FACTORS ASSOCIATED WITH OCCURRENCE AND EARLY DETECTION OF PRESSURE ULCERS FOLLOWING TRAUMATIC SPINAL CORD INJURY

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Pressure ulcers (PUs) are serious secondary complications occurring in individuals with spinal cord injury (SCI). PUs not only decrease quality of life, but can adversely affect physical, psychological, emotional and financial status. Although studies have investigated general risk factors for PUs, few have focused on the time period immediately following SCI when the inflammation associated with SCI may influence the body's ability to tolerate secondary tissue damage leading to occurrence of PUs. Biomarkers obtained from plasma and urine biofluids are commonly used to characterize inflammation.

The Rehabilitation Engineering Research Center on SCI (RERC on SCI) recruited individuals with new traumatic SCI (TSCI). Data were collected at predetermined time points in acute care, inpatient rehabilitation and after discharge on the risk factors and incidence of PUs, and on plasma and urine inflammatory mediators. A secondary analysis was performed on data obtained from the 104 individuals with TSCI.

The purpose of this study was to investigate the association of clinical, demographic and inflammatory factors with the formation of PUs following TSCI during acute care hospitalization and inpatient rehabilitation. Severity of SCI (ASIA A) and presence of pneumonia were determined to predict PU incidence. Plasma concentrations of IL-1RA, GM-CSF, MIP-1α, IFN-γ, IL-5, IL-17, MIG and MIP-1β; and urine concentrations of IL-6, IL-8, IL-13, IP-10, MCP-1,

IFN-γ, IL-5, IL-17, MIG and TNF-α were associated with formation of PU, immediately after SCI. An increase in the plasma concentrations of IP-10 and a decrease in the urine concentrations of IFN-α were observed just before formation of the first PU. A significant association between presence of pneumonia and formation of PU was observed as compared to no pneumonia. This association between PU and pneumonia could be linked through inflammation. Increased plasma synthesis of IFN-α and urine synthesis of IL-1RA were associated with formation of PU in individuals with pneumonia.

The findings of this study suggest that an imbalance in the inflammatory response after SCI may be associated with formation of PUs. The findings also suggest an association between the presence of pneumonia and formation of PU, which could be linked through inflammation.

TABLE OF CONTENTS

LIST OF TABLES

xviii

LIST OF FIGURES

LIST OF EQUATIONS

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1.0   INTRODUCTION

Pressure ulcers (PUs) are the most frequent secondary complication in individuals with spinal cord injury (SCI) that affects their quality of life, length of stay during hospitalization and increases the mortality and morbidity [1]. Although numerous risk factors are associated with the occurrence of PUs, there is limited evidence for the increased incidence of PUs and factors associated with the same in individuals with newly traumatic SCI during hospitalization. In addition, an acute inflammatory response by the immune system is inherent after traumatic SCI due to increased synthesis of inflammatory factors that spills into the systemic circulation. The effects of this response can be both beneficial and detrimental [2]. Studies also report an increase in the inflammatory biomarkers in individuals with SCI having comorbidities such as pneumonia [3], urinary tract infections (UTIs) [4] and PUs [4, 5]. The complex cascade of secondary complications after traumatic SCI can be attributed to this intrinsic inflammatory response after SCI. Thus, identifying the clinical and inflammatory factors associated with the development of PUs in this population during acute care hospitalization and inpatient rehabilitation may help prevent this secondary complication and may reduce the risk of rehospitalization and improve the quality of life.

1.1   OBJECTIVE AND SPECIFIC AIMS

The objective of this study was to identify the clinical factors and inflammatory biomarkers that are associated with the risk of developing pressure ulcers during acute care and inpatient rehabilitation. The specific aims of this dissertation were:

1. To identify the clinical and demographic risk factors that predict the formation of PUs in individuals with traumatic SCI;

2. To identify the inflammatory mediators in plasma and urine immediately after traumatic SCI that may predict future PU incidence;

3. To identify changes in the inflammatory mediators in plasma and urine that can detect pressure ulcers just before clinical diagnosis in individuals with traumatic SCI; and

4. (A) To investigate an association between presence of pneumonia and PU incidence in individuals with traumatic SCI in the Rehabilitation Engineering Research Center on SCI (RERC on SCI) and Spinal Cord Injury Model Systems (SCIMS) database; and

    (B) To identify a change in the inflammatory mediators in urine and plasma before and after occurrence of pneumonia; that predict formation of PUs in subjects having pneumonia preceding or coinciding with PU as compared to subjects having pneumonia who do not develop PU.

1.1.1   Hypothesis and Rationale for Specific Aim 1

Despite a large amount of evidence recommending preventive strategies, pressure ulcers remain the most common secondary complication in individuals with SCI that increases the risk of

rehospitalization [6, 7]. The extrinsic environment plays an important role in the development of PUs. A majority of studies focus on the risk factors associated with the development of PUs in community care in individuals with SCI [8-15], however only a few address the risk factors during hospitalization or acute care [16]. Hence, identifying the predictors for development of PUs in high-risk settings (e.g., during hospitalization) is essential. Most studies on risk factors associated with PUs used self-report or retrospective chart review methods [8, 10]. This may result in underreporting of the data due to recall bias or insufficient data. Those who are likely to develop PUs are individuals who have SCI of traumatic origin [17], males [18], have a history of smoking, alcohol or drug use [10], have medical comorbidities such as diabetes mellitus [19, 20], decreased oxygenation or hypotension [16], infections such as pneumonia, urinary tract infections, osteomyelitis, and other bacterial infections[8, 21], those on mechanical ventilators [22] and use of steroids [16]. The current study is a prospective longitudinal cohort and focused on identifying the clinical and demographic risk factors associated with the occurrence of PUs in individuals with traumatic SCI during the time of initial hospitalization- acute care and inpatient rehabilitation.

Hypothesis 1: Pneumonia, urinary tract infection, use of steroids, gender, age, and ASIA score will indicate increased risk for the formation of PUs in individuals with traumatic SCI.

1.1.2 Hypothesis and Rationale for Specific Aim 2

Activation of an inflammatory response by the immune system is inherent after traumatic SCI (TSCI). After the initial SCI; damage to the axonal and neuronal cells is followed by a complex secondary cascade of events [4, 23, 24]. The disruption of the blood spinal cord barrier (BSCB)

after SCI increases the synthesis of the pro-inflammatory and anti-inflammatory cytokines and other inflammatory mediators. This disrupted BSCB further increases the passage of these inflammatory markers into the circulation. The inflammatory response after SCI can be detrimental and predispose the individuals to secondary complications and tissue damage [25, 26]; on the other hand, inflammatory response after SCI also helps reduce injury by activating the process of wound healing, thus initiating repair [27]. Hence, inflammation after SCI is described as a "double-edged sword", and early inflammatory response may be detrimental whereas the late inflammatory response is likely beneficial [2]. Inflammatory response may be beneficial by maintaining finely tuned balance between the pro-inflammatory and the anti-inflammatory mediators [28]. Pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α increase immediately after injury until 5 hours post injury [29]. The inflammatory response can be explained by the early expression of the pro-inflammatory mediators from 24 hours until 4 days after injury [30]. The synthesis of pro-inflammatory cytokines such as TNF-α increased after subjecting mice to SCI in the lumbar region due to the disruption of the BSCB [24]. Increased serum concentrations of pro-inflammatory cytokines such as TNF-α, IL-2, and IL-1RA were found in individuals with SCI as compared to able-bodied individuals [4]. The synthesis of anti-inflammatory cytokines such as IL-10 suppresses the microglial activity and helps to decrease inflammation after SCI [27]. Hence, the purpose of this aim was to identify the inflammatory mediators in urine and plasma immediately (within four days) following SCI associated with subsequent risk for formation of PUs during acute care and inpatient rehabilitation.

