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Objectives: The purpose of this pilot study was to investigate the immediate effects of Osteopathic Manipulative Treatment (OMT) on immune function in a healthy population.

Methods: This was a randomized, blinded and controlled clinical trial. 50 healthy individuals, ages 18 to 40, were recruited. Subjects were randomly assigned to one of two groups: OMT or Rest (control). Blood and saliva samples were collected pre and post-intervention (thirty minutes of OMT or Rest). Samples were analyzed for a CBC, salivary IgA, and various lymphocyte populations.

Results: This study successfully demonstrated the feasibility of this protocol. No statistically significant differences in outcome measures were identified between the two groups, nor were any apparent trends identified.

Conclusion: This study established a framework for future research investigating the effects of OMT on acute and chronic infection, chronic pain, and immunocompromised populations in human and/or animal populations.

IMMEDIATE EFFECTS OF OSTEOPATHIC MANIPULATIVE TREATMENTS ON IMMUNE FUCTION IN A HEALTHY POPULATION: A PILOT STUDY

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Immediate Effects of Osteopathic Manipulative Treatment on Immune Function in a Healthy Population: A Pilot Study

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LIST OF ABBREVIATIONS

ANS	Autonomic Nervous System
BALT	Bronchial Associated Lymphoid Tissue
CAM	Complementary and Alternative Medicine
CBC	Complete Blood Count
CD	Cluster of Differentiation
CRP	C- Reactive Protein
ELISA	Enzyme Linked Immunosorbant Assay
GALT	Gut Associated Lymphoid Tissue
GUALT	Genito-Urinary Associated Lymphoid Tissue
IgA	Immunoglobulin A
LPT	Lymphatic Pump Techniques
MALT	Mucosal Associated Lymphoid Tissue
NALT	Nasal Associated Lymphoid Tissue
NK Cells	Natural Killer Cells
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
OMM	Osteopathic Manipulative Medicine
OMT	Osteopathic Manipulative Treatment
PMN	Polymorphonuclear lymphocytes
SALT	Skin Associated Lymphoid Tissue
TBGP	Total Blood Granulocyte Pool
TLP	Thoracic Lymph Pumps

CHAPTER I

BACKGROUND AND INTRODUCTION

It has been taught that Osteopathic Manipulative Treatment (OMT) enhances immune function. For years, anecdotal evidence has sustained the belief that OMT does indeed improve immune function by removing restrictions thereby optimizing blood flow, lymph flow, respiratory mechanics and restoring balance within the sympathetic and parasympathetic nervous systems.[1, 2] In fact, there are numerous case studies that demonstrate the efficacy of OMT in patients with acute infections. For example, Purse reported on a patient with acute bronchitis suffering from congestion that started to expectorate immediately following 5-10 minutes of thoracic lymphatic pump (TLP).[3] However, as the public is beginning to demand evidence-based medicine, it is critical to conduct scientific investigations to test the claims made about the potential benefits of OMT, especially as it pertains to immune function. The purpose of this research is to conduct a thorough literature review of how OMT and other forms of manual medicine influence immune function, design a clinical trial and protocol that is feasible and reproducible, and finally investigate the immediate effects of OMT on immune cells in a healthy population.

The average healthy individual is exposed to hundreds of potentially harmful pathogens daily, but the human immune system defends against and resists infection. Occasionally, however, even healthy immune systems fail and infection ensues.

According to the Center for Disease Control (CDC), the latest data on emergency department visits show that upper respiratory infections are among the most frequently reported diagnoses.[4] Another common infectious process is pneumonia, with approximately 4.5 million cases of community-acquired pneumonia (CAP) occurring each year. Twenty percent of these cases result in hospitalization. Lost days of work due to CAP were 8.9 days per 100 adult employees. In addition, the annual cost to treat patients with CAP in the United States was 9.7 billion dollars in 1994 and 92% of these costs were secondary to hospitalization.[5] Infections are common and affect all people; unfortunately some infections, though less common, have life threatening consequences such as the human immunodeficiency virus. Between 800,000 and 900,000 individuals infected with HIV live in the United States;[6] these individuals are at increased risk for fatal opportunistic infections and are faced with exorbitant medical expenses.

As indicated above, the economic burden of infectious processes such as upper respiratory infections, urinary tract infections, pneumonia, otitis media, and especially HIV is enormous.[6] If osteopathic manipulative treatments could indeed optimize or even boost immune function thereby minimizing lost workdays, shortening hospital stays, preventing infection, decreasing dosages/frequency of medications and most importantly improving quality of life, it is definitely worth investigating.

Osteopathic Manipulative Treatment (OMT) is a type of manual medicine based on an understanding of the musculoskeletal, circulatory, lymphatic and nervous systems of the body. OMT helps alleviate pain, restore motion, support the body's natural functions and influence the body's structure to help it function more efficiently.[1, 2] The

principles that guide the osteopathic approach to patient care are: 1) The body is a unit, the person is a unit of body, mind, and spirit; 2) The body is capable of self regulation, self-healing, and health maintenance; 3) Structure and function are reciprocally interrelated; and 4) Rational treatment is based upon an understanding of the basic principles of body unity, self regulation and the interrelationship of structure and function. An osteopathic approach to patients recognizes and appreciates the aforementioned fundamental principles.[1, 2] Osteopathic physicians utilize medications and surgery when appropriate, but can also offer OMT.

IMMUNE SYSTEM: COMPONENTS and FUNCTION

This section is included to provide background information on the immune system including lymphatics and to stress the significance of the outcome measures selected for this study. The immune system functions to protect against bacteria, viruses, fungi, parasites, and foreign substances; it also serves a role in destroying cancer cells. This system is divided into two separate categories: Innate and Adaptive immunity.

Innate immunity includes protection against pathogens without previous exposure to the host.[7, 8] Innate immunity can be thought of as first lines defense, working both quickly and generically irrespective to the pathogen. Among the components that make up the innate immune system are: epithelial and mucosal barriers, normal flora, certain cytokines, cells such as macrophages, neutrophils, natural killer cells (NK cells), intraepithelial T cells, mast cells, and dendritic cells.[8] Within natural immunity, the first

line of defense are the physical (e.g. skin) and chemical barriers (e.g. gastric juices), followed by natural antibodies and immune cells. Natural antibodies are present in healthy states and are not stimulated by any specific antigen. Natural antibodies inhibit viral spread to parenchymal organs and the spread of bacteria to lymphoid organs.[7, 8] The adaptive immune system is antigen specific; it is responsible for the production of antigen-specific antibodies. It offers the added benefits of protecting against future re-infections. The first adaptive immune response can take anywhere from four to seven days, therefore innate immune responses are more important during this window of time.[7]

Both innate and adaptive immunity depend on the activities of white blood cells, also known as leukocytes.[7] There are five types of white blood cells. Three of them contain granules and are therefore referred to as granulocytes. These granulocytes are neutrophils (polymorphonuclear lymphocytes- PMN's), basophils and eosinophils. The two non-granule WBCs are lymphocytes and monocytes.[7]

Neutrophils are the most abundant type of white blood cells making up anywhere from 50-60% of circulating white blood cells in a healthy individual and are a significant component of the innate immune response.[9] They are the first cells to arrive after an inflammatory response has been stimulated. They attack and phagocytize bacteria, fungi, protozoa, viruses, as well as cells that are infected by virus. They also attack tumor cells. Neutrophils undergo elaborate development that occurs in the bone marrow and take approximately 2 weeks for proliferation and differentiation. Healthy adults produce anywhere from 10^{11} to 10^{12} (in acute inflammatory processes) neutrophils each day. Interestingly, these cells remain as non-activated cells even after being released from the

bone marrow into the blood. Neutrophils have a half-life of only 4 to 10 hours before they marginate and enter tissue pools; They live for 1 to 2 days in the tissue pools.[9] There is evidence supporting the existence of neutrophil populations that are neither dormant nor activated but are rather in a state of preactivation and that these neutrophil populations may be able to respond robustly secondary to a priming mechanism.[9]

Clinical Correlate: Neutrophilia refers to increased neutrophils; neutropenia is abnormally low neutrophil counts.[10] Shift neutrophilia occurs when cells migrate from the marginal pool to the circulating pool; there is no increase in the total blood granulocyte pool (TBGP). In contrast, true neutrophilia occurs when there is an increase in TBGP size. Shift neutrophilia occurs with strenuous exercise or epinephrine injection and is usually transient.[9] True neutrophilia is primarily associated with infection. In these cases, the TBGP may be 5-6 times normal. Initially, in the setting of acute infection, neutrophil counts may actually decrease briefly due to margination of cells from the blood. Soon after, cells are released from the marrow, leading to an increase in the TBGP and blood neutrophilia. A left shift occurs if demand for these granulocytes is high. This means more immature forms appear in blood. Other causes of neutrophilia are noninfectious inflammation (such as burns, autoimmune disease, postoperatively), metabolic conditions (DKA, preeclampsia, and uremia), poisoning (lead, mercury, digitalis), acute hemorrhage, malignant neoplasms, physiologic neutrophilia and several other rare conditions. Physiologic neutrophilia may be caused by strenuous exercise, epinephrine injection, pregnancy, labor, and may be found in newborns.[9] Neutropenia is a decrease in circulating neutrophils in the blood. This condition is defined by having an

absolute neutrophil count (ANC) of less than 1500 cells per mm^3.[9] When individuals are neutropenic they risk being susceptible to bacterial infections and recurrent infections.[10]

In general there are two types of lymphocytes: B and T lymphocytes. T-lymphocytes, also known as T cells because most T cells originate in the thymus, are vital to the immune response because they participate in antigen-specific cellular immunity as well as facilitate antigen-specific B-cell dependant humoral immunity. B-lymphocytes arise in bone marrow. B-lymphocytes synthesize and secrete immunoglobulins. Immunoglobulins (antibodies) are antigen-specific proteins that are able to neutralize toxins and viruses. Antibodies can also prevent colonization of pathogens. Antibodies are involved in the opsonization of bacteria and fungi; these opsonizing antibodies facilitate the ability of phagocytic cells to destroy the bacteria/fungi. Finally, antibodies may also trigger the complement cascade and facilitate lysis of gram-negative bacteria, and accentuate opsonization. There are five basic classes of antibodies. The five classes are IgA, IgD, IgE, IgG, and IgM. Because this research uses IgA as an outcome measure, a more thorough discussion of IgA follows.[8]

The primary outcome measure of this research is salivary IgA. Though this particular antibody is produced and secreted primarily in the mucous epithelium of the intestinal and respiratory tracts, it is also produced and secreted by the lactating breast, salivary glands and tear glands.[7] The primary function of IgA is to protect epithelial surfaces from infectious agents by preventing the attachment of bacteria or toxins to the epithelial cells or by absorbing foreign substances.[7] Within the human nasal mucosa, secretory IgA is produced as a J-chain containing dimmers, that are then conjugated with

the glandular epithelium and then incorporated in the mucous coat of the epithelial lining.[8]

Clinical Correlate: Salivary IgA can indicate immune function. IgA is the most common immunoglobulin made by plasma cells in the sinopulmonary, gastrointestinal and genitourinary tracts. Therefore it is often the first line of defense against many pathogens including those that cause respiratory, gastrointestinal and genitourinary infections. Cohen stated, "Up to 95% of all infections are initiated at the mucosal surfaces."[11]

Natural Killer cells (NK cells) are essential to innate immunity. They attack viruses, intracellular bacteria and parasites. NK cells have cytotoxicity against tumor cells, immature hematopoietic cells and most cultured cell lines. These cells make up approximately 5- 15% of T lymphocytes.[7,10]

Cytotoxic T cells, also known as CD8+ T cells, destroy cells that have already been infected by recognizing specific antigens on these target cells. Helper T cells, also known as CD4 T cells, assist in lymphocyte function. After being activated, they divide and secrete cytokines. Each kind of T cell has a unique activation mechanism. However, the following is a general framework that they all use. First the T cell binds to an MHC/antigen complex on an antigen presenting cell (APC). By binding the cell recognizes it is specific for. Then, with costimulation, the T cell is activated. CD4, CD8, or whatever surface markers the T cell has makes contact with the APC's membrane and gets confirmation that this is the right antigen. Then the cell divides and differentiates, and does its designated duties.[7, 8, 10]

