	-0.01	0.930	-0.10	0.369

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Swallowing

Descriptions of patients' self-reported difficulties with swallowing solids as assessed by the VHNSS Swallowing Solids cluster score at baseline and changes from baseline at 6-months and 12-months post treatment are presented in Table 23. There was considerable variability in reported difficulties with swallowing solids over the duration of this study. This variability likely made it possible to identify associations with other variables such as cytokines.

Table 23: Reported Difficulties with Swallowing Solids from Baseline and Changes from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	0.63	0.63	0.38
IQR	0.03, 2.63	-0.38, 2.91	-0.38, 1.16
Min, Max	0.00, 8.00	-3.50, 5.63	-4.63, 6.13

Associations of initial levels of cytokines with changes in symptoms related to swallowing solids between pre-treatment and 6- and 12-months post treatment are presented in Table 24. Higher initial levels of IL-6, IL-10 and TNF-α were associated with an increase in difficulty swallowing solids from baseline to 6- and 12-months post-treatment. [IL-6: (6-months: *beta*= 0.34, p=0.009; 12-months: *beta*= 0.36, p=0.006), IL-10: (6-months: *beta*= 0.31, p=0.023; 12-months: *beta*= 0.39, p=0.003), and TNF-α: (6-months: *beta*= 0.31, p= 0.019)]. Lower levels of albumin pre-treatment were both associated with increased difficulty swallowing solids one-year later (*beta*= -0.27, p=0.048).

Table 24: Initial Biomarker Levels and Changes in Swallowing Solids from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.26	0.057	0.25	0.060
IL-6	0.34	0.009	0.36	0.006
IL-8	0.22	0.107	0.21	0.116
IL-10	0.31	0.023	0.39	0.003
IL-12p70	-0.04	0.770	-0.11	0.394
MMP2A	0.03	0.828	0.16	0.233
MMP9A	0.16	0.262	0.11	0.416
TGF-β1	-0.17	0.223	-0.11	0.445
TGF-β2	-0.12	0.399	-0.14	0.303
TGF-β3*	-0.17	0.448	-0.10	0.628
TNF-α	0.31	0.019	0.23	0.091
IFN-y	-0.07	0.625	-0.05	0.711
CRP	-0.09	0.509	-0.15	0.253
RBC	0.18	0.198	0.24	0.085
Albumin	-0.15	0.292	-0.27	0.048

* N=~25 with data for TGF-β3 pre-treat, 6- and 12-months post

Summaries of patient-reported VHNSS Swallowing Liquids cluster scores at baseline and changes in that score from baseline to 6- and 12-months post treatment are presented in Table 25. The median score was 0 at baseline and the median IQR change values also indicated was also 0 or less than 0.5 point change on a scale of 0-10 from baseline to 6- and 12-months post treatment.

Table 25: Reported Difficulties with Swallowing Liquids from Baseline and Changes from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	0.00	0.00	0.00
IQR	0.00, 0.38	0.00, 0.13	0.00, 0.00
Min, max	0.00, 9.00	-9.00, 6.00	-8.50, 10.00

Associations of initial levels of biomarkers with changes in the patient-reports of changes in difficulty with swallowing liquids from baseline to 6- and 12-months post treatment were considerably weaker than those observed for swallowing solids (see Table 26). A single inverse association was statistically significant (IL-12p70: *beta*= -0.27, p=0.025).

Table 26: Initial Biomarker Levels and Changes in Swallowing Liquids from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.24	0.058	0.15	0.221
IL-6	0.09	0.483	0.03	0.822
IL-8	0.09	0.507	-0.12	0.349
IL-10	<0.01	0.989	0.15	0.231
IL-12p70	-0.01	0.948	-0.27	0.025
MMP2A	-0.12	0.347	0.01	0.928
MMP9A	0.20	0.114	0.05	0.682
TGF-β1	-0.23	0.078	-0.16	0.201
TGF-β2	-0.11	0.413	-0.14	0.297
TGF-β3*	-0.31	0.171	-0.04	0.865
TNF-α	0.11	0.411	<0.01	0.990
IFN-y	-0.20	0.132	-0.15	0.221
CRP	-0.01	0.936	-0.13	0.282
RBC	0.13	0.330	0.15	0.249
Albumin	0.10	0.465	-0.02	0.889

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Mucositis

Descriptions of the patient-reported VHNSS Mucositis cluster scores at baseline and changes from baseline at 6- and 12-months post treatment are presented in Table 27. The median baseline score was <0.2 on a 0-10 scale and there was no change in the median score over the duration of the study. Additionally, 75% of the participants did not increase more than 0.50 over the

course of this study period. Due to the limited variability in mucositis self-reported scores, detecting any associations with cytokines and mucositis will be difficult.

Table 27: Reported Difficulties with Mucositis Symptoms at Baseline and Changes from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	0.13	0.00	0.00
IQR	0.00, 1.00	-0.25, 0.50	-0.75, 0.50
Min, Max	0.00, 10.00	-10.00, 9.75	-10.00, 7.00

Associations of initial levels of biomarkers with changes from baseline to 6- and 12-months post treatment in patient-reported mucous symptoms are summarized in Table 28. Initial levels of IL-1β indicated a statistically significant positive association with increases in such symptoms 6-months post-treatment (*beta*= 0.26, p=0.035).

Table 28: Initial Biomarker Levels and Changes in Mucous Symptoms from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.26	0.035	0.11	0.333
IL-6	0.21	0.097	0.02	0.861
IL-8	0.12	0.322	0.02	0.834
IL-10	0.08	0.540	0.08	0.501
IL-12p70	0.10	0.405	-0.01	0.968
MMP2A	0.04	0.762	0.09	0.446
MMP9A	0.10	0.409	0.05	0.698
TGF-β1	-0.14	0.277	-0.10	0.370
TGF-β2	-0.04	0.783	-0.18	0.122
TGF-β3*	-0.25	0.244	-0.29	0.153
TNF-α	0.14	0.257	-0.02	0.880
IFN-y	-0.09	0.487	0.06	0.600
CRP	-0.06	0.612	-0.07	0.535
RBC	0.21	0.093	0.20	0.081
Albumin	-0.13	0.328	0.05	0.683

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Generalized Pain

Descriptions of the patient-reported VHNSS generalized pain cluster scores at baseline and changes from baseline at 6- and 12-months post treatment are presented in Table 29. Due to the limited variability in generalized pain self-reported scores, detecting any associations with cytokines and generalized pain scores will be difficult.

Table 29: Reported Difficulties with Generalized Pain at Baseline and Change from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	0.83	0.00	0.00
IQR	0.00, 4.67	-2.08, 0.21	-3.00, 0.33
Min, Max	0.00, 9.00	-9.33, 2.33	-9.33, 6.67

Associations of initial levels of cytokines with changes in patient-reports of pain levels from baseline to 6-and 12-months post treatment are presented in Table 30. Higher levels of IL-1β and RBC pre-treatment were associated with greater increases in pain symptoms from pre-treatment to 6-months post-treatment (IL-β1: *beta*= 0.25, p=0.031, RBC: *beta*= 0.23 p=0.048).

Table 30: Initial Biomarker Levels and Changes in General Pain Symptoms from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.25	0.031	0.07	0.585
IL-6	0.20	0.094	-0.12	0.345
IL-8	0.21	0.078	0.16	0.198
IL-10	0.20	0.092	0.07	0.613
IL-12p70	-0.02	0.873	-0.11	0.355
MMP2A	0.07	0.592	0.04	0.742
MMP9A	0.11	0.342	-0.06	0.617
TGF-β1	-0.01	0.964	0.10	0.420
TGF-β2	0.02	0.854	-0.13	0.306
TGF-β3*	-0.19	0.370	-0.23	0.217
TNF-α	0.16	0.169	0.07	0.583
IFN-y	-0.07	0.555	0.16	0.198
CRP	-0.10	0.429	-0.16	0.210
RBC	0.23	0.048	0.20	0.105
Albumin	-0.01	0.930	0.13	0.293

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Mouth Pain

Descriptions of the patient-reported VHNSS mouth pain cluster scores at baseline and changes from baseline at 6- and 12-months post treatment are presented in Table 31. At baseline, the overall median was <0.3 on a scale where 10 indicates severe pain and 1 indicates mild discomfort. There was no change in the median pain score from baseline at both 6- and 12-months. Due to the limited variability in mouth pain self-reported scores, detecting any associations with cytokines and mouth pain will be difficult. Similar patterns of association for pre-treatment IL-1β and RBCs as that found for general pain were observed for changes in mouth pain symptoms from pre- to 6-months post (IL-β1: *beta*= 0.30, p=0.021; RBC: *beta*= 0.30, p=0.027, see Table 32).

Table 31: Reported Difficulties with Mouth Pain at Baseline and Change from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	0.25	0.00	0.00
IQR	0.00, 1.63	-0.21, 1.13	-0.33, 0.42
Min, Max	0.00, 10.00	-10.00, 8.83	-8.33, 6.00

Table 32: Initial Biomarker Levels and Changes in Mouth Pain Symptoms from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.30	0.021	0.14	0.260
IL-6	0.22	0.093	0.15	0.233
IL-8	0.15	0.260	0.13	0.296
IL-10	0.17	0.201	0.14	0.261
IL-12p70	0.07	0.631	0.01	0.961
MMP2A	0.09	0.496	0.09	0.453
MMP9A	0.17	0.193	-0.01	0.925
TGF-β1	-0.04	0.789	-0.06	0.662
TGF-β2	0.11	0.470	-0.05	0.686
TGF-β3*	-0.18	0.356	-0.21	0.203
TNF-α	0.08	0.541	0.02	0.865
IFN-y	-0.15	0.254	0.02	0.899
CRP	-0.15	0.270	-0.13	0.307
RBC	0.30	0.027	0.25	0.051
Albumin	-0.05	0.697	-0.18	0.164

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Dental Discomfort

Descriptions of the VHNSS Dental cluster scores at baseline and changes from baseline at 6- and 12-Months post treatment are presented in Table 33. The baseline median score was 0 on a 0-10 scale and there was little to no change from this level over the course of the study. No statistically significant

associations of pre-treatment biomarker levels with changes in dental symptoms were observed (see Table 34).

Table 33: Reported Difficulties with Dental Discomfort at Baseline and Change from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	44	43	44
Median	0.00	0.00	0.00
IQR	0.00, 0.50	0.00, 0.75	0.00, 1.25
Min, Max	0.00, 5.00	0.00, 6.00	0.00, 5.00

Table 34: Initial Biomarker Levels and Changes in Dental Discomfort Symptoms from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.13	0.426	-0.01	0.935
IL-6	0.17	0.280	0.13	0.423
IL-8	0.07	0.648	0.08	0.620
IL-10	0.12	0.437	0.04	0.795
IL-12p70	-0.22	0.167	-0.15	0.350
MMP2A	0.09	0.581	-0.12	0.499
MMP9A	0.21	0.189	-0.21	0.192
TGF-β1	0.10	0.551	0.12	0.472
TGF-β2	-0.28	0.082	-0.26	0.126
TGF-β3*	-0.12	0.652	0.29	0.256
TNF-α	0.01	0.952	-0.08	0.629
IFN-y	-0.14	0.388	0.17	0.274
CRP	-0.09	0.592	0.10	0.534
RBC	0.11	0.508	-0.06	0.718
Albumin	-0.22	0.196	0.05	0.749

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Hearing

Descriptions of the VHNSS Hearing cluster scores at baseline and changes from baseline at 6-months and 12-months post treatment are presented in Table 35. The baseline median score was 0 on a 0-10 scale and again there was little to no change from this level over the course of the study. A single

statistically significant inverse association of pre-treatment TGF-β2 levels with the amount of change in hearing symptoms from pre-treatment to 6-months post-treatment was observed (*beta*= -0.23, p=0.028, see Table 36).

Table 35: Reported Difficulties with Hearing at Baseline, and Changes from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	0.00	0.00	0.00
IQR	0.00, 3.00	-2.00, 0.00	-1.25, 0.00
Min, Max	0.00, 10.00	-6.00, 6.00	-8.00, 8.00

Table 36: Initial Biomarker Levels and Changes in Hearing from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	-0.02	0.876	0.05	0.670
IL-6	0.06	0.616	0.24	0.071
IL-8	0.14	0.208	0.11	0.368
IL-10	0.10	0.362	0.21	0.084
IL-12p70	-0.15	0.165	-0.15	0.235
MMP2A	-0.09	0.425	0.01	0.969
MMP9A	-0.10	0.366	0.06	0.656
TGF-β1	-0.10	0.325	0.11	0.358
TGF-β2	-0.23	0.028	0.02	0.877
TGF-β3*	0.09	0.566	0.04	0.822
TNF-α	0.06	0.577	0.18	0.144
IFN-y	0.13	0.231	0.04	0.741
CRP	0.05	0.664	<0.01	0.985
RBC	-0.08	0.479	-0.14	0.263
Albumin	-0.17	0.171	-0.04	0.769

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Xerostomia

Descriptions of the VHNSS Dry Mouth (Xerostomia) cluster scores at baseline and change from baseline at 6 and 12-month are presented in Table 37.

There was considerable variation in xerostomia scores over the duration of the study. This variation is important for the detection of associations with other variables.

Table 37: Reported Difficulties with Xerostomia at Baseline and Change from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	0.40	1.10	0.80
IQR	0.00, 1.75	0.15, 3.20	0.00, 2.20
Min, Max	0.00, 10.00	-2.60, 7.80	-6.00, 8.00

Associations of initial levels of cytokines and changes in xerostomia symptoms as assessed by the VHNSS Dry Mouth cluster scores from baseline to 6- and 12-months post treatment are presented in Table 38. Statistically significant associations of higher initial IL-6, IL-10, and RBCs with worsening xerostomia were found at 6-months (IL-6: *beta*= 0.28, p=0.042; IL-10: *beta*=0.32, p=0.021; RBCs: *beta*= 0.28, p= 0.042). Of those, only baseline IL-10 levels remained statistically significantly associated with the amount of increase in VHNSS Dry Mouth (xerostomia) scores at 12-months relative to baseline (*beta*=0.31, p=0.024).

Table 38: Initial Biomarker Levels and Changes in Xerostomia Symptoms from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.10	0.458	0.05	0.716
IL-6	0.28	0.042	0.14	0.298
IL-8	0.08	0.550	0.04	0.802
IL-10	0.32	0.021	0.31	0.024
IL-12p70	-0.15	0.280	-0.23	0.094
MMP2A	-0.10	0.499	0.04	0.773
MMP9A	0.04	0.756	-0.04	0.778
TGF-β1	-0.08	0.551	-0.07	0.622
TGF-β2	-0.18	0.194	-0.20	0.152
TGF-β3*	0.04	0.865	0.05	0.842
TNF-α	<0.01	0.998	-0.07	0.613
IFN-y	-0.09	0.510	-0.03	0.815
CRP	0.09	0.521	0.01	0.946
RBC	0.28	0.042	0.08	0.576
Albumin	<0.01	0.989	0.02	0.868

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Taste-Smell

Descriptions of the VHNSS Taste/Smell cluster scores at baseline and changes from baseline to 6-months and 12-months post treatment are presented in Table 39. The median baseline score was 0 on a 0-10 scale and did not change more than a point over the course of the study. There was moderate variability in self-reported taste/smell scores throughout the duration of the study. As shown in Table 40, higher baseline levels of IL-1β and IL-6 were associated with worsening taste/smell symptoms at 6- and 12-months post-treatment (all *betas* > 0.30, $p < 0.05$).

Table 39: Reported Changes in Taste/Smell from Baseline and Changes from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	53	54
Median	0.00	0.67	0.00
IQR	0.00, 1.33	0.00, 2.33	-0.42, 1.00
Min, Max	0.00, 8.00	-5.50, 7.00	-5.17, 6.50

Table 40: Initial Biomarker Levels and Changes in Taste/Smell Symptoms from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.37	0.005	0.32	0.003
IL-6	0.31	0.022	0.36	0.001
IL-8	0.02	0.897	0.16	0.148
IL-10	0.26	0.055	0.16	0.154
IL-12p70	0.02	0.867	0.05	0.680
MMP2A	0.14	0.312	0.04	0.719
MMP9A	0.04	0.749	0.16	0.148
TGF-β1	-0.24	0.079	-0.06	0.563
TGF-β2	0.19	0.169	-0.02	0.870
TGF-β3*	-0.02	0.946	0.30	0.149
TNF-α	0.20	0.150	0.19	0.088
IFN-y	-0.27	0.050	-0.14	0.217
CRP	-0.17	0.209	0.01	0.904
RBC	-0.03	0.811	-0.07	0.512
Albumin	0.17	0.221	-0.05	0.685

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Jaw Movement / Trismus

Descriptions of the VHNSS Jaw Movement cluster scores at baseline, and changes from baseline to 6-months and 12-months post treatment are presented in Table 41. There was limited variability in reported difficulties with opening and moving the jaw over the duration of this study. This lack of variability makes it difficult to identify associations with other variables such as cytokines. A single

statistically significant inverse association of pre-treatment IL-12p70 levels with the amount of change in jaw/trismus symptoms from pre-treatment to 6-months post-treatment was observed (*beta*= -0.23, p=0.049, see Table 42).

Table 41: Reported Difficulties with Opening and Movement of the Jaw at Baseline and Change from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	0.00	0.00	0.00
IQR	0.00, 2.00	0.00, 1.00	-1.00, 0.00
Min, Max	0.00, 10.00	-8.00, 10.00	-10.00, 7.00

Table 42: Initial Biomarker Levels and Changes in Jaw/Trismus Function from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.05	0.647	0.02	0.875
IL-6	0.10	0.375	0.01	0.964
IL-8	0.12	0.315	0.14	0.174
IL-10	0.15	0.206	0.12	0.216
IL-12p70	-0.23	0.049	-0.12	0.232
MMP2A	0.04	0.766	<0.01	0.994
MMP9A	0.05	0.696	0.02	0.884
TGF-β1	0.07	0.526	0.13	0.197
TGF-β2	-0.11	0.346	-0.17	0.085
TGF-β3*	0.19	0.277	0.15	0.362
TNF-α	0.20	0.080	0.16	0.108
IFN-y	0.06	0.618	0.16	0.112
CRP	-0.02	0.852	-0.01	0.935
RBC	0.05	0.701	-0.06	0.578
Albumin	0.07	0.566	-0.01	0.962

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Voice

Descriptions of the VHNSS Voice cluster scores at baseline and changes in those scores from baseline at 6-months and 12-months post treatment are presented in Table 43. The median baseline score was <0.7 on a 0-10 scale

There was limited variability in reported difficulties with voice over the duration of this study. This lack of variability makes it difficult to identify associations with other variables such as cytokines. No statistically significant associations of pre-treatment biomarker levels with changes in dental symptoms were observed (see Table 44).

Table 43: Reported Difficulties with Voice from the VHNSS at Baseline and Changes from Baseline at 6- and 12-Months

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	0.67	0.00	0.00
IQR	0.00, 2.25	-1.00, 1.33	-1.00, 0.33
Min, Max	0.00, 10.00	-8.67, 7.00	-9.00, 7.00

Table 44: Initial Biomarker Levels and Changes in Voice Function from Baseline at 6- and 12-Months

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.21	0.086	0.04	0.771
IL-6	0.10	0.423	0.09	0.452
IL-8	0.12	0.331	0.15	0.205
IL-10	0.05	0.700	0.07	0.544
IL-12p70	-0.18	0.134	-0.19	0.112
MMP2A	-0.09	0.493	0.08	0.488
MMP9A	0.13	0.301	-0.02	0.894
TGF-β1	-0.09	0.507	-0.09	0.473
TGF-β2	-0.16	0.219	-0.21	0.097
TGF-β3*	0.02	0.920	0.04	0.833
TNF-α	0.14	0.262	0.14	0.248
IFN-y	-0.01	0.965	-0.01	0.932
CRP	-0.01	0.946	-0.08	0.533
RBC	0.24	0.059	0.10	0.427
Albumin	-0.08	0.521	-0.10	0.402

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

AIM 4. To examine the association between cytokines and changes in musculoskeletal function in HNC patients.

Question 4: *Are baseline cytokines associated with musculoskeletal impairment (neck and shoulder movement) as measured by shoulder and neck ranges of motion and neck disability at 12-months?*

Descriptions of the Neck Disability Index values (NDI) values at baseline and changes from baseline at 6- and 12-months post treatment are presented in Table 45. The median score at baseline was 10 on a scale of 0-50. There was significant variability of the data over the 12-month time period of the study. While the median score did not change throughout the study period, there was variation within the IQR.