Hypothesis 2(a): Levels of TNF-α, IL-1β, IL-2, IL-6, IL-10 and IL-1RA measured within four days after traumatic SCI will indicate increased risk for formation of PUs.

Hypothesis 2(b): Inflammatory markers in urine that predict occurrence of PUs will be similar to those in plasma.

1.1.3   Hypotheses and Rationale for Specific Aim 3

In vitro studies have shown increased synthesis and proliferation of inflammatory cytokines such as IL-1, IL-6, IL-8, GM-CSF and TNF-α after loading the tissue before the tissue damage is barely visible [31]. Hence, the tissue damage due to inflammation can be termed as a sub-clinical disease that is not visible on the skin. Increase in the plasma concentrations of pro-inflammatory mediators such as interleukin 2 receptor (IL-2R), and intercellular adhesion molecule 1 (ICAM-1) were noted in individuals with SCI having PUs or slow healing PUs as compared to able-bodied subjects or subjects with SCI having no PUs [32]. Pro-inflammatory cytokines and chemokines such as IL-8, IL-1β and TNF-α are increased in subjects having slow healing or Stage III and IV PUs when compared to able-bodied individuals or individuals with healing ulcers [5, 33]. Clinically it is not known why certain individuals with SCI develop PUs and some do not despite having similar risk factors that predispose them to develop PU. However, this could be attributed to the differences in the intrinsic inflammatory state in the individual, since evidence supports an increase in serum concentrations of pro-inflammatory cytokines in individuals with PUs, [34]. A multidisciplinary expert panel sponsored by SCI Quality Enhancement Research Initiative (SCI QUERI) identified "research on biomarkers" for the development of PUs in individuals with SCI as "highest priority risk factor research" [34]. There are only a few objective tools to measure the risk assessment for PUs and studies have shown that there is an increase in the concentration of these serum biomarkers during skeletal muscle

damage in response to a deep tissue injury (DTI) induced by pressure on a chronic SCI rat model [35]. Also, serum protein alterations were found in subjects with PUs that were not specific for individuals with SCI. These alterations also tend to disappear with the healing of the PU [36]. It is therefore essential to identify the inflammatory biomarkers that may alter before the tissue damage is visible. This can aid in earlier diagnosis and treatment measures for PUs. The intrinsic risk factors can be identified by analyzing the plasma and urine levels of these inflammatory mediators just before the individuals develop PUs. Hence, aim of this study was to identify the inflammatory mediators in plasma and urine, associated with formation of PU, before the PU is clinically diagnosed and is in its incipient stage, in individuals with TSCI and PUs, during acute care through inpatient rehabilitation.

Hypothesis 3(a): A change in the level of TNF-α, IL-1β, IL-8, IL-6 and GM-CSF measured immediately after SCI and just before clinical diagnosis of PU will indicate increased risk for the formation of PUs.

Hypothesis 3(b): Inflammatory markers in urine that predict imminent occurrence of PUs will be similar to those in plasma.

1.1.4   Hypotheses and Rationale for Specific Aim 4(A)

Approximately 200 risk factors have been associated with the formation of PUs in individuals with SCI [8]. As reported by the SCI QUERI panel in 2011, the identification of all risk factors in individuals with SCI and implementing corresponding preventive techniques can be challenging in the clinical practice. Hence, it is essential to identify dependent and independent risk factors associated with development of PUs in this population [34]. Significant correlations

between individuals having PUs and pneumonia (or atelectasis) were observed after 1, 2 and 5 years of injury in the SCI population [37]. Subjects with high-level traumatic SCI are dependent on mechanical ventilation, and report an increase in incidence of pneumonia within days of intubation [22]. A preliminary study of 91 subjects from the RERC on SCI database showed that the subjects were twice as likely to develop PUs when the ulcer was preceded by or coincided with the presence of pneumonia as compared to subjects who did not have pneumonia Hence, the presence of pneumonia could indicate possible future pressure ulcer development, in individuals with SCI during acute care hospitalization and inpatient rehabilitation [38]. It was worthwhile to explore the data of the SCI Model Systems to investigate if this association holds true in a larger population. Hence, the first part of this aim investigated the association between the presence of pneumonia and occurrence of pressure ulcers in the SCIMS database population. The study was intended to confirm the RERC SCI study's results.

Hypothesis 4(a): Individuals having pneumonia will have a higher incidence or presence of pressure ulcers than individuals who do not have pneumonia.

1.1.5   Hypotheses and Rationale for Specific Aim 4(B)

The synthesis of the inflammatory biomarkers increases in individuals with the presence of PUs [5], and also in individuals having respiratory infections such as pneumonia [39]. However, an apparent association linked through inflammation has not yet been explored. Presence of pneumonia activates an acute inflammatory response to counteract the infection, which when not in balance, may result in secondary complications such as PUs (Figure 1). TNF-α is one of the early mediators in the inflammatory process and induces activation of pro- and anti-

inflammatory mediators and is responsible for the protective response in pneumonia. Cytokines such as IL-1β, IL-6, and IL-8 were correlated with TNF-α in subjects having pneumonia [40]. The inflammatory response in individuals with SCI may be different during the inception of pneumonia and days after the onset of pneumonia; as previous studies have shown significant impact of time from onset of pneumonia on inflammatory response [40]. Increased concentrations of IL-1β and IL-10 were also found to be associated in individuals with pneumonia [3, 39]. The differences in the inflammatory response before and after the presence of pneumonia may explain the formation of PUs in these individuals. The aim of this study was to identify the inflammatory cytokines and chemokines in urine and plasma, before and after the presence of pneumonia that will indicate increased risk for formation of PUs in acute care and inpatient rehabilitation.

Hypothesis 4(b): Differences in inflammatory mediators measured before and after the presence of pneumonia of TNF-α, IL-1β, IL-6, IL-8 and IL-10 will indicate increased risk for the formation of PUs.

1.2   BACKGROUND

Every year, more than 2.5 million individuals in the United States develop pressure ulcers (PUs). In 2006, the total cost of hospitalization in an adult diagnosed with PUs was around $11.0 billion. Cost of individual patient care ranges from $20,900 to $151,700 per PU depending upon the stage, severity or extent of the PU [41, 42]. Overall prevalence of PUs during hospitalizations

from 2006-2011 ranged from 11.2% to 13.8%. Long-term acute care had the highest prevalence of PUs (29.2%), acute care setting had an overall prevalence of 10.8% to 13.3% and the acute care facility-acquired prevalence of PUs was 4.5% to 6.4%. Rehabilitation facilities showed an overall prevalence of 13.3% to 19.4% and facility-acquired prevalence of 3.7% to 6.6% from the years 2006 to 2011 [43]. In hospitals, the mortality rate in individuals with PUs as a secondary diagnosis was five times higher than individuals with no PUs [42].

Spinal Cord Injury (SCI) is a devastating neurologic disorder and has profound impact on physical, psychological and socioeconomic status [44]. Increase in age and severity of injury are associated with rise in long-term complications after traumatic SCI. Pneumonia accounts for approximately 67% of respiratory complications, and is the most frequent secondary complication causing mortality and morbidity in individuals with SCI [45]. Respiratory condition accounts for 50% of mortality rate during the first year post SCI [46, 47].The second leading cause of death in individuals with SCI is septicemia (88.6%); usually associated with urinary tract infections (UTI's), pneumonia, and presence of PUs [45].