Clinical Correlate: A low lymphocyte count is known as lymphopenia, whereas, high lymphocyte count is called lymphocytosis.[10] Lymphopenia may indicate defects in cellular immunity.[8] Lymphocytes make up approximately 20 to 40 percent of the leukocytes count circulating in the blood. Viral infections and chronic bacterial infections are often associated with lymphocytosis.[10] In patients infected by the HIV, T lymphocytes are attacked. $CD4^+$ T cells along with viral load are used to monitor the progresssion of this condition. Normal CD4+ T cell counts in adults range from 600-1500 cells. When this number is less than 200 in an HIV positive patient, they are considered to have acquired immunodeficiency syndrome (AIDS).[10]

Clinical Correlate: A Complete Blood Count (CBC) gives a total white blood cell count and can report the breakdown of the particular types of WBCs. In addition, a CBC also reports hemoglobin, hematocrit, and platelet levels. A normal white cell count is between 4,500 and 10,000 cells per microliter. Several factors may cause an elevated white count such as infection, certain medications (i.e. steroids, antibiotics or anti-seizure drugs), severe physical or emotional stress, chronic bone marrow diseases such as a myeloproliferative disorder and leukemia.[10]

LYMPHATICS

Knowledge about the role and function of the lymphatic system continues to expand. The lymphatic system may be defined as "an organized network, composed of functionally interrelated lymphoid tissue and transportation pathways of tissue fluid/

lymph and lymphoid cells."[12] Lymph components vary based on the tissue the lymph sample is taken from. Nonetheless, lymph is essentially made up of water, proteins, lipids, carbohydrates, enzymes, urea, minerals, hormones, some dissolved gases (i.e. carbon dioxide), cells (such as lymphocytes, macrophages), toxins, bacteria, body waste, and cellular debris.[13] Lymph capillaries originate in almost all vascular tissues; these capillaries feed into precollectors, which feed into ducts and ultimately into the thoracic duct and finally empties lymph into the venous circulation via the left subclavian and internal jugular vein and rarely at the brachiocephalic vein.[13, 2]

If the lymphatic system failed to recover the protein rich liquid into the cardiovascular system, the body would likely develop major systemic edema.[13] If lymphatic vessels were ligated and lymph flow was completely obstructed, tissue necrosis would occur.[14] The lymphatic system is vital to homeostasis and immune function.[12, 13, 14, 15] Despite, the significance of the lymphatic system to human health, most medical professionals fail to appreciate its overall value and know very little about this system.

Lymph vessels are characterized by size and location.[13] Pre-lymphatic pathways and lymph capillaries have neither valves nor muscular units. Pre-collector vessels have an internal valve but lack muscular units. Collector vessels, the primary transporting vessels in the system, have both internal vessels and muscular units, as do lymph trunks and ducts. This may explain why early research showed multidirectional flow in lymph capillaries whereas lymph flow toward the heart was unidirectional.[13] The anatomy of the terminal pathway of lymph flow is of particular importance as it relates to OMT and

other manual medicine. The largest lymphatic vessel is the thoracic duct that divides into a right and left thoracic duct.[12, 13, 15]

The various lymphoid organs are divided into primary and secondary lymphoid organs.[7, 8] The primary organs are bone marrow and thymus. Bone marrow is the primary site of lymphocyte production. The thymus is more significant during the first years of life; it secretes hormones called thymosin and thymopoietin, which causes T-cells to become immunocompetent.[7, 8]

Antigens and lymphocytes eventually encounter each other in the secondary lymphoid organs.[7] Secondary lymphoid organs and tissues include the lymph nodes, spleen, tonsils, the appendix, mucosal associated lymphoid tissues (MALT), gut associated lymphoid tissues (GALT), nasal associated lymphoid tissues (NALT), skin associated lymphoid tissues (SALT), bronchial associated lymphoid tissue (BALT), genitourinary associated lymphoid tissues (GUALT), appendix and Peyer's patches.[7,8, 12] Both the lymph nodes and spleen serve as sites for lymphocytes to proliferate. They have high concentrations of macrophages and lymphocytes and therefore these organs provide a convenient place for exposure to antigens. Lymph nodes serve to filter lymph returning to blood and to activate immune cells. The spleen is the largest lymphoid organ, which functions similarly to the lymph nodes. Its primary functions are lymphocyte proliferation, immune surveillance and response. The spleen filters blood by removing old erythrocytes, platelets, cellular debris, foreign matter, bacteria, toxins, and other waste products.[7,8]

Clinical Correlate: Lymphadenopathy may be caused by increased lymphocytes and/or macrophages secondary to an immune response to an antigen as in a viral infection. Lymphadenitis is the infection of a lymph node itself. Clinically, lymphadenitis is associated with erythema, tenderness, warmth, and fluctuance. Lymph nodes may become inflamed and tender to touch when overwhelmed by antigen. This phenomenon is known as lymphadenopathy.[15]

OMT and IMMUNE FUNCTION

How does manual medicine, specifically OMT, affect the immune system When evaluating the potential mechanisms of how OMT can influence immune function, it is helpful organize the information into three categories. 1) Lymphatic Flow 2) Autonomic Nervous System and 3) Psychoneuroimmunology

OMT and Lymph Flow

A further review of lymphatic physiology and anatomy may be helpful. Anatomists concur that fascia is the primary support structure for lymphatic vessels.[16] The walls of lymphatic vessels are relatively thin and therefore their function is affected by external stress especially by surrounding myofascia. When lymphatic flow is obstructed by myofascial restrictions, surrounding tissues can become edematous. Toxic materials accumulate in edematous tissues inhibiting normal cellular activity.[1,2, 12, 14]

Lymph is propelled through the vessels and back into the venous circulation via both active and passive pumps.[12, 13] Passive pumps of lymph include respiration, skeletal muscle contraction, peristalsis of visceral smooth muscles, contractions of adjacent arteries, active or passive movement of extremities, and external compression.[13] It is apparent that OMT can augment lymph flow by directly affecting many if not all of these passive pumps. OMT influences passive pumps by applying external compression, mobilizing extremities and occasionally requiring patients to cooperate in the therapy by contracting muscles (as in muscle energy techniques). According to Olszewski, external pressure applied to tissues causes tissue pressure to increase encouraging interstitial fluid into lymph vessels.[12] This fluid contains filtered plasma proteins, products of tissue cells and migrating immune cells.[12]

Techniques such as doming the diaphragm may remove myofascial restrictions surrounding the muscle thereby increasing the amplitude of diaphragmatic excursions during respiration; treating rib somatic dysfunction may free rib motion during respiration. This may further amplify another passive pump- respiration.[1, 2] Finally, OMT is theorized to optimize blood circulation, and this again may influence another passive pump.[1,2] Lymphatic pumps, a specific type of OMT, have demonstrated increased lymph flow in the animal model.[17, 18, 19] Lymphatic OMT research has also demonstrated that manual medicine can mobilize lymph and affect immunity in the human body.[20, 21, 22, 23, 24, 25 ,26, 27]

Historically, scientists who studied lymphology asserted that lymph flow completely depended on muscle contraction and movement of the extremities. Both

animal and human studies in the middle to late 1900s failed to demonstrate signs of lymph flow in a still appendage, while active or passive movement caused a continuous stream of lymph.[16] The mechanism offered by Kubik described multiple dilated ampullae throughout the lymphatic system. He stated that as muscle contracted, these ampullae were compressed, driving lymph through the vessels and with relaxation the surrounding connective tissue would then draw apart the vessel walls suctioning more lymph to that area.[28] Basically, the pressure change caused by external compression propels fluids from higher to lower pressure areas.

Recently, as research in this field has progressed, the intrinsic contractility of lymphatic vessels was confirmed by more sophisticated studies (although the rhythmic contraction of lymphatic vessels in animal models were recognized as early as 1774). A lymphangion is the segment between two lymph vessel valves; it is made up of 2-3 layers of spiral muscles. These segments are considered to be the active lymph pumps, because they contract after distending past a certain threshold. This contraction or systole occurs anywhere from 5 to 18 times a minute.[12, 13, 16] The contraction of one lymphangion may stimulate consecutive contraction of adjacent lymphangions, therefore moving lymph towards the terminal lymphatic ducts. This peristaltic wave of contractions is mediated by the ANS.[12, 13]

Autonomic Nervous System and Immune Function

OMT is theorized to modulate the autonomic nervous system and balance the tone of the sympathetic and parasympathetic nervous systems. It is well known that autonomic

nerve fibers do indeed innervate lymphatic vessels.[13] Manipulation of the vertebrae, paraspinal muscles associated with spinal nerves and the sympathetic chain ganglia that innervate areas of lymphatic congestion may theoretically improve the active pump of lymph flow.[16] Several studies illustrate the relationship between immune function and the nervous system. Allen submits the following points that demonstrate this relationship in his review of the literature.[29, 30, 31, 32, 33, 34]

- Organs and tissues of the immune system such as the thymus, bone marrow, spleen, lymph nodes, and other lymphoid tissue receive neural innervation, thereby signifying neural influence on cellular function and blood flow to these tissues and organs.
- Lesions of the hypothalamus, pituitary gland, and peripheral nerve sectioning can result in depressed function of the bone marrow, thymus, spleen, and other lymphoid tissues. Conversely, stimulation of peripheral nerves also affects immune response.
- Lymphocytes and other WBCs have receptors on the cell surface for a multitude of neurotransmitters such as adrenergic, cholinergic, and histamine substances, endorphins and other neurally active peptides

Psychoneuroimmunology

Fundamental to the osteopathic approach to patient care is the concept that each individual has a physical, mental, and spiritual component and that these parts influence each other.[1, 2] The newly immerging field of psychoneuroimmunology studies this very

relationship. Psychological stress may cause increased susceptibility to cancer, lower lymphocytes' cytotoxicity, lower natural killer cell activity, change immunoglobulin levels, alter interferon production and decrease other immune function.[35] Recent studies have identified a link between chronic psychological stress to upper respiratory infection and disease.[36, 11] In addition, the intimate relationship between the ANS and immune function is demonstrated in a clinical trial, n=36, conducted by Redwine et. al.[37] Redwine measured autonomic measures, heart rate, vagal tone, blood pressure, and the hormones of the hypothalamus-pituitary-adrenal axis for their relationship in mediating lymphocyte proliferation in postpartum and non–postpartum subjects. She discovered that lactation and parturition can effect lymphocyte proliferation and that vagal activity can influence the stress response of lymphocytes.[37] If OMT does indeed have relaxing effects and can balance ANS tone, perhaps this is another mechanism by which OMT can affect immune function.

CHAPTER II

LITERATURE REVIEW of PRELIMINARY STUDIES

In this information age, society values and even demands evidence based medicine. Therefore, it is imperative to continue answering questions relating to osteopathic medicine, its mechanism of action and its efficacy. There is a scarcity of literature on how osteopathic medicine affects the human immune system. However, careful examination of osteopathic research and further review of other fields of manual medicine and its influence on immune cells provided valuable insight and has guided the design and protocol used in this study.

OMT LITERATURE

In 1918, America suffered a devastating influenza epidemic.[38] Kolata reports that there were approximately 30 million fatalities related to this two-year outbreak. More than twenty-eight percent of the American population lost their lives.[39] This outbreak gave osteopathic physicians visibility while the profession was still in its infancy. D.O.s used OMT to help their patients in a time before the use of antibiotics and other forms of modern medicine.[38] Osteopathic physicians appreciated significant benefits in their patients who received OMT, specifically the thoracic lymphatic pump (TLP). The American Osteopathic Association decided to investigate the potential efficacy of OMT especially to patients who had been affected by the epidemic. Of the100,000 cases of

influenza that were reviewed, the mortality rate among the patients of osteopathic physicians who received OMT was 0.25%, substantially lower than the 5% mortality rate of patients who did not receive OMT. In addition, of the 6000 cases of epidemic-related pneumonia that were treated with OMT, the mortality rate was 10%; this was significantly less than the 30% mortality rate of patients affected by epidemic-related pneumonia treated by allopathic physicians.[38]

These interesting findings inspired further research in the role of OMT on immune function during the 1930s. In 1932, Castlio and Ferris-Swift studied the effects of splenic manipulation in one hundred healthy individuals by measuring a complete blood count and the opsonic index before treatment and after treatment.[20] Their results showed that five minutes of splenic manipulation increased leukocyte counts in 80% of subjects, increased the opsonic index in over 80% of subjects and finally decreased red blood cell counts in over 75% of the subjects.[20] Two years later, they studied the specific effects of splenic manipulation in subjects with acute infectious disease (n=100) and found that the WBC counts of the subjects had a mean increase of 2.2×10^3 in about 80% of the subjects after manipulation and a mean increase of 7% in PMN counts.[21]

There are a few more recent studies exploring the influence of OMT on the immune system. Measel, 1982, recruited healthy male students (n=21) to study the antibody response to pneumococcal polysaccharide after receiving lymphatic pump.[22] He reported that subjects who received thoracic lymphatic pumps twice daily for one week after receiving the Pneumovax had a significantly higher concentration of antibodies against inoculated bacterial polysaccharides (types 3,4,25,51,1, 6, 14, and 19).[22] Later in

1986, Measel, et al studied the effect of the lymphatic pump on the B cells and T cells in peripheral blood. He then found that TLP increased total white blood cell counts, decreased lymphocyte counts and increased relative percentages of T cells and B cells.[23] Jackson, 1998, conducted a pilot study to investigate the efficacy of lymphatic and splenic pumps on the antibody response to a series of Hepatitis B vaccine given at 0, 5, and 25 weeks.[24] Thirty-nine subjects were recruited; 20 were assigned to the experimental group while 19 individuals were controls. The experimental group received OMT three times a week for two weeks after each vaccination. There treatment groups showed consistently higher antibody titers than the control.[24] These studies point out that lymphoid organs and lymphatic flow are key to the activation and distribution of lymphocytes in the body. In an effort to continue research in this area, this study is designed to investigate the affects of OMT using various techniques with a special emphasis on techniques removing lymphatic restrictions and mobilizing lymph.