Table 45: Neck Disability Index as Reported at Baseline and Changes from Pre-Treatment to 6- and 12-Months Post-Treatment

	BL	6 Month Change	12 Month Change
N	56	54	54
Median	10.00	0.00	0.00
IQR	2.50, 22.00	-8.50, 6.50	-10.00, 4.00
Min, Max	0.00, 59.00	-43.11, 42.00	-38.00, 44.00

Associations of pre-treatment biomarker levels with changes in scores on the neck disability index are presented in Table 46. As shown, higher pre-treatment levels of IL-β1, IL-6, IL-8, MMP9A, and TNF-α were statistically significantly associated with increasing disability at 6-months relative to pre-treatment. Those patterns of association remained statistically significant with more long-term increasing disability for IL-8 and MMP9A. Lower pre-treatment TGF-β2 levels were statistically associated with greater increases in disability at 12-months relative to levels of disability pre-treatment (*beta*= -0.26, p=0.035, see Table 46).

Table 46: Initial Biomarker Levels with Changes in NDI from Pre-Treatment to 6- and 12-Months Post-Treatment

Cytokines	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.22	0.048	0.16	0.170
IL-6	0.26	0.019	0.18	0.118
IL-8	0.27	0.015	0.24	0.040
IL-10	0.12	0.286	0.07	0.564
IL-12p70	0.03	0.789	0.07	0.587
MMP2A	0.12	0.296	0.13	0.270
MMP9A	0.23	0.044	0.25	0.030
TGF-β1	0.10	0.399	0.13	0.279
TGF-β2	-0.20	0.093	-0.26	0.035
TGF-β3*	-0.19	0.304	-0.29	0.143
TNF-α	0.24	0.036	0.17	0.144
IFN-y	0.07	0.516	0.03	0.823
CRP	<0.01	0.983	-0.07	0.573
RBC	0.12	0.314	0.12	0.358
Albumin	-0.22	0.053	-0.20	0.089

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Descriptive statistics for the Cervical Range of Motion (CROM) scores at baseline and changes in those scores from baseline to 6- and 12-months for all areas of measurement (flexion, extension, left lateral, right lateral, left lateral rotation and right lateral rotation) are presented in Table 47. There was not a high degree of variability among neck movement throughout the first year with the exception of neck flexion. It was not uncommon to see many patients with zero detectable neck measurements for right lateral neck movement throughout the study.

Table 47: Neck-related Function Measured by Cervical Range of Motion at Baseline and Changes from Baseline at 6- and 12-Months (CROM)

Flexion			
	BL	6 Month Change	12 Month Change
N	56	54	54
Median	47.0	-1.0	1.5
IQR	37.1, 52.8	-9.0, 8.1	-4.3, 9.0
Min, Max	19.0, 74.0	-23.1, 32.0	-33.0, 32.0
Extension			
	BL	6 Month Change	12 Month Change
N	56	54	54
Median	50.0	-3.0	-3.8
IQR	44.0, 62.8	-9.3, 1.5	-10.1, 5.3
Min, Max	19.0, 80.0	-33.0, 18.0	-29.0, 34.0
Left Lateral			
	BL	6 Month Change	12 Month Change
N	56	54	54
Median	33.5	-1.0	0.0
IQR	30.0, 42.8	-6.0, 7.3	-8.6, 5.0
Min, Max	13.0, 54.0	-23.0, 17.0	-28.0, 23.0
Right Lateral			
	BL	6 Month Change	12 Month Change
N	56	54	54
Median	33.0	-1.5	-2.0
IQR	27.4, 41.8	-6.3, 2.6	-7.0, 4.0
Min, Max	9.0, 60.0	-28.0, 23.0	-30.0, 30.0
Left Lateral Rotation			
	BL	6 Month Change	12 Month Change
N	56	54	54
Median	53.5	1.5	1.8
IQR	46.3, 61.0	-5.5, 10.0	-4.9, 9.6
Min, Max	29.0, 71.0	-45.0, 26.0	-40.0, 35.0
Right Lateral Rotation			
	BL	6 Month Change	12 Month Change
N	56	54	54
Median	57.0	0.0	0.0
IQR	47.3, 63.0	-7.3, 8.0	-8.0, 6.0
Min, Max	12.0, 75.0	-29.0, 58.0	-20.0, 60.0

Associations of pre-treatment biomarker levels with changes in CROM scores from baseline to 6- and 12-months post treatment are summarized in

Tables 48 to 53. No clear patterns of associations were observed. The only biomarker with more than one statistically significant association among the 6 CROM measures was MMP2A. Higher pre-treatment levels of the cytokine was associated with greater decreases (relative to pre-treatment) in extension (*beta*= -0.31, p=0.020) and left lateral movement (*beta*= -0.28, p=0.032) at 6-months post-treatment (see Tables 48 to 53).

Table 48: Initial Biomarker Levels and Changes in Forward Flexion from Pre-Treatment to 6- and 12-Months Post-Treatment

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	-0.13	0.240	-0.03	0.793
IL-6	-0.10	0.344	-0.07	0.525
IL-8	-0.11	0.315	0.09	0.460
IL-10	-0.08	0.452	-0.14	0.204
IL-12p70	-0.10	0.364	-0.10	0.396
MMP2A	-0.07	0.546	-0.17	0.140
MMP9A	-0.07	0.533	0.11	0.347
TGF-β1	0.21	0.056	0.09	0.408
TGF-β2	0.12	0.290	-0.18	0.125
TGF-β3	0.14	0.405	0.21	0.297
TNF-α	-0.18	0.108	-0.16	0.148
IFN-y	0.12	0.264	0.14	0.229
CRP	0.03	0.762	0.10	0.384
RBC	0.01	0.903	0.09	0.457
Albumin	-0.15	0.186	<0.01	0.993

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Table 49 : Initial Biomarker Levels and Changes in Extension from Pre-Treatment to 6- and 12-Months Post-Treatment

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.02	0.892	0.07	0.571
IL-6	-0.13	0.354	-0.12	0.364
IL-8	-0.08	0.531	0.01	0.919
IL-10	-0.20	0.133	0.01	0.954
IL-12p70	0.15	0.263	0.01	0.940
MMP2A	-0.31	0.020	-0.06	0.646
MMP9A	0.04	0.766	0.06	0.646
TGF-β1	0.04	0.773	<0.01	0.974
TGF-β2	-0.20	0.141	-0.05	0.717
TGF-β3	0.13	0.495	0.07	0.736
TNF-α	-0.07	0.620	-0.05	0.731
IFN-y	0.14	0.304	-0.04	0.750
CRP	0.20	0.122	0.04	0.770
RBC	-0.14	0.300	-0.05	0.726
Albumin	0.18	0.186	0.11	0.433

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Table 50: Initial Biomarker Levels and Changes in Left Lateral Flexion from Pre-Treatment to 6- and 12-Months Post-Treatment

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	0.09	0.518	-0.09	0.483
IL-6	0.03	0.823	-0.05	0.715
IL-8	-0.04	0.774	-0.05	0.696
IL-10	0.03	0.822	-0.11	0.425
IL-12p70	0.26	0.058	0.20	0.127
MMP2A	-0.07	0.586	-0.13	0.325
MMP9A	<0.01	0.974	-0.12	0.366
TGF-β1	0.10	0.490	0.06	0.645
TGF-β2	0.06	0.689	-0.09	0.527
TGF-β3	-0.05	0.800	-0.05	0.812
TNF-α	-0.08	0.562	-0.23	0.083
IFN-y	-0.05	0.706	-0.04	0.780
CRP	-0.02	0.870	0.15	0.268
RBC	0.14	0.298	0.14	0.286
Albumin	0.02	0.915	0.14	0.307

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Table 51: Initial Biomarker Levels and Changes in Right Lateral Flexion from Pre-Treatment to 6- and 12-Months Post-Treatment

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	-0.02	0.882	-0.05	0.713
IL-6	-0.22	0.096	0.04	0.737
IL-8	-0.01	0.948	0.13	0.282
IL-10	-0.11	0.409	-0.08	0.529
IL-12p70	0.03	0.844	0.14	0.282
MMP2A	-0.06	0.646	-0.11	0.371
MMP9A	-0.21	0.114	-0.06	0.622
TGF-β1	-0.08	0.563	0.04	0.740
TGF-β2	-0.26	0.059	-0.09	0.508
TGF-β3	0.02	0.917	0.02	0.912
TNF-α	-0.17	0.190	-0.13	0.290
IFN-y	0.14	0.310	0.16	0.200
CRP	0.04	0.770	0.25	0.040
RBC	0.03	0.819	0.04	0.744
Albumin	0.15	0.279	-0.13	0.326

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Table 52: Initial Biomarker Levels and Changes in Left Lateral Rotation from Pre-Treatment to 6- and 12-Months Post-Treatment

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	-0.05	0.723	-0.21	0.100
IL-6	-0.02	0.903	-0.11	0.409
IL-8	-0.05	0.715	0.08	0.507
IL-10	-0.06	0.665	-0.06	0.636
IL-12p70	0.15	0.227	-0.07	0.592
MMP2A	-0.28	0.032	-0.15	0.263
MMP9A	0.08	0.530	-0.08	0.515
TGF-β1	0.06	0.670	-0.01	0.934
TGF-β2	-0.09	0.483	-0.20	0.115
TGF-β3	0.34	0.104	0.11	0.602
TNF-α	-0.23	0.079	-0.20	0.120
IFN-y	-0.16	0.218	-0.07	0.584
CRP	0.14	0.284	0.15	0.228
RBC	<0.01	0.999	-0.09	0.480
Albumin	0.25	0.057	0.12	0.342

* N=~25 with data for TGF-β3 pre-treatment, 6- and 12-months post-treatment

Table 53: Initial Biomarker Levels and Changes in Right Lateral Rotation from Pre-Treatment to 6- and 12-Months Post-Treatment

Biomarker	6 Month Change		12 Month Change	
	Beta	p-value	Beta	p-value
IL-β1	-0.10	0.449	-0.21	0.099
IL-6	-0.04	0.780	-0.14	0.319
IL-8	-0.19	0.157	-0.13	0.297
IL-10	-0.05	0.707	0.06	0.659
IL-12p70	0.05	0.722	0.17	0.190
MMP2A	-0.18	0.197	0.11	0.435
MMP9A	0.06	0.658	-0.04	0.780
TGF-β1	-0.06	0.679	-0.21	0.106
TGF-β2	-0.10	0.452	-0.28	0.029
TGF-β3	0.07	0.752	0.12	0.575
TNF-α	-0.24	0.084	-0.14	0.316
IFN-y	-0.07	0.582	-0.12	0.346
CRP	0.13	0.330	0.03	0.838
RBC	-0.25	0.065	-0.23	0.074
Albumin	0.02	0.902	0.01	0.915

* N=~25 with data for TGF-β3 pre-treat, 6- and 12-months post

CHAPTER V

DISCUSSION

This chapter presents a summary and discussion of the study finding in the following five sections: (a) sample characteristics, (b) aims and questions, (c) study strengths and limitations, (d) implications, and (e) recommendations for future research.

Sample Characteristics

Due to the complexity of the original study requiring several blood draws and patient self-reporting on tools such as the VHNSS, CESD and NDI, a completion total of 56 patients is similar in size compared with other HNC studies where frequent blood draws and multiple cytokine analysis were conducted (Jager-Wittenaar et al., 2011) (Liu et al., 2010) (Heimdal et al., 2008) (Druzgal et al., 2005). Larger studies have occurred, but have primarily focused on fewer cytokines and fewer symptoms (Duffy et al., 2008) (Pai et al., 2012) (Shen et al., 2012). The average age of persons completing one-year of this study was 57.8 (N=56). The average age for a HNC patient is between 50-70 years (American Cancer Society, 2012). The mean age within this study is similar to the mean ages in other studies such as 59 in Duffy et al., (2008) and 60 in Jager-Wittenaar et al., (2011) study. Globally, there is a trend with younger persons more commonly being afflicted with HNC (Wang et al., 2012), primarily due to an increase in HPV and other viral mediated factors. Statistically, there was a

difference between the 56 individuals analyzed within this study compared to the parent study. This difference was in the context of more persons with a viral etiology causing their HNC completing one-year of the study and thus viral HNC was more represented in the secondary analysis group. This is perhaps why the mean age was slightly lower than the parent study (although not statistically significant). Having more patients with viral mediated HNC does correlate with current trends in the HNC literature. Studies have indicated a survival advantage for those individuals with a viral mediated HNC compared to smoking and alcohol linked HNC (Ihloff et al., 2010).

The majority of participants enrolled in the study were male 72.9% as well as those who completed one year of the study (71.4%). The predominance of males in this study is similar to other studies such as (Liu et al., 2010) (70.7%) and (Ehrsson et al., 2010) (70.3%). This is consistent with the national 3:1, male to female, ratio of HNC diagnosis (American Cancer Society, 2012). Most participants who completed one-year of the study were either married or partnered at the time of their enrollment (75.0%). Additionally, a majority of those in the study identified as White (N=51, 91.1%). According to the US Census bureau, the average race in the United States is White (77.7%)(2014). The average person diagnosed with HNC is White. Additionally, there is also a higher rate of HPV associated HNC in White males compared to White females and Black males and females (American Cancer Society, 2015). However, Blacks have a higher rate of mortality from the disease (Gourin & Podolsky, 2006). This study adequately reflected the racial differences seen in HNC diagnosis.

Within Tennessee, the median income in 2013 was $42,764 (City-data.com, 2015). Within this study, there was a higher propensity for individuals to have a higher income compared to the parent study, but this difference was not statistically significant. In total 28 (29.2%) of enrollees at the start of the parent study made less than $30,000 annually. Of this original group only 13 people completed their 48-week assessment. Similarly, 28 people (29.2%) of enrollees at the start of the parent study made greater than $50,000 annually. Of this group, 18 persons finished one-year of the study. It is interesting to note that the median income in the United States for the 55-64 year old group in 2013 was $57,538, which is slightly higher than the national median of $51,758 (US Census Bureau, 2014).

Overall, there was no statistical difference between the parent and secondary analysis groups in terms of income. Within this study a majority had a history of smoking (N=38, 67.9%). Slightly fewer persons had a history of alcohol intake (N=32, 57.1%). The majority of persons also indicated that they lived in the country as opposed to the city (N=31, 55.4%). The demographics of the study participants within this secondary analysis resemble other HNC studies (Liu et al., 2010).

Aims and Research Questions

Aim 1. To examine cytokines, RBCs and albumin in HNC patients.

Question: Do baseline RBCs and albumin correlate with changes in cytokines at 6- and 12-months?

Within this study, higher baseline levels of RBCs associated with decreased MMP9A over a 6- and 12-month period and TNF-α over a 12-month period. Higher MMP-9 levels are linked with increased inflammation, diabetic micro-vascular complications, extracellular matrix degradation and synthesis, and cardiac dysfunction (Halade, Jin & Lindsey, 2013). The association between higher levels of RBCs at baseline and long-term levels of MMP9A could theoretically be associated with the possibility that persons with higher RBCs were less sick at the time of their enrollment in the study and perhaps had less long-term inflammation. However, if this were the precise reason, one would expect to see this association at 6-months. Further studies examining MMP9A levels in head and neck cancer patients will need to be conducted to examine this association.

In regards to TNF-α, there was no association between TNF-α and RBCs at 6-months with a p-value of 0.501. The statistically significant association at 12-months is likely spurious. TNF-α was one of the first cytokines that was associated with cancer cachexia (Oliff et al., 1987). However, TNF-α has not consistently demonstrated a cause and effect on the development and severity of cachexia (Maltoni et al., 2001). TNF-α appears to be a stimulus for the production of other catabolic cytokines that subsequently cause the development of

cachexia (Argiles et al., 2014). The Association between RBCs and decreased TNF-α level over time is intriguing. There are several possibilities for why this may be the case.

Higher levels of RBCs may be associated with healthier individuals at the time of enrollment in the study. It is a possibility that persons with higher RBCs were less sick at baseline, had less inflammation and subsequently had lower TNF-α. Overtime, these healthier individuals with higher red blood cells may have experienced less overall systemic inflammation and therefore incurred a decrease in TNF-α production. If these patients were healthier at the time of enrollment in the study, it may have conferred a benefit to them post-treatment, allowing for quicker healing time due to less generalized inflammation. However, further studies of this association must occur, as blood delivery of oxygen under certain conditions can be toxic secondary to free radical production such as acute illness, sepsis and cardiogenic shock.

Higher albumin levels in general are associated with healthier individuals. It has traditionally been a marker of long-term nutrition, with higher levels indicating better overall nourishment. Lower albumin levels at the time of admission to the hospital have been associated with worse prognosis (Ferguson et al., 1993) (Baltazar et al., 2015). Within this study, lower levels of IL-1β at 12-months was associated with higher baseline levels of albumin at baseline. IL-1β is associated with inflammatory pain responses in the central nervous system as well as cellular matrix formation. It is generally considered a pro-inflammatory cytokine.

Perhaps, overtime, patients with higher albumin levels have the reserves to mount a better immunological response compared to individuals with lower albumin levels because they are overall healthier due to their nutritional status. Subsequently their IL-1β levels begin to decrease. However, if this were completely true, one would expect that persons with higher albumin levels should have other cytokines decrease proportionally. Potentially, there is a metabolic process that influences IL-β1, but not such physiological process has yet been identified. A further exploration of the association between increased RBCs and albumin levels will need to occur to elucidate what these findings mean in the HNC population.

Aim 2: To examine the role of cytokines, RBCs, albumin and severity of weight loss in HNC patients.

Question 2a: Are baseline cytokines, RBCs and albumin correlated with weight loss at 6- and 12-months?

There was only one statistically significant finding when examining for an association between baseline cytokines, RBCs and albumin with weight loss. Patients with higher IL-6 levels at baseline had greater decrease in weight through the study period than patients with lower IL-6 pre-treatment values. By 12-months post-treatment a similar direction yet slightly smaller coefficient and no longer statistically significant coefficient was demonstrated. IL-6 levels have been correlated with cachexia in patients in several studies (Duffy, et al, 2008) (Bayliss, et al., 2011). IL-6 is an acute phase protein that is associated with inflammation during cancer and increased tumor genesis (Taniguchi & Karin,

2014). Within HNC patients, higher circulating levels of IL-6 has been significantly associated with muscle wasting, weight loss and higher mortality (Duffy et al., 2008) (Moses et al., 2009). The findings within this study reflect other findings regarding higher levels of IL-6 and weight loss.

In regards to IL-6, at 12-months, as IL-6 levels decreased it appears that individuals weight increased. Several smaller HNC studies have also demonstrated this trend, but it was not until Duffy et al., (2008), had a large enough sample size to demonstrate that there was a statistical difference between levels of IL-6 and weight loss. Within this study, no other cytokine demonstrated any close relation to weight loss at 6- and 12-months. This is perhaps due to the significant variability in cytokine levels for individuals as well as due to the small sample size used in this study.

Initial cytokines may not have any association with long-term weight loss and what may be more important is the overall trend of these cytokines as demonstrated by Duffy et al., (2008). Additionally, the study group as a whole had a BMI median >25kg/m^2. Studies have suggested that patients with HNC and BMIs > 25kg/m^2 are associated with improved outcomes and better long-term survival (Gaudet et al., 2012). Perhaps the lack of significance with other cytokines in these findings is suggestive of the obesity paradox conferring a benefit to the overall progression of disease in the study patients (Gonzalez et al., 2014). Future studies examining the obesity paradox in HNC patients should occur.

Question 2b: Does a change in cytokine levels, RBCs and albumin from baseline to 6-months correlate with weight loss at 6- and 12-months?

Increasing IL-10 levels were associated with decreasing weight and BMI over the same time period. This correlates with similar results found at 15 months by Mydlarz et al., (2014). IL-10 is involved in the regulation of the JAK-STAT signaling pathway. Through inhibition of this pathway, the body increases its consumption of muscle protein while at the same time begins to decrease muscle production through increased release of myostatin. The question and answer that is unknown is why would IL-10 remain elevated in some individuals? It is possible that there may be an unknown genetic component influencing IL-10 production. Certain IL-10 genotypes appear to contribute to oral cancer susceptibility (Tsai, et al., 2014). What is less clear is what the IL-10 elevation means beyond the 12-month period of this study. The same questions can be posited for TGF-β2 and TFN-y.