1.2.1 Pressure Ulcers in Individuals with Spinal Cord Injury

Individuals with SCI are at a great risk for the formation of PUs from the time of acute hospitalization throughout their life [7, 12]. PUs are the most frequent secondary complication occurring after SCI that decrease the quality of life and life expectancy of these individuals [48]. They also increase the length of stay, cases of re-hospitalization, and admission to long-term care [49]. Studies indicate that around 47% of individuals with SCI develop at least one PU during the period of acute care hospitalization and rehabilitation [50]. The prevalence of PUs following

TSCI increases after every annual follow-up [37]. The 2011 annual statistical report for the Spinal Cord Injury Model Systems (SCIMS) reported PUs to be the second most frequent complication and third leading cause of death following SCI. Around 34% of individuals with SCI develop PUs in acute care or rehabilitation. The report also indicated that 53.4% of subjects with neurologically complete tetraplegia develop PUs [45]. The prevalence rate of PUs in veterans with SCI was reported to be 39% during a period of 3 years [13].

1.2.2   Risk Factors for Pressure Ulcers

There are several risk factors that contribute to the formation of PUs. Individuals with SCI with traumatic origin have higher occurrence of PUs when compared to other etiologies [37]. Males and subjects with complete tetraplegics are at a higher risk to develop PUs than females and complete paraplegics [18]. Smoking, alcohol or drug use, were also reported to be associated with presence of PUs. [10].

Numerous intrinsic and extrinsic characteristics were related to the risk for developing a PU. Moisture and/or urinary, fecal incontinence and type of bowel management [8, 16], temperature (hypo/hyperthermia), friction, shear, are some of the extrinsic risk factors associated with the formation of PUs in individuals with SCI during acute care and intensive care unit (ICU) [19, 51-55]. Decreased nutrition or low serum albumin levels, decreased mobility and sensation, medical comorbidities such as diabetes mellitus, cardiac, pulmonary, renal disease and impaired cognitive function contribute towards the intrinsic risk factors for the development of PUs in individuals with SCI [19, 20]. A retrospective study in a surgical intensive care unit (ICU), reported 9.6% individuals with SCI developed PUs. Hypotension and decreased

oxygenation were found to be the primary risk factors for the development of PUs. Additionally medications such as use of steroids were also associated with the development of PU. [16].

Infections such as septicemia, pneumonia, urinary tract infections (UTIs), osteomyelitis, and bacterial or any other skin infection were found to increase the risk of development of pressure ulcers. [8, 21]. Of the other clinical risk factors for development of PUs after SCI, strong evidence was reported for individuals with pneumonia and deep vein thrombosis (DVT) [11]. Three cohort studies found pneumonia as a significant risk factor for the formation of PUs in individuals with SCI [15, 19, 37]. Pneumonia was the only medical complication that was a predictor for occurrence of PUs during primary inpatient rehabilitation in individuals with SCI, and the other predictors that were significant were completeness of injury, mobility status and previous PU [56]. The individuals on mechanical ventilation are at a risk of developing PUs, and the risk increases with length of time of using mechanical ventilation. A study reported an incidence of 13.4% for occurrence of PUs, in individuals on mechanical ventilators [22].

1.2.3   Pneumonia in Individuals with Spinal Cord Injury

Pneumonia is an inflammatory condition of the lung, specifically inflammation of the alveoli (microscopic air sacs in the lungs). Although there are many causes of pneumonia, infection is the most common etiology. The infecting agents can be bacteria, viruses, fungi, or parasites [57, 58]. Pneumonia is the most frequent respiratory complication (66.9% cases) that occurs weeks after the SCI [45, 59, 60]. Around 30% of individuals with SCI develop pneumonia during the time of hospitalization [50]. Of all the secondary conditions following SCI, pneumonia is one of the most frequent causes of death [46, 47].

11

The diaphragm is the main inspiratory muscle of respiration and is supplied by the phrenic nerve that originates from third and extends to fifth cervical nerve (C3-C5). The muscles of respiration such as the diaphragm, abdominals and intercostal are affected with progressively higher level of SCIs, resulting in a higher incidence of pneumonia in individuals with tetraplegia than paraplegia [61]. Individuals with complete tetraplegia have altered lung volumes and ineffective cough, and present with a restrictive ventilatory pattern. The alteration in lung volumes decreases the lung compliance and increases the cost of energy, predisposing the individual to respiratory fatigue. Therefore, higher-level SCIs (tetraplegics at C3-C5) may produce complete respiratory paralysis [7, 37, 59, 62].

Overall 5% to 20% of individuals with SCI develop pneumonia during initial rehabilitation. Pneumonia was found to be associated with atelectasis in these subjects that alters the pattern of respiration. Studies have shown that prior anesthesia increases the risk of developing pneumonia [61]. Studies also indicated that the incidence of pneumonia increases within days of intubation for individuals who are on mechanical ventilation [63]. Hence, critically ill subjects on prolonged mechanical ventilation are predisposed to ventilator-associated pneumonia. These subjects who are on mechanical ventilators in either supine or semi recumbent positions also had incidence of PUs in the heel and sacral regions [63, 64].

1.2.4   Inflammation

Inflammation involves a complex process of cellular activation resulting in release of pro-inflammatory mediators such as cytokines, activation of neutrophils, monocytes and microvascular endothelial cells, involvement of neuroendocrine reflexes, and activation of the

12

complement, coagulation, and fibrinolytic system [65]. Inflammation is a necessary process by which the body restores its baseline function after tissue insult like trauma or sepsis, at the same time it can be destructive to otherwise healthy tissue resulting in tissue injury and further stimulating inflammatory response. Thus, finely tuned balance between pro-inflammatory and anti-inflammatory cytokines is necessary for the host [28] (Figure 1).
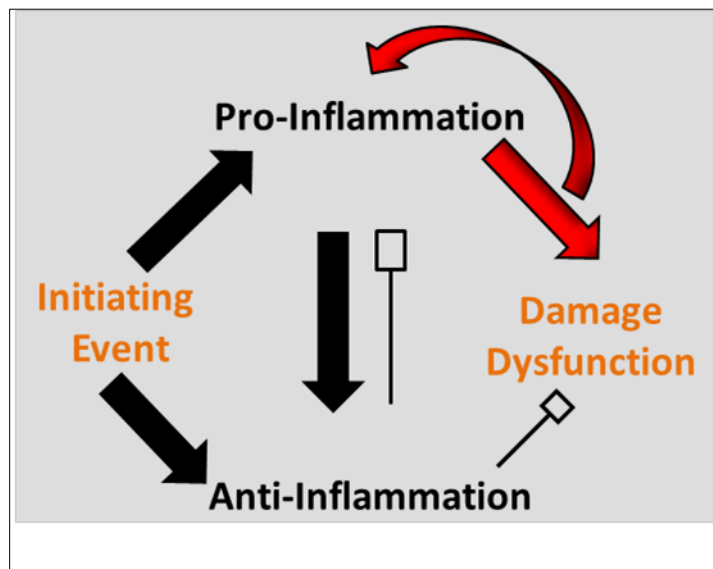
Figure 1. Inflammatory response post trauma (Source: Vodovotz, 2012)

1.2.4.1 Cytokines and Chemokines

The human immune system is complex and a large array of pro and anti-inflammatory cytokines are produced at the time of inflammation. The balance and the net interaction effect between these cytokines determine the nature of this immune response. [28]. The human body is capable of producing innate immune response, which is a pre-programmed activity independent of the antibodies produced by the body. This innate response acts as the first line of defense during

trauma or infection. The adaptive immune response usually acts or follows after the innate immune response [66].