According to concurrent research done at the University of North Texas Health Science Center, an immunologist partnered with an osteopathic physician to develop a rat OMT model to further study the efficacy of the lymphatic pump technique (LPT) in improving antigen-specific antibody responses during immunization.[40] In this study, the rats were divided into control and LPT treatment groups. These rats were immunized with ovalbumin (OVA) antigen or PBS (control). The animals in the treatment group were given eight minutes of LPT under anesthesia daily for 3 days straight. Seven days after immunization, blood and spleens from the animals were analyzed for OVA-specific antibody responses. Hodge reported that OVA-specific IgG titers were approximately 2-

fold higher in rats given LPT.[40] This indicates LPT enhanced antigen-specific adaptive immune response.

Taking the animal model further, Knot et. al. canulated the thoracic duct of five mongrel dogs to measure flow and concentrations of various leukocytes before and after receiving OMT, specifically, lymphatic pump. The pre-OMT leukocyte concentration in the lymph was $1.7 \times 10^6 \pm 0.57 \times 10^6$ cells/ml. This figure increased significantly following lymphatic pump to $6.2 \times 10^6 \pm 1.6 \times 10^6$ cells/ml. In addition, macrophages, neutrophils, lymphocytes, T cells and B cells were significantly enhanced ($p \leq 0.05$) during LPT and lymphatic flow rate increased around five-fold. This study indicates that OMT does enhance lymphatic flow, increase WBC output in lymph, which eventually disseminates to venous circulation. Hodge concluded "this release may be one of the immune mechanisms responsible for the increased immune responses observed in patients given OMT." [41]

MANUAL MEDICINE

Beyond OMT research, there is increasingly more research supporting the immunological benefits of other manual medicine modalities and alternative medicine such as chiropractic, massage therapy, Reike touch, and others. Many of these efforts have indicated that manual medicine can influence specific mechanisms within the immune system.

According to a Japanese investigation done in 2002 by Iwama et al, a skin rubdown with a dry towel activated natural killer cells in bedridden elderly patients.[42] "Kanpu- mastsu" is a traditional therapy used in Japan to enhance the immune system and improve the autonomic nervous system.[42] The theory supporting this practice is that by rubbing skin you provide compression and remove cutaneous sebum thereby enhancing lymph circulation and peripheral blood flow. Iwama and Akama studied the effects of skin rubdown on lymphocyte counts, gamma globulin levels, CRP, neutrophil and natural killer cell counts in a geriatric population.[42] Though there was no significant change in lymphocyte, gamma-globulin counts or in CRP levels, the study did indicate increased neutrophil and natural killer cells counts. It was speculated that the elevation in natural killer cells may have an effect of certain mediators released from the T lymphocytes and/or the stimulated effect on the sympathetic nervous system. They concluded that daily skin rubdown activates natural killer cells in geriatric patients who are confined to the bed.[42] This is a cost effective way to enhance cell-mediated immunity in patients who may be more susceptible to nosocomial infections.

In general, all health professionals seek better, more cost effective, less risky, more personable and compassionate methods of providing effective quality care to patients, especially in an environment where technology is slowly replacing touch.[43] In a single group repeated measures designed study using 23 healthy subjects conducted by the University of Texas Houston Health Science Center-School of Nursing, the physiologic and biochemical effects of Reike Touch were investigated.[44] Reike touch is a form of manual medicine that balances the body's energy through the hands of an

experienced Reike healer or master. This form of touch therapy originated in Tibet, approximately 3000 years ago. The protocol involved thirty minutes of Reiki touch. The outcome measures studied were state anxiety, Salivary IgA and cortisol levels, blood pressure, galvanic skin response, muscle tension, and skin temperature. The results of this study demonstrated significant decreases in state anxiety and blood pressure, and significant increases in salivary IgA. It is theorized that the relaxation response may have contributed to increased salivary IgA.[44]

In another study conducted by nursing professionals, Groer et al, 1994, measured salivary IgA and state anxiety following a 10-minute nursing back rub.[43] Thirty-two older adults volunteered to participate in this study, and there were no exclusion criteria. These subjects gave a five-minute unstimulated saliva sample, received a 10-minute massage, and then gave a second saliva sample using the same method. Jensenm Nelson developed the manual therapy utilized in this study. The back rub was theorized to stimulate lymphatic flow by using warm lotion and making slow effleurage circles near the vertebrae, over the paraspinal muscles, across the upper chest region, and spanning the back all the way to the iliac crest. Physical contact was maintained throughout the session. Of the 32 subjects, 14 were controls. The average age of the control group was 64 years old, and the average age of the experimental group was 67 years old. The results of this study showed that state anxiety levels did not change, but salivary IgA concentrations increased in both groups. The concentrations of salivary IgA in the experimental group nearly doubled after the back rub.[43]

When investigating the effects of chiropractic on the immune system, it is critical to understand the origin of this field of manual medicine. Fundamental to chiropractic care is the idea that disease and health are dependant on the nervous system and that chiropractic alters and balances autonomic tone.[29, 30, 45, 46] Based on a separate literature review studying mechanisms of physiological responses to chiropractic adjustments, the majority of the consistent observations in inflammatory function indicate a firm relationship between the sympathetic nervous system and exacerbations of disease states.[45] Also, according to chiropractic research there is evidence that the sympathetic nervous system participates in the modulation of the immune response.[45] A new area of interest in clinical research and especially in chiropractic studies is the effect of chiropractic care on immune deficient patients such as those who are HIV positive. A randomized, controlled clinical trial used 10 HIV positive patients to study the potential benefits of chiropractic reduction of subluxations.[30] In this 1994 study, Selano reported that the adjusted group experienced a 48% increase in CD4+ T lymphocytes over a six month period. It is theorized that this enhanced immune function may be related to stress and its relationship to the autonomic nervous system.[30] Stress stimulates the release of catecholamines and norepinephrine; this may enhance replication of HIV and suppress the release of cytokine, interferon gamma, and interleukin 10. Therefore it is hypothesized that chiropractic care may in fact improve immune function in HIV patients by decreasing stress and in doing so, decreasing sympathetic tone.[30]

Another very popular mode of manual therapy is massage therapy. According to a study conducted by the Office of Alternative Medicine, approximately 60 % of those

patients infected with HIV who seek alterative treatments for their condition get massage therapy.[47] Ironson reported that HIV positive men benefited from daily massage therapy over a one month period of time; the natural killer cell counts, cytotoxicity and soluble CD8+ T cells all increased and the cytotoxic subset of CD 8+ T cells significantly increased.[48] In addition, Diego, 2001, studied the effects of massage therapy in HIV positive adolescents, n=24, and found increases in natural killer cells, CD4/CD8 T cell ratio, and CD 4+ T cell numbers.[49] In Field's study, twenty pediatric patients with leukemia received daily massage therapy provided by their parents at bedtime for one month.[50] State anxiety and depressed mood of patient and parent and patient's complete blood counts were measured at the beginning and end of a 30-day trial and were compared to the control group who did not receive any manual medicine. The investigators reported that there was an increase in both WBC and neutrophil counts, and a decrease in the parents' depressed mood.[50]

According to the above studies, research results indicate that manual manipulation may improve immune function by enhancing the effects of vaccinations, preventing primary and secondary infections, increasing antibody levels, boosting leukocytes in the setting of immune-compromised individuals. Furthermore, osteopathic literature emphasizes the benefits of manipulation of lymphoid organs and enhancing lymphatic flow to increase immune function. Additional research is necessary to better understand the mechanism by which OMT affects immune function.

SPECIFIC AIMS

This study was conducted with many goals in mind. The purpose of this prospective, randomized and controlled clinical trial was to design a protocol that was feasible, reproducible and preserved the integrity of the scientific method. It aimed to investigate the immediate effects of OMT on immune function in a healthy population by measuring a broad spectrum of immune cells. The outcome measures for this research were selected based on previous research conducted in the area of manual medicine and its influence on immune function; they were: salivary IgA, serum white blood cell counts with a differential, B lymphocytes, CD4+ T lymphocytes, CD8+ T lymphocytes and natural killer cells. No other osteopathic research has examined the influence of OMT on salivary IgA or used a global OMT protocol with a lymphatic emphasis. This study was constructed so that other studies may be modeled after it using different outcome measures, different OMT techniques, and different populations. Finally, this study aimed to test the following hypotheses:

1. Hypothesis 1: OMT will increase salivary immunoglobulin A (IgA) as compared to the control group.
2. Hypothesis 2: OMT will enhance white blood cell count, specifically granulocyte count as compared to the control group.
3. Hypothesis 3: OMT will increase B and T lymphocyte percentages, increase T lymphocyte subsets CD4, CD8 and natural killer cells as compared to the control group.

CHAPTER III

METHODS

This was a prospective, randomized, blinded and controlled clinical trial designed to study the immediate effects of OMT on immune function in a healthy population. A power analysis was calculated using Analysis of Variance (ANOVA) to determine how many subjects needed to be recruited. A total of 42 subjects (21 per group) was needed to demonstrate 85% power at a .05 alpha level of significance, however 50 were recruited to allow for a 15% attrition rate. Fifty healthy subjects were recruited to participate and 42 successfully completed the study.

INCLUSION / EXCLUSION CRITERIA

The inclusion criteria for this study included that subjects must be between 18 and 40 years of age. A narrow age range was studied to minimize confounding factors and variability such as undiagnosed chronic disease in older individuals. Children were excluded because they may have not reached the full potential of their immune function and are often considered an immune compromised group.[51] Likewise, the geriatric population were excluded as they may have declined immune function. There is evidence indicating that as patients age their immunological responses weaken and may not be as efficient; this is a phenomenon known as immunosenescence.[52] For this trial subjects

must have been healthy devoid of chronic or acute illnesses or infections. Both male and female subjects, as well as subjects from all ethnic backgrounds were recruited for the study.

The exclusion criteria for this study included the use of medications that directly affect immune function such as antibiotics, antivirals, steroids, or non-steroidal anti-inflammatory drugs. These medications cannot be used by subjects within the two weeks preceding the study. Individuals who were undergoing chemotherapy and/or radiation therapy were excluded. Individuals with known medical conditions affecting immune function (including, but not limited to rheumatoid arthritis, systemic lupus erythematosis, hepatitis, cirrhosis of the liver, leukemia, multiple myeloma, ataxia-telangectasia, nephrotic syndrome, HIV infection, other autoimmune diseases or infectious processes) were excluded. Chronic medical conditions (including but not limited to diabetes mellitus, cardiovascular disease, chronic obstructive pulmonary disease, thyroid disorders, cancer, or chronic renal failure) were also exclusion criteria. Individuals were excluded if they had any conditions for which OMT is contradicted such as bone metastasis, severe osteoporosis, osteomyelitis, and fractures. Consumption of alcohol within twenty-four hours prior to treatment and smoking within 12 hours prior to treatment also served as exclusion criteria because these factors may alter immune function .[36, 53, 54] Subjects were also asked not to exercise 12 hours prior to treatment, again because of the potential influence on immune function.[55] Finally, subjects were excluded if they were unable to give informed consent.

RECRUITMENT

50 subjects were recruited to participate in this study from the University of North Texas Health Science Center (UNTHSC). Advertisements were posted on campus and in the waiting rooms of the clinics in the Patient Care Center. Participation in this clinical trial was completely voluntary. At any point during the study, subjects were able to withdraw with no consequence to their employment and/or academic standing.