Patients', who had lower levels of TGF-β1 overtime, appear to have experienced increased weight loss. This may be attributed to the transforming growth factor ability to promote tissue repair. Perhaps in persons with higher TGF-β1 there is a propensity for improved tissue repair thereby decreasing an individual's overall inflammatory status? The ability to promote tissue repair may decrease the metabolic demands of the body. The loss of TGF-β1 signaling has been associated with worse outcomes for patients with HNC (Bian et al., 2012). Overall, as a whole, patients within this study lost the most weight between baseline and 6-months and then in fact began to increase their weight by the 12th month. The reasons for this increase are presently unclear. However, it is likely

indicative of improving health and function. Additionally, as treatment progresses and tumors are either resected or eradicated, it is possible that fewer tumorkines were produced allowing for a decrease in myostatin production allowing for weight gain to occur. The decrease in tumorkines would also shift the body back to normal physiological homeostasis allowing for weight to be added. However, lower levels of IFN-y and increasing weight loss does not match the literature.

Higher levels of IFN-y have been strongly associated with cachexia in both mice and human models (Matthys et al., 1991) (Ito, et al., 2006). However, within this study, this association only occurred at the month 12. It is possible that this association was spurious as at 6-months the Beta for this weight association was zero. However, a recent study indicated that patients with lower levels of IFN-y and higher levels of IL-10 with oral squamous cell carcinoma had worse long-term outcomes (Wang, et al., 2014). The Wang, et al., study did not explore an association with IFN-y, IL-10 and weight loss. Like IL-10, it is possible that within HNC patients, IFN-y levels may be a prognosticator of other clinical manifestations other than weight loss and cachexia. Overall, IFN-y and IL-10 appear to associate with negative outcomes in HNC patients. Further studies differentiating these cytokines at the cellular and genetic level should occur.

AIM 3: To examine the association between cytokines, RBCs, albumin and reported symptoms in HNC patients?

Question 3: Are initial cytokines, RBCs and albumin associated with changes in reported symptoms such as: depression, nutrition consumption, swallowing difficulties, mucositis, generalized pain, mouth pain, dental discomfort, hearing, xerostomia, taste, jaw movement and voice at 6- and 12-months?

A primary goal of this study was to assess how initial baseline cytokines correlated with long-term symptom development in patients post-treatment for HNC. Throughout the literature, most studies are conducted for a short-term duration of 3 to 6-months during cancer treatment. Furthermore, limited studies to date examine how baseline cytokines affect the development of long-term symptoms. Many patients within this study had advanced cancer at the time of diagnosis (Stage 3 or greater) therefore the inflammatory mediators responsible for the development of cachexia and symptoms were already promoting metabolic derangements at the time of enrollment. Due to the fact that cytokines have variable half-lives ranging from seconds to a few minutes, examining how these biomarkers influence long-term symptom development was important so that future research can target modalities that may interfere with cytokines that appear to promote worse symptoms. The caveat to understanding these cytokines is that the ability to understand the development of cytokines in human cancer patients during early stages is limited because cancers are generally not detected at the onset of the disease. Thus determining the progression of cytokine initiation from the tumor is difficult to elucidate. Presently, researchers must rely upon animal models to direct the understanding of cytokine progression

in early cancer states. Consecutively, animals cannot fill out forms in regards to their symptoms. Overall, associating symptoms and cytokines is a difficult endeavor. However, through identifying those cytokines that associate with symptoms, the goal is to further study these associations at the cellular and genetic levels in future animal studies in order to begin fully understanding the potential biological processes that cytokines influence upon each other.

Depression

Within this study two significant associations with increased levels of depression were lower baseline levels of TGF-β2 and higher initial levels of CRP. No similar associations of TGF-β2 have been found, in regards to depression, in previous research. With regards to CRP, an association with increased CRP levels and increased levels of depressive symptoms has previously been described in the literature (Archer, et al, 2014). A recent study comparing different alleles associated with CRP production found that elevated CRP was associated with increased risk of depression, but genetically elevated CRP was not concluding that CRP per se is not a causal risk factor for depression (Wium-Andersen, Orsted, Nordestgaard, 2014).

Depression is a common symptom that is associated with cancer, cancer cachexia and associated cytokines (Moubayed et al., 2014) (Archer et al., 2012) (Kamath, 2012). Several studies indicate an association between increasing cytokines and the development of depression in cancer patients. However, the association within this study is opposite of what has been consistently described in the literature. With the exception of CRP (which isn't a cytokine) no other cytokine correlated with worsening symptoms of depression. It remains unclear

as to the possibility of why a growth factor would correlate with depression. The statistically significant association between TGF-β2 and depression only occurred at the 12-month interval, indicating that this may be a spurious finding. A further exploration of this possible connection will need to be conducted.

CRP is a generic marker of inflammation within the body and has been linked with not only depressive symptoms, but that of fatigue and pain in cancer patients (Myers, 2008). Most studies report a negative association with outcomes and cancer when CRP levels are elevated (Chung & Chang, 2003) (Wallengren et al., 2013). Within this study, only elevated CRP levels at baseline was associated with depression. While this finding is not new, there was no association with any other symptom. However, this study did not examine the symptom of fatigue, and generally throughout the literature, depression and fatigue have correlated with elevated CRP.

Nutrition consumption

Several baseline cytokines were statistically significant at the 6-month point, which were significantly associated with the VHNSS nutrition cluster. They were IL-6, IL-10, MMP2A, TGF- β1 and IFN-y.

Head and neck cancer patients are prone to developing difficulties obtaining adequate nutritional intake due to the location of their tumor. Furthermore, there is a direct link between lack of caloric intake and increased mortality (Jager-Wittenaar et al., 2011) (O'Neill & Shaha, 2011) and decreased quality of life (Caro et al., 2007). The role of cytokines and their mediation of caloric intake through influencing of the brain, specifically the hypothalamus, have become increasingly understood. Increased inducible nitric oxide synthase

production in the hypothalamus leads to severe anorexia, which is possibly a pathway through which pro-inflammatory cytokines produce anorexia (Morley & Farr, 2008). Various cytokines influence several neuropeptides that directly affect the desire to eat (Patra & Arora, 2012). Therefore, understanding the cytokines that are influential in potentially regulating these various neuropeptides is important in developing future interventional modalities that may be used to thwart the progression of cachexia.

Individuals, who had higher levels of IL-6, IL-10, MMP2A, TGF-β1 and IFN-y at baseline, were prone to having greater difficulties with nutrition symptoms such as anorexia and losing weight. The precise role of IL-6, IL-10, MMP2A, TGF- β1 and IFN-y on the progression of anorexia and cachexia is not fully understood, but there are two hypotheses. 1) cytokines influence neuropeptide interaction through the blood brain barrier promoting early satiety and anorexia (Banks, 2001), 2) IL-6 specifically alters the JAK/STAT pathway promoting muscle catabolism and decreases muscle production through promoting an increase in circulating levels of myostatin which inhibits muscle regeneration.

The role of IL-10 and MMP2 on cachexia is less clear than IL-6. In a large study of 203 patients examining multiple cytokine polymorphisms, only IL-10-1082 polymorphism was linked to the development of cachexia in gastric cancer (Deans et al., 2009). Increased IL-10 serum expression was significantly associated with worse survival in early stage oral squamous cell carcinoma (Chen et al., 2013). However, this same association was not witnessed in

myelofibrosis. Another recent study indicates that certain IL-10 genotypes increase the susceptibility of oral cancer (Tsai, et al., 2014). What is additionally problematic is that IL-10 is generally considered an anti-inflammatory cytokine and it inhibits other cytokines such as TNF-α, which can promote cachexia. The association of IL-10 and worsening nutrition symptoms is perplexing and needs further exploration.

MMP2 has generally been correlated with tumor promotion and progression as its role in the healthy individual is tissue repair and regulation of vascularization. An association with MMP2 and cachexia remains unclear presently. However, MMP2 may play a role in vascularization of tumors, which help them grow and subsequently enhances release of cachectic inducing cytokines.

The TGF-Beta superfamily cytokine MIC-1/GDF15 circulates in all humans and when overproduced in cancer leads to anorexia/cachexia, by direct action on brain feeding centers, specifically the hypothalamus. However, within this study, lower levels of TGF- β1 was associated with worsening nutrition symptoms. This finding was only statistically significant at the 6 month mark. Additionally, lower levels of IFN-y were also associated with worsening nutrition symptoms at the 6-month mark and not at the 12-month mark. Both higher levels of IFN-y and TGF-β1 have been associated with worsening cachexia (Argiles, et al, 2005) (Bing, 2011)(Blum et al, 2011)(Cahlin, 2000)(Donohoe, 2011)(Patra &Arora, 2012). The findings within this study are likely spurious in regards to TGF-β1 and IFN- y.

By the 12-month the effects of IL-6, IL-10, MMP2A, TGF-β1 and IFN-y were all less significant with only the association with IL-6 maintaining statistical significance. Additionally, at this same time the median weight began to increase, cytokine levels decreased as well as study participants reported less issues with nutrition symptoms. This is perhaps attributed to post-surgery patients beginning to improve their overall states of health and having less inflammation as the tumorkines production in these patients should theoretically be zero.

Swallowing related symptoms

Within this study, higher levels of 3 cytokines correlated with increased difficulty swallowing solids at both 6- and 12-months. They were IL-6, IL-10 and TNF-α. Lower levels of albumin at baseline were also associated with worsening ability to swallow. Additionally, lower levels of IL-12p70 at baseline were associated with worsening ability to swallow liquids at the 12-month.

Difficulties swallowing are directly related to several factors such as the direct impact of the tumor, cancer resection, chemotherapy and radiotherapy (Raber-Durlacher et al., 2012). What is not well described in the literature is the association between cytokines and swallowing dysfunction in the HNC patient over time. Interestingly, IL-6 and IL-10 also correlated with difficulties in regards to nutrition consumption. However, in this case IL-6 and IL-10 were associated with difficulty swallowing at 12-months, but this was not the case when patients self-reported difficulties obtaining nutrition on the VHNSS. This perhaps indicates that some patients were able to adapt to their difficulty obtaining nutrition over the year by adapting to different foods. Their perception of obtaining nutrition may

have changed over the duration of their illness, but they still experienced "trouble" swallowing. IL-6 inhibits muscle production and promotes degradation of muscle mass through inhibition of the JAK/STAT pathway as well as altering the mitochondrial functioning of muscle (White et al., 2012). The body employs over fifty muscles when it produces the act of swallowing. Further studies quantifying muscle tissue degradation with IL-6 and other cytokines in the HNC patient should occur to determine if muscle wasting in the specific muscles of swallowing is occurring. IL-10 at the early stage of cancer has been associated with worse outcomes in HNC patients (Chen et al., 2013). The role of IL-10 in regards to muscle breakdown and dysfunction is not well understood.

TNF-α has been implicated in the development of cachexia in several studies (Tisdale, 2005). TNF-α appears to be a stimulus for the production of other catabolic cytokines that subsequently cause the development of cachexia (Argiles, Busquets, Stemmler, & Lopez-Soriano, 2014). Additionally, TNF-α appears to affect the hypothalamus through pro-inflammatory signals that affects neurotransmitters and subsequent hunger signaling to patients perpetuating the cycle of cachexia by inducing anorexia (Amaral et al., 2006). TNF-α also promotes fat lipolysis and fat depletion (Tsoli & Robertson, 2013). TNF-α has also been recently identified to regulate lipolysis through the GO/G1 switch gene 2 (GOS2). By inhibiting expression of GOS2, an increased level of lipolysis occurs (Yang, Zhang, Heckmann, Lu, & Liu, 2011). Presently, it appears that TNF-α directly relates to adipose tissue loss, but not directly to the loss of muscle protein (van Hall, 2012). With limited effects on muscle loss, the association with

decreased ability to swallow and increased TNF- α levels in the HNC is not clear. It is possible that the elevation of TNF- α promoted the metabolic effects that promoted muscle degradation through promotion of myostatin.

Lower levels of albumin at baseline are associated with worse swallowing symptoms. Low albumin is a marker of the inability to obtain adequate nutrition for approximately 12-weeks prior to the lab draw. Persons with lower albumin levels may have been sicker at baseline and perhaps did not recover by the 12th month. If the dysphagia was already problematic pre-treatment, this could have affected the patient's albumin levels initially as the patient would have had difficulty consuming adequate calories prior to enrollment. Finding ways to optimize nutritional intake to combat many negative cachexia symptoms is complex and not fully understood. However, increasing caloric intake is not the solution to thwarting cancer cachexia.

Mucositis, Generalized Pain and Mouth Pain

Within this study, higher baseline IL-1β levels were associated with worse mucositis, generalized pain and mouth pain at 6-months but not at 12-months. Higher initial baseline RBC levels were associated with worse general and mouth pain at 6-months but not at 12-months. It should be noted that within this study, mucositis, generalized pain and mouth pain were measured on a 0-10 point scale with 10 being the most severe level of pain. At baseline, the median level of mucositis was 0.13, generalized pain was 0.83 and mouth pain was 0.25. While mucositis and mouth pain are well-documented negative sequela of HNC cancer

treatment and progression, it is perplexing as to why the average pain scores remained low for this study population (Nicolatou-Galitis, et al., 2013).

Inflammation has consistently been correlated with pain in several cancer studies (McClement, 2005) (Laird et al., 2011b) (Myers, 2008). IL-1β has been associated with worsening pain in patients through its up-regulation of pro-nociceptive mediators (Ren & Torres, 2009). Through this action, this makes the body more prone to perceiving the presence of pain. Additionally, there appears to be an association with individuals with a genetic disposition towards producing IL-1β having increased pain during cancer (Oliveira et al., 2014). Oncologists and nurses treating pain could use the association between IL-1β to potentially anticipate or titrate pain medication needs. Further studies testing the validity of this idea should occur.

Again, higher levels of RBCs at baseline were also associated with worsening generalized pain and mouth pain, but not mucositis. Interestingly, it is generally anemia that is associated with increased bone pain and angina due to lack of oxygen delivery. It is possible that the ability of the body to deliver oxygen to the tissue and cellular level promotes free radical damage that perpetuates the demise of the IL-6 altered mitochondria. Most studies within cancer attribute an association between lower levels of RBCs and increased pain and other symptoms such as fatigue (Holzner, et al., 2002). This study did not examine a change in RBCs over the 12-month period. It is possible that patients with higher RBC levels at the start of the study had a greater decrease in their RBC levels,

which is associated with worsening pain. A further exploration of this result and HNC patients should occur.

Xerostomia

Similarly to swallowing and nutrition, increased levels of IL-6 and IL-10 were associated with worse reports of xerostomia. However, IL-6 was only statistically significant at 6-months with a shared variance of less than 15. The IL-6 results need to be interpreted cautiously. Higher RBCs were associated with worse xerostomia at the 6-month mark, but not the 12-month mark.

Overall, these associations are not surprising in that it takes saliva in order to swallow and digest solid foods. It is known that xerostomia directly impacts the ability to swallow solids (Raber-Durlacher et al., 2012). Furthermore, the same association between elevated baseline RBCs was appreciated for xerostomia as it was for swallowing solids. This association indicates a plausible link between the two symptoms. The question that is not fully elucidated then is are the cytokines responsible primarily for inhibiting saliva production which then makes swallowing solids more difficult, or are their two distinct pathways that promote both a decrease in saliva production and a decrease in neck muscle functioning inhibiting the ability to swallow? Due to the fact that IL-10 also remained statistically significant for associating with increased issues with xerostomia at month-12, while it did not do so for swallowing solids at month-12, could suggest that two distinct pathways mediated by cytokines are responsible for a decrease in saliva as well as decreased muscle functioning. This notion should be explored further.

Taste dysfunction

Higher levels of baseline IL-1β and IL-6 correlated with increased taste dysfunction at both 6- and 12- months. IL-1β is involved in olfactory abilities (Poretti et al., 2015). An examination of the influence of IL-1β on the influence of smell and taste in HNC patients has not been conducted. Increasing levels of IL-1β may inhibit the ability to smell, which is the predominant sense that humans utilized for taste. In a recent study, an injury to the tongue correlated with higher IL-1β levels approximately 2-days after an initial injury (Shi, et al., 2012). This same process could also be occurring in the HNC population both through tumor mediation as well as through the negative sequela of treatment. Additionally, increased IL-6 levels have been found in the plasma, saliva and nasal mucus of patients with hyposmia (Henkin, Schmidt & Velicu, 2013). Overall, the association with IL-6 and impaired taste and smell has not been well studied. However, it appears that increased IL-6 and IL-1β does negatively affect the symptom of decreased taste and smell.

Voice dysfunction

Within this study, no significant associations between cytokines and voice were found. However, a higher baseline level of RBCs significantly correlated with increased problems in voice at 6-months. This result is perplexing. Without An association for an inflammatory mediator, it is possible that there is some other untested cytokine that may be promoting inflammation of the vocal cords in conjunction with the presence of increased RBCs. A review of the literature has

not identified any other associations between RBCs and dysphonia in HNC patients.

Non-significant findings

There were no significant findings for either hearing impairment or jaw/trismus dysfunction. Conductive and sensorineural hearing impairment can occur in HNC patient due the region of the tumor as well as treatment modalities utilized to eradicate the disease (Hitchcock, Tward, Szabo, Bentz, & Shrieve, 2009). However, within this study, the median hearing problem for the entire 12-months was zero. While lower levels of TGF-β2 were associated with worsening hearing loss at 6-months, this did not hold true for 12-months when in fact higher levels of TGF-β2 were associated with worsening hearing though not significantly. It appears that these findings were spurious. This study was not a good representation of those patients with affected hearing secondary to their HNC.

AIM 4. To examine the Association between cytokines and changes in musculoskeletal function in HNC patients.

Question 4: Are baseline cytokines associated with musculoskeletal impairment (neck and shoulder movement) as measured by shoulder and neck ranges of motion and neck disability at 12-months?

Neck Disability Index

The study group as a whole had high self-reported rates of neck movement dysfunction at baseline with a median score of 10. By the 12th month, the median score had dropped to 6. Higher levels of IL-β1, IL-6, IL-8, MMP9 and TNF-α at baseline associated with worsening scores on the NDI. Lower initial TGF-β2 levels were significantly associated with worse NDI scores at 12-months.

Dysfunction of muscle movement of the head and neck is common in HNC patients (Teymoortash et al., 2010). However, the relationships between cytokines and long-term abilities to move the head and neck over the long term have not been conducted. Within this study both the subjective, Neck Disability Index scale, and objective, Cervical Range of Motion test was utilized to evaluate and compare the associations with cytokines with long-term problems with head and neck movement.

Higher levels of MMP9 have been correlated with worse prognosis for nasopharyngeal cancer patients and higher mortality (Liu et al., 2010). MMP9 has also been associated with worsening myocardial muscle functioning (Chiao et al., 2012) (Pan et al., 2014). Under normal circumstances, MMP9 promotes muscle healing and tissue repair. An alteration of MMP9 however may be promoting muscle dysfunction at the cellular level that has not been identified

presently. Additionally, the body may be producing more MMP9 in order to promote tissue healing, specifically at the site of the muscle, but other metabolic processes are hindering the normal tissue repair that MMP9 orchestrates. However, it is possible that higher levels of MMP9 were directly related to the tumorgenesis that was occurring at the time of enrollment in the study. It is possible that higher levels of MMP9 were associated with tumor progression. However, this study lacks the ability to address that hypothesis. Overall, the association with cytokines and long-term muscle dysfunction in HNC is not well understood. Further studies of this finding will need to be made in the future; perhaps specifically examining the MMP9 within the tissue at the time treatment begins and looking for correlations among neck motility dysfunction over time.

IL-6 inhibits muscle production and promotes degradation of muscle mass through inhibition of the JAK/STAT pathway as well as altering the mitochondrial functioning of muscle (White et al., 2012). An association with higher levels of IL-6 and worsening neck movement over time correlates with the known degradation of muscle that IL-6 can produce. However, IL-6 was only significantly associated with worsening neck movement at the 6-month mark. This is likely attributed to the decreasing levels of IL-6 in general for this population and the likely decrease in the effects IL-6 would have on this patient population as they continued to recover in the post-treatment period.

IL-1β had a similar pattern to that of IL-6 in that it was only statistically significantly associated with worse neck movement at the 6-month mark. However, the correlation with movement of muscle and IL-1β is not well

documented in the literature, what is documented is a strong correlation between IL-1β and increasing muscle pain (Willemen, et al., 2014) (Chen, et al., 2015). It is possible that neck movement was hindered by pain, which contributed to this association. The association with IL-1β with muscle dysfunction symptoms in HNC patients will need further research, specifically around movement of the head and neck in association with pain. It is possible that IL-1β is contributing to a potential symptom cluster.