1.2.4.2 Pro-Inflammatory Cytokines and Chemokines

Pro-inflammatory cytokines are important in maintaining both acquired and innate immunity and are released in response to stress or trauma. At the time of injury the outcome of the inflammatory response depends upon the extent and duration of these cytokines released. The interleukin-1 (IL)-1, IL-2, IL-6, tumor necrosis factor-α (TNF- α), and interferon gamma (IFN-γ) are included in the family of these cytokines. The interferons are mainly concerned with innate and acquired immunity. The pro-inflammatory cytokines, TNF-α, IL-1, IL-6, IL-12, IL-15, IL-18 and chemokines such as IL-8, monocyte chemotactic protein-1 (MCP-1), and interferons, are produced by local and circulating macrophages. In response to injury the lymphocytes and macrophages are activated at the site of injury to reduce the excess inflammation and decrease the damage cells. Too little or not enough response (maladaptive response) delays the healing process. Thus, inflammation is the process by which body tries to restore hemostasis following an insult [67]. On the other hand, excess activation of these cells can lead to tissue destruction [2].

1.2.4.3 Anti-Inflammatory Cytokines and Chemokines

The anti-inflammatory cytokines or the cytokine inhibitors such as IL-10 and IL-1RA are one of the most important mediators of this immune system. These cytokines limit the deleterious effects of excessive inflammation caused by the pro-inflammatory mediators after an injury. Studies report that therapeutic administrations of the anti-inflammatory cytokines are known to

have clinical benefits [28]. During a traumatic event the synthesis of the anti-inflammatory cytokines may be beneficial as they help combat the excessive effects of the pro-inflammatory mediators, thus reducing the pathology. On the other hand, excessive synthesis of these anti-inflammatory cytokines can over compensate the state of pathology, leading to systemic damage. The anti-inflammatory cytokines such as IL-6 sometimes have properties of pro-inflammatory cytokines depending upon the timing they are released and the sensitivity of the tissue that responds. [28, 68].

1.2.4.4 Inflammatory response to trauma

Traumatic injury induces an early inflammatory immune response that further releases pro-inflammatory cytokines such as IL-1, IL-2, IL-6, and TNF-α, predisposing the body to infection and increased mortality. The release of these cytokines depends upon the severity of the traumatic injury. It was initially believed that with initial pro-inflammatory response there is release of compensatory anti-inflammatory cytokines. At present, research suggests that there is rapid and simultaneous alterations in the pro-inflammatory and anti-inflammatory mediators after trauma [69]. Anti-inflammatory responses help attenuate the infection and induce healing and, when excessive, may lead to immunosuppression further aggravating the infection injury and increased mortality. Immediately following trauma, the inflammatory response starts locally. Gradual increase in inflammation may spill the inflammatory mediators into the systemic circulation, causing multiple organ damage including soft tissue injuries (Figure 2) [70].
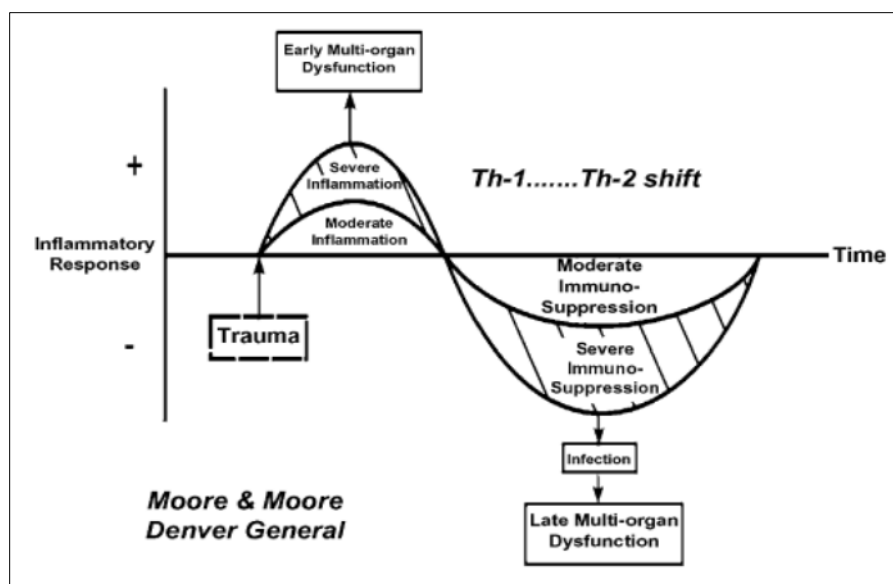
Figure 2. Inflammatory response post trauma (Moore, 1995)

1.2.4.5 Inflammation in Individuals with Spinal Cord Injury

In individuals who sustain traumatic SCI, production of cytokines and chemokines results in developing a robust inflammatory response that is characterized by neutrophil infiltration (being the first inflammatory cells to arrive) at the site of injury followed by macrophages [23]. The resident macrophages also known as resting microglia are diffusely spread in the central nervous system (CNS). These macrophages that are activated post-SCI, results in secretion of cytotoxic substances and pro-inflammatory mediators such as TNF-α, IL-1, IL-6, nitric oxide, reactive free radicles and oxygen free radicals. Post-traumatic SCI, inflammatory response further leads to deterioration in the functional outcome and predisposes the host to secondary complications such as tissue damage and other infections [25, 26]. These cytotoxic substances can lead to destruction resulting in secondary complications or production of growth factors essential for tissue repair. The anti-inflammatory mediators such as the IL-10 suppress most of the microglial activity. The

16

beneficial role of this inflammatory process can be attributed to the dual nature of the post inflammatory process [27].

Most of the cytokines such as IL-1α, IL-1β, IL-1ra, IL-6, and TNF-α pass freely through the blood brain barrier (BBB). Traumatic SCI elicits up-regulation of this transport due to the disruption of the blood-spinal cord barrier (BSCB). Studies have shown that the secondary complications and infections after SCI can be associated with the increase in the serum concentrations of mediators such as TNF-α and IL-2 [4, 24]. The pro-inflammatory mediators such as IL-1β, IL-6, TNF-α increase immediately after injury until 5 hours post injury, and from the second day post injury the expression of these cytokines declines to baseline [29]. Previous studies showed that the pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α and circulating levels of antibodies and immunoglobulins such as IgG and IgM were increased in individuals with SCI and were further increased in this population with complications such as UTIs and PUs as compared to able bodied subjects [4]. This suggests an increase in the active inflammatory process in individuals with SCI and other medical complications such as PUs and UTI's.

1.2.4.6 Inflammation in Individuals with Pressure Ulcers

Increase in the local inflammatory process at the PU site is observed in individuals with PUs. Keratinocytes present in the epidermis play an important role in the immune system. Upon mechanical skin injury *in vitro* via a loading device these keratinocytes lead to increase in production of pro-inflammatory cytokines such as IL-1 and granulocyte macrophage stimulating factor (GM-CSF). The IL-1 further produces the production of other cytokines and chemokines such TNF-α, IL-6, IL-8 [71]. Elevated levels of IL-8 were found in the tissue biopsies of the slow healing wounds as compared to healed wounds or skin and suggested that IL-8 retards

keratinocyte replication and thus delays the process of wound healing [33]. Moreover increase in pro-inflammatory cytokines such as TNF-α and IL-1β have been observed in stage III and IV PUs. [5]. These pro-inflammatory cytokines were increased in response to low mechanical loading when the tissue damage is barely visible. There was a threefold increase in these cytokines *in vitro* after a short loading with high pressure [31]. In addition, an *in vivo* study indicated morphological signs of inflammation in stage I PUs. [72]. Hence, these inflammatory markers can play an important role in the early detection of occurrence of PUs.