Potential subjects underwent preliminary screening over confidential electronic mail and/or phone conversations, and were scheduled for further screening depending on the initial interaction. Once eligibility was confirmed, a member of the research team obtained informed consent from the subjects. Subjects were informed on the nature of the study, the risks, the protocol, rights to withdraw and given contact information, verbally and in writing. After informed consent was signed, the subject was scheduled for the protocol and reminded of the inclusion/exclusion criteria.

COLLABORATIONS

This research was a collaborative effort between osteopathic clinicians, basic scientists, and technologists. Resources from UNTHSC Immunology Department included:

- Fully equipped laboratories in the Department of Immunology and Microbiology – used to prepare and analyze samples

- A flow cytometer - used to analyze serum samples for B lymphocytes, T lymphocyte subpopulations, and NK cells.
- A freezer set at -70 degrees Celsius – used to freeze and store saliva samples

Dr. Jones, an immunologist, along with an experienced research technician, Tejaswi Belawadi supervised the student technologists from Tarleton State University (TSU)

TSU's department of Clinical Laboratory Sciences also offered their resources and personnel to make this research possible. They provided a Coulter Counter that was used to measure the CBCs of the subjects. Two of TSU's faculty members, Sally Lewis, a Masters leveled immunologist and Virginia Reyes, a hematology instructor, analyzed the saliva samples using ELISA. These professors also oversaw student technologists who conducted the CBCs. Finally, both phlebotomists were students in this program.

Finally, the OMM department at the Texas College of Osteopathic Medicine (TCOM) and the Osteopathic Research Center (ORC) provided the vast majority of the infrastructure that was utilized for this clinical trial. This study was completely funded by the ORC. Two clinic rooms in the medical school's primary care center were used for the study protocol. In addition, osteopathic physicians from TCOM and the ORC were consulted regarding the OMT protocol.

PROCEDURES

The protocol used an experimental design with 50 subjects assigned in a random fashion to two groups - either an osteopathic manipulative treatment (OMT) group or

Rest only (no treatment) group, group A and group B respectively (25 in each group). Subjects were randomized using block permutation on a statistical program called SPSS.

Prior to collecting initial blood and saliva samples, all subjects had their temperature, blood pressure, pulse, respiratory rate and pain level (0-10) taken at the start of the session. Subjects were excluded if they had abnormal vital signs for this could indicate illness.

Both groups had their blood drawn by a student technologist from Tarleton State University. Approximately 3 milliliters of blood were collected in two small test tubes each, totaling 6 milliliters. The blood was drawn using a 23-gauge needle to minimize discomfort without compromising the integrity of the cells. After wiping out the oropharynx with dry sterile gauze, subjects then produced a non-stimulated salivary sample by spitting into 2 mL microcentrifuge tubes. All saliva and blood samples were immediately labeled with a random code number (linked to the identity of each subject and their group assignment) and stored in a cooler. Then group A received thirty-minutes of OMT while group B rested for the same period of time. After the thirty minutes, the student technologist drew a second blood sample using the same technique. Again, subjects produced a saliva sample in the same manner. Pre-treatment blood samples were taken from the right arm and the post-treatment blood draws from the left arm. There were four exceptions, which occurred because the phlebotomist did not feel like there were "good veins" on the appropriate arm. Also, there were two subjects whom post blood samples were not successfully obtained secondary to phlebotomy failure. After completion of the study protocol the subjects were verbally questioned to assess overall

satisfaction and sense of well being. All subjects were reimbursed $25.00 for their time and travel regardless if they completed the entire protocol.

TABLE 1: PROTOCOL

Group A			
(n=25)	BD/SS	OMT 30 min.	BD/SS
Group B			
(n=25)	**BD/SS**	**Rest 30 min.**	**BD/SS**

BD =Blood Draw
SS = Saliva Sampling

All samples were stored in a cooler until they were analyzed. All blood samples were analyzed within 12 hours of being drawn. The salivary samples were stored and frozen at -70 degrees Celsius analyzed within 2-8 weeks of collection using an ELISA.

SAMPLE ANALYSIS

In this study, flow cytometry was used to analyze blood samples to determine the amount of B lymphocyte percentages, T lymphocyte percentages, and subpopulation concentrations (CD4+ cells, CD8+ cells, and natural killer cells). Student technologists

from TSU under the direct supervision of an experienced research technician conducted all of the flow cytometry. This technique uses one particular frequency of light to analyze, count, and sort microscopic particles suspended in a stream of fluid based on the unique characteristics of the cells/particles.[10] Detectors are directed to the point where the light meets this stream. There are several detectors aimed perpendicalar to the light and these are referred to as side scatter; there is also one detector that is parallel to the beam of light known as forward scatter. Based on the scattered light as well as the fluorescent light emitted by the particles, flow cytometry makes it possible to analyze these particles. Among the measurable parameters are:cell surface antigens (CD markers), intracellular antigens (various cytokines, secondary mediators etc.), enzymatic activity, oxidative burst among many other.[10] This data was viewed in 2D as dot plots and gated were implemented by an immunolgist.

The Enzyme Linked Immunosorbant Assay (ELISA) technique was used to identify and quantify the salivary secretory IgA (SIgA) in the saliva samples. Salivary secretory IgA indirect enzyme immunoassay kits were purchased through Salimetrics. Goat anti-human SIgA conjugated to horseradish peroxidase was added to the saliva samples. Antibody-conjugate binds to the SIgA and concentrations were determined based on the remaining free antibody.

Obviously, the OMM predoctoral fellow who assessed and provided treatment was not blinded. However, all other members of the research team including the research technologists from Tarleton State University who performed phlebotomy and analyzed the serum and saliva were blinded to the subject's group assignment. All subjects were

specifically directed not to disclose details of their treatment group status to those who were blinded in this study. Finally, an OMM predoctoral fellow who was blinded to group assignment completed all of the data entry.

OMT PROTOCOL

A fourth year osteopathic medical student who is also an OMM predoctoral fellow in the Department of Osteopathic Manipulative Medicine performed the assessment and treatment of all subjects. A NMM-OMM board certified physician approved the OMT protocol. In addition, he trained two OMM predoctoral fellows on the techniques used in the study protocol. One fellow was the OMT provider for all subjects in the study; the other fellow was trained for backup purposes only. The OMM specialist attended an interim sessions to asses the quality and consistency of the OMT protocol.

The OMM predoctoral fellow provided the OMT session, which lasted approximately 30 minutes. OMT was implemented to resolve somatic dysfunction in the general areas of the neck, back, ribs, diaphragm, and thoracic inlet. These treatments focused on removing lymphatic obstructions, enhancing mobilization of lymph, optimizing respiratory mechanics, increasing venous drainage and balancing the autonomic nervous system.

Each individual received a standardized treatment addressing the eight regions/areas of emphasis as outlined below. However, because each individual has unique somatic dysfunction and responds differently to various OMT techniques, the

predoctoral fellow individualized the 30-minute session by using one or many OMT techniques (see appendix B) to treat each of the eight areas. In an effort to maintain the integrity of the standardized protocol the predoctoral fellow treated all eight regions/areas of emphasis in this order. See appendix A for pictures and descriptions of these treatments; the following list only provides the order, time frame and rationale for the OMT protocol.

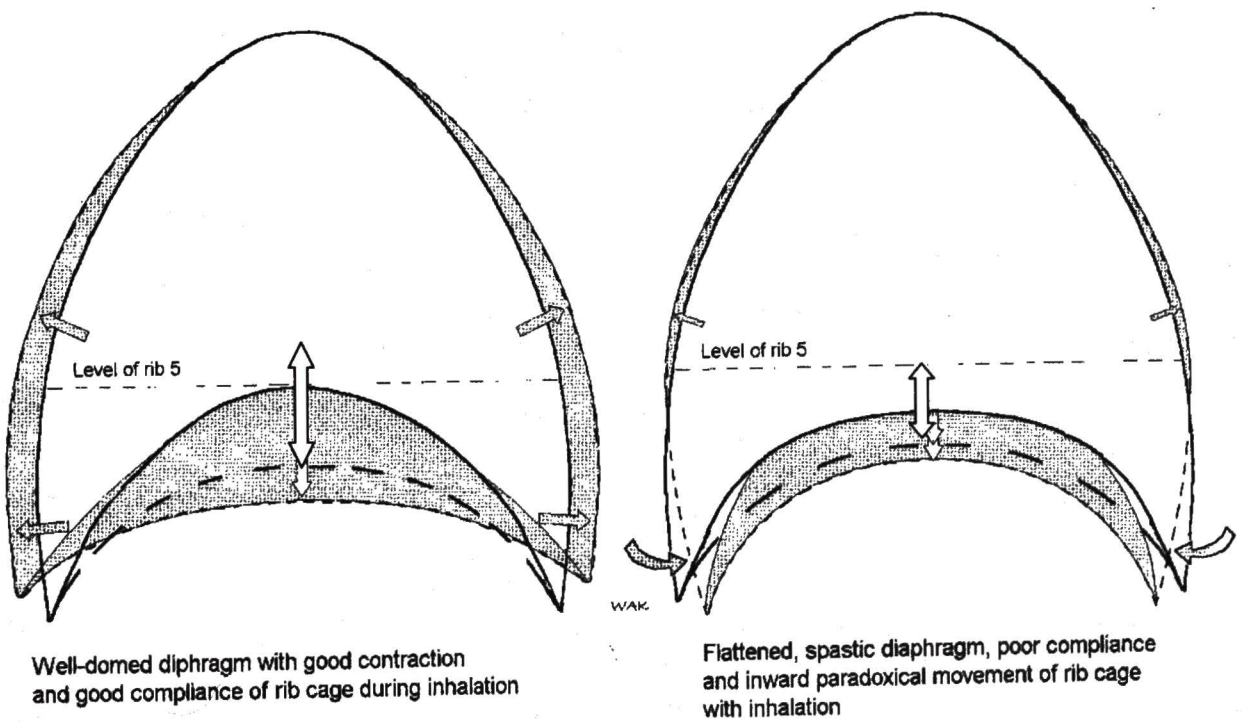
1. Occipito-atlantal (OA) Decompression (2-4 minutes) – Anatomically, the occipito-atlantal joint is positioned very close to the vagus nerve.[56] Therefore, because of its relationship with the vagus nerve the OA joint is theorized to be especially important to parasympathetic tone in the body.[4, 5, 57] This technique was used because of the previously described relationship between the autonomic nervous system, lymph flow and immune function. See appendix B for description of this technique. The cervical region tissue was prepared by using soft tissue techniques prior to OA decompression.[58]
2. Sibson's Fascia Release (2-3 minutes) - The cervicothoracic diaphragm, also known as Sibson's fascia, is associated with the thoracic inlet. The thoracic inlet is defined by the following anatomical boundries: the manubrium, clavicles, 1st ribs and the body of the first thoracic vertebrae.[56] Based on the anatomical position of the thoracic duct it is theorized that by removing myofascial and bony obstructions from this region lymph

drainage is optimized.[4, 5, 46] See appendix B for description of this technique.

3. Doming the Diaphragm (2-4 minutes) –This myofascial release of the thoracoabdominal diaphragm is used to remove areas of restriction thereby increasing the amplitude of diaphragmatic excursion. By optimizing this movement of the diaghragm, an important passive lymph pump, lymphatic flow is enhanced.[57] See appendix B for description of this technique.

Figure 1: Effect of Doming the Diaphragm

Picture from *Practical Osteopathic Procedure*



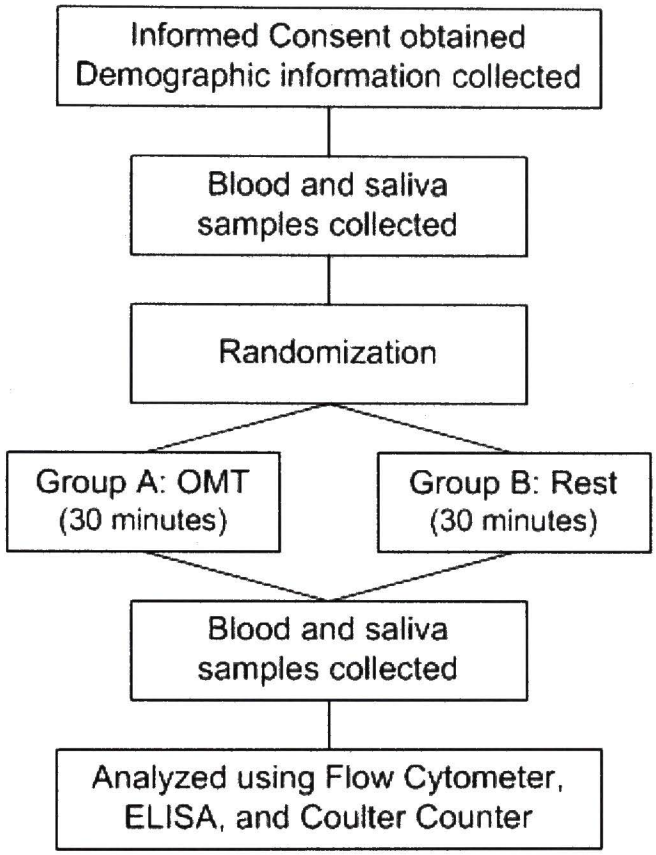
4. Rib Raising (2-3 minutes) – Because of the relationship between the rib heads and the sympathetic chain ganglion, rib raising is used to decrease

hypersympathetic tone thus increasing lymphatic and venous flow.[4, 5, 58] See appendix B for description of this technique.