TNF-α also had a similar pattern to IL-6 and IL-1β in that it was only statistically significantly associated with decreased neck movement at 6-months. TNF-α was originally called cachectin and has been associated with a decrease in muscle mass in cancer patients. This decrease in muscle mass may be contributing to the difficulties HNC patients were reporting with neck movement within this study. However, this study did not directly examine muscle mass changes over time. As the patients continue to recover post-treatment, it is likely that the initial levels of TNF-α became less important. However, the correlation with TNF-α and neck movement does indicate that HNC patients with higher levels of TNF-α are likely to have increased neck disability immediately post-treatment due to increased muscle mass loss. However, TNF-α has not consistently demonstrated a cause and effect on muscle wasting. Further studies should examine TWEAK in association to neck dysfunction as it appears that TWEAK is associated more strongly with overall muscle wasting (Kumar, Bhatnagar, & Paul, 2012)

Higher initial levels of IL-8 were associated with increased neck impairment at both 6- and 12-months post-treatment. The role of IL-8 in cancer appears to be one of promoting muscle wasting (Gioulbasanis, Patrikidou & Kitikidou, 2012). It is probable that increased IL-8 levels promoted some muscle wasting for patients in this study, however as stated previously, this study did not directly measure muscle mass changes. It is probable that any muscle wasting that did occur in turn likely promoted neck dysfunction. Within this study it appears that the effects of IL-8 had a longer-term effect compared to other cytokines. Studies about the effects of IL-8 on HNC patients are inconclusive presently (Mojtahedi, et al., 2014). Further research into IL-8 levels in HNC patients need to occur, specifically measuring muscle dysfunction and any wasting simultaneously.

Lower levels of TGF-β2 and worsening muscle dysfunction of the neck correlates with other recent findings. When TGF-β2 is inhibited, muscle atrophy is worse (Ohsawa, et al., 2012). Further studies indicate that TGF-β2 is responsible for conduction at the nerve synaptic function (Kutsano, et al., 2010). Less TGF-β2 would cause a decrease in electrical conduction and theoretically would decrease muscle movement abilities. Lower levels of TGF-β2, in conjunction with location of tumor and treatment, would likely cause neck motility dysfunction in the HNC patient. In a recent mouse model study, providing TGF-β2 helped increase muscular function and prevented muscle wasting. Exploring ways to increase TGF-β2 levels in the HNC patient may help to alleviate some of the muscle wasting and dysfunction that occurs within this population.

Lower albumin level at baseline is possibly indicative of overall poorer health at the beginning of the study. This may translate into worse muscle functioning in patients for several reasons. Lower albumin levels indicate decreased long-term caloric consumption, specifically of protein. For muscle to regenerate and heal, protein is required. Several studies indicate that lower albumin levels generally lead to worse outcomes in patients (Jiang et al., 2014) (Yang, Zhang, Hou, Xie, & Cao, 2014). Additionally, a recent study examining outcomes in association with serum albumin and CRP found that those with lower albumin and elevated CRP at the time of surgery had worse outcomes post-operatively (Farhan-Alanie, McMahon, & McMillan, 2015). Possibly, decreased albumin levels could affect musculoskeletal dysfunction. Further evaluation in cancer patients needs to occur to examine this association.

Cervical Range of Motion

Overall, this objective measurement provided inconsistent results. No clear patterns of associations were observed. The only biomarker with more than one statistically significant association among the 6 CROM measures was MMP2A. Higher pre-treatment levels of MMP2A were associated with greater decreases (relative to pre-treatment) in extension. In a recent study, the reliability and validity of measuring CROM in healthy volunteers demonstrated a wide range of intra-class-association coefficients, suggesting that its validity in healthy individuals is questionable, while it appeared valid for those with known neck movement disability (Wibault, Vaillant, Vuillerme, Dedering, & Peolsson, 2013). Perhaps in future studies determining who is having neck difficulty verses those who do not may need to be examined. Overall, a sound conclusion regarding

cytokines and neck movement disability measured by the CROM cannot be made from this study. Furthermore, patients in this study received lymphedema therapy, physical therapy and corrective posture instruction as part of the parent study. This could have directly improved CROM as well as NDI scores.

Synthesis of Findings

A primary goal of this dissertation research was to examine for the associations with cytokine production and weight loss as a marker of cachexia. Increasing levels of IL-10 was associated with both increasing weight loss at 6- and 12-months as well as worsening swallowing of solids and xerostomia at 6- and 12-months and worse nutrition symptoms as measured by the VHNSS at 6-months. Additionally, patients who had higher albumin levels at the time of enrollment had lower IL-10 levels. It is possible that the inflammatory cascade promoted by IL-10 caused anorexia in patients prior to treatment. This anorexia would have theoretically resulted in albumin levels to decrease. IL-10 is involved in the regulation of the JAK-STAT signaling pathway. Through inhibition of this pathway, the body increases its consumption of muscle protein while at the same time begins to decrease muscle production through increased release of myostatin. This imbalance likely was exacerbated by the ensuing decrease in albumin levels, which was inhibited by xerostomia and difficulty swallowing. The question and answer that is unknown is why would IL-10 remain elevated in some individuals? It is possible that there may be an unknown genetic

component influencing IL-10 production. Certain IL-10 genotypes appear to contribute to oral cancer susceptibility (Tsai, et al., 2014). What is less clear is what the IL-10 elevation means beyond the 12-month period of this study. Overall, it appears that IL-10 may be a potential cytokine that can help identify patients early during the course of their treatment that may be at greater risk for the development of cachexia and difficulties obtaining nutrition. Other cytokines, which were elevated at baseline also associated with difficulty obtaining nutrition, they were IL-β1, IL-6, TNF-α, MMP2A and TGF-β1. Of these, IL-6 matched similarly to that of IL-10.

Patients with higher IL-6 levels at baseline had greater decrease in weight through the study period than patients with lower IL-6 pre-treatment values. However, unlike IL-10, increasing levels of IL-6 did not associate with weight loss, but it did associate with worse ability to swallow solids and nutrition symptoms at 6- and 12-months as well as worse xerostomia at 6-months. Unlike IL-10, IL-6 baseline levels also associated with worse ability to taste and smell at 6- and 12-months. The likely difference between IL-6 and IL-10 is the pattern in which IL-6 increases first in an acute illness. It is possible that the initial levels of IL-6 are most important in terms of long-term symptom development. In short, IL-6 may be a marker for how acute a cancer is for a particular patient at the time treatment begins or more precisely at the initial stages of cancer. Therefore, the change overtime is not as important for a patient's IL-6 levels in comparison to IL-10, which is likely a cytokine that is more indicative of how a patient is

progressing with their illness. Another cytokine that may be indicative of how a patient is progressing through their disease is IL-β1.

IL-1β has been increasingly identified as an interleukin that is positively associated with worse pain levels in cancer patients. Within this study, generalized pain, mouth pain and worse mucositis associated with higher levels of this cytokine at baseline. Unlike IL-6, there was no association with weight loss. However, while there was an association with increased pain scores with IL-β1, this did not associate with the inability to consume nutrition. It is likely that the IL-1β physiologically is responsible for pain signaling, but not with the physiological processes that IL-6 and IL-10 appear to associate with in terms of saliva production and swallowing. Furthermore, pain often times correlates with increased depression scores. IL-1β did not associate with worsening depressive symptoms. Within this study higher CRP levels and lower TGF-β2 levels correlated with depression. The association with lower TGF-β2 levels and less depressive symptoms is not completely understood presently. Interestingly, higher TGF-β2 levels associated with worse scores on the NDI. It is possible that muscle dysfunction in general contributes to an overall decrease in quality of life for patients and increases their self-reporting of depression. Further studies regarding this finding are warranted. Another perplexing finding within this study is the association with higher RBC levels at baseline and worse generalized pain, mouth pain and xerostomia at 6-months.

It is possible that the ability of the body to deliver oxygen through hemoglobin to the tissue and cellular level promotes free radical damage that

perpetuates the demise of the IL-6 altered mitochondria. Most studies within cancer attribute an association between lower levels of RBCs and increased pain and other symptoms such as fatigue (Holzner, et al., 2002). A further exploration of this result and HNC patients should occur.

Overall, this study demonstrated some new results not previously reported in the literature as well as correlated with previous published results. While IL-10 has generally been considered an anti-inflammatory cytokine, recent studies, in conjunction with results in this study, demonstrate that IL-10 may promote not only muscle wasting and weight loss, but also correlates with worse symptoms over 12-months specifically those symptoms surrounding nutrition and swallowing. Additionally, IL-1β appears to associate with worse pain symptoms over time. This association will need further research as a potential target for helping cancer patients manage their pain. Finally, IL-6 associated with weight loss within this study, and this finding while not new, highlights the fact that the IL-6 is integral to the metabolic derangements that promote cachexia.

Strengths and Limitations

Strengths

This dissertation research is unique in the fact that it is the first known study to examine the relationship between cytokines and the development of long-term symptoms in head and neck cancer patients. The long-term development of symptoms and the effects on patient's lives is not well

understood. A predominance of studies within the HNC literature are short term studies that examine how cytokines effect outcome and symptoms generally over three to six months during cancer treatment. Overall, the study population within this group matched other HNC cohorts in terms of age, gender and race. Furthermore, this study examined the relationship between RBCs, albumin and cytokines. No studies examining these correlates within HNC have been found. Several of the instruments utilized within this study demonstrated sound internal consistency and test-retest reliability. Overall, this was a unique study that will be able to inform several future studies.

Limitations

One of the paramount weaknesses of this study was the fact that it was a secondary analysis. While the parent study utilized in this secondary analysis examined the development, nature, progression and prevalence of late-effect fibrosis and/or lymphedema as well as biological correlates such as cytokines and genetic polymorphisms and psychological stressors in patients with head and neck cancer, this study analyzed data specific to cachexia and potential long term effects of cytokines on symptom development. A limitation of this study was that the original purpose of the study was not intended to specifically answer the questions posited in this dissertation. However, the original study evaluated HNC patients and this dissertation continued to focus on HNC patients. Another limitation of this study was the small number of participants (N=56). While this matches other HNC studies, it may not have been large enough to see the effects of cytokines on symptom development and musculoskeletal dysfunction. Furthermore, due to the multiple associations conducted during this study, it is

possible that the findings were simply statistical “noise”. This is evident in that many scores overall had a low median baseline score, such as pain, without significant associations with pain and baseline cytokines and the changes in those cytokines over 6- and 12-months. Additionally, it appears that the CROM may not be the best measure of musculoskeletal dysfunction in this patient population. Due the nature of this secondary analysis, as well as understanding the fact that patients received physical therapy and corrective posture instruction, it will be difficult to interpret those results.

Implications

Generally, the trend for patients within this study was improvement in their overall symptoms burden at both 6- and 12-months post-treatment. These overall findings indicate that eradication of the tumor burden appears to positively impact patients. This correlates with the theory that the tumor initiates the metabolic derangements that the body experiences. Through the removal of the tumor, this tumor burden decreases and the body is able to return to homeostasis. As the body returns to homeostasis, the body is able to heal and symptoms burden declines. This knowledge can help nurses and providers work with their patients as they progress through the cancer treatment spectrum and enter into their post-treatment phase.

The association with IL-10 and IFN-y and negative outcomes within this study supports relatively new research indicating that these two cytokines

negatively affect cancer patients. This study demonstrates that there is also an association with symptoms even during the recovery period following treatment. These findings, in conjunction with other findings, could be utilized to specifically conduct animal studies to determine the biological processes that promote the interplay between these two cytokines. Through this research, it is possible that future translational research at the bedside could be conducted to help providers thwart the many negative symptoms that accompany cancer.

Nurses are excellent patient advocates that utilize scientific evidence to implement holistic nursing care plans aimed at improving patient's symptoms and quality of life. Understanding which patients are at the highest risk for having long-term difficulties with symptoms is a venue through which nurses can provide this important holistic care. While the applicability of cytokines and RBCs on long-term symptom development is not fully understood, this study demonstrated that low albumin levels at the time of enrollment generally correlated with more difficulty long-term with symptom problems surrounding nutrition and swallowing. These study findings correlate with other cancer studies (Gupta and Lis, 2010). Nurses can apply the knowledge of albumin directly to patient care today by identifying those patients with low albumin levels and initiating nursing care plans that may help promote healing with a focus on improving symptoms. Through this endeavor it may be possible help HNC patients recover from their illnesses more effectively. Nurse practitioners may also utilize the knowledge from this and similar research to help treat cancer patients.

It is possible that nurse practitioners may someday be utilizing the measurement of some cytokines to help direct the care and treatment of their patients. For example, the result that IL-1β appears to drive pain over a 12-month period could potentially be utilized by the nurse practitioner to treat pain in the future once a further understanding of the biological processes that promote IL are further understood. This knowledge may help the nurse practitioner direct the prescribing of pain medications with the understanding that patients with higher IL-1β may actually have increased pain medication needs for a longer period when compared to those with lower IL-1β levels. It will be important to continue with studies of associations with cytokines and symptoms so that future translational research can be conducted at the bedside to help promote healing and recovery for patients with HNC.

Recommendations for Future Research

Presently, there are six domains that will require future research: 1) further evaluation of cytokines in a larger sample, 2) genetics, 3) cancer associations, 4) viral verses non-viral, 5) translational research, 6) the role of RBCs in cancer.

Larger Sample: Several findings within this study were statistically significant. A larger sample size investigating initial cytokine levels and long-term symptom development is warranted based upon these results. Through such studies, it may be possible to further highlight the associations between cytokines and symptoms. This knowledge could ultimately direct laboratory research that examines the biological pathways and influences of these cytokines, which may

help to distinguish the pathways through which these cytokines influence symptom development. These studies may identify cytokines that can be targeted from a therapeutic perspective with the ultimate goal of helping to decrease symptom burden in the cancer patient.

Genetics: The scientific understanding of the role that genetics have within health continues to increase. Future studies need to continue to examine the genetic differences within individuals' production of cytokines under various illnesses including cancer. Through further understanding of these factors, it may be possible to highlight the cytokines that give survival advantage in some diseases and perhaps not in others.

Cancer associations: Cancer by its very nature is heterogeneous. However, cancers cannot remain within their silos if we are to develop a sound understanding of cachexia and the role of cytokines in cancer. To understand the differences and similarities in symptom development, a study following cytokines and symptoms in other cancers over a long time period should occur. Through this research, it may be possible to identify the common links between negative and positive symptom development in all cancers.

Viral verses non-viral: Evidence continues to increase that many cancers are virally mediated. Are there certain cytokine mediators that can attenuate the development of cancer and or the development of cachexia and other negative cancer symptoms in virally induced cancers? Further large-scale examinations of the differences in these cancers need to occur to inform our understanding of the pathways of not only cancer progression, but also the progression of cachexia.

Translational research: Throughout the research that comprised much of this dissertation, what became clear is that the many studies that have been conducted surrounding cachexia have not made its way to the nurse or medical provider at the bedside. While there is no curative modality available to stop cachexia, there are other ideas that could inform how patients are treated presently. Through better understanding of cytokines and cachexia by bedside providers it may be possible to provide better treatment.

The role of RBCs in cancer: This study provided some very interesting associations with initial higher levels of RBCs and worse long-term symptoms. Future studies examining this association need to occur. It is possible that these findings were secondary to the multiple associations conducted during this analysis. However, these findings support recent recommendations to not transfuse chronically ill patients with hemoglobin levels greater than 7.

Summary

This dissertation study was a secondary analysis of data from a four-year prospective longitudinal, descriptive study that was recently completed. The following assumptions guided this study: 1) many patients with HNC develop cachexia; 2) the physiological underpinnings of cachexia in this population are not well articulated; 3) inflammatory processes contribute to cachexia; 4) other symptoms besides weight loss can accompany cachexia; and 5) long-term associations between baseline cytokines and symptoms progression are not well understood. In order to examine these assumptions, this secondary analysis

examined cytokines, red blood cells (RBCs), albumin and changes in symptoms and weight over a 12-month period. It assessed how changes in cytokines over time correlated with variables of cachexia and cancer symptoms as well as changes in musculoskeletal functioning over the 12-month period.

Overall, symptom burden declined over the 6- and 12-month period in patients. As cytokine levels decreased, so too did symptoms. However, a interesting finding was obtained with patients who had higher levels of RBCs at baseline. Higher RBCs were correlated with worse symptoms burden at 6- and 12-month, such as with swallowing and pain. A further exploration of these results will need to be conducted.

Cachexia and symptom burden in head and neck cancer patients is an understudied area of research. This study resulted in associations between cytokines, RBCs and albumin levels with changes in symptoms. Further studies and explorations of these results will need to be conducted to clarify these results to further the understanding of cachexia and cytokines in head and neck cancer patients.

REFERENCES

Adams, V., Anker, S. D., & Schuler, G. (2011). Muscle metabolism and exercise capacity in cachexia. *Curr Pharmaceutical Design*, *17*(35), 3838-3845.

Amaral, M. E., Barbuio, R., Milanski, M., Romanatto, T., Barbosa, H. C., Nadruz, W., Velloso, L. A. (2006). Tumor necrosis factor-alpha activates signal transduction in hypothalamus and modulates the expression of pro-inflammatory proteins and orexigenic/anorexigenic neurotransmitters. *Journal of Neurochemistry*, *98*(1), 203-212.

American Cancer Society (2012). Cancer Facts and Figures 2012. Retrieved February 1, 2013 from http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/d

American Cancer Society. (2014). Economic Impact of Cancer. Retrieved October 8, 2014 from http://www.cancer.org/cancer/cancerbasics/economic-impact-of-cancer

Andl, T., Kahn, T., Pfuhl, A., Nicola, T., Erber, R., Conradt, C., .Bosch, F. X. (1998). Etiological involvement of oncogenic human papillomavirus in tonsillar squamous cell carcinomas lacking retinoblastoma cell cycle control. *Cancer Research*, *58*(1), 5-13.

Antoun, S., Birdsell, L., Sawyer, M. B., Venner, P., Escudier, B., & Baracos, V. E. (2010). Correlation of skeletal muscle wasting with treatment with sorafenib in patients with advanced renal cell carcinoma: results from a placebo-controlled study. *Journal of Clinical Oncology*, *28*(6), 1054-1060.

Archer, J. A., Hutchison, I. L., Dorudi, S., Stansfeld, S. A., & Korszun, A. (2012). Interrelationship of depression, stress and inflammation in cancer patients: a preliminary study. *Journal of Affective Disorders*, *143*(1-3), 39-46.

Argiles, J. M., Busquets, S., Felipe, A., & Lopez-Soriano, F. J. (2005). Molecular mechanisms involved in muscle wasting in cancer and ageing: cachexia versus sarcopenia. *International Journal of Biochemical Cell Biology*, *37*(5), 1084-1104.

Argiles, J. M., Busquets, S., Stemmler, B., & Lopez-Soriano, F. J. (2014). Cancer cachexia: understanding the molecular basis. *Nature Reviews Cancer*, *14*(11), 754-762.

Argiles, J. M., Lopez-Soriano, F. J., Toledo, M., Betancourt, A., Serpe, R., & Busquets, S. (2011). The cachexia score (CASCO): a new tool for staging cachectic cancer patients. *Journal of Cachexia Sarcopenia and Muscle*, *2*(2), 87-93.

Attner, P., Du, J., Nasman, A., Hammarstedt, L., Ramqvist, T., Lindholm, J., Munck-Wikland, E. (2010). The role of human papillomavirus in the increased incidence of base of tongue cancer. *International Journal of Cancer*, *126*(12), 2879-2884.

Aviles-Jurado, F. X., Terra, X., Gomez, D., Flores, J. C., Raventos, A., Maymo-Masip, E., Chacon, M. R. (2014). Low blood levels of sTWEAK are related to locoregional failure in head and neck cancer. *European Archives of Otorhinolaryngology*.

Baldwin, C., Spiro, A., Ahern, R., & Emery, P. W. (2012). Oral nutritional interventions in malnourished patients with cancer: a systematic review and meta-analysis. *Journal of the National Cancer Institute*, *104*(5), 371-385.

Balkwill, F. (2002). Tumor necrosis factor or tumor promoting factor? *Cytokine Growth Factor Review*, *13*(2), 135-141.

Baltazar, G. A., Pate, A. J., Panigrahi, B., LaBoy, S., Prosniak, R., Mody, A., & Chendrasekhar, A. (2015). Malnutrition as measured by albumin and prealbumin on admission is associated with poor outcomes after severe traumatic brain injury. *Am Surg*, *81*(2), 61-63.