The cytokines such as IL-1α, IL-1ra and IL-8 were found to be significantly increased after 1 hour of mechanical loading of tissue-engineered epidermal equivalents (*in vitro*), whereas TNF-α was reported to be released after 4 hours of epidermal loading and remains constant for 16 hours of loading as compared to the unloaded group. These pro-inflammatory cytokines released within four hours of epidermal loading, was much before the tissue damage was visible. Thus, measuring pro-inflammatory cytokines may help as an objective measure to determine risk for formation of PUs [73].

Some researchers reported systemic elevation of inflammatory mediators in individuals with PUs. Segal et al., found an increase in plasma concentrations of interleukin 2 receptor (IL-2R), IL-6 and ICAM-1 following SCI, in individuals with slow healing PUs as compared to able-bodied subjects or healthy subjects with SCI without PUs [32]. The increase in pro-inflammatory cytokines in individuals with SCI having formation of PUs and presence of other infections inhibits the wound health and are associated with slow healing of these ulcers [4]. A recent study compared the variations in the plasma biomarkers in three groups, individuals with SCI, PU in individuals with SCI and able-bodied subjects, and the results indicated decrease in the trend of creatine kinase (CK) in the individuals with SCI with PU as compared to the other groups.

Increased trend was noted in in the C-reactive protein; marker of inflammation and tissue damage, and heart type fatty acid binding protein (H-FABP) in subjects with SCI with PU as compared to the other two groups. The biomarkers analyzed in this study were the biomarkers associated with skeletal muscle damage and cardiac muscle damage [74]. Thus, increase in concentration of the inflammatory mediators in individuals with SCI and PUs. The synthesis of these inflammatory mediators was increased both locally at the tissue level and systemically. The initial inflammatory response increases the blood flow to heal the injured tissue. Prolonged activation and synthesis of this inflammatory response predisposes the body to chronic inflammation, and upturns the concentrations of these mediators systemically.

1.2.4.7 Inflammation in Individuals with Pneumonia

The complex and dynamic network of pro and anti-inflammatory mediators contributes to the initiation and resolution of the infectious process involved with presence of pneumonia. On one hand, the local synthesis of pro-inflammatory cytokines is essential to fight against the pathogens in pneumonia; on the other hand the synthesis of the anti-inflammatory cytokines if in excess impairs the host defense. Although the inflammatory response is essential to eliminate the pathogen in pneumonia this response in excess may worsen the damage to the lung and may make it difficult to eliminate the pathogens. Any bacteria or other pathogen entering the lung is killed by the alveolar macrophages before the pathogens reach the alveoli. These macrophages activate the synthesis of the pro-inflammatory cytokines and neutrophils and further increase the response of the pro-inflammatory cytokines such as the TNF-α and IL-1. Plasma levels of IL-1β and TNF were found to be elevated in subjects with local pneumonia irrespective of the etiology [39]. Experimental studies show that TNF-α eliminates the pathogens by inhibiting the

outgrowth of bacteria. Administrating anti-TNF in experimental animals was found to decrease their survival rate [75]. Hence, the synthesis of TNF-α is essential to maintain the host defense against different microorganisms. Excess of IL-10 was found to impair the host defense, and increases the pathogen production in subjects with pneumonia. Treatment with anti-IL-10 improves the host defense to eliminate the pathogens by increasing the production of the pro-inflammatory cytokines. At the same time inhibition of IL-10 seemed to be lethal since it is essential to control the excessive inflammation. Hence, the effect of IL-10 in infections such as pneumonia seems to be complex and is dependent upon the source of infection and is based on the balance in the dynamic network of the pro and anti-inflammatory cytokines [3].

1.3   SIGNIFICANCE OF THE STUDY

Pressure ulcers are the most frequent secondary complication and are one of the leading causes of death after Spinal Cord Injury (SCI) [8]. Individuals with PUs experience increased pain, discomfort and distress that increase the hospital stay and the ongoing treatment time [76]. Loss of independence, body image, and burden to others were some of the concerns expressed by these individuals further contributing towards anxiety emotional and psychological complications. These individuals avoid moving their body parts due to the pain and continue to stay in one position. This further increases conflicts with their healthcare professionals and interfere with their rehabilitation care [77]. A recent literature review discusses the lack of evidence in the medical risk factors during hospitalization associated with developing PUs as compared to the care related risk factors [6]. This study investigated and determined the demographic and clinical risk factors in newly injured individuals following traumatic SCI associated with the formation of PUs during hospitalization. Individuals with TSCI having PUs and pneumonia possibly will have an imbalance in the inflammatory response. Reduced life expectancy and decreased quality of life was reported in individuals with SCI having secondary complications such as pneumonia and septicemia [78]. This study identified the inflammatory mediators associated with the formation of PUs, following SCI and just before the formation of PUs was visible. Although studies indicate pneumonia to be correlated with the occurrence of PUs in individuals with SCI [37, 42], none of them explore the mechanism of the presence of pneumonia that may be a risk factor for the formation of PUs in this population. This study highlighted the relationship between presence of pneumonia and incidence of PU linked through inflammation.

1.4   DISSERTATION STRUCTURE

Chapter 2 investigates the demographic and medical risk factors associated with the formation of pressure ulcers in individuals with traumatic spinal cord injury (TSCI). In Chapter 3, we explored and investigated whether the concentrations of the inflammatory mediators measured in both plasma and urine biofluids immediately following TSCI, could predict the occurrence of pressure ulcers, by building a stepwise multivariate logistic regression model. Chapter 4 investigates whether there are changes in the concentrations of the inflammatory mediators in both plasma and urine biofluids from immediately after spinal cord injury to just before formation of pressure ulcer. In Chapter 5, the association between pneumonia and occurrence of pressure ulcer was investigated in the Rehabilitation Engineering Research Center on SCI and Spinal Cord Injury Model Systems populations. Chapter 6 explored and investigated whether changes in the concentrations of inflammatory mediators before and after formation of pneumonia, in both plasma and urine biofluids could predict the occurrence of pressure ulcers. Chapter 7 summarizes the results and conclusions obtained from this dissertation study and venues for future work.

2.0 DEMOGRAPHIC FACTORS AND CLINICAL COMORBIDITIES ASSOCIATED WITH FORMATION OF PRESSURE ULCERS IN INDIVIDUALS WITH TRAUMATIC SPINAL CORD INJURY

2.1 INTRODUCTION

Spinal cord injury (SCI) is a devastating neurologic disorder that has profound impact from physical, psychological and socioeconomic perspectives [44]. Increase in age and severity of injury are associated with rise in long-term complications after traumatic SCI [79]. The second leading cause of death in individuals with SCI is septicemia (88.6%); usually associated with urinary tract infections (UTI's), pneumonia, and/ or presence of PUs [45]. Pressure ulcers (PUs) are the most frequent secondary complication in individuals with SCI from the time of acute hospitalization through community reintegration PUs affect quality of life, length of stay during hospitalization and increases the mortality and morbidity [1, 7, 12, 48, 49]. The 2011 annual statistical report for the Spinal Cord Injury Model Systems (SCIMS) identify PUs as the second most frequent complication and third leading cause of death for people with SCI [45].

Many risk factors have been associated with the formation of pressure ulcers. Medical complications such as cardiac or renal disease were associated with the risk of developing PUs [8]. Individuals likely to develop PUs were those who have SCI of traumatic origin [17], males [18], have a history of smoking, alcohol or drug use [10], have medical comorbidities such as

diabetes mellitus [19, 20], decreased oxygenation or hypotension [16], infections such as pneumonia, urinary tract infections, osteomyelitis, and other bacterial infections [8, 21], those on mechanical ventilators [22] and use of steroids [16]. Moisture and/or urinary and fecal incontinence, hypo/hyperthermia, friction, shear, were reported to be the extrinsic risk factors for the formation of PUs in individuals in acute care and intensive care unit (ICU) [16, 19, 51-55]. Decreased nutrition or low serum albumin levels, decreased mobility and sensation, and impaired cognitive function contribute towards the intrinsic risk factors for the development of PUs in individuals with SCI [19, 20].