5. Pelvic Diaphragm Release (2 minutes) – The pelvic diaphragm works with the abdominal diaphragm to mobilize fluids in the body.[4, 5, 57] See appendix B for description of this technique.
6. Assessment and Treatment of T1-L2 Somatic Dysfunction (7 minutes)- This is the region of sympathetic innervation to the entire body.[4, 5, 57] According to the nature of the somatic dysfunction found in this area, HVLA, ME, indirect and/or myofascial release techniques were used.[58] Prior to correction, the region was prepared with soft tissue techniques.
7. Thoracic Pump (2-3 minutes)- Pumps are used to augment and promote lymph flow. The thoracic pump uses physiological principles to create a negative pressure gradient to improve lymphatic flow.[5, 57] Oscillatory pressure applied to the thorax is synchronized to breathing cycles; this pressure progressively increases in intensity. As pressure is released, the negative intrathoracic pressure pulls lymph into central circulation; as positive pressure is applied the valves in the lymphatic vessels prevent retrograde flow.[57] See appendix B for description of this technique.
8. Pedal Pump – (1 minute) - This pump was also used to augment and promote lymph flow. External pressure on the sole increases tissue fluid pressure and thus encourages fluid into to lymph vessels.[12, 13] Repetitious, pumping motion further promotes fluid from the periphery towards central

lymphatic circulation. This pump was given for one full minute. See appendix B for description of this technique.

FIGURE 2: FLOW CHART OF PROTOCOL



DATA ANALYSIS

Statistical analyses consisted of descriptive statistics for demographic characteristics. To determine how successful randomization was, statistical calculations

were done to compare the demographics and baseline characteristics between the two groups.

An analysis of covariance (ANCOVA) was computed to evaluate six of the continuous variables. Due to skewed distributions, a nonparametric test, Mann Whitney U was used to analyze the remaining three outcome measures. Change scores between pre and post outcome measures were used. All analyses were conducted using the SPSS v12.0 for Windows statistical software package with an alpha value of 0.05.

Finally, exploratory data analysis was conducted to further investigate the influence of OMT on these outcome measures. Beyond the outcome measures of this study, other components of the CBC such as red blood cells, platelets, hemoglobin and hematocrit were examined to see if and what immediate influence OMT had on these markers. Also, change scores and means were evaluated to identify any trends.

CHAPTER IV

RESULTS

A total of 50 subjects were recruited to participate in the study. Twenty-five subjects were randomly assigned into the OMT group and 25 were assigned to the control group. The majority of the participants were male (64%). The ethnic breakdown was approximately 46% White, 34% Asian, 10% Latino, 8% African American and 2% Other. The mean age of the participants was 26.9 (SD = 4.54).

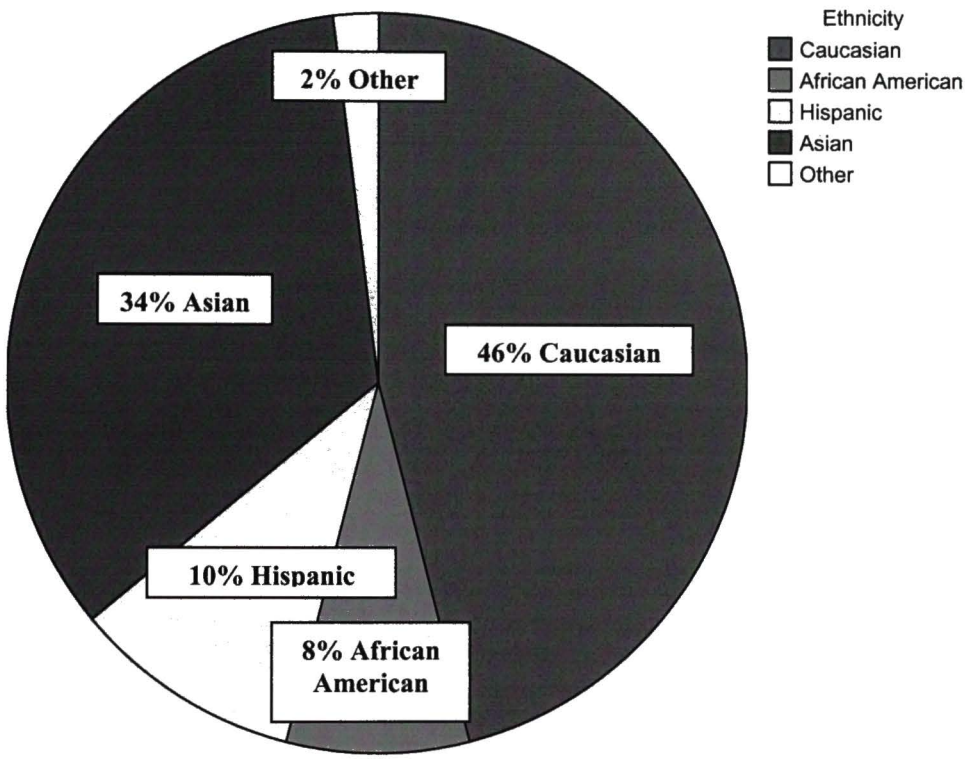
The groups were compared using chi squared and revealed that they were not statistically different with regard to gender or ethnicity. However, an independent T test revealed that there was as statistical difference in age between the two groups, p= 0. 05.

Therefore, to control for this difference, an analysis of covariance (ANCOVA) was performed for 6 of the outcome measures with the OMT group as the independent variable and age as the covariate. This analysis revealed no significant differences between groups with lymphocyte counts, $F(1, 41) = 1.21$, $p = 0.28$, monocyte counts, $F(1, 41) = 2.36$, $p = 0.13$ and granulocyte counts, $F(1, 41) = 0.27$, $p = 0.60$.

CD8+ T cell counts did not differ significantly between groups, $F(1, 39) = 0.47$, $p = 0.50$ and this trend continued for CD4+ T cell counts, $F(1, 39) = 1.80$, $p = 0.19$. Finally, the primary outcome measure, salivary IgA concentrations did not differ significantly between groups, $F(1, 40) = 0.14$, $p = 0.71$.

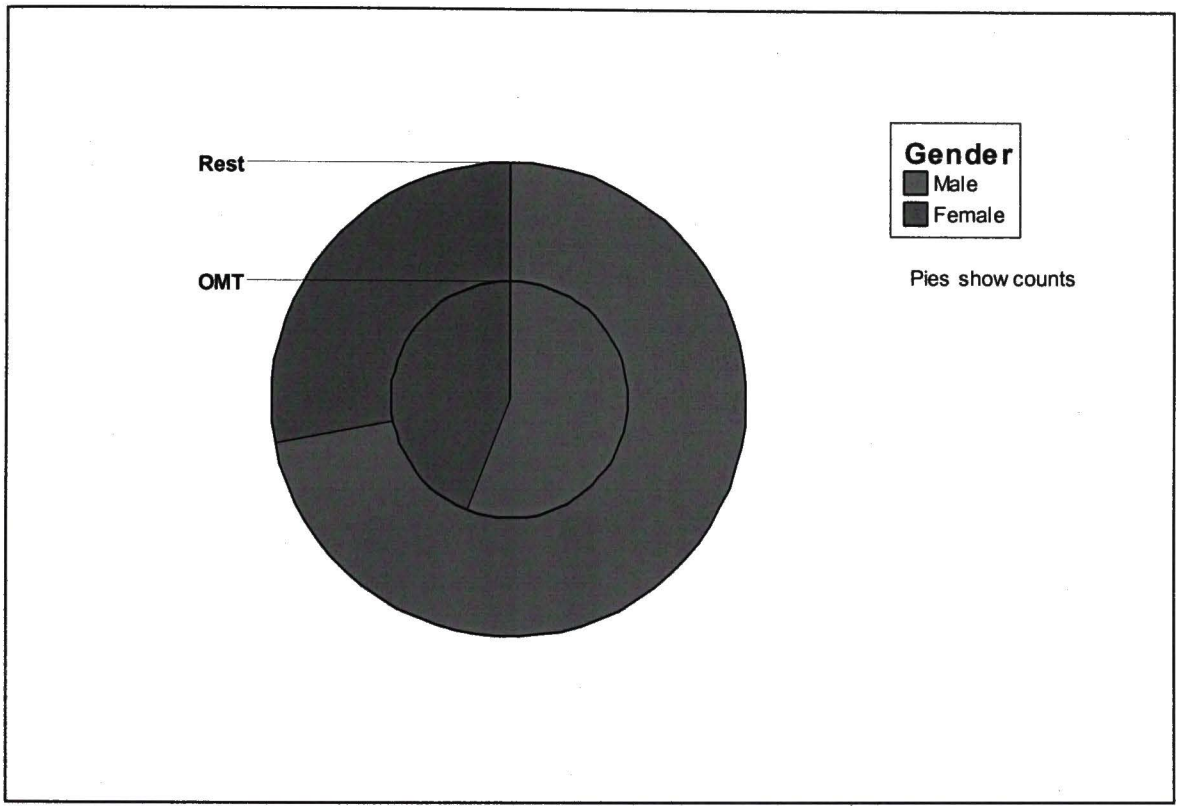
Three dependent variables had distributions that were skewed and therefore were submitted to nonparametric analyses. Mann-Whitney U tests revealed that white blood cells, $z = -1.17$, $p = 0.24$, natural killer cells, $z = -0.99$, $p = 0.33$ and B lymphocytes, $z = -0.67$, $p = 0.51$. These outcome measures also confirmed a null hypothesis and were not significantly different between the groups.