Baltgalvis, K. A., Berger, F. G., Pena, M. M., Davis, J. M., Muga, S. J., & Carson, J. A. (2008). Interleukin-6 and cachexia in ApcMin/+ mice. *American Journal of Physiology*, *294*(2), R393-R401.

Banks, W. A. (2001). Anorectic effects of circulating cytokines: role of the vascular blood-brain barrier. *Nutrition*, *17*(5), 434-437.

Barrera, T. L., & Norton, P. J. (2009). Quality of life impairment in generalized anxiety disorder, social phobia, and panic disorder. *Journal of Anxiety Disorders*, *23*(8), 1086-1090.

Bayliss, T. J., Smith, J. T., Schuster, M., Dragnev, K. H., & Rigas, J. R. (2011). A humanized anti-IL-6 antibody (ALD518) in non-small cell lung cancer. *Expert Opinion on Biological Therapy*, *11*(12), 1663-1668.

Ben-Noun, L. L. (2004). The disease that caused weight loss in King David the Great. *Journal of Gerontology*, *59*(2), 143-145.

Bennani-Baiti, N., & Walsh, D. (2009). What is cancer anorexia-cachexia syndrome? A historical perspective. *The Journal of the Royal College of Physicians Edinburgh*, *39*(3), 257-262.

Benny Klimek, M. E., Aydogdu, T., Link, M. J., Pons, M., Koniaris, L. G., & Zimmers, T. A. (2010). Acute inhibition of myostatin-family proteins preserves skeletal muscle in mouse models of cancer cachexia. *Biochemical and Biophysical Research Communications*, *391*(3), 1548-1554.

Beutler, B., & Cerami, A. (1986). Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature*, *320*(6063), 584-588.

Bhattacharya, S., Katlinski, K. V., Reichert, M., Takano, S., Brice, A., Zhao, B., .Fuchs, S. Y. (2014). Triggering ubiquitination of IFNAR1 protects tissues from inflammatory injury. *EMBO Molecular Medicine*, *6*(3), 384-397.

Bian, Y., Hall, B., Sun, Z. J., Molinolo, A., Chen, W., Gutkind, J. S., Kulkarni, A. B. (2012). Loss of TGF-beta signaling and PTEN promotes head and neck squamous cell carcinoma through cellular senescence evasion and cancer-related inflammation. *Oncogene*, *31*(28), 3322-3332.

Bing, C. (2011). Lipid mobilization in cachexia: mechanisms and mediators. *Current Opinion in Supportive Palliative Care*, *5*(4), 356-360.

Bing, C., & Trayhurn, P. (2009). New insights into adipose tissue atrophy in cancer cachexia. *Proc Nutr Soc*, *68*(4), 385-392.

Blomberg, M., Nielsen, A., Munk, C., & Kjaer, S. K. (2011). Trends in head and neck cancer incidence in Denmark, 1978-2007: focus on human papillomavirus associated sites. *International Journal of Cancer*, *129*(3), 733-741.

Blum, D., Omlin, A., Baracos, V. E., Solheim, T. S., Tan, B. H., Stone, P., Strasser, F. (2011). Cancer cachexia: a systematic literature review of items and domains associated with involuntary weight loss in cancer. *Critical Reviews in Oncology/Hematology*, *80*(1), 114-144.

Bond, S. M., Dietrich, M. S., Shuster, J. L. J., & Murphy, B. A. (2012). Delirium in patients with head and neck cancer in the outpatient treatment setting. *Supportive Care in Cancer*, *20*(5), 1023-1030.

Bond, S. M., Hawkins, D. K., & Murphy, B. A. (2014). Caregiver-reported neuropsychiatric symptoms in patients undergoing treatment for head and neck cancer: a pilot study. *Cancer Nursing*, *37*(3), 227-235.

Bonetto, A., Penna, F., Aversa, Z., Mercantini, P., Baccino, F. M., Costelli, P., .Muscaritoli, M. (2013). Early changes of muscle insulin-like growth factor-1 and myostatin gene expression in gastric cancer patients. *Muscle and Nerve*, *48*(3), 387-392.

Bonomi, M., Patsias, A., Posner, M., & Sikora, A. (2014). The role of inflammation in head and neck cancer. *Advanced Experiments in Medical Biology*, *816*, 107-127.

Bosaeus, I., Daneryd, P., Svanberg, E., & Lundholm, K. (2001). Dietary intake and resting energy expenditure in relation to weight loss in unselected cancer patients. *International Journal of Cancer*, *93*(3), 380-383.

Bragelmann, J., Dagogo-Jack, I., El Dinali, M., Stricker, T., Brown, C. D., Zuo, Z., Seiwert, T. Y. (2013). Oral cavity tumors in younger patients show a poor prognosis and do not contain viral RNA. *Oral Oncology*, *49*(6), 525-533.

Braun, T. P., & Marks, D. L. (2010). Pathophysiology and treatment of inflammatory anorexia in chronic disease. *Journal of Cachexia Sarcopenia and Muscle*, *1*(2), 135-145.

Braun, T. P., Zhu, X., Szumowski, M., Scott, G. D., Grossberg, A. J., Levasseur, P. R., Marks, D. L. (2011). Central nervous system inflammation induces muscle atrophy via activation of the hypothalamic-pituitary-adrenal axis. *Journal of Experimental Medicine*, *208*(12), 2449-2463.

Brink, M., Price, S. R., Chrast, J., Bailey, J. L., Anwar, A., Mitch, W. E., & Delafontaine, P. (2001). Angiotensin II induces skeletal muscle wasting through enhanced protein degradation and down-regulates autocrine insulin-like growth factor I. *Endocrinology*, *142*(4), 1489-1496.

Busquets, S., Toledo, M., Orpi, M., Massa, D., Porta, M., Capdevila, E., . Argiles, J. M. (2012). Myostatin blockage using actRIIB antagonism in mice bearing the Lewis lung carcinoma results in the improvement of muscle wasting and physical performance. *Journal of Cachexia Sarcopenia and Muscle*, *3*(1), 37-43.

Bye, A., Jordhoy, M. S., Skjegstad, G., Ledsaak, O., Iversen, P. O., & Hjermstad, M. J. (2013). Symptoms in advanced pancreatic cancer are of importance for energy intake. *Supportive Care Cancer*, *21*(1), 219-227.

Cahlin, C., Korner, A., Axelsson, H., Wang, W., Lundholm, K., & Svanberg, E. (2000). Experimental cancer cachexia: the role of host-derived cytokines interleukin (IL)-6, IL-12, interferon-gamma, and tumor necrosis factor alpha evaluated in gene knockout, tumor-bearing mice on C57 Bl background and eicosanoid-dependent cachexia. *Cancer Research*, *60*(19), 5488-5493.

Caro, M. M., Laviano, A., Pichard, C., & Candela, C. G. (2007). Relationship between nutritional intervention and quality of life in cancer patients. *Nutrition Hospital*, *22*(3), 337-350.

Cerezo, L., Lopez, C., de la Torre, A., Suarez, D., Hervas, A., Ruiz, A., Sandoval, P. (2014). Incidence of human papillomavirus-related oropharyngeal cancer and outcomes after chemoradiation in a population of heavy smokers. *Head and Neck*, *36*(6), 782-786.

Chacon-Cabrera, A., Fermoselle, C., Urtreger, A. J., Mateu-Jimenez, M., Diament, M. J., de Kier Joffe, E. D., .Barreiro, E. (2014). Pharmacological strategies in lung cancer-induced cachexia: effects on muscle proteolysis, autophagy, structure, and weakness. *Journal of Cell Physiology*, *229*(11), 1660-1672.

Chai, S. J., Pua, K. C., Saleh, A., Yap, Y. Y., Lim, P. V., Subramaniam, S. K., Yap, L. F. (2012). Clinical significance of plasma Epstein-Barr Virus DNA loads in a large cohort of Malaysian patients with nasopharyngeal carcinoma. *Journal of Clinical Virology*, *55*(1), 34-39.

Chaturvedi, A. K., Engels, E. A., Pfeiffer, R. M., Hernandez, B. Y., Xiao, W., Kim, E., Gillison, M. L. (2011). Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *Journal of Clinical Oncology*, *29*(32), 4294-4301.

Chen, C. J., Sung, W. W., Su, T. C., Chen, M. K., Wu, P. R., Yeh, K. T., Lee, H. (2013). High expression of interleukin 10 might predict poor prognosis in early stage oral squamous cell carcinoma patients. *Clinica Chimica Acta*, *415*, 25-30.

Chen, H., Jian, Y.S., Sun, Y., & Xiong, Y.C (2015). p38 and interleukin-1 beta pathway via toll-like receptor 4 contributed to the skin and muscle incision and retraction-induced allodynia. *Journal of Surgical Research*.

Chen, S. C., Tsai, M. C., Liu, C. L., Yu, W. P., Liao, C. T., & Chang, J. T. (2009). Support needs of patients with oral cancer and burden to their family caregivers. *Cancer Nursing*, *32*(6), 473-481.

Chiao, Y. A., Ramirez, T. A., Zamilpa, R., Okoronkwo, S. M., Dai, Q., Zhang, J., Lindsey, M. L. (2012). Matrix metalloproteinase-9 deletion attenuates myocardial fibrosis and diastolic dysfunction in ageing mice. *Cardiovasc Res*, *96*(3), 444-455.

Chung, Y. C., & Chang, Y. F. (2003). Serum C-reactive protein correlates with survival in colorectal cancer patients but is not an independent prognostic indicator. *European Journal of Gastroenterology and Hepatology*, *15*(4), 369-373.

Citrin, D. E., Hitchcock, Y. J., Chung, E. J., Frandsen, J., Urick, M. E., Shield, W., & Gaffney, D. (2012). Determination of cytokine protein levels in oral secretions in patients undergoing radiotherapy for head and neck malignancies. *Radiation Oncology*, *7*, 64.

Clark, M. M., Atherton, P. J., Lapid, M. I., Rausch, S. M., Frost, M. H., Cheville, A. L., .Rummans, T. A. (2014). Caregivers of patients with cancer fatigue: a high level of symptom burden. *American Journal of Hospice and Palliative Care*, *31*(2), 121-125.

Cleeland, C. S., Bennett, G. J., Dantzer, R., Dougherty, P. M., Dunn, A. J., Meyers, C. A., Lee, B. N. (2003). Are the symptoms of cancer and cancer treatment due to a shared biologic mechanism? A cytokine-immunologic model of cancer symptoms. *Cancer*, *97*(11), 2919-2925.

Cohen, S., Brault, J. J., Gygi, S. P., Glass, D. J., Valenzuela, D. M., Gartner, C., Goldberg, A. L. (2009). During muscle atrophy, thick, but not thin, filament components are degraded by MuRF1-dependent ubiquitylation. *Journal of Cell Biology*, *185*(6), 1083-1095.

Conway, D. I., Stockton, D. L., Warnakulasuriya, K. A., Ogden, G., & Macpherson, L. M. (2006). Incidence of oral and oropharyngeal cancer in United Kingdom (1990-1999) -- recent trends and regional variation. *Oral Oncol*, *42*(6), 586-592.

Cooperstein, E., Gilbert, J., Epstein, J.B., Dietrich, M.S., Bond, S.M., Ridner, S.H., Wells, N., Cmelak, A., Murphy, B.A. (2012). Vanderbilt head and neck symptom survey version 2.0: report of the development and initial testing of a subscale for assessment of oral health. *Head and Neck,* 24(6), 797-804.

Costelli, P., Muscaritoli, M., Bossola, M., Penna, F., Reffo, P., Bonetto, A., .Rossi Fanelli, F. (2006). IGF-1 is downregulated in experimental cancer cachexia. *Am J Physiol Regul Integr Comp Physiol*, *291*(3), R674-R683.

Couch, M. E., Dittus, K., Toth, M. J., Willis, M. S., Guttridge, D. C., George, J. R., .Der-Torossian, H. (2014). Cancer cachexia update in head and neck cancer: Definitions and diagnostic features. *Head and Neck*.

Dalal, S., Hui, D., Bidaut, L., Lem, K., Del Fabbro, E., Crane, C., .Bruera, E. (2012). Relationships among body mass index, longitudinal body composition alterations, and survival in patients with locally advanced pancreatic cancer receiving chemoradiation: a pilot study. *Journal of Pain Symptom Management*, *44*(2), 181-191.

Dalamaga, M. (2013). Interplay of adipokines and myokines in cancer pathophysiology: Emerging therapeutic implications. *World Journal of Experimental Medicine*, *3*(3), 26-33.

Dallapiazza, R., Bond, A. E., Grober, Y., Louis, R. G., Payne, S. C., Oldfield, E. H., & Jane, J. A. J. (2014). Retrospective analysis of a concurrent series of microscopic versus endoscopic transsphenoidal surgeries for Knosp Grades 0-2 nonfunctioning pituitary macroadenomas at a single institution. *Journal of Neurosurgery*, *121*(3), 511-517.

Das, S. K., Eder, S., Schauer, S., Diwoky, C., Temmel, H., Guertl, B., Hoefler, G. (2011). Adipose triglyceride lipase contributes to cancer-associated cachexia. *Science*, *333*(6039), 233-238.

de Carvalho, T. M., Miguel Marin, D., da Silva, C. A., de Souza, A. L., Talamoni, M., Lima, C. S., & Monte Alegre, S. (2013). Evaluation of patients with head and neck cancer performing standard treatment in relation to body composition, resting metabolic rate, and inflammatory cytokines. *Head and Neck*.

Deans, D. A., Tan, B. H., Ross, J. A., Rose-Zerilli, M., Wigmore, S. J., Howell, W. M., Fearon, K. C. (2009). Cancer cachexia is associated with the IL10 -1082 gene promoter polymorphism in patients with gastroesophageal malignancy. *Americna Journal of Clinical Nutrition*, *89*(4), 1164-1172.

DeBoer, M. D. (2010). Update on melanocortin interventions for cachexia: progress toward clinical application. *Nutrition*, *26*(2), 146-151.

Del Fabbro, E., Dev, R., Hui, D., Palmer, L., & Bruera, E. (2013). Effects of melatonin on appetite and other symptoms in patients with advanced cancer and cachexia: a double-blind placebo-controlled trial. *Journal of Clinical Oncology*, *31*(10), 1271-1276.

DeNavas-Walt, C., Proctor, B.D. Income and poverty in the United States 2013. Accessed February 11, 2015 from: http://www.census.gov/content/dam/Census/library/publications/2014/demo/p60-249.pdf

Denis, F., Garaud, P., Bardet, E., Alfonsi, M., Sire, C., Germain, T., Calais, G. (2004). Final results of the 94–01 French Head and Neck Oncology and Radiotherapy Group randomized trial comparing radiotherapy alone with concomitant radiochemotherapy in advanced-stage oropharynx carcinoma. *Journal of Clinical Oncology*, *22*(1), 69-76.

Derynck, R., Akhurst, R. J., & Balmain, A. (2001). TGF-beta signaling in tumor suppression and cancer progression. *Nature Genetics*, *29*(2), 117-129.

Dewys, W. D., Begg, C., Lavin, P. T., Band, P. R., Bennett, J. M., Bertino, J. R., .Ezdinli, E. Z. (1980). Prognostic effect of weight loss prior tochemotherapy in cancer patients. *The American journal of medicine*, *69*(4), 491-497.

Dillon, E. L., Basra, G., Horstman, A. M., Casperson, S. L., Randolph, K. M., Durham, W. J., Hatch, S. S. (2012). Cancer cachexia and anabolic interventions: a case report. *Journal of cachexia, sarcopenia and muscle*, *3*(4), 253-263.

Dodson, S., Baracos, V. E., Jatoi, A., Evans, W. J., Cella, D., Dalton, J. T., & Steiner, M. S. (2011). Muscle wasting in cancer cachexia: clinical implications, diagnosis, and emerging treatment strategies. *Annual review of medicine*, *62*, 265-279.

Donnelly, M., Anderson, L. A., Johnston, B. T., Watson, R. G. P., Murphy, S. J., Comber, H., Murray, L. J. (2008). Oesophageal cancer: caregiver mental health and strain. *Psycho-Oncology*, *17*(12), 1196-1201.

Donohoe, C. L., Ryan, A. M., & Reynolds, J. V. (2011). Cancer cachexia: mechanisms and clinical implications. *Gastroenterology research and practice*, *2011*.

Druzgal, C. H., Chen, Z., Yeh, N. T., Thomas, G. R., Ondrey, F. G., Duffey, D. C.,Van Waes, C. (2005). A pilot study of longitudinal serum cytokine and angiogenesis factor levels as markers of therapeutic response and survival in patients with head and neck squamous cell carcinoma. *Head Neck*, *27*(9), 771-784.

Duffy, S. A., Taylor, J. M., Terrell, J. E., Islam, M., Li, Y., Fowler, K. E., Teknos, T. N. (2008). Interleukin-6 predicts recurrence and survival among head and neck cancer patients. *Cancer*, *113*(4), 750-757.

Ehrsson, Y. T., Hellström, P. M., Brismar, K., Sharp, L., Langius-Eklöf, A., & Laurell, G. (2010). Explorative study on the predictive value of systematic inflammatory and metabolic markers on weight loss in head and neck cancer patients undergoing radiotherapy. *Supportive care in cancer*, *18*(11), 1385-1391.

Evans, W. J., Morley, J. E., Argilés, J., Bales, C., Baracos, V., Guttridge, D., Mantovani, G. (2008). Cachexia: a new definition. *Clinical nutrition*, *27*(6), 793-799.

Fakhry, C., Rosenthal, B. T., Clark, D. P., & Gillison, M. L. (2011). Associations between oral HPV16 infection and cytopathology: evaluation of an oropharyngeal “pap-test equivalent in high-risk populations. *Cancer Prevention Research*.

Farhan-Alanie, O. M., McMahon, J., & McMillan, D. C. (2015). Systemic inflammatory response and survival in patients undergoing curative resection of oral squamous cell carcinoma. *Br J Oral Maxillofac Surg*, *53*(2), 126-131.

Fearon, K., Arends, J., & Baracos, V. (2013). Understanding the mechanisms and treatment options in cancer cachexia. *Nature Reviews in Clinical Oncology*, *10*(2), 90-99.

Fearon, K., Strasser, F., Anker, S. D., Bosaeus, I., & Bruera, E. (2011). Definition and classification of cancer cachexia: an international consensus. *The lancet oncology*.

Fearon, K. C. H. (2012). The 2011 ESPEN Arvid Wretlind lecture: cancer cachexia: the potential impact of translational research on patient-focused outcomes. *Clinical Nutrition*, *31*(5), 577-582.

Fearon, K. C. H., Glass, D. J., & Guttridge, D. C. (2012b). Cancer cachexia: mediators, signaling, and metabolic pathways. *Cell metabolism*, *16*(2), 153-166.

Ferguson, R. P., O'Connor, P., Crabtree, B., Batchelor, A., Mitchell, J., & Coppola, D. (1993). Serum albumin and prealbumin as predictors of clinical outcomes of hospitalized elderly nursing home residents. *J Am Geriatr Soc*, *41*(5), 545-549.

Gaudet, M. M., Patel, A. V., Sun, J., Hildebrand, J. S., McCullough, M. L., Chen, A. Y., & Gapstur, S. M. (2012). Prospective studies of body mass index with head and neck cancer incidence and mortality. *Cancer Epidemiol Biomarkers Prev*, *21*(3), 497-503.

Germolec, D. R., Frawley, R. P., & Evans, E. (2010). Markers of inflammation. *Methods in Molecularl Biology*, *598*, 53-73.

Giannousi, Z., Gioulbasanis, I., Pallis, A. G., & Xyrafas, A. (2012). Nutritional status, acute phase response and depression in metastatic lung cancer patients: associations and association prognosis. *Supportive Care in Cancer*.

Gillison, M. L., Alemany, L., Snijders, P. J. F., Chaturvedi, A., Steinberg, B. M., Schwartz, S., & Castellsagué, X. (2012). Human papillomavirus and diseases of the upper airway: head and neck cancer and respiratory papillomatosis. *Vaccine*, *30*, F34-F54.

Gillison, M. L., Koch, W. M., Capone, R. B., Spafford, M., Westra, W. H., Wu, L., Symer, D. E. (2000). Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *Journal of the National Cancer Institute*, *92*(9), 709-720.