The extrinsic environment plays an important role for the development of PUs. A majority of studies focus on the risk factors associated with the development of PUs in community care in individuals with SCI [8-15], however only a few address the risk factors in this population during hospitalization or acute care [16]. Hence, identifying the factors for development of PUs in high-risk settings (e.g., during hospitalization) is essential. Studies on risk factors associated with PUs mostly used self-report or retrospective chart review methods [8, 10]. This may result in underreporting of the data due to recall bias or misclassification of the data. Although numerous risk factors are associated with the occurrence of PUs, the increased incidence of PUs for newly injured individuals with SCI during hospitalization have not been similarly determined.

Thus, identifying the clinical factors associated with the development of PUs during acute care hospitalization and inpatient rehabilitation may help prevent this secondary complication and may reduce the risk of re-hospitalization and improve the quality of life. Since this study is a secondary analysis few factors were included to predict the formation of first PU due to limited availability of data.

24

We hypothesized medical factors such as presence of pneumonia, urinary tract infection (UTI), use of steroids, and demographic factors such as gender, age, and severity of injury measured by ASIA score will be associated with increased risk for the formation of PUs in individuals with traumatic SCI during acute care hospitalization and inpatient rehabilitation.

2.2   METHODS

2.2.1   Research Design

The study was conducted at the Rehabilitation Engineering Research Center on Spinal Cord Injury (RERC on SCI). The RERC on SCI serves individuals with SCI by research and development of new technologies to improve treatment and thus help with reintegration into society. This protocol received approval from the University of Pittsburgh Institutional Review Board (IRB). The Clinical Core of the Center enrolled acute patients with SCI to collect blood, urine, demographic information and medical information during their acute and inpatient stay through their rehabilitation stay at UPMC and post discharge. The focus of the RERC project was to obtain information related to the incidence of pressure ulcers in the SCI population, the occurrence of urinary tract infections and data associated with depression and pain status, and develop a protocol was developed generate data needed to develop a model of inflammation and healing in PU development following SCI

Once informed consent was obtained, clinical data, plasma and urine samples were collected three times per week when the subject was in acute care, weekly in inpatient

rehabilitation and annually after discharge to outpatient care until the duration of study; that occurred for 5 years (2008-2012). The RERC on SCI was funded by the National Institute for Disability and Rehabilitation Research (NIDRR), grant #H133E070024. The RERC database was used in this study in a secondary analysis to explore and examine the relationship among the clinical and demographic variables and PU outcome [80].

2.2.2 Inclusion and Exclusion Criteria

The individuals with SCI were recruited within 24-72 hours of admission into the University of Pittsburgh Medical Center, neurotrauma centers. The subjects were eligible for the study if they met the following inclusion criteria:

1. Received acute medical and surgical treatment at UPMC hospitals

2. Received acute rehabilitation at Institute for Rehabilitation and Research (IRR) South Side / UPMC Mercy after acute medical/surgical treatment

3. Were 18 years and older

4. Presented with acute newly traumatic SCI (such as motor vehicle accident, fall or sport injury)

The subjects were excluded if they had the following comorbidities:

1. Pre-existing diseases (such as autoimmune or demyelinating diseases) that affected the inflammatory response to SCI

2. Previous SCI or other neurological diseases that affected the motor and sensory function

Individuals with any pre-existing disease (e.g. diabetes) were initially excluded from the study but later included after a year in an effort to increase recruitment rate.

2.2.3   Data Collection

The Clinical Core of the RERC on SCI collaborated with the University of Pittsburgh Medical Center (UPMC) and UPMC Model System on SCI to recruit, screen and retain subjects. Data related to risk and incidence of PUs and UTIs were recorded. PU risk variables included age, gender, and level of injury, other injury associated with trauma, ASIA grade, smoking, alcohol use and comorbidities [12, 81]. The American Spinal Cord Impairment scale (AIS) was used to grade severity of motor and sensory impairment [82]. Injury severity score (ISS), (Table 3) was used to record the combined severity of the possible multiple other traumatic injuries [83]. The AIS and ISS were noted from the UPMC electronic health records by the research staff. The extent of the injury that was recorded for this study in listed in Table 1. Comorbidities in all systems for individuals with TSCI were noted by the research staff. The research staff measured the intensity of pain using the pain scale [84]. The severity of depression was assessed by the research staff using the patient health questionnaire (PHQ)-9 [85]. If a subject developed PU, the research coordinators recorded the severity, size, shape and progression of the wound healing. Severity was determined using National Pressure Ulcer Advisory Panel (NPUAP) staging system, Table 2. Shape and size was recorded by the research staff using digital photograph and quantified using length, width and depth measurement technique [86]. The characteristics of the PU such as site, shape, necrotic tissue, amount, wound base, ulcer edges, drainage, surrounding tissue, and steroid protocol were also recorded by the recorded. The medications such as steroids,

NSAIDs, pain, anti-inflammatory and any other mediation these individuals were on were noted from their UPMC electronic health records. The data collected is summarized in (Table 1).

Table 1. Data Collected for the RERC on SCI

Variables	*Specifics*
Demographics	Admission date, Consent date, Date of Injury, Age Sex, Marital Status, Ethnicity, Height, Weight, past medical history (PMH) of smoking.
Injury	Cause of Injury, Level of Injury: Cervical / thoracic/ Lumbar/ Sacral/ Coccyx; Also Bilateral v/s left and right, Complete v/s Incomplete, American Spinal Injury Classification (ASIA) score, Lower Extremity Score (This is a subset of ASIA score, where the motor score of the individual is tested by manual Muscle test), Injury Severity Score (ISS- This is a score which asses the severity of injury).
Medical Information	- Alcohol use & tobacco use, musculoskeletal pain, PHQ scale (Scale to assess depression; 1-4 – Minimal depression, 5-9 – Mild depression, 10-14 – Moderate Depression, 15-19 – Moderately severe depression, 20-27- Severe Depression) - Medical Co Morbidities such as cardiovascular, hematopoietic, respiratory, ENT, gastrointestinal, rheumatologic, musculoskeletal, neurologic, endometric/metabolic, immunological, psychiatric, malignancy, substance Abuse, UTI, integumentary. - Number of PUs, Braden Score (pressure ulcer risk score) - Bladder and Bowel Management - Medications: Pain, Non-steroidal anti-inflammatory drugs (NSAIDs), Steroids, Antibiotics and Others. - Ambulation Status were recorded only at the time of inpatient discharge and Outpatient phases
Pressure Ulcer Information	Size, shape, severity and progression of the PU are recorded. Severity was recorded using the National Pressure Ulcer Advisory Panel (NPUAP) staging system

Table 2. Stages of Pressure Ulcer[87]

Category/ Stage	Description
I "Non-blanchable erythema"	"Intact skin with non-blanchable redness of a localized area usually over a bony prominence. Darkly pigmented skin may not have visible blanching; its color may differ from the surrounding area. The area may be painful, firm, soft, warmer or cooler as compared to adjacent tissue. Category I may be difficult to detect in individuals with dark skin tones. May indicate "at risk" persons".
II "Partial thickness"	"Partial thickness loss of dermis presenting as a shallow open ulcer with a red pink wound bed, without slough. May also present as an intact or open/ruptured serum-filled or sero-sanginous filled blister".
III "Full thickness skin loss"	"Subcutaneous fat may be visible but bone, tendon or muscle are *not* exposed. Slough may be present but does not obscure the depth of tissue loss. *May* include undermining and tunneling. Bone/tendon is not visible or directly palpable".
IV "Full thickness tissue loss"	"Full thickness tissue loss with exposed bone, tendon or muscle. Slough or eschar may be present. Often includes undermining and tunneling. Exposed bone/muscle is visible or directly palpable".
"Unstageable: Full thickness skin or tissue loss– depth unknown"	"Full thickness tissue loss in which actual depth of the ulcer is completely obscured by slough (yellow, tan, gray, green or brown) and/or eschar (tan, brown or black) in the wound bed. Until enough slough and/or eschar are removed to expose the base of the wound, the true depth cannot be determined; but it will be either a Category/Stage III or IV".
"Suspected Deep Tissue Injury – depth unknown"	"Purple or maroon localized area of discolored intact skin or blood-filled blister due to damage of underlying soft tissue from pressure and/or *shear.* The area may be preceded by tissue that is painful, firm, mushy, boggy, warmer or cooler as compared to adjacent tissue".