FIGURE 3: ETHNIC BREAKDOWN



OMT GROUP: 13 Caucasian, 12 Minority
REST GROUP:10 Caucasian, 15 Minority

FIGURE 4: GENDER BREAKDOWN BETWEEN GROUPS



OMT GROUP: 72% Male, 28% Female
REST GROUP: 56% Male, 44% Female

FIGURE 5: AGE BREAKDOWN BETWEEN GROUPS

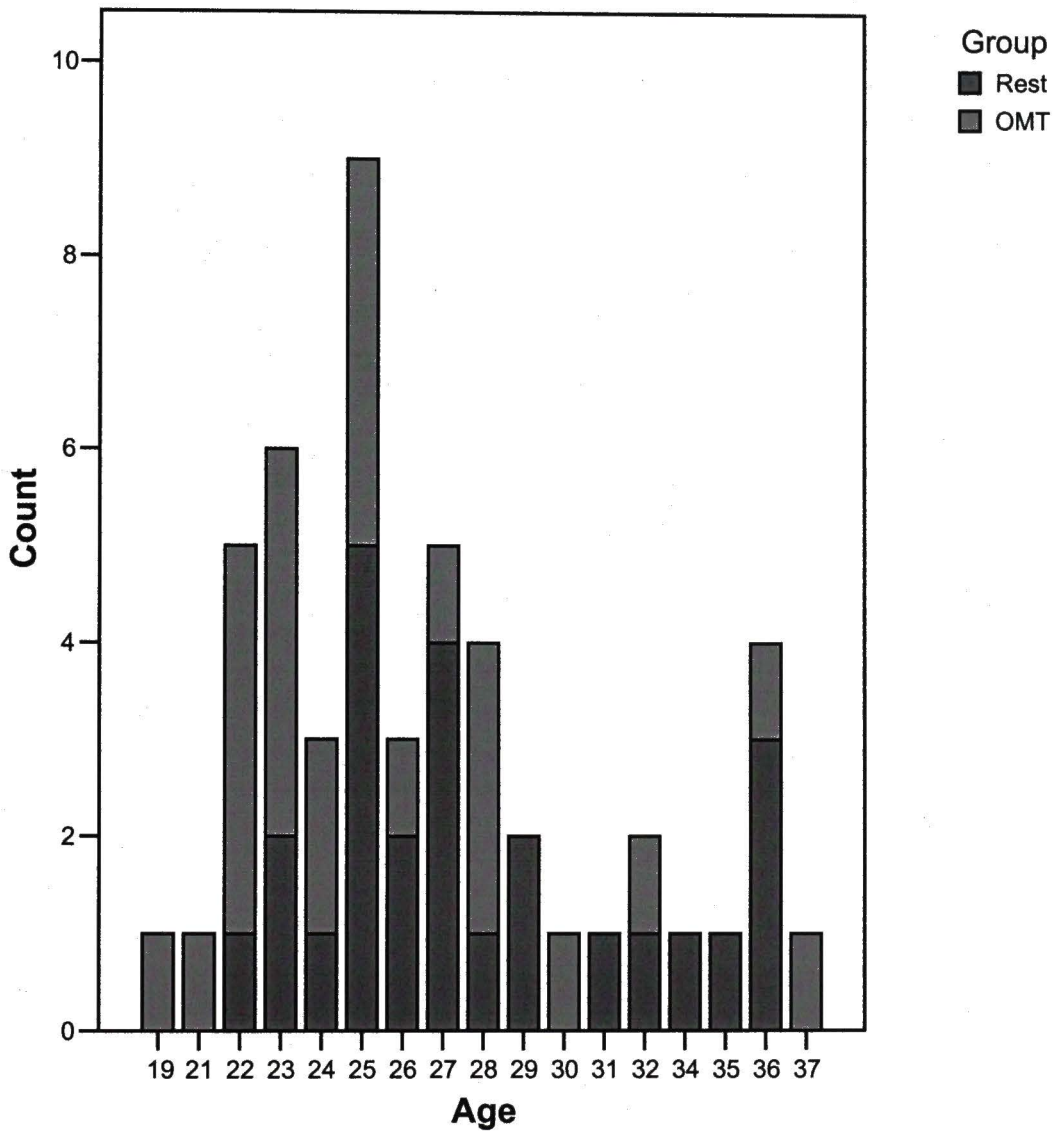


Table 2. Demographics and Baseline values of OMT and Control group

	Control	OMT	P value
Age	25.60	28.12	.05
Gender	18 ♂, 7 ♀	14 ♂, 11 ♀	.24
Ethnicity			.29
White	13	10	
Asian	9	8	
Latino	1	4	
Black	1	3	
Other	1	0	
S IgA µg/ml	214.82	281.45	.00
Total WBC x 10^9/L	6.03	5.88	.47
Lymphocytes %	39.93	36.75	.18
Monocytes %	10.70	9.44	.28
Granulocytes %	49.14	53.67	.37
CD4+ T Cells	25.55	32.71	.04
CD8+ T Cells	21.92	24.19	.59
B Lymphocytes	10.53	10.56	.49
NK cells	12.83	11.37	.86

Table 3. Outcome Measures

Outcome measure	CONTROL			OMT			
	Pre	Post	Δ (post-pre)	Pre	Post	Δ (post-pre)	ANCOVA# p-value
S IgA µg/ml	214.82	143.42	-71.40	281.45	222.72	-48.09	0.71
Total WBC x 10^9/L	6.03	5.88	-0.12	5.88	5.84	0.16	0.24*
Lymphocytes %	39.93	39.42	-0.41	36.75	36.18	-1.16	0.28
Monocytes %	10.70	9.11	-1.90	9.44	8.39	-0.30	0.32
Granulocytes %	49.14	51.24	2.31	53.66	55.21	1.41	0.60
CD4+ T Cells	25.55	27.56	2.28	32.70	29.71	-1.90	0.19
CD8+ T Cells	21.92	20.58	-0.42	24.19	21.80	-2.32	0.50
B Lymphocytes	10.53	9.99	-0.59	10.56	11.37	0.60	0.51*
NK cells	12.83	7.00	-5.74	11.37	8.04	-2.45	0.33*

*p value calculated using Mann-Whitney U nonparametric test
#ANCOVA was calculated to account for difference in age between groups

CHAPTER V

DISCUSSION

Although a total of fifty subjects were recruited, data was only collected for 48 individuals, 24 in each group. In addition, complete data sets were not collected for all of the subjects. After one vial of blood was collected, one subject reported that she had a chronic condition so she was withdrawn from the study because of having met an exclusion criterion. One subject stated she did not feel comfortable having her blood drawn a second time, but still gave a post-saliva sample. The phlebotomists were not able to get a post blood sample in 2 subjects. (Phlebotomists attempted a maximum of three times, to avoid excess discomfort to the subjects.) Finally, two subjects experienced syncopal events related to phlebotomy; these adverse events were documented and reported. These subjects were immediately placed in the supine position, given juice and blood pressures were taken immediately. Both subjects were stable and feeling better within minutes. Finally, a couple of errors occurred with labeling samples, so to avoid any guesswork, the data was thrown out for those two individuals. For these reasons complete data sets were collected on only 42 subjects.

The demographic breakdown somewhat represented that of the university campus from which subjects were recruited. The ethnic breakdown was about 46% White, 34% Asian, 10% Latino, 8% African American and 2% Other (West Indies). The mean age of the participants was 26.9 with a standard deviation of 4.53, once again indicative of the

graduate students who participated in this study. It would have been interesting to see how younger subjects compared with older subjects in how OMT elicited an immune response, but the age range was quite narrow.

The randomization process was successful in that there were no significant ethnic, or gender differences between groups. There were 25 subjects in each group. The OMT group had 14 male subjects while the rest group had 18 male subjects. The average age of the OMT group was 26 years and 28 for the rest group. The ethnic breakdown of the OMT group was: 13 White, 1 Black, 1 Hispanic, 9 Asian and 1 Other. The ethnic breakdown of the rest group was: 10 White, 3 Black, 4 Hispanic, and 8 Asian. A Chi Squared test comparing the gender and ethnic breakdown between groups indicated that they were not statistically different with $p = 0.24$ and $p = 0.29$, respectively. Independent variable T tests were calculated to compare the age and baseline outcome measure values. Age was statistically different between groups with a borderline p value of 0.05. In addition the baseline salivary IgA levels and the CD 4+ T cells were different between groups, but because change scores were used in determining the significance, this should not change the results.

The ANCOVAs run on the six outcome measures did not demonstrate any significant differences between OMT and control groups. A Mann Whitney U test was computed for three of the outcome measures because these variables had skewed distributions; these variables also failed to show any significant differences between groups. After exploratory data analysis, no significant trends were apparent nor were there any differences between RBCs, hemoglobin, hematocrit or platelets.

The purpose of this research was to investigate the short-term effects of OMT on immune function in healthy individuals. The scope of this study was limiting in that only healthy subjects were recruited and only immediate effects of one thirty minute OMT session were investigated. The inclusion and exclusion criteria were stringent in an effort to exclude as many confounding factors and to minimize variability. However, is investigating efficacy or mechanism of action more valuable? Clinically, perhaps demonstrating efficacy is more valuable.

The overall design of this study was confined to looking at the immediate effects of a single OMT session. The majority of manual medicine studies looking at immune function examine effects over a longer period of time using multiple treatments.[22, 24, 30, 42, 48, 49, 50] In practice, physicians who utilize OMT or even other manual therapies advise patients that it may take several treatments to experience any benefits. On the other hand, there is some evidence that minimal OMT can cause transient basophilia.[26] In this study, potential transient changes in immune factors secondary to OMT were not captured in the blood or saliva samples collected immediately after a thirty-minute session of OMT. Although this study confirmed a null hypothesis, immediate effects of OMT on immune function have not been disproven. Furthermore, based on a recent study conducted at the UNTHSC, it has been demonstrated that OMT alters the concentrations of leukocytes in lymph collected directly from the lymphatic duct.[41]

The primary outcome measure was salivary IgA. The process by which saliva specimens were collected must be critiqued. Saliva samples were collected after the subjects wiped out the entire oropharynx including the surface of the tongue and the

sublingual cavity with sterile gauze. Then subjects were asked to spit into a 2 ml microcentrifuge tube. If subjects filled the tube half way, this was considered a sufficient sample. Other research using saliva samples have used other techniques, such that subjects were asked to produce saliva over a set period of time.[29] Using a set period of time resulted in larger saliva samples. Saliva samples obtained by using lemon drops or citric acid crystals are considered stimulated samples; these typically produce larger samples, but may dilute the samples.[43] Dimitriou et. al. demonstrated a morning circadian lowering of IgA[59] but according to other studies this circadian influence has not been demonstrated consistently.[60] All subjects gave saliva and serum samples between 8 a.m. and noon. Therefore, although it may be possible that IgA concentrations fluctuate during the day, the circadian influence of any of the outcome measures would be minimal.

Another point of interest is the autonomic nervous system control over the salivary reflex. Parasympathetic excitation causes copious salivary secretion and enlargement of the tight junctions within the secretory end pieces, whereas stimulation of the sympathetic nervous system causes vasoconstriction of blood vessels supplying the salivary glands.[60] Though this was a subjective observation, in general it was noted that post saliva samples were faster and easier to collect in all subjects; flow of saliva appeared faster. This may be attributed to the fact that the pre-saliva sample was the first time subjects performed this awkward maneuver, and it simply got easier with experience. However, it may be related to autonomic tone, and the relaxing effects of both OMT and rest. Many manual medicine styles, most notably OMT and chiropractic,

suggest an influence on the autonomic nervous system.[4,5,31] Although the mechanisms are still being studied, it is reasonable to state that relaxation and decreased sympathetic tone contributed to this observation.

LIMITATIONS

The OMT protocol used in this study, though thorough, did not incorporate the splenic pump technique. This can be viewed as a limitation, taking into account that other research that successfully demonstrated positive effects of OMT on immune function used splenic pump.[20,21,24]

Perhaps, outcome measures other than those selected in this study would have shown valuable information. In a study by Castilio and Ferris-Swift, red blood cells were examined and splenic manipulation decreased total RBCs.[20] This decrease was attributed to increased sequestration and destruction of RBCs by the spleen. In the exploratory data analysis, RBC, hemoglobin, hematocrit, and platelets were analyzed, but showed no differences between groups.

A medical student, who was also an OMM predoctoral fellow, performed the OMT protocol. Although the practitioner had more experience than the average fourth year osteopathic medical student, OMT is a skill that is refined with time and experience. It is possible that a seasoned osteopathic physician may have been more effective with their OMT. Also, the student technologists who did the phlebotomy and laboratory analysis may be considered novice. Although they were trained well and had some

experience, phlebotomy and other laboratory skills also take experience to develop.

The largest limitation of the study was the use of rest as the control. Another observation that was made during the protocol is that the majority of the subjects assigned to the control group did more than rest; they fell asleep. Studies have concluded that sleep is vital to health and especially immune function.[61] Perhaps an alternative control should have been used, such as sitting and reading, or watching television.

MERITS

This research brought the distinct, but overlapping worlds of immunology, lymphology and osteopathic manipulative medicine together. This study should be commended for using the expertise and experience of osteopathic physicians, immunologists, and technologists to conduct a unique and high quality clinical trial. The rather seamless collaboration between the Osteopathic Research Center, The University of North Texas Health Science Center and Tarleton State University was key to the success of this research. It is this partnership of clinicians, basic scientists and technologists that provides high quality research that ultimately leads to better health care and quality of life.

This study was based on a fairly comprehensive literature search. It was tightly controlled and high powered with a good sample size for a pilot study. In addition, it had a well thought out and highly organized protocol. This study established the feasibility of this protocol, one that can be utilized again in future studies. No side effects or

detrimental effects of OMT were demonstrated. Finally, this study provided useful information that can be used to guide future research.

CHAPTER VI

CONCLUSIONS

Although this study confirmed a null hypothesis, immediate effects of OMT on immune function have not been disproven; it may be possible that transient changes occurred but were not detected. This research provided vital information that can be used to guide future research. Also, this clinical trial demonstrated the feasibility of a protocol of this caliber. The protocol used in this study was well planned, organized, and controlled and required the insight and participation of osteopathic physicians, basic scientists and technologists. It may be replicated in future studies. Because there is a paucity of osteopathic research, truly all research in this field is significant and insightful.

Future studies should investigate the effects of OMT in sick populations with acute infections, chronic infections, chronic pain, and those who are immuno-compromised. It would be prudent to use multiple OMT treatment sessions and over a longer period of time when investigating efficacy. In addition, osteopathic researchers should continue to form an alliance and nurture relationships with basic scientists, research technologists and technicians.