Gioulbasanis, I., Patrikidou, A., & Kitikidou, K. (2012). Baseline plasma levels of interleukin-8 in stage IV non-small-cell lung cancer patients: relationship with nutritional status and prognosis. *Nutrition and Cancer*.

Gonzalez, M. C., Pastore, C. A., Orlandi, S. P., & Heymsfield, S. B. (2014). Obesity paradox in cancer: new insights provided by body composition. *American Journal of Clinical Nutrition*, *99*(5), 999-1005.

Gourin, C. G., & Podolsky, R. H. (2006). Racial disparities in patients with head and neck squamous cell carcinoma. *Laryngoscope*, *116*(7), 1093-1106.

Green, V. L., Irune, E., Prasai, A., Alhamarneh, O., Greenman, J., & Stafford, N. D. (2012). Serum IL10, IL12 and circulating CD4+CD25high T regulatory cells in relation to long-term clinical outcome in head and neck squamous cell carcinoma patients. *Int J Oncol*, *40*(3), 833-839.

Grossberg, A. J., Scarlett, J. M., & Marks, D. L. (2010). Hypothalamic mechanisms in cachexia. *Physiological Behavior*, *100*(5), 478-489.

Gullett, N. P., Mazurak, V., & Hebbar, G. (2011). Nutritional interventions for cancer-induced cachexia. *Current problems in Cancer*.

Guntinas-Lichius, O., Wendt, T., & Buentzel, J. (2010). Head and neck cancer in Germany: a site-specific analysis of survival of the Thuringian cancer registration database. *Journal of cancer research and clinical oncology*.

Gupta, D. & Lis, C.G. (2010) Pretreatment serum albumin as a predictor of cancer survival: A systematic review of the epidemiological literature. *Journal of Nutrition,* 9:69.1-16.

Gupta, S. C., Kim, J. H., Kannappan, R., Reuter, S., Dougherty, P. M., & Aggarwal, B. B. (2011). Role of nuclear factor kappaB-mediated inflammatory pathways in cancer-related symptoms and their regulation by nutritional agents. *Experimental Biological Medicine*, *236*(6), 658-671.

Halade, G.V., Jin, Y.F., Lindsey, M.L. (2013). Matrix metalloproteinase (MMP)-9: a proximal biomarker for cardiac remodeling and a distal biomarker for inflammation. *Pharmacology Therapy,* 139(1): 32-40.

Hains, F., Waalen, J., & Mior, S. (1998). Psychometric properties of the neck disability index. *Journal of Manipulative Physiological Therapy*, *21*(2), 75-80.

Hammarstedt, L., Dahlstrand, H., Lindquist, D., Onelöv, L., Ryott, M., Luo, J., Munck-Wikland, E. (2007). The incidence of tonsillar cancer in Sweden is increasing. *Acta oto-laryngologica*, *127*(9), 988-992.

Han, B. L., Xu, X. Y., Zhang, C. Z., Wu, J. J., Han, C. F., Wang, H., Xie, Y. (2012). Systematic review on Epstein-Barr virus (EBV) DNA in diagnosis of nasopharyngeal carcinoma in Asian populations. *Asian Pacific Journal of Cancer Prevention*, *13*(6), 2577-2581.

Han, H. Q., & Mitch, W. E. (2011). Targeting the myostatin signaling pathway to treat muscle wasting diseases. *Current Opinion in Supportive Palliative Care*, *5*(4), 334-341.

He, W. A., Berardi, E., Cardillo, V. M., Acharyya, S., Aulino, P., Thomas-Ahner, J., .Guttridge, D. C. (2013). NF-kappaB-mediated Pax7 dysregulation in the muscle microenvironment promotes cancer cachexia. *Journal of Clinical Investigation*, *123*(11), 4821-4835.

Heimdal, J. H., Aarstad, H. J., Klementsen, B., & Olofsson, J. (1999). Ex vivo interleukin (IL)-1 beta, IL-6, IL-12 and tumor necrosis factor-alpha responsiveness with monocytes from patients with head and neck carcinoma. *European Archives of Otorhinolaryngology*, *256*(5), 250-256.

Heimdal, J.-H., Kross, K., Klementsen, B., Olofsson, J., & Aarstad, H. J. (2008). Stimulated monocyte IL-6 secretion predicts survival of patients with head and neck squamous cell carcinoma. *BMC cancer*, *8*(1), 34.

Henkin, R.I., Schmidt, L., & Velicu, L. (2013). Interleukin 6 in hyposmia. *JAMA Otolaryngology Head & Neck Surgery.* 139(7), 728-34.

Hitchcock, Y. J., Tward, J. D., Szabo, A., Bentz, B. G., & Shrieve, D. C. (2009). Relative contributions of radiation and cisplatin-based chemotherapy to sensorineural hearing loss in head-and-neck cancer patients. *Int J Radiat Oncol Biol Phys*, *73*(3), 779-788.

Holzer, P. (2010). Opioid antagonists for prevention and treatment of opioid-induced gastrointestinal effects. *Current Opinion in Anesthesiology*, *23*(5), 616-622.

Hopkinson, J. B. (2010). The emotional aspects of cancer anorexia. *Current opinion in supportive and palliative care*, *4*(4), 254-258.

Hotamisligil, G. S. (2000). Molecular mechanisms of insulin resistance and the role of the adipocyte. *International journal of obesity.*, *24*(4), 23-27.

Huhmann, M. B., & Cunningham, R. S. (2005). Importance of nutritional screening in treatment of cancer-related weight loss. *Lancet Oncology*, *6*(5), 334-343.

Hursting, S. D., & Berger, N. A. (2010). Energy balance, host-related factors, and cancer progression. *Journal of Clinical Oncology*, *28*(26), 4058-4065.

Ihloff, A. S., Petersen, C., Hoffmann, M., Knecht, R., & Tribius, S. (2010). Human papilloma virus in locally advanced stage III/IV squamous cell cancer of the oropharynx and impact on choice of therapy. *Oral Oncology*, *46*(10), 705-711.

Imayama, I., Alfano, C. M., Neuhouser, M. L., George, S. M., Smith, A. W., Baumgartner, R. N., & McTiernan, A. (2013). Weight, inflammation, cancer-related symptoms and health-related quality of life among breast cancer survivors. *Breast Cancer Researc & Treatment*.

Isayeva, T., Li, Y., Maswahu, D., & Brandwein-Gensler, M. (2012). Human papillomavirus in non-oropharyngeal head and neck cancers: a systematic literature review. 2013. *Head Neck Pathology*, *6 Suppl 1*, S104-S120.

Ito, R., Shin-Ya., M., Kishida, T., Urano, A., Takada, R, Sakadami, J., Imanishi, J., Kita, M., Ueda, Y., Iwakura, Y, Kataoka, K., Okanoue, T., & Mazda, O. (2006). Interferon-gamma is causatively involved in experimental inflammatory bowel disease in mice. *Clinical Experimental Biology,* 146(2), 330-338.

Jager-Wittenaar, H., Dijkstra, P. U., Vissink, A., Langendijk, J. A., van der Laan, B. F., Pruim, J., & Roodenburg, J. L. (2011). Changes in nutritional status and dietary intake during and after head and neck cancer treatment. *Head & Neck*, *33*(6), 863-870.

Jatoi, A., Ritter, H. L., Dueck, A., Nguyen, P. L., Nikcevich, D. A., Luyun, R. F., Loprinzi, C. L. (2010). A placebo-controlled, double-blind trial of infliximab for cancer-associated weight loss in elderly and/or poor performance non-small cell lung cancer patients (N01C9). *Lung Cancer*, *68*(2), 234-239.

Jemal, A., Siegel, R., Xu, J., & Ward, E. (2010). Cancer statistics, 2010. *CA Cancer J Clin*, *60*(5), 277-300.

Jiang, N., Deng, J. Y., Ding, X. W., Ke, B., Liu, N., Zhang, R. P., & Liang, H. (2014). Prognostic nutritional index predicts postoperative complications and long-term outcomes of gastric cancer. *World J Gastroenterol*, *20*(30), 10537-10544.

Jimenez, A., Madero, R., Alonso, A., Martinez-Marin, V., Vilches, Y., Martinez, B., Feliu, J. (2011). Symptom clusters in advanced cancer. *Journal of Pain and Symptom Management*, *42*(1), 24-31.

Johnen, H., Lin, S., Kuffner, T., Brown, D. A., Tsai, V. W., Bauskin, A. R., .Breit, S. N. (2007). Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. *Natural Medicine*, *13*(11), 1333-1340.

Johns, N., Stephens, N. A., & Fearon, K. C. (2013). Muscle wasting in cancer. *Int ernational Journal of Biochemistry and Cell Biology*, *45*(10), 2215-2229.

Kamath, J. (2012). Cancer-related fatigue, inflammation and thyrotropin-releasing hormone. *Current aging science*, *5*(3), 195-202.

Kanat, O., Cubukcu, E., Avci, N., Budak, F., Ercan, I., Canhoroz, M., & Olmez, F. (2012). Comparison of three different treatment modalities in the management of cancer cachexia. *Tumori*, *99*(2), 229-233.

Kanzaki, M., Soda, K., Gin, P. T., Kai, T., Konishi, F., & Kawakami, M. (2005). Erythropoietin attenuates cachectic events and decreases production of interleukin-6, a cachexia-inducing cytokine. *Cytokine*, *32*(5), 234-239.

Karayiannakis, A. J., Syrigos, K. N., Polychronidis, A., Pitiakoudis, M., Bounovas, A., & Simopoulos, K. (2001). Serum levels of tumor necrosis factor-alpha and nutritional status in pancreatic cancer patients. *Anticancer Research*, *21*(2B), 1355-1358.

Kempf, T., von Haehling, S., Peter, T., Allhoff, T., Cicoira, M., Doehner, W., Wollert, K. C. (2007). Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. *Journal of American Collge of Cardiology*, *50*(11), 1054-1060.

Khal, J., Hine, A. V., Fearon, K. C., Dejong, C. H., & Tisdale, M. J. (2005). Increased expression of proteasome subunits in skeletal muscle of cancer patients with weight loss. *International Journal of Biochemistry and Cell Biology*, *37*(10), 2196-2206.

Kim, H. J., Kim, H. J., Yun, J., Kim, K. H., Kim, S. H., Lee, S. C., Hong, D. S. (2012). Pathophysiological role of hormones and cytokines in cancer cachexia. *Journal of Korean Medical Science*, *27*(2), 128-134.

Knudtson, M. D., Klein, B. E. K., Klein, R., & Shankar, A. (2005). Associations with weight loss and subsequent mortality risk. *Annals of epidemiology*, *15*(7), 483-491.

Koyama, S., Hata, S., Witt, C. C., Ono, Y., Lerche, S., Ojima, K., . . . Sorimachi, H. (2008). Muscle RING-finger protein-1 (MuRF1) as a connector of muscle energy metabolism and protein synthesis. *Journal of Molecular Biology*, *376*(5), 1224-1236.

Krasnow, S. M., & Marks, D. L. (2010). Neuropeptides in the pathophysiology and treatment of cachexia. *Current Opinion in Supportive and Palliative Care*, *4*(4), 266-271.

Kumar, A., Bhatnagar, S., & Paul, P. K. (2012). TWEAK and TRAF6 regulate skeletal muscle atrophy. *Currenty Opinion in Clinical Nutrition and Metabolic Care*, *15*(3), 233-239.

Kurz, A., & Sessler, D. I. (2003). Opioid-induced bowel dysfunction. *Drugs*, *63*(7), 649-671.

Laine, A., Iyengar, P., & Pandita, T. K. (2013). The role of inflammatory pathways in cancer-associated cachexia and radiation resistance. *Molecular Cancer Research*, *11*(9), 967-972.

Laird, B. J., Scott, A. C., Colvin, L. A., McKeon, A. L., Murray, G. D., Fearon, K. C., & Fallon, M. T. (2011a). Cancer pain and its relationship to systemic inflammation: an exploratory study. *Pain*, *152*(2), 460-463.

Laird, B. J., Scott, A. C., Colvin, L. A., McKeon, A. L., Murray, G. D., Fearon, K. C., & Fallon, M. T. (2011b). Pain, depression, and fatigue as a symptom cluster in advanced cancer. *Journal of Pain Symptom Management*, *42*(1), 1-11.

Landis, S. H., Muellerova, H., Mannino, D. M., Menezes, A. M., Han, M. K., van der Molen, T., Davis, K. J. (2014). Continuing to Confront COPD International Patient Survey: methods, COPD prevalence, and disease burden in 2012-2013. *International Journal of Chronic Obstruct ive Pulmonary Disorders*, *9*, 597-611.

Larsson, M., Hedelin, B., Johansson, I., & Athlin, E. (2005). Eating problems and weight loss for patients with head and neck cancer: a chart review from diagnosis until one year after treatment. *Cancer Nursing*, *28*(6), 425-435.

Laugsand, E. A., Kaasa, S., & Klepstad, P. (2011). Management of opioid-induced nausea and vomiting in cancer patients: systematic review and evidence-based recommendations. *Palliative medicine*, *25*(5), 442-453.

Laviano, A., Seelaender, M., Rianda, S., Silverio, R., & Rossi Fanelli, F. (2012). Neuroinflammation: a contributing factor to the pathogenesis of cancer cachexia. *Critical Reviewes in Oncogenesis*, *17*(3), 247-251.

Lewis, J. S. J., & Chernock, R. D. (2014). Human papillomavirus and Epstein Barr virus in head and neck carcinomas: suggestions for the new WHO classification. *Head and Neck Pathology*, *8*(1), 50-58.

Liu, Y., Cheng, H., Zhou, Y., Zhu, Y., Bian, R., Chen, Y., Zhu, D. (2013). Myostatin induces mitochondrial metabolic alteration and typical apoptosis in cancer cells. *Cell Death Disease Journal*, *4*, e494.

Liu, Z., Li, L., Yang, Z., Luo, W., Li, X., Yang, H., Fang, W. (2010). Increased expression of MMP9 is correlated with poor prognosis of nasopharyngeal carcinoma. *BMC Cancer*, *10*, 270.

Longacre, M. L., Ridge, J. A., Burtness, B. A., Galloway, T. J., & Fang, C. Y. (2012). Psychological functioning of caregivers for head and neck cancer patients. *Oral Oncology*, *48*(1), 18-25.

Lucia, S., Esposito, M., Fanelli, F. R., & Muscaritoli, M. (2012). Cancer Cachexia: From Molecular Mechanisms to Patient's Care. *Critical Reviews in Oncogenesis*, *17*(3).

Mahmoud, F. A., & Rivera, N. I. (2002). The role of C-reactive protein as a prognostic indicator in advanced cancer. *Current oncology reports*, *4*(3), 250-255.

Maltoni, M., Nanni, O., Scarpi, E., Rossi, D., Serra, P., & Amadori, D. (2001). High-dose progestins for the treatment of cancer anorexia-cachexia syndrome: a systematic review of randomised clinical trials. *Annals of Oncology*, *12*(3), 289-300.

Marcell, T. J. (2003). Sarcopenia: causes, consequences, and preventions. *Journal of Gerontology and Biological Science Medicine*, *58*(10), M911-M916.

Martin, F., Santolaria, F., Batista, N., Milena, A., Gonzalez-Reimers, E., Brito, M. J., & Oramas, J. (1999). Cytokine levels (IL-6 and IFN-γ amma), acute phase response and nutritional status as prognostic factors in lung cancer. *Cytokine*, *11*(1), 80-86.

Martin, L., Birdsell, L., Macdonald, N., Reiman, T., Clandinin, M. T., McCargar, L. J., .Baracos, V. E. (2013). Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. *Journal of Clinical Oncology*, *31*(12), 1539-1547.

Matthys, P., Dijkmans, R., Proost, P., Van Damme, J., Heremans, H., Sobis, H., & Billiau, A. (1991). Severe cachexia in mice inoculated with interferon-gamma-producing tumor cells. *International Journal of Cancer*, *49*(1), 77-82

.

McClement, S. (2005). Cancer anorexia-cachexia syndrome: psychological effect on the patient and family. *Journal of Wound Ostomy Continence Nursing*, *32*(4), 264-268.

Mehendale, S. R., & Yuan, C.-S. (2006). Opioid-induced gastrointestinal dysfunction. *Digestive Diseases*, *24*(1-2), 105-112.

Mirza, K. A., Wyke, S. M., & Tisdale, M. J. (2011). Attenuation of muscle atrophy by an N-terminal peptide of the receptor for proteolysis-inducing factor (PIF). *British Journal of Cancer*, *105*(1), 83-88.

Mitch, W. E., & Goldberg, A. L. (1996). Mechanisms of muscle wasting. The role of the ubiquitin-proteasome pathway. *New England Journal of Medicine*, *335*(25), 1897-1905.

Mittal, A., Bhatnagar, S., Kumar, A., Lach-Trifilieff, E., Wauters, S., Li, H., Kumar, A. (2010). The TWEAK-Fn14 system is a critical regulator of denervation-induced skeletal muscle atrophy in mice. *Journal of Cell Biology*, *188*(6), 833-849.

Mojtahedi, Z., Khademi, B., Erfani, N., Taregh, Y., Rafati, Z., Malekzadeh, M. * Ghaderi, A. (2014). Serum levels of interleukin-7 and interleukin-8 in head and neck squamous cell carcinoma. *Indian Journal of Cancer,* 51(3), 227-230.

Moore-Carrasco, R., Busquets, S., Almendro, V., Palanki, M., Lopez-Soriano, F. J., & Argiles, J. M. (2007). The AP-1/NF-kappaB double inhibitor SP100030 can revert muscle wasting during experimental cancer cachexia. *International Journal of Oncology*, *30*(5), 1239-1245.

Morley, J. E., & Farr, S. A. (2008). Cachexia and neuropeptide Y. *Nutrition*, *24*(9), 815-819.

Moses, A. G., Maingay, J., Sangster, K., Fearon, K. C., & Ross, J. A. (2009). Pro-inflammatory cytokine release by peripheral blood mononuclear cells from patients with advanced pancreatic cancer: relationship to acute phase response and survival. *Oncology Reports*, *21*(4), 1091-1095.

Moubayed, S. P., Sampalis, J. S., Ayad, T., Guertin, L., Bissada, E., Gologan, O. E., .Christopoulos, A. (2014). Predicting Depression and Quality of Life among Long-term Head and Neck Cancer Survivors. *Otolaryngology- Head and Neck Surgery*.

Munshi, A., Pandey, M. B., Durga, T., Pandey, K. C., Bahadur, S., & Mohanti, B. K. (2003). Weight loss during radiotherapy for head and neck malignancies: what factors impact it? *Nutrition and Cancer*, *47*(2), 136-140.

Murphy, B. A., Dietrich, M. S., Wells, N., Dwyer, K., Ridner, S. H., Silver, H. J., .Netterville, J. (2010). Reliability and validity of the Vanderbilt Head and Neck Symptom Survey: a tool to assess symptom burden in patients treated with chemoradiation. *Head and Neck*, *32*(1), 26-37.

Mydlarz, W. K., Hennessey, P. T., Wang, H., Carvalho, A. L., & Califano, J. A. (2014). Serum biomarkers for detection of head and neck squamous cell carcinoma. *Head Neck*.

Myers, J. S. (2008). Proinflammatory cytokines and sickness behavior: implications for depression and cancer-related symptoms. *Oncology Nursing Forum*, *35*(5), 802-807.

Naeim, A., Dy, S. M., Lorenz, K. A., Sanati, H., Walling, A., & Asch, S. M. (2008). Evidence-based recommendations for cancer nausea and vomiting. *Journal of Clinical Oncology*, *26*(23), 3903-3910.

Nagase, H., & Woessner, J. F. J. (1999). Matrix metalloproteinases. *The Journal of Biological Chemistry*, *274*(31), 21491-21494.

National Cancer Institute. (2013). Definition of head and neck cancer. Retrieved May 28, 2013 from http://www.cancer.gov/cancertopics/types/head-and-neck

National Institute of Health (2015). RBC Count. Retrieved January 20, 2015 from http://www.nlm.nih.gov/medlineplus/ency/article/003644.htm.

National Institute of Medicine and Health (2013). What is Depression? Retrieved June 25, 2013, from http://www.nimh.nih.gov/health/topics/depression/index.shtml

Nicolatou-Galitis, O., Sarri, T., Bowen, J., Di Palma, M., Kouloulias, V. E., Niscola, P., .Lalla, R. V. (2013). Systematic review of anti-inflammatory agents for the management of oral mucositis in cancer patients. *Supportive Care in Cancer*.