Table 3. ISS Scale Score

ISS Score	Severity
1-9	Minor
10-15	Moderate
16-24	Moderate/Severe
≥25	Severe/Critical

2.2.4 Procedure

The primary outcome is occurrence of first PU. All stages of PUs (Stage I- IV, deep tissue injury and Unstageable) were considered. Through expert opinion of a group of therapists and clinicians, and review of the literature, the list of possible variables was reduced to the following for analysis:

1. Age

2. ASIA Impairment Scale (AIS) Score

3. Gender

4. Use of Steroids

5. Urinary Tract Infection diagnosis

6. Pneumonia diagnosis

7. Diabetes

2.2.5 Data Analysis

All data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistics using means, frequencies, and standard error of mean were computed.

2.2.5.1 Logistic Regression

Univariate Logistic Regression

A univariate logistic regression analysis was conducted to assess individual risk factors ability to predict the probability of the outcome (PU, yes/no). The model is[88]:

$$\textbf{logit (Y)} = \frac{\textbf{1}}{\textbf{1} + \boldsymbol{e}^{-(\beta_0 + \beta_1 X_1)}} = \boldsymbol{\beta_0} + \boldsymbol{\beta_1 X_1}$$

- β_0 is a constant

- β_1 is the coefficient of the individual predictor variable.

- X_1 is the clinical (predictors) variable in question

Our hypotheses are:

H_0 (Null Hypothesis): $\beta_1 = 0$

H_1 (Alternate Hypothesis): $\beta_1 \neq 0$

The significance level was set at $\alpha = 0.05$.

Multivariate Logistic Regression

A multivariate logistic regression was conducted with the independent variables selected from univariate analysis and by reviewing the literature that previously identified these risk factors. The outcome for the model was occurrence pressure ulcer (yes/no). The model is: [88]

$$\textbf{logit (Y)} = \frac{\textbf{1}}{\textbf{1} + \boldsymbol{e}^{-(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots \beta_k X_k)}} = \boldsymbol{\beta_0} + \boldsymbol{\beta_1 X_1} + \boldsymbol{\beta_2 X_2} + \cdots \ldots + \boldsymbol{\beta_k X_k}$$

- β_1, $\beta_{2\ldots}$ β_k are the regression coefficients for the independent variables of the regression equation.

- X_1, X_2…X_k are the independent variables (risk factors in question).

31

Our hypotheses are:

H₀ (Null Hypothesis): $\beta_1 = \beta_2 = \ldots = \beta_k$ (or) $\beta_i = 0$

H₁ (Alternate Hypothesis): $\beta_1 \neq 0$ (or) $\beta_i \neq 0$

The significance level was set at $\alpha = 0.05$.

2.2.5.2 Logistic Regression Coefficients and Wald statistic

The logistic regression coefficients, $\hat{\beta}_0$, $\hat{\beta}_1 \ldots$, $\hat{\beta}_k$ $\hat{\beta}_0$ and their standard errors are estimated using maximum likelihood methods. These values, in turn, are used to evaluate the fit of one or more models. Maximum likelihood estimation provides estimates of regression coefficients that make a sample as likely as possible, given values on the predictors and the outcome. The computed likelihood of a sample given the maximum likelihood estimates is termed the maximum likelihood of the sample, typically denoted by L. If an acceptable model is found, the statistical significance of each of the coefficients is evaluated using the Wald test where the squared coefficient is divided by the squared standard error. The Wald statistic is a χ^2 statistic with one degree of freedom. Wald statistic has different forms and can be defined as in Equation 1 and Equation 2, and it has standard normal (z) distribution [89].

Equation 1. Wald Statistic

$$W_i = \frac{\hat{\beta}_i^2}{s_{\hat{\beta}_i}^2}$$

Equation 2. Different form of Wald statistic

$$W_i = \frac{\hat{\beta}_i}{s_{\hat{\beta}_i}}$$

2.2.5.3 Interpretation of β's

β's cannot be interpreted directly. $\hat{\beta}_j$ is the amount of change in logit for each unit increase in X_j. e^{β_j} is equal to odds ratio; change in odds between a baseline group and a single unit increase in X_j. e^{β_j} can be interpreted [88] (Table 4).

Table 4. Interpretation of Odds Ratio

Odds Ratio(e^{β_j})	Interpretation
$e^{\beta_j} = 1$	No change in odds ratio
$e^{\beta_j} < 1$	Odds ratio decrease
$e^{\beta_j} > 1$	Odds ratio increase

The sample sizes were not estimated by using power analysis to obtain a desired power prior to data collection. Since this study was a secondary analysis, to have a good model fit and a good power the general "rule of thumb" to have 10 cases per predictor variable was considered [90]. The assumptions of logistic regression such as multicollinearity, linearity and outliers were checked to minimize the number of predictors and include the best set of variables to maximize the strength of prediction.

2.2.5.4 Hosmer-Lemeshow goodness of fit

The Hosmer-Lemeshow goodness of fit was computed. This evaluates the goodness of fit by comparing the observed model by creating ten groups of subjects (based on estimated probability) to the predicted logistic regression model. When there are one or more continuous predictors in the model, Hosmer-Lemeshow statistic can be used to assess goodness of fit.

Subjects are first put in order by their estimated probability on the outcome variable. Then subjects are divided into 10 groups according to their estimated probability: those with estimated probability below .1 (in the lowest decile) form one group, and so on, up to those with estimated probability 0.9 or higher (in the highest decile). The next step is to further divide the subjects into two groups on the outcome variable to form a 2×10 matrix of observed frequencies. If the logistic regression model is good, then most of the subjects with outcome 1 are in the higher deciles of risk and most with outcome 0 in the lower deciles of risk. If the model is not good, then subjects are roughly evenly spread among the deciles of risk for both outcomes 1 and 0. A nonsignificant Hosmer-Lemeshow chi-square statistic indicates a good model. The test statistic of Hosmer-Lemeshow is given in Equation 3 [91].