Certainly, animal studies are vital to mechanistic investigations and should continue to pave the way for future human research. As data from animal research is collected, theories about the mechanism of action of OMT will evolve and human studies can be designed based on these maturing theories. Animal models should lay the

framework for mechanistic studies while human studies using a sick population should lay the framework for discovering the efficacy of OMT.

Over all, osteopathic research has repeatedly demonstrated that OMT is safe, has minimal risks and produces no major side effects. In addition, compared to the sky rocketing cost of pharmaceuticals, OMT is relatively affordable. As modern day health care relies more and more on technology, it is imperative that the personal touch in the delivery of health care not be forgotten. In this information era, as much as evidence based medicine is valued, patients desire compassionate and personalize care.

Lastly, there is evidence that OMT along with other forms of manual medicine can positively influence immune function. However, there are still many questions about osteopathic manipulative treatments that have yet to be answered. If osteopathic manipulative treatments could indeed optimize or even boost immune function thereby minimizing lost workdays, shortening hospital stays,[25, 62] preventing infection,[38] enhancing effects of immunizations [24, 63-65], decreasing dosages/frequency of medications and most importantly improving quality of life, it is definitely worth investigating. Moreover, in a time when society faces the fear of epidemics, be it the Avian Influenza or even acts of bioterrorism, OMT must be considered a safe therapy to enhance immune function. Needless to say, the potential benefits of OMT on health and particularly on the immune system is exciting and must continue to be explored.

APPENDIX A

OMT TECHNIQUES

Articulatory Treatment: The region of somatic dysfunction is gently carried through its full range of motion with the therapeutic goal of increased range of motion. The operator will employ compression at the wrist with circumduction /counter-circumduction in an attempt to re-articulate the carpal bones of the wrist. The motion of this technique is continued until the operator achieves improvement in the mobility of the carpal bones.

Balanced Ligamentous Tension(BLT) /Balanced Membranous Tension/Ligamentous Articular Strain (LAS): This technique is used to treat any area of somatic dysfunction. The operator places his/her hands on the area of somatic dysfunction and applies a gentle pressure combined with a gentle push or pull until a balance of ligamentous (membranous) tension is felt. This tension is maintained and gently modified to the changes in somatic dysfunction through a period of release. When there is a feeling of restoration of normal motion through the tissue, the tension is gently released.

Combined Treatment: This consists of a combination of direct and indirect techniques. One may start to treat a region of somatic dysfunction with an indirect technique, and without stopping or breaking contact, applies a direct technique. Articulatory, muscle energy, and range of motion techniques are direct; all other techniques are indirect.

Functional Positional Release (FPR): A modification of an indirect myofascial release treatment. The region of somatic dysfunction is placed in a neutral position in all planes of motion and an activating force (compression and/or torsion) is added.

High Velocity Low Amplitude (HVLA): A direct method technique that utilizes a quick but small thrust. The operator will contact an area of restriction and isolate the body to that are of somatic dysfunction by side bending, rotating, flexing and/or extending.

Indirect: The restrictive barrier is disengaged and the dysfunctional body part is moved away from the restrictive barrier until tissue tension is equal in one or all planes and directions.

Muscle Energy (ME): This technique is used to treat areas of somatic dysfunction in a direct fashion to engage restrictive barriers. The patient is positioned in a manner where the restrictive barrier is engaged. The patient provides an isometric muscular contraction against the force provided by the operator for approximately 3 to 5 seconds. The patient then relaxes the muscular contraction and operator moves the patient into a new restrictive barrier. This is repeated approximately three to five times.

Myofascial Release (MFR)Treatment: This technique is used to treat areas of fascial restriction in either an indirect, direct or combined fashion. The operator's hands apply a very gentle pressure until a barrier or point of ease of the fascia is felt. The operator holds that position until tissue response is felt, generally between 1 and 3 minutes.
Range of Motion Treatment: This technique is used to increase the range of motion of a joint. The limb is gently taken through its passive range of motion. This is repeated until an increase in the range of motion occurs.

Strain / Counterstrain: This technique uses treatment of tender points to relieve somatic dysfunction. The operator contacts the tender point and gently folds that body region over the tender point until the patient reports a significant decrease in the tenderness. This position is held for 90 to 120 seconds and then gently returned to a resting position.

Soft Tissue Techniques: These are typically direct techniques that treat myofascial structures and related neural and vascular components. Soft tissue techniques include stretching, kneading, and inhibition.

APPENDIX B

Pictures used in this appendix were taken from either: Kimberly P. An Outline of Osteopathic Manipulative Procedures. Kirksville, MMO: KCOM Press; 2000
or taken by the OMM Predoctoral Fellows at the UNTHSC- TCOM Department of OMM

1. O-A Release

The operator contacts the occiput close to the condyles, applies anterior-lateral pressure and holds till a tissue change is palpated.

2. Sibson's Fascia Release

This technique with the patient in the supine position. The operator grasps the clavicle while the fingers progressed behind the clavicle to the point of fascial tension. Meanwhile, the other hand grasped above the subject's elbow and traction was applied inferiorly. Tension was held until tissues relaxed. An alternate method was to apply caudad force on the medial aspect of the shoulders bilaterally to the barrier and maintain pressure until a release was palpated.

3. Doming the Diaphragm

The operator's hands applied a very gentle pressure on the diaphragm until a barrier or point of ease of the fascia was felt. To accentuate the results, the individual was asked to take deep inhalations and exhalations while the operator followed the respirations in restricted areas. The operator continued until a tissue response was palpated.

4. Rib Raising

Pressure was applied to the rib heads until anterior chest wall movement was observed and the pressure was held until the surrounding tissue relaxed. Rib-raising was applied rhythmically for several cycles to both sides of the rib cage.

5. Assessment and Treatment of T1-L2

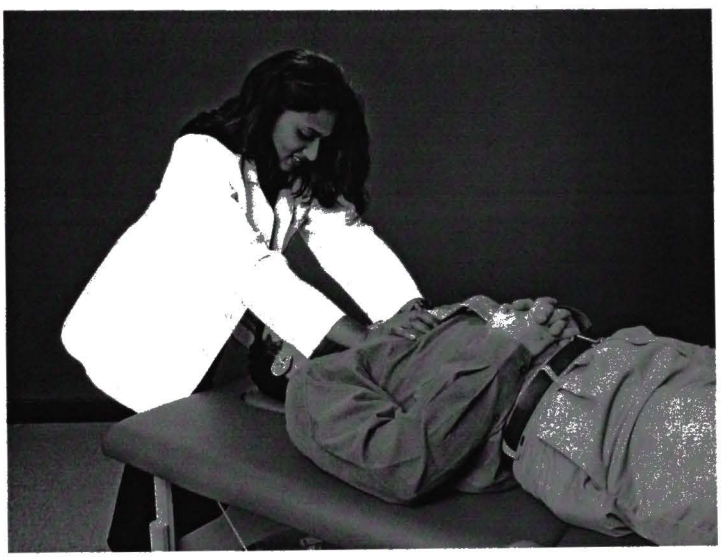
This region was assessed for somatic dysfunction and based on the subjects' unique dysfunction, various areas were treated using the techniques described in Appendix A.

6. Pelvic Diaphragm Release

The operator contacted the pelvic diaphragm, asked the subject to take deep breaths, while resisting on inhalation and following the tissue cephalad on exhalation, till a myofascial release was palpated.

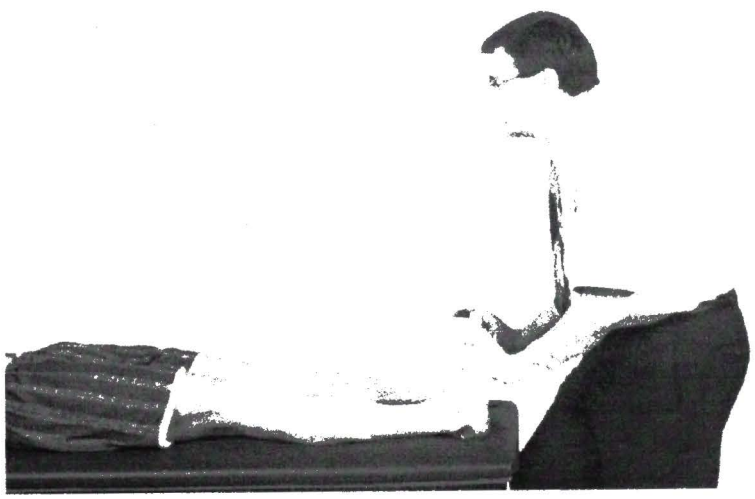
7. Thoracic Pump

Palms are placed over the subject's chest below the clavicles and the subject was instructed to, "Take a deep breath and let it all out." As the subject exhaled, the physician followed the thorax to full exhalation and applied a gentle springing. Compressive force was maintained while the subject takes another deep breath and inhalation was resisted until sufficient respiratory force accumulated. Then compression was abruptly released. The operator did this for 3 cycles.



8. Pedal Pump

This technique was done by having the subject lay in the supine position with the legs completely extended. The feet were dorsiflexed and the operator applied pressure to the soles of the feet bilaterally further dorsiflexing and causing the entire body to move gently with each application of pressure. This pressure was applied rhythmically, creating a pump like motion. This technique was applied for one whole minute.



APPENDIX C

VIII. KEY PERSONNEL.

1. Janice Thomas, MSIV, Principal Investigator, Pre-doctoral Fellow, Department of Manipulative Medicine, UNTHSC, TCOM – Recruited, obtained informed consent, performing OMT and coordinated and led entire research team
2. Scott Stoll, D.O., Ph.D., Co-Investigator, Chair of Department of Osteopathic Manipulative Medicine, UNTHSC, TCOM – provided training and consultation to OMM Pre-doctoral fellows in OMT study protocol.
3. Jennifer White CCRP and Kimberly Fulda MPH both served as Clinical Research Coordinator, Osteopathic Research Center, UNTHSC, TCOM – provided guidance on issues regarding the IRB and obtaining informed consent from subjects
4. Sally Lewis, M.S., Department Head of Clinical Laboratory Sciences, Tarleton State University Department of Clinical Laboratory Sciences– supervised technologists in the correct procedure to analyze serum, provided guidance regarding laboratory procedures and analyzed saliva samples
5. Harlan Jones, Ph. D., Department of Molecular Science and Immunology, UNTHSC at Fort Worth, Graduate School of Biomedical Sciences - provided instruction and supervision for technologists when operating flow cytometer for serum analysis
6. Virginia Reyes, M ed, Instructor of Immuno-hematology and Genetics and Hematology, Tarleton State University Department of Clinical Laboratory Sciences - supervised technologists in the correct procedure to analyze serum samples, provided guidance regarding laboratory procedures and analyzed the saliva samples
7. Daisha Cipher, Ph.D., Assistant Professor- Department of Biostatistics, UNTHSC, School of Public Health– provided statistical consultation and guidance
8. Matthew Blackburn, MSIII, Pre-doctoral Fellow, Department of Manipulative Medicine, UNTHSC, TCOM – served as a backup OMT provider and did data entry
9. The following individuals conducted the phlebotomy and necessary analysis of serum samples. They have been trained in phlebotomy and other clinical laboratory skills and will graduate with a Bachelors Degree in Clinical Laboratory Sciences from the Tarleton State University- School of Clinical Laboratory Sciences located in Fort Worth.

Phlebotomy and Coulter Counter

- Sam McCellan – senior student technologist at Tarleton State University- School of Clinical Laboratory Sciences.
- Merrie Wimmer - senior student technologist at Tarleton State University- School of Clinical Laboratory Sciences.
- Steven Mason - senior student technologist at Tarleton State University- School of Clinical Laboratory Sciences

Flow Cytometer

- Anita Lam - senior student technologist at Tarleton State University- School of Clinical Laboratory Sciences.
- Shayla Tucker – senior student technologist at Tarleton State University- School of Clinical Laboratory Sciences.
- Janelle Vanderburg - senior student technologist at Tarleton State University- School of Clinical Laboratory Sciences.

APPENDIX D

ADVERTISEMENT FOR VOLUNTEERS for a RESEARCH STUDY

Volunteers needed for a Research Study
to be conducted at:

Department of Osteopathic Manipulative Medicine
University of North Texas Health Science Center

This will be a pilot study to evaluate
The effects of Osteopathic Manipulative Treatment on the body's ability to produce factors that fight infection and protect from infection

Principal Investigator:
Scott Stoll, D.O., Ph.D.