O'Neill, J. P., & Shaha, A. R. (2011). Nutrition management of patients with malignancies of the head and neck. *Surgical Clinics of North America*, *91*(3), 631-639.

Oi-Ling, K., Man-Wah, D. T., & Kam-Hung, D. N. (2005). Symptom distress as rated by advanced cancer patients, caregivers and physicians in the last week of life. *Palliative Medicine*, *19*(3), 228-233.

Oliff, A., Defeo-Jones, D., Boyer, M., Martinez, D., Kiefer, D., Vuocolo, G., Socher, S. H. (1987). Tumors secreting human TNF/cachectin induce cachexia in mice. *Cell*, *50*(4), 555-563.

Oliveira, A., Dinis-Oliveira, R. J., Nogueira, A., Goncalves, F., Silva, P., Vieira, C.,Medeiros, R. (2014). Interleukin-1beta genotype and circulating levels in cancer patients: metastatic status and pain perception. *Clin Biochem*, *47*(13-14), 1209-1213.

Op den Kamp, C. M., Langen, R. C., Snepvangers, F. J., de Theije, C. C., Schellekens, J. M., Laugs, F., Schols, A. M. (2013). Nuclear transcription factor kappa B activation and protein turnover adaptations in skeletal muscle of patients with progressive stages of lung cancer cachexia. *American Journal of Clinical Nutrition*, *98*(3), 738-748.

Oz, V., Theilla, M., & Singer, P. (2008). Eating habits and quality of life of patients receiving home parenteral nutrition in Israel. *Clinical Nutrition*, *27*(1), 95-99.

Pai, P. C., Chuang, C. C., Tseng, C. K., Tsang, N. M., Chang, K. P., Yen, T. C., Chang, J. T. (2012). Impact of pretreatment body mass index on patients with head-and-neck cancer treated with radiation. *Int J Radiat Oncol Biol Phys*, *83*(1).

Palus, S., Haehling, S. V., Flach, V. C., & Tschirner…, A. (2013). Simvastatin reduces wasting and improves cardiac function as well as outcome in experimental cancer cachexia. *International journal of Cardiology*.

Pan, H. Y., Sun, H. M., Xue, L. J., Pan, M., Wang, Y. P., Kido, H., & Zhu, J. H. (2014). Ectopic trypsin in the myocardium promotes dilated cardiomyopathy after influenza A virus infection. *Am J Physiol Heart Circ Physiol*, *307*(6), H922-H932.

Parmar, M. P., Swanson, T., & Jagoe, R. T. (2013). Weight changes correlate with alterations in subjective physical function in advanced cancer patients referred to a specialized nutrition and rehabilitation team. *Supportive Care in Cancer*, *21*(7), 2049-2057.

Patra, S. K., & Arora, S. (2012). Integrative role of neuropeptides and cytokines in cancer anorexia-cachexia syndrome. *Clinica Chimica Acta*, *413*(13-14), 1025-1034.

Patterson, J. M., Rapley, T., Carding, P. N., Wilson, J. A., & McColl, E. (2012). Head and neck cancer and dysphagia; caring for carers. *Psychooncology*.

Penna, F., Costamagna, D., Pin, F., Camperi, A., Fanzani, A., Chiarpotto, E. M., .Costelli, P. (2013). Autophagic degradation contributes to muscle wasting in cancer cachexia. *The American journal of pathology*, *182*(4), 1367-1378.

Penner, J. L., McClement, S., Lobchuk, M., & Daeninck, P. (2012). Family members' experiences caring for patients with advanced head and neck cancer receiving tube feeding: a descriptive phenomenological study. *Journal of Pain Symptom Management*, *44*(4), 563-571.

Perboni, S., & Inui, A. (2006). Anorexia in cancer: role of feeding-regulatory peptides. *Philosophical Transactions of the Royal Society*, *361*(1471), 1281-1289.

Pollak, M. (2008). Targeting insulin and insulin-like growth factor signalling in oncology. *Current Opinion in Pharmacology*, *8*(4), 384-392.

Poretti, M. B., Rask-Andersen, M., Kumar, P., Rubiales de Barioglio, S., Fiol de Cuneo, M., Schioth, H. B., & Carlini, V. P. (2015). Ghrelin effects expression of several genes associated with depression-like behavior. *Prog Neuropsychopharmacol Biol Psychiatry*, *56*, 227-234.

Prado, C. M., Antoun, S., Sawyer, M. B., & Baracos, V. E. (2011). Two faces of drug therapy in cancer: drug-related lean tissue loss and its adverse consequences to survival and toxicity. *Current Opinion in Clinical Nutrition and Metabolic Care*, *14*(3), 250-254.

Precious, E., Haran, S., Lowe, D., & Rogers, S. N. (2012). Head and neck cancer patients' perspective of carer burden. *British Journal of Oral Maxillofacial Surgert*, *50*(3), 202-207.

Pries, R., & Wollenberg, B. (2006). Cytokines in head and neck cancer. *Cytokine Growth Factor Review*, *17*(3), 141-146.

Raber-Durlacher, J. E., Brennan, M. T., Verdonck-de Leeuw, I. M., Gibson, R. J., Eilers, J. G., Waltimo, T., .Spijkervet, F. K. (2012). Swallowing dysfunction in cancer patients. *Supportive Care in Cancer*, *20*(3), 433-443.

Reed, S. A., Sandesara, P. B., Senf, S. M., & Judge, A. R. (2012). Inhibition of FoxO transcriptional activity prevents muscle fiber atrophy during cachexia and induces hypertrophy. *FASEB Journal*, *26*(3), 987-1000.

Ren, K., & Torres, R. (2009). Role of interleukin-1beta during pain and inflammation. *Brain Res Rev*, *60*(1), 57-64.

Rhoads, M. G., Kandarian, S. C., Pacelli, F., Doglietto, G. B., & Bossola, M. (2010). Expression of NF-kappaB and IkappaB proteins in skeletal muscle of gastric cancer patients. *Eur J Cancer*, *46*(1), 191-197.

Rhondali, W., Chisholm, G. B., Daneshmand, M., Allo, J., Kang, D. H., Filbet, M., Bruera, E. (2012). Association Between Body Image Dissatisfaction and Weight Loss Among Patients With Advanced Cancer and Their Caregivers: A Preliminary Report. *Journal of Pain Symptom Management*.

Rhoten, B. A., Murphy, B., & Ridner, S. H. (2013). Body image in patients with head and neck cancer: a review of the literature. *Oral Oncology*, *49*(8), 753-760.

Richey, L. M., George, J. R., Couch, M. E., Kanapkey, B. K., Yin, X., Cannon, T., Shores, C. G. (2007). Defining cancer cachexia in head and neck squamous cell carcinoma. *Clinical Cancer Research*, *13*(22 Pt 1), 6561-6567.

Ridner, S. H., & Dietrich, M. S. (2008). Self-reported comorbid conditions and medication usage in breast cancer survivors with and without lymphedema. *Oncology Nursing Forum*, *35*(1), 57-63.

Roberts, B. M., Frye, G. S., Ahn, B., Ferreira, L. F., & Judge, A. R. (2013). Cancer cachexia decreases specific force and accelerates fatigue in limb muscle. *Biochemical and Biophysical Research Communications*.

Roshani, R., McCarthy, F., & Hagemann, T. (2014). Inflammatory cytokines in human pancreatic cancer. *Cancer Letters*, *345*(2), 157-163.

Ross, S., Mosher, C. E., Ronis-Tobin, V., Hermele, S., & Ostroff, J. S. (2010). Psychosocial adjustment of family caregivers of head and neck cancer survivors. *Supportive Care in Cancer*, *18*(2), 171-178.

Russell, S. T., Siren, P. M., Siren, M. J., & Tisdale, M. J. (2010). Mechanism of attenuation of protein loss in murine C2C12 myotubes by D-myo-inositol 1,2,6-triphosphate. *Experimental Cell Research*, *316*(2), 286-295.

Saini, A., Al-Shanti, N., & Stewart, C. E. (2006). Waste management - cytokines, growth factors and cachexia. *Cytokine Growth Factor Review*, *17*(6), 475-486. 6

Sandri, M., Sandri, C., Gilbert, A., Skurk, C., Calabria, E., Picard, A., Goldberg, A. L. (2004). Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell*, *117*(3), 399-412.

Schakman, O., Kalista, S., Barbe, C., Loumaye, A., & Thissen, J. P. (2013). Glucocorticoid-induced skeletal muscle atrophy. *International Journal of Biochemistry and Cell Biology*, *45*(10), 2163-2172.

Schulmeister, L., & Gobel, B. H. (2008). Symptom management issues in oncology nursing. *Nursing Clinics of North America*, *43*(2), 205-220.

Scott, H. R., McMillan, D. C., Crilly, A., McArdle, C. S., & Milroy, R. (1996). The relationship between weight loss and interleukin 6 in non-small-cell lung cancer. *British Journal of Cancer*, *73*(12), 1560-1562.

Scott, H. R., McMillan, D. C., Forrest, L. M., Brown, D. J. F., McArdle, C. S., & Milroy, R. (2002). The systemic inflammatory response, weight loss, performance status and survival in patients with inoperable non-small cell lung cancer. *British journal of cancer*, *87*(3), 264-267.

Sharma, A., Mendez, E., Yueh, B., Lohavanichbutr, P., Houck, J., Doody, D. R., Chen, C. (2012). Human papillomavirus-positive oral cavity and oropharyngeal cancer patients do not have better quality-of-life trajectories. *Otolaryngology- Head and Neck Surgery*, *146*(5), 739-745.

Shen, G. P., Xu, F. H., He, F., Ruan, H. L., Cui, C., Chen, L. Z., Jia, W. H. (2012). Pretreatment lifestyle behaviors as survival predictors for patients with nasopharyngeal carcinoma. *PLoS One*, *7*(5), e36515.

Shi, L, He., Sarvepalli, P., &McCluskey, L.P (2012). Functional role for the interleukin-1 in the injured peripheral taste system. *Journal of Neuroscience Research,* 90(4). 816-30.

Siddiqui, R., Pandya, D., Harvey, K., & Zaloga, G. P. (2006). Nutrition modulation of cachexia/proteolysis. *Nutrition in Clinical Practice*, *21*(2), 155-167.

Sishi, B. J. N., & Engelbrecht, A.-M. (2011). Tumor necrosis factor alpha (TNF-α) inactivates the PI3-kinase/PKB pathway and induces atrophy and apoptosis in L6 myotubes. *Cytokine*, *54*(2), 173-184.

Smith, H. J., Khal, J., & Tisdale, M. J. (2005). Downregulation of ubiquitin-dependent protein degradation in murine myotubes during hyperthermia by eicosapentaenoic acid. *Biochemistry and Biophysical Research Communications*, *332*(1), 83-88.

Solheim, T. S., Blum, D., Fayers, P. M., Hjermstad, M. J., Stene, G. B., Strasser, F., & Kaasa, S. (2014). Weight loss, appetite loss and food intake in cancer patients with cancer cachexia: three peas in a pod? - analysis from a multicenter cross sectional study. *Acta Oncology*, *53*(4), 539-546.

Steinman, J., & Deboer, M. D. (2012). Treatment of cachexia: melanocortin and ghrelin interventions. *Vitamins and hormones*.

Stephen, J. K., Chen, K. M., Shah, V., Havard, S., Lu, M., Schweitzer, V. P., Worsham, M. J. (2012). Human papillomavirus outcomes in an access-to-care laryngeal cancer cohort. *Otolaryngology- Head and Neck Surgery*, *146*(5), 730-738.

Suh, S. Y., Choi, Y. S., Yeom, C. H., Kwak, S. M., Yoon, H. M., Kim, D. G., Yim, E. (2013). Interleukin-6 but not tumour necrosis factor-alpha predicts survival in patients with advanced cancer. *Support ive Care in Cancer*, *21*(11), 3071-3077.

Sukhanov, S., Semprun-Prieto, L., Yoshida, T., Michael Tabony, A., Higashi, Y., Galvez, S., & Delafontaine, P. (2011). Angiotensin II, oxidative stress and skeletal muscle wasting. *American Journal of Medical Science*, *342*(2), 143-147.

Suzuki, H., Asakawa, A., Amitani, H., Nakamura, N., & Inui, A. (2013). Cancer cachexia-pathophysiology and management. *Journal of Gastroenterology*, *48*(5), 574-594.

Tamayo, G. J., Broxson, A., Munsell, M., & Cohen, M. Z. (2010). Caring for the caregiver. *Oncology Nursing Forum*, *37*(1), E50-E57. Tan, B. H., Deans, D. A., Skipworth, R. J., Ross, J. A., & Fearon, K. C. (2008). Biomarkers for cancer cachexia: is there also a genetic component to cachexia? *Supportive Care in Cancer*, *16*(3), 229-234.

Tan, B. H., & Fearon, K. C. (2008). Cachexia: prevalence and impact in medicine. *Current Opinion in Clinical Nutrition and Metabolic Care*, *11*(4), 400-407.

Taniguchi, K., & Karin, M. (2014). IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. *Semin Immunol*, *26*(1), 54-74.

Tefferi, A., Vaidya, R., Caramazza, D., Finke, C., Lasho, T., & Pardanani, A. (2011). Circulating interleukin (IL)-8, IL-2R, IL-12, and IL-15 levels are independently prognostic in primary myelofibrosis: a comprehensive cytokine profiling study. *Journal of Clinical Oncology*, *29*(10), 1356-1363.

Teymoortash, A., Hoch, S., Eivazi, B., & Werner, J. A. (2010). Postoperative morbidity after different types of selective neck dissection. *Laryngoscope*, *120*(5), 924-929.

Thomas, S. S., & Mitch, W. E. (2013). Mechanisms stimulating muscle wasting in chronic kidney disease: the roles of the ubiquitin-proteasome system and myostatin. *Clinical Experimentation in Nephrology*, *17*(2), 174-182. Thompson, M. P., & Kurzrock, R. (2004). Epstein-Barr virus and cancer. *Clinical Cancer Research*, *10*(3), 803-821.

Tisdale, M. J. (2005). Molecular pathways leading to cancer cachexia. *Physiology (Bethesda)*, *20*, 340-348.

Tisdale, M. J. (2009). Mechanisms of cancer cachexia. *Physiological Reviews*, *89*(2), 381-410.

Torelli, G. F., Meguid, M. M., Moldawer, L. L., Edwards, C. K., Kim, H. J., Carter, J. L., Rossi Fanelli, F. (1999). Use of recombinant human soluble TNF receptor in anorectic tumor-bearing rats. *American Journal of Physiology*, *277*(3 Pt 2), R850-R855.

Trendelenburg, A. U., Meyer, A., Jacobi, C., Feige, J. N., & Glass, D. J. (2012). TAK-1/p38/nNFkappaB signaling inhibits myoblast differentiation by increasing levels of Activin A. *Skeletak Muscle*, *2*(1), 3.

Tribius, S., & Hoffmann, M. (2013). Human papilloma virus infection in head and neck cancer. *Deutshces Arzteblatt International*, *110*(11), 184-90, 190e1.

Trivedi, B. P. (2014). Neuroscience: Dissecting appetite. *Nature*, *508*(7496), S64-S65.

Trobec, K., von Haehling, S., Anker, S. D., & Lainscak, M. (2011). Growth hormone, insulin-like growth factor 1, and insulin signaling-a pharmacological target in body wasting and cachexia. *Journal of Cachexia Sarcopenia and Muscle*, *2*(4), 191-200.

Tsai, S. (2012). Importance of lean body mass in the oncologic patient. *Nutrition in Clinical Practice*, *27*(5), 593-598.

Tsai, V. W., Husaini, Y., Manandhar, R., Lee-Ng, K. K., Zhang, H. P., Harriott, K., Breit, S. N. (2012). Anorexia/cachexia of chronic diseases: a role for the TGF-beta family cytokine MIC-1/GDF15. *Journal of Cachexia Sarcopenia and Muscle*, *3*(4), 239-243.

Tsoli, M., & Robertson, G. (2013). Cancer cachexia: malignant inflammation, tumorkines, and metabolic mayhem. *Trends in Endocrinology and Metabolism*, *24*(4), 174-183.

Turi, K., Barabas, P., Csurgay, K., Lehner, G., Lorincz, A., & Nemeth, Z. (2013). An Analysis of the Epidemiological and Etiological Factors of Oral Tumors of Young Adults in a Central-Eastern European Population. *Pathology and Oncology Research*.

van Hall, G. (2012). Cytokines: muscle protein and amino acid metabolism. *Current Opinion in Clinical Nutrition and Metabolic Care*, *15*(1), 85-91.

Vaughan, V. C., Martin, P., & Lewandowski, P. A. (2012). Cancer cachexia: impact, mechanisms and emerging treatments. *Journal of Cachexia Sarcopenia and Muscle*.

Vernon, H., & Mior, S. (1991). The Neck Disability Index: a study of reliability and validity. *Journal of Manipulative Physiological Therapy*, *14*(7), 409-415.

Vidall, C., Dielenseger, P., Farrell, C., Lennan, E., Muxagata, P., Fernández-Ortega, P., & Paradies, K. (2011). Evidence-based management of chemotherapy-induced nausea and vomiting: a position statement from a European cancer nursing forum. *Ecancermedicalscience*, *5*.

Vigano, A., Del Fabbro, E., Bruera, E., & Borod, M. (2012). The cachexia clinic: from staging to managing nutritional and functional problems in advanced cancer patients. *Critical Reviews in Oncogenesis*, *17*(3), 293-303.

Vissink, A., Jansma, J., Spijkervet, F. K., Burlage, F. R., & Coppes, R. P. (2003). Oral sequelae of head and neck radiotherapy. *Critical Reviews in Oral Biology and Medicine*, *14*(3), 199-212.

von Haehling, S., & Anker, S. D. (2010). Cachexia as a major underestimated and unmet medical need: facts and numbers. *Journal of Cachexia Sarcopenia and Muscle*, *1*(1), 1-5.

Wakefield, L. M., & Hill, C. S. (2013). Beyond TGFbeta: roles of other TGFbeta superfamily members in cancer. *Nat Rev Cancer*, *13*(5), 328-341.

Wallengren, O., Lundholm, K., & Bosaeus, I. (2013). Diagnostic criteria of cancer cachexia: relation to quality of life, exercise capacity and survival in unselected palliative care patients. *Supportive Care in Cancer*, *21*(6), 1569-1577.

Walter, L., Vidaurre, T., Gilman, R. H., Poquioma, E., Olaechea, C., Gravitt, P. E., & Marks, M. A. (2013). Trends in head and neck cancers in Peru between 1987-2008: Experience from a large public cancer hospital in Lima. *Head and Neck*.

Walz, D. A. (2010). Cancer-related anorexia-cachexia syndrome. *Clinical Journal of Oncology Nursing*, *14*(3), 283-287.

Wang, S., Sun, M., Gu, C., Wang, X., Chen, D., Zhao, E., Jiao, X., & Zheng, J. (2014). Expression of CD163, interleukin-10, and interferon-gamma in oral squamous cell carcinoma: mutual relationships and prognostic implications. *European Journal of Oral Science.* 122(3): 202-209.

Wang, X. I., Thomas, J., & Zhang, S. (2012). Changing trends in human papillomavirus-associated head and neck squamous cell carcinoma. *Annals of Diagnostic Pathology*, *16*(1), 7-12.

Wang, Z., Corey, E., Hass, G. M., Higano, C. S., True, L. D., Wallace, D. J., Vessella, R. L. (2003). Expression of the human cachexia-associated protein (HCAP) in prostate cancer and in a prostate cancer animal model of cachexia. *International Journal of Cancer*, *105*(1), 123-129.

Weinberger, P. M., Yu, Z., Haffty, B. G., Kowalski, D., Harigopal, M., Brandsma, J., Psyrri, A. (2006). Molecular classification identifies a subset of human papillomavirus--associated oropharyngeal cancers with favorable prognosis. *Journal of Clinical Oncology*, *24*(5), 736-747.

White, J. P., Baynes, J. W., Welle, S. L., Kostek, M. C., Matesic, L. E., Sato, S., & Carson, J. A. (2011). The Regulation of Skeletal Muscle Protein Turnover during the Progression of Cancer Cachexia in the ApcMin/+ Mouse. *Plos One*, *6*(9), e24650.