Co- Investigator:
Janice Thomas, MSIV

Subjects should be between 18 and 40 years of age, healthy and not currently taking any medications.

All subjects must be capable of giving informed consent.

For more information, please call or email:
Janice Thomas
(817) 735-5161
jathomas@hsc.unt.edu

APPENDIX E

INFORMED CONSENT
TO PARTICIPATE IN A RESEARCH PROJECT AND AUTHORIZATION TO USE AND DISCLOSE HEALTH INFORMATION FOR RESEARCH

TITLE:	Immediate Effects of Osteopathic Manipulative Treatment on Immune Function in a Healthy Population: A Pilot Study
PRINCIPAL INVESTIGATOR:	Scott Stoll, D.O., Ph.D. Chairman and Associate Professor, Department of Manipulative Medicine
INSTITUTION:	University of North Texas Health Science Center (UNTHSC) Ft. Worth, Texas
PARTICIPANT'S NAME:	_____
DATE:	_____

Before agreeing to participate in this research study, it is important that you read and understand the following explanation of the proposed procedures. It describes the procedures, benefits, risks, and discomforts of the study. It also describes your right to withdraw from the study at any time. It is important that no guarantees or assurances can be made as to the results of the study.

You should also understand that if you decide to sign this form, you are giving permission for use and disclosure of your health information for this research study.

PURPOSE
The purpose of this study is to determine how a specific type of manual medicine called Osteopathic Manipulative Treatment (OMT) can affect a person's ability to produce factors in the blood that can protect you from infection.

STUDY PROCEDURES
After signing this form, you will be randomly assigned to one of two groups: 1) OMT, or 2) Rest only. This means that you are put into a group by chance. It is like flipping a coin. Neither you nor the researcher will choose what group you will be in.

First, a student technologist from Tarleton State University will collect a blood sample. The blood sample will be obtained from a vein in your arm. Approximately 6-10 milliliters (1 ½ - 2 teaspoons) of blood will be collected into two tubes. You will also give a small saliva sample by spitting into a small container after wiping the inside of your mouth with a piece of gauze. Then depending on your group placement you will

either receive thirty minutes of OMT or be asked to lie down and simply rest for thirty minutes. After that thirty minute period a saliva sample, and a second blood sample will be collected into two additional tubes. The second blood sample will not be collected from the same arm as the first blood sample. The blood and saliva will be tested for specific immune factors that may indicate the body's ability to fight infection and protect against infection.

Osteopathic Manipulative Medicine Predoctoral fellows (3rd or 4th year medical students), who are skilled in osteopathic manipulation and have been trained in the study protocols, will give you the 30 minute OMT session. If you are assigned to Group B you will be asked to lie down on a manipulative medicine bed and simply rest for thirty minutes.

Study protocol treatments (OMT) will be provided at no charge to you or your insurance company. For your time and travel expenses, you will be reimbursed twenty-five (25) dollars. As applicable, reimbursement to you may be withheld and credited to any outstanding debts you may have with the University of North Texas Health Science Center.

OMT PROCEDURES

If you are in this group you will receive an osteopathic structural exam of your neck, back, ribs, diaphragm (breathing muscle) and other muscles and tissues of the body at the Osteopathic Manipulative Medicine (OMM) Clinic on the sixth floor of the Patient Care Center. The treatment will last about 30 minutes. OMT will be done to the neck, back, ribs, diaphragm (breathing muscle), other muscles, tissues, and joints. All of these techniques are given with you lying on your back or seated on the treatment table. All treatments are done with you fully clothed.

RISKS

This study carries minimal risk to you; such risks are not significantly increased over that ordinarily encountered in daily life for a healthy person. If you are injured or suffer adverse effects while participating in this study, you will be immediately referred to the most appropriate health care resource. Osteopathic Manipulative Medicine Predoctoral fellows (3rd or 4th year medical students), who are skilled in osteopathic manipulation and have been trained in the study protocols, will conduct all intervention protocols. Trained and licensed Osteopathic Physicians will oversee the Osteopathic Manipulative Medicine Predoctoral Fellows in order to guarantee an absolute minimal possibility for injury or other adverse reaction.

- Osteopathic Manipulation: There are minimal risks associated with osteopathic manipulative treatments (OMT) due to the non-invasive nature to these techniques. There is a risk that you may experience muscle and/or joint soreness and/or tenderness during or after receiving an OMT treatment.

There is an OMT treatment called High Velocity/Low Amplitude (HVLA) which may be used during an OMT treatment visit. HVLA is an additional force applied during some OMT treatments to the neck, back, or ribs that involves a rapid, forceful, briefly applied thrust or push to that area. There is a small risk that HVLA can result in a bone fracture. Please inform the study staff if you wish to not receive this particular treatment.

Blood Draw: Obtaining blood samples for the immune markers can result in localized infection, hematoma (blood clot) and/or ecchymosis (bruising). Sterile techniques will be used to prevent infection. A student technologist with at least 8 months of experience in phlebotomy will perform the blood draws. You may experience mild discomfort and/or pain when the blood is drawn. If, during blood analysis, you are found to have any abnormalities, we will notify you immediately so that you may make an appointment with your primary care physician for further evaluation.

BENEFITS

While taking part in this study, there may or may not be a direct medical benefit to you. By participating in this study, you may experience improved immune function, increased resistance to potential infections, relief of structural strains/somatic dysfunctions, and/or soothing/relaxing effects. You may not experience any personal benefit but may contribute to identifying the mechanism by which OMT affects immune function.

COMPENSATION FOR INJURY

Neither the investigator conducting this study nor the University of North Texas Health Science Center at Fort Worth or Tarleton State University Department of Clinical Laboratory Sciences are able to offer financial compensation nor absorb the cost of medical treatment should you be injured as a result of your participation in this research. If required, medical care will be made available to you in the case of such injury, but you (or your private insurer, Medicare, Medicaid or other government health care program) will be responsible for the expense of any medical care, including hospitalization, that is needed.

You should know that by signing this form you are neither waiving any of your legal rights against nor releasing the principal investigator, the University of North Texas Health Science Center at Fort Worth, Tarleton State University Department of Clinical Laboratory Sciences or any of their respective agents from liability for negligence with respect to the conduct of this study. If you are injured and feel that your injury justifies pursuing a legal remedy, you have the right to do so.

PROCEDURE FOR MAINTAINING CONFIDENTIALITY OF RESEARCH RECORDS

Study records that identify you will be kept confidential as required by law. Federal Privacy regulations provide safeguards for privacy, security, and authorized access. Except when required by law, you will not be identified by name, social security number, address, telephone number or any other direct personal identifier in study records

disclosed outside UNT Health Science Center. For records disclosed outside of UNT HSC, you will be assigned a unique code number. This code number will be used to label all blood and saliva samples as well as medical charts and written documentation. The key to the code will be kept in a locked file cabinet in the Research Coordinator's office. During the course of the study, any medical information obtained will be kept confidential.

USE AND DISCLOSURE OF PROTECTED/PERSONAL HEALTH INFORMATION (PHI)

WHO MAY USE OR DISCLOSE PHI

Under federal privacy regulations, you have the right to determine who has access to your PHI. If you chose to take part in this study, you will be giving you permission to or authorizing the investigators and the research staff (individuals involved in carrying out the study) to collect and use your PHI for this research study.

INFORMATION THAT WILL BE USED OR DISCLOSED

In carrying out this research, the PHI we will collect and use about you will include:

- Information obtained from procedures used to determine your eligibility to participate in the study such as medical history, current medications and vital signs.
- Information that is created or collected from you during your participation in the study including treatment records, vital signs, and current medications
- Demographic information such as age, sex and race.

WHO WILL RECEIVE PHI

As part of the study, Scott Stoll, D.O., Ph.D., Janice Thomas, PDF, and the research team will maintain the results of blood and saliva analysis at UNTHSC-FW, TCOM and Tarleton State University Department of Clinical Laboratory Sciences. This will be done using the above mentioned confidentiality procedures. In addition, your records may be reviewed in order to meet federal or state regulations. Reviewers may include representatives from the University of North Texas Health Science Center Institutional Review Board (IRB) and the Tarleton State University Department of Clinical Laboratory Sciences School of Clinical Laboratory Sciences Institutional Review Board.

PURPOSE OF EACH USE OR DISCLOSURE

The purposes of disclosing your PHI to these entities are to collect the data necessary to complete the research, to properly monitor how this study is carried out and to answer research questions proposed by the study.

EXPIRATION OF AUTHORIZATION

This authorization will expire at the end of the study.

RIGHT TO REVOKE AUTHORIZATION

You are free to choose not to participate in this study or to withdraw from this study at any time. You are also free not to authorize the use and disclosure of your PHI. If you choose not to authorize these uses and disclosures of your PHI, you will not be able to participate in the research study and there will be no penalties. In other words, you will not be denied any treatments you may need. If you revoke your authorization during the study, your participation in the study will end and the study staff will stop collecting medical information from you and about you. However, the study will continue to use the PHI already collected to the extent that it is needed to complete the research and to preserve the scientific integrity of the study. If you decide to withdraw from the study or withdraw authorization to use/disclose PHI, we ask that you notify the principal investigator, Scott Stoll, D.O., in writing that you are withdrawing at the following address:

Osteopathic Research Center
3500 Camp Bowie Blvd.
Fort Worth, TX 76107
Atten: Scott Stoll, D.O.

ACCESS TO PHI BY PARTICIPANT

Federal regulations allow you to obtain access to your PHI collected or used in this study. While the research study is in progress, you access to the PHI in your study records will be temporarily suspended. You will be able to access you information when the research study is completed. At that time, you will have the right to see and copy the medical information collected from you in the course of the study.

POTENTIAL FOR RE-DISCLOSURE OF PHI

You need to be aware that these organizations receiving your PHI may not have the same obligations to protect your PHI and may further disclose your PHI to groups not named here. Information released to these parties is no longer under the control of the study doctor and can no longer be protected by Federal Privacy Rules.

STUDY-RELATED QUESTIONS

For study related questions, please call the principal investigator, Scott Stoll, D.O., at (817) 735-2004.

STUDY-RELATED INJURIES

For questions regarding a research-related injury, please call Scott Stoll, D.O. at (817) 735-2004.

HUMAN SUBJECTS RESEARCH PARTICIPANT RIGHTS

Your participation in this study is voluntary. You may discontinue your participation at any time. Leaving the study will not result in any penalty or loss of benefit in which you are entitled. It will not affect the level of care you receive. For questions regarding your

rights as a research participant, please call Jerry McGill, PhD, Chair of the UNTHSC IRB, at (817) 735-5458.

CONSENT

I have read or have had read to me all of the above. _____ has explained the study to me and answered all of my questions. I have been told the risks and/or discomforts as well as the possible benefits of the study. I have been told of other choices of the treatment to me. I have been told how my PHI will be used and disclosed for the study.

I understand that I do not have to take part in this study or authorize use and disclosure of my health information, and my refusal to participate or my decision to withdraw will involve no penalty, loss of rights, or legal recourse to which I am entitled. If I decide to withdraw from the study, the study personnel may only use and disclose information already collected. If I decide to revoke my authorization to use and disclose PHI, I may not be allowed to continue in the study. The study personnel may choose to stop my participation at any time.

UNTHSC employees/students' participation is completely voluntary. Participation (or non-participation) will in no way affect my academic standing or employment status.

In case problems arise, I have been told I can contact the Principal Investigator at (817) 735-2004.

I understand my rights as a research subject and I voluntarily consent to participate in this study. I understand what the study is about, how the study will be conducted, and why it is being performed. I will receive a copy of this consent and authorization after it has been signed.

Subject's Name or (Printed) Subject's Legal Representative — Date

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Subject's Signature Subject's Legal Representative — Date

I certify that I have reviewed the contents of this form with the subject signing above. I have explained the known benefits and risks of the research and the use and disclosure of PHI. It is my opinion that the subject understands the explanation.

Signature of Person Discussing Informed Consent — Date

APPENDIX F

Complete Blood Count (CBC) – Normal Values	
Red Blood Cells (RBCs)	4.1-6.2 x 10^6/ mm^3
Platelets	150-400 x 10^3/mm^3
White Blood Cell (WBCs)	**4,000-10,000/mm^3**
	Percentage of Total WBC
Granulocytes - Polymorphonuclear leukocytes (PMNs)	
Neutrophils	47 - 77%
Segmented Neutrophils	60 - 70%
Banded Neutrophils	0 - 5%
Basophils	0 - 2%
Eosinophils	0 - 7%
Monocytes	2 - 10%
Lymphocytes	16 - 43%

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