White, J. P., Puppa, M. J., Sato, S., Gao, S., Price, R. L., Baynes, J. W., Carson, J. A. (2012). IL-6 regulation on skeletal muscle mitochondrial remodeling during cancer cachexia in the ApcMin/+ mouse. *Skeletal Muscle*, *2*, 14.
Whitehouse, A. S., Smith, H. J., Drake, J. L., & Tisdale, M. J. (2001). Mechanism of attenuation of skeletal muscle protein catabolism in cancer cachexia by eicosapentaenoic acid. *Cancer Research*, *61*(9), 3604-3609.

Wibault, J., Vaillant, J., Vuillerme, N., Dedering, A., & Peolsson, A. (2013). Using the cervical range of motion (CROM) device to assess head repositioning accuracy in individuals with cervical radiculopathy in comparison to neck-healthy individuals. *Man Ther*, *18*(5), 403-409.

Wiley, S. R., & Winkles, J. A. (2003). TWEAK, a member of the TNF superfamily, is a multifunctional cytokine that binds the TweakR/Fn14 receptor. *Cytokine Growth Factor Review*, *14*(3-4), 241-249.

Willemen, H.L, Eijelkamp, N., Garza-Carbajal, A., Wang, H., Mack, M., Zijistra, J., Heijnen, C.J & Kavellars, A.(2014). Monocyte/macrophages control resolution of transient inflammatory pain. *Journal of Pain,* 15(5) 496-506.

Williams, M. L., Torres-Duarte, A., Brant, L. J., Bhargava, P., Marshall, J., & Wainer, I. W. (2004). The relationship between a urinary cachectic factor and weight loss in advanced cancer patients. *Cancer Investigation*, *22*(6), 866-870.

Wing, S. S. (2013). Deubiquitinases in skeletal muscle atrophy. *International Journal of Biochemistry and Cell Biology*, *45*(10), 2130-2135.

Wium-Andersen, M.K., Orsted, D.D., & Nordestgaard, B.G. (2014). Elevated C-reactive protein, depression, somatic diseases, and all-cause mortality: A Mendelian randomization study. *Biological Psychiatry.* 76 (3), 249-257.

Wysong, A., Couch, M., Shadfar, S., Li, L., Rodriguez, J. E., Asher, S., Willis, M. S. (2011). NF-kappaB inhibition protects against tumor-induced cardiac atrophy in vivo. *American Journal of Pathology*, *178*(3), 1059-1068.

Yang, X., Zhang, X., Heckmann, B. L., Lu, X., & Liu, J. (2011). Relative contribution of adipose triglyceride lipase and hormone-sensitive lipase to tumor necrosis factor-alpha (TNF-α)-induced lipolysis in adipocytes. *Journal of Biological Chemistry*, *286*(47), 40477-40485.

Yang, Z., Zhang, B., Hou, L., Xie, Y., & Cao, X. (2014). Pre-operative prognostic nutritional index predicts the outcomes for triple-negative breast cancer. *Tumour Biol*, *35*(12), 12165-12171.

Yavuzsen, T., Davis, M. P., Walsh, D., LeGrand, S., & Lagman, R. (2005). Systematic review of the treatment of cancer-associated anorexia and weight loss. *Journal of Clinical Oncology*, *23*(33), 8500-8511.

Zechner, R., Zimmermann, R., Eichmann, T. O., Kohlwein, S. D., Haemmerle, G., Lass, A., & Madeo, F. (2012). FAT SIGNALS--lipases and lipolysis in lipid metabolism and signaling. *Cell Metabolism*, *15*(3), 279-291.

Zhao, J., Brault, J. J., Schild, A., Cao, P., Sandri, M., Schiaffino, S., Goldberg, A. L. (2007). FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. *Cell Metabolism*, *6*(6), 472-483.

Appendix
Cervical Range of Motion (CROM)

VISIT _____

The subject's CROM parameters:

(1) Forward flexion_____degrees (normal: 0-45 degrees)

(2) Extension _____ degrees (normal: 0-45 degrees)

(3) Left lateral flexion_____degrees (normal: 0-45 degrees)

(4) Right lateral flexion_____degrees (normal: 0-45 degrees)

(5) Left lateral rotation_____degrees (normal: 0-80 degrees)

(6) Right lateral rotation____degrees (normal: 0-80 degrees)

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(1) Forward flexion_____degrees (normal: 0-45 degrees)

(2) Extension _____ degrees (normal: 0-45 degrees)

(3) Left lateral flexion_____degrees (normal: 0-45 degrees)

(4) Right lateral flexion_____degrees (normal: 0-45 degrees)

(5) Left lateral rotation_____degrees (normal: 0-80 degrees)

(6) Right lateral rotation____degrees (normal: 0-80 degrees)

Please check the following symptoms if the subject has them:

Pain____, weakness____, spasm____, tenderness____, lack of endurance____

Postural abnormalities____, fixed deformity of cervical spine musculature__

Head and Neck Disease and

Treatment Additional Information

Form

Primary Specific Tumor Location
_____
_____
Secondary Specific Tumor Location
_____
_____
Tertiary Specific Tumor Location
_____
_____

Cancer Type
(Type of Cancer according to Pathology)
___(1) Squamous Cell Carcinoma
___(2) Nasopharyngeal Carcinoma
___(3) Salivary Duct Carcinoma
___(4) Acinic Cell Carcinoma
___(5) Spindle Cell Carcinoma
___(6) Olfactory Neuroblastoma (esthesioneuroblastoma)
___(7) Adenoid Cystic Carcinoma
___(8) Buccal Cell Carcinoma
___(9) Adenocarcinoma
___(10) Mucoepidermoid Carcinoma
___(11)Teratoma
___(12) Carcinoma, Unknown Type
___(13)
Other_____
_____
Other_____

Local Metastasis
___(0) No
___(1) Yes
Date of Diagnosis: ___/___/____
Type:
___(1) Squamous Cell Carcinoma
___(2) Nasopharyngeal Carcinoma
___(3) Salivary Duct Carcinoma
___(4) Acinic Cell Carcinoma
___(5) Spindle Cell Carcinoma
___(6) Olfactory Neuroblastoma (esthesioneuroblastoma)
___(7) Adenoid Cystic Carcinoma
___(8) Buccal Cell Carcinoma
___(9) Adenocarcinoma
___(10) Mucoepidermoid Carcinoma
___(11)Teratoma
___(12) Unknown
___(13)
Other_____
_____
Other_____
Location:
___(1) Neck
___(2) Lungs
___(3) Bone
___(4) Other
_____
_____
Other_____

Distant Metastasis
___(0) No
___(1) Yes
Date of Diagnosis: ___/___/____
Type:
___(1) Squamous Cell Carcinoma
___(2) Nasopharyngeal Carcinoma
___(3) Salivary Duct Carcinoma
___(4) Acinic Cell Carcinoma
___(5) Spindle Cell Carcinoma
___(6) Olfactory Neuroblastoma (esthesioneuroblastoma)
___(7) Adenoid Cystic Carcinoma
___(8) Buccal Cell Carcinoma
___(9) Adenocarcinoma
___(10) Mucoepidermoid Carcinoma
___(11)Teratoma
___(12) Unknown

___(13)
Other_____
_____
Other_____
Location:
___(1) Neck
___(2) Lungs
___(3) Bone
___(4) Other
_____
_____
Other_____

Known Lymphatic Spread
___(0) No
___(1) Yes

Tumor-Expressed Virus
___(0) No known virus
___(1) HPV
___(3) EBV
___(8) Other
_____

Known Viral Infection
___(0) No known virus
___(1) Human Papilloma Virus (HPV)
___(2) HIV
___(3) Epstein-Barr Virus (EBV)
___(4) Cytomegalovirus (CMV)
___(5) Herpes Simplex Virus (HSV)
___(6) Hepatitis C (HCV)
___(7) Other Herpes virus_____
___(8) Other virus_____
___(9) Hepatitis A (HAV)
___(10) Hepatitis B (HBV)
___(11) Hepatitis, Unknown type
_____
Other_____

Secondary Known Viral Infection
___(0) No known virus
___(1) Human Papilloma Virus (HPV)
___(2) HIV
___(3) Epstein-Barr Virus (EBV)
___(4) Cytomegalovirus (CMV)
___(5) Herpes Simplex Virus (HSV)
___(6) Hepatitis C (HCV)
___(7) Other Herpes virus_____
___(8) Other virus_____
___(9) Hepatitis A (HAV)
___(10) Hepatitis B (HBV)
___(11) Hepatitis, Unknown type
_____
Other_____

Head and Neck Cancer
Medical Record Extraction-To be used if unable to pull directly from electronic record.

Date of diagnosis: _____

Medical History:

Cardiovascular
Excretory
Digestive
Respiratory
Integumentary

Nervous
Skeletal
Muscular
Endocrine
Reproductive
Immune

Type of treatment [check all that apply]

❑ Radiation ❑ Chemotherapy ❑ Biopsy ❑ Surgery

Date of Biopsy: _____

Dates of Chemotherapy: _____

Dates of Radiation: _____

Stage HNC
- ❑ 1
- ❑ 2
- ❑ 3
- ❑ 4

Site of HNC
- ❑ Larynx
- ❑ Pharynx
- ❑ Oral Cavity
- ❑ Salivary Glands
- ❑ Perinasal Sinus
- ❑ Other_____

Total Radiation Dose: _____

Schedule of Radiation Therapy (days on/off, etc.)

_____

_____

_____

_____

_____

_____

Structures within Radiation Field: (check all that apply)

Structure	Check	Structure	Check
Base of Tongue			
Posterior Pharyngeal Wall			
Epiglottis			
Pharyngoepiglottic Folds			
Aryepiglottic Folds			

Interarytenoid Space			
Cricopharyngeal Prominence			
Arytenoids			
False Vocal Folds			
True Vocal Folds			
Anterior Commissure			

Surgery

Type	Date

Medication regimen at time of study enrollment and during study:

Drug	Dose	Frequency

Performance Status at each study assessment

	Notes	**Date**
Baseline		
EOT		
6wk		
12wk		
18wk		
24wk		
30wk		
36wk		
42wk		
15mth		
18mth		

Infection/Injury at each study assessment

	Notes	**Date**
Baseline		
EOT		
6wk		
12wk		
18wk		
24wk		

30wk		
36wk		
42wk		
15mth		
18mth		

Toxicity Criteria at Each Visit

	Notes	**Date**
Baseline		
EOT		
6wk		
12wk		
18wk		
24wk		
30wk		
36wk		
42wk		
15mth		
18mth		

Comments

CT Findings

Date	Findings

Barium Swallow Results

	Notes	Date
Baseline		
48wk		

18mth		

Laryngoscopy Results

	Notes	**Date**
Baseline		
EOT		
6wk		
12wk		
18wk		
24wk		
30wk		
36wk		
42wk		
15mth		
18mth		

CESD

Below is a list of ways you might have felt or behaved. Please tell me how often you have felt this way during the past week.

	Hardly ever or none of the time (less than 1 day)	Some or a little of the time (1-2 days)	Occasionally or a moderate amount of the time (3-4 days)	Most or all of the time (5-7days)
1. I was bothered by things that usually don't bother me.	0	1	2	3
2. I did not feel like eating; my appetite was poor.	0	1	2	3
3. I felt that I could not shake off the blues even with help from my family or friends.	0	1	2	3
4. I felt I was just as good as other people.	0	1	2	3
5. I had trouble keeping my mind on what I was doing.	0	1	2	3
6. I felt depressed.	0	1	2	3
7. I felt that everything I did was an effort.	0	1	2	3
8. I felt hopeful about the future.	0	1	2	3
9. I thought my life had been a failure.	0	1	2	3
10. I felt fearful.	0	1	2	3
11. My sleep was restless.	0	1	2	3
12. I was happy.	0	1	2	3

13. I talked less than usual.	0	1	2	3
14. I felt lonely.	0	1	2	3
15. People were unfriendly.	0	1	2	3
16.I enjoyed life.	0	1	2	3
17. I had crying spells.	0	1	2	3
18. I felt sad.	0	1	2	3
19. I felt that people disliked me.	0	1	2	3
20. I could not get "going".	0	1	2	3

Patient Interview;
1. What is your birthdate? ____/____/_____ (month/day/year)

2. Gender:
(1) Female___(2) Male____
(3) Other____(4) Do not care to respond___

3. What is your race?
(1) American Indian/Alaskan Native____
(2) Asian____
(3) Native Hawaiian or Other Pacific Islander
_____
(4) Black or African American_____
(5) White_____
Nation of Origin: _____

4. What is the highest grade of education you completed? (Please circle)
1 2 3 4 5 6 7 8 9 10 11 12 (high school) 13 14 15 16 (college)
17 18 (master) 19 20 (doctorate)

5. What is your marital status?
(1) Single____
(2) Single, living with partner_____
(3) Married_____
(4) Widowed____
(5) Other____

6. What is your current employment status?
(1) Employed full time____
(2) Employed part time____
(3) Homemaker____
(4) Retired_____
(5) Unemployed_____
(6) Other____
Vocation_____

7. What best describes your area of residence?
(1) City___ (2) Country ___ (3) Other___

Chart Review

8. Health Maintenance
8.1 Smoking/Dipping/Chewing
(1) No ____
(2) Yes ___(Tobacco__or Marijuana__or ___)
_____years _____ cigarettes per day
_____ dips per day ____ plugs/chaw per day
(3) Quit _____When_____
(4) Not Quit _____
_____cigarettes per day (Current)

8.2 Drinking Alcohol
(1) No ____
(2) Yes ____
_____years _____ times per week
(3) Quit _____When_____
(4) Not Quit _____
_____ times per week (Current)

9. What is your insurance coverage?
(1) Medicare____ (2) Medicaid____
(3) TennCare____ (4) Private Insurance____
(5) HMO____ (6) None_____
(7) Other_____

10. Do you have any medical problems?
(1) No
(2) Yes (e.g., HBP, DM, Obesity, Injury History)
_____

11. What is your yearly household income?
(1) $10,000 or less____
(2) $10,001 to $ 20,000____
(3) $20,001 to $ 30,000____
(4) $30,001-$40,000___
(5) $ 40,001 to $50,000____
(6) $50,001 to $60,000 ____
(7) Over $60,000____
(8) Do not care to respond____

The Vanderbilt Head and Neck Cancer Symptom Survey (version 2.0)

Name:_____-
_____Date:_____

Directions: Please answer the following questions by checking the appropriate box.

1. I currently have a feeding tube in place. □ Yes □ No 2. I have teeth □ Yes □ No
3. I use dentures □ Yes □ No

Directions: Please read all questions and circle the number that best describes your symptoms over the past week. In general, a "0" indicates the least amount of problems with a particular symptom and "10" indicates the most problems.

1. I have been losing weight

   0 1 2 3 4 5 6 7 8 9 None

2. I have lost my appetite

   0 1 2 3 4 5 6 7 8 9 Normal

10
A lot

10
No appetite

3. I have to use liquid supplements (like Ensure® or Boost®) to maintain my weight

0 1 2 3 4 5 6 7 8 9 None

supplements

4. I have trouble maintaining my weight because of swallowing problems

0 1 2 3 4 5 6 7 8 9 None

10
All liquid

10
A lot

5. I have trouble eating certain solid foods (like hard to chew, crumbly, or sticky foods)

0 1 2 3 4 5 6 7 8 9 None

6. I have trouble drinking thin liquids (like water, tea and Ensure®)

10
A lot

10
A lot

10 Always

10 Always

0 1 2 3 4 None

7. Food gets stuck in my mouth

   0 1 2 3 4 Never

8. Food gets stuck in my throat

   0 1 2 3 4 Never

9. I choke or strangle on liquids

5 6 7 8 9

5 6 7 8 9

5 6 7 8 9

0 1 2 3 4 5 6 7 8 9 10

Never

10. I choke or strangle on solid foods

    0 1 2 3 4 Never

11. I cough after I swallow

    0 1 2 3 4 Never

12. Swallowing takes great effort

    0 1 2 3 4 Never

Always

5 6 7 8 9 10 Always

5 6 7 8 9 10 Always

5 6 7 8 9 10 Always

13. It takes me longer to eat because of my swallowing problem

    0 1 2 3 4 5 6 7 8 9 10 Never Always

14. I have problems with dry mouth

    0 1 2 3 4 5 6 7 8 9 10 Never Severe

15. Problems with dry mouth make chewing and swallowing difficult

    0 1 2 3 4 5 6 7 8 9 10 Never Always

16. Problems with dry mouth affect my ability to sleep

    0 1 2 3 4 5 6 7 8 9 10 Never Always

17. Problems with dry mouth affect my ability to talk

0 1 2 3 4 5 Never

18. I have thick mucous or phlegm

    0 1 2 3 4 5 Never

19. Mucous causes me to choke or gag

    0 1 2 3 4 5 Never

20. Mucous causes difficulty swallowing

    0 1 2 3 4 5 Never

21. Mucous causes difficulty sleeping

    0 1 2 3 4 5 Never

6 7 8 9 10 Always

6 7 8 9 10 Always

6 7 8 9 10 Always

6 7 8 9 10 Always

6 7 8 9 10 Always

22. I have sores in my mouth or throat that cause pain

0 1 2 3 4 5 6 7 8 9 No Pain

23. Mouth or throat pain causes difficulty swallowing

0 1 2 3 4 5 6 7 8 9 Never

24. Mouth or throat pain causes difficulty speaking

0 1 2 3 4 5 6 7 8 9 Never

10
Severe pain

10 Always

10 Always

10
Severe pain

10
Severe pain

25. My average pain level over the last week has been.....

0 1 2 3 4 5 6 7 No pain

26. My worst pain level over the last week has been....

0 1 2 3 4 5 6 7 No pain

27. The average relief from my pain medication is....

pain medications

0 1 2 3 4 5 6 7 No relief

28. Pain causes difficulty sleeping

0 1 2 3 4 5 6 7 Never

29. I have trouble speaking

0 1 2 3 4 5 6 7 Never

30. My voice is hoarse

0 1 2 3 4 5 6 7 Not at all

8 9 8 9

31. I have trouble being understood because of my speaking or hoarse voice

0 1 2 3 4 5 6 7 8 9 Never

32. I have trouble with my hearing

0 1 2 3 4 5 6 7 8 9 None

33. My taste is altered

0 1 2 3 4 5 6 7 8 9 None

34. I have less desire to eat due to taste change

0 1 2 3 4 5 6 7 8 9 Never

10 Always

10 Severe

10
A lot

10 Always

□ Not Applicable, I am not on

8 9

8 9

8 9

8 9

10
Total relief

10 Always

10 Always

10
Very Hoarse

35. My taste changes have altered the foods that I choose to eat

0 1 2 3 4 5 6 7 8 9 Never

36. My taste changes have caused me to decrease the amount of food I eat

0 1 2 3 4 5 6 7 8 9 Never

37. My sense of smell has changed

0 1 2 3 4 5 6 7 8 9 Not at all

38. I have altered what I eat due to a change in my sense of smell

0 1 2 3 4 5 6 7 8 9 Not at all

10 Always

10 Always

10
Very much

10
Very much

39. I have difficulty chewing because of my teeth or dentures... Not applicable, I do not have teeth or dentures

0 1 2 3 4 5 6 7 8 9 10 None Severe

40. My teeth are sensitive to hot, cold or sweet foods Not applicable, I do not have teeth

0 1 2 3 4 5 6 7 8 9 Not at all

41. My teeth feel looser Not applicable, I do not have teeth

0 1 2 3 4 5 6 7 8 9 Not at all

10
Very Sensitive

10
Very Loose

42. My teeth are cracking or chipping Not applicable, I do not have teeth

0 1 2 3 4 5 6 7 8 9 10 Not at all Severe

43. I have trouble with my dentures Not applicable, I do not have dentures

0 1 2 3 4 5 6 7 8 9 None

44. I have a burning sensation in the lining of my mouth and throat

0 1 2 3 4 5 6 7 8 9 None

10
A lot

10
Very Painful

45. The lining of my mouth and throat is sensitive to spicy, hot or acidic foods

0 1 2 3 4 5 6 7 8 9 Not at all

46. The lining of my mouth and throat is sensitive to dryness

0 1 2 3 4 5 6 7 8 9 Not at all

47. Burning pain in the lining of my mouth and throat changes what I eat

10
Very Sensitive

10
Very Sensitive

0 1 2 3 4 5 6 7 8 9 10 Never Always

48. Burning pain in the lining of my mouth and throat prevents me from brushing my teeth

0 1 2 3 4 5 6 7 8 9 10 Never Always

49. I have limitations in the ability to open or move my jaw

0 1 2 3 4 5 6 7 8 9 10 Never Severe

50. I have limitations in the ability to move my neck and shoulders

0 1 2 3 4 5 6 7 8 9 10 Never Severe