Equation 3. Hosmer-Lemeshow test statistic

$$\chi^2_{HL} = \sum_{k=1}^{g} \frac{(o_k - n_k \overline{\pi}_k)^2}{n_k \overline{\pi}_k (1 - n_k \overline{\pi}_k)}$$

- g is the number of groups
- o_k is the total frequency of event outcomes in the kth group
- $\overline{\pi}_k$ is the average estimated predicted probability of an event outcome for the kth group
- n_k is the frequency of subjects in the kth group
- degrees of freedom = g – 2

2.2.5.5 Area under the Receiver Operating Characteristic (ROC) curve

The ROC curve was plotted for the multivariate logistic regression model. The ROC curve analysis allows us to evaluate the multivariate logistic regression model. It plots the probability of detecting true signal (sensitivity) and false signal (1 - specificity) for an entire range of

possible cutoff values. The area under the ROC curve, which ranges from 0 to 1, provides a measure of discrimination (classification). The ROC analysis thus helps to determine the power of the model's predicted values to discriminate between those with and without the disease (pressure ulcer). A complete description of ROC classification accuracy is given by the area under the receiver operating characteristic (ROC) curve (AUC). The area under the ROC curve (AUC) helps us to quantify the power and evaluate the accuracy of the multivariate logistic regression model. An area under the curve varies from 0.5 (represents discriminating power not better than chance) to 1.0 (represents a perfect discriminating power). A guide for classifying the accuracy of a diagnostic test is given in Table 5 [92].

Table 5. Area under the Receiver Operating Characteristic (ROC) curve classification

Area under ROC	Accuracy
0.90-1	excellent
0.80-0.90	good
0.70 -0.80	fair
0.60 -0.70	poor
0.50 -0.60	fail

2.3   RESULTS

2.3.1   Baseline Characteristics

One hundred and four individuals were included and enrolled in the RERC on SCI study. The demographics, characteristics, location of the subjects and medical comorbidities are shown in Table 6. Majority of SCIs were due to falls (38%) or due to motor vehicle accident (32%). Thirty-five percent of the individuals were followed from acute care through discharge, while the other individuals were either withdrawn by the study team, withdrew themselves from the study, or were discharged at different time points. Eighty-one percent of the individuals in the population were Caucasians. Almost 93% of individuals in acute care and 83% of individuals' in inpatient rehabilitation used catheters for their bladder management.

2.3.2   Pressure Ulcers

Forty-four individuals (42%) with traumatic spinal cord developed at least one pressure ulcer. The demographics, characteristics, location of subjects and medical comorbidities of these subjects are shown in Table 6. The severity of first pressure ulcer for most of the individuals in this population was Stage II (67%). The mean number of days from the time of injury until onset of the first PU was 19.2 ± 1.8. 42% of males and 29% of females developed pressure ulcers. 45% of individuals between the ages 18-30 years developed PUs.

2.3.3 Risk factors for the Formation of Pressure Ulcer

2.3.3.1 Univariate analysis

A univariate logistic regression was performed for age, gender, ASIA, pneumonia, UTI, steroids and diabetes (Table 7). The analysis was significant for individuals with pneumonia; p=0.014 and for injury severity (ASIA scale score); p=0.011. The univariate analysis was not significant for any other variables. The average number of days from the time of injury to develop pneumonia in subjects with PUs in this study was 11 days (Table 6). The receiver operator characteristic (ROC) curve was plotted for all the individual predictors (Figure 3) and the area under the curve (AUC) was computed (Table 8). The AUC for individuals who had pneumonia is 0.626 and significant p= 0.035. The AUC was not significant for any other variables.

2.3.3.2 Multivariate Logistic Regression Model

A multivariate logistic regression was performed with pressure ulcer as the outcome and six predictors: Age, ASIA classification, gender, steroid use, pneumonia, and urinary tract infection. The PU outcome was dichotomized into 2 levels (PU present (1) or not PU (0)). There were 2 levels in UTI, steroids, and pneumonia (yes (1), no (0)), 2 levels in gender (male and female) and 4 levels in ASIA (ASIA A (1), ASIA B (2), ASIA C (3) and ASIA D (4)). (Table 9) shows the results of the multivariate logistic regression. All assumptions were met. There was a significant prediction of PU outcome by the clinical and demographic factors that were included in the multivariate model, $\chi2$ (8) = 17.925, p=0.022. The odds of formation of a first PU in individuals with ASIA A was 4.7 times greater individuals with ASIA B, p=0.04 and the odds of formation of a PU in individuals with ASIA A was 5.5 times greater individuals with ASIA C, p= 0.01.

There were no significant prediction of pressure ulcer occurrence by medical factors such as pneumonia (p=0.25), urinary tract infection (p=0.25), use of steroids (p=0.36), and demographic factors such as age (p= 0.94) and gender, (p= 0.411).

2.3.3.3 Hosmer-Lemeshow goodness of fit

In this study, there was no significant difference between the observed and predicted group membership, Hosmer-Lemeshow χ^2 (8) = 9.912, p= 0.271. This means that there is a good overall fit of the logistic regression model and that there is no misspecification of the predictors.

2.3.3.4 Area under the Receiver Operating Characteristic (ROC) curve

In this study, the area under the ROC curve is 0.735 with 95% confidence interval (0.636, 0.835). Also, the area under the curve is significantly different from 0.5 since p value = 0.000 (Table 10). This means that the variables in the multivariate logistic regression model significantly classifies formation of PU the RERC on SCI population.

Table 6. Baseline Characteristics of Individuals in the RERC on SCI during acute care and inpatient rehab

Variables	All subjects (n=104) #of subjects (%)	Subjects with PUs (n=39)	Subjects without PU (n=65)	P value
Gender				0.65
Male	83 (80%)	32 (82%)	51 (79%)	
Female	21 (20%)	7 (18%)	14 (22%)	
Age – years (Mean ± SEM)	40.9 ± 1.7	38.1±2.7	42.5±2.2	0.19
18-30	40 (38%)	16 (41%)		
31-50	28 (27%)	10 (27%)		
51-65	25 (24%)	10 (27%)		
>65	11 (11%)	3 (8%)		
Level of Injury				0.49
C2-C7	51 (49%)	19 (49%)	32 (49%)	
T1- T6	14(14%)	6 (12%)	8(12%)	
T6-T12	21 (20%)	9 (23%)	12 (19%)	
L1-L5	13 (13%)	2 (5%)	11 (17%)	
Other	3 (3%)	2 (5%)	1 (2%)	
Unknown	2 (2%)	1 (3%)	1 (2%)	
ASIA Score				< 0.001
A	42 (40%)	25 (64%)	17 (26%)	
B	13 (13%)	3 (8%)	10 (15%)	
C	24 (23%)	5 (13%)	19 (29%)	
D	21 (20%)	5 (13%)	15 (23%)	
E	1 (1%)	0 (0%)	1 (2%)	
Unknown	5 (5%)	1 (3%)	3 (5%)	
Education				0.48
High School	75 (72%)	30 (77%)	45 (69%)	
4 year College	10 (10%)	2 (5%)	8 (12%)	
Post-Graduate	3 (3%)	0 (0%)	3 (5%)	
Tech/2year degree	11 (11%)	4 (10%)	7 (11%)	
In High School	2 (2%)	1 (3%)	1(2%)	
Unknown	3 (3%)	2 (5%)	1 (2%)	
Marital Status				
Single	55 (53%)	19 (49%)	36 (55%)	0.77
Married	37 (36%)	16 (41%)	21 (32%)	
Divorced	10 (10%)	3 (8%)	7 (11%)	
Widowed	2 (2%)	1 (3%)	1 (2%)	
Ethnicity				0.18
African-American	19 (18%)	4 (10%)	15 (23%)	
Asian-American	1 (1%)	0 (0%)	1 (2%)	
Caucasian/White	84 (81%)	35 (90%)	49 (75%)	
Past Medical History of Smoking	43 (41%)	16 (41%)	27 (42%)	0.85
Diabetes	15 (14%)	7 (18%)	8 (12.3%)	0.4
Secondary Conditions				
UTI	53 (51%)	24 (63%)	29 (44%)	0.06
Onset of UTI (Mean no. of days ± SEM)	26.1 ± 3.3	34.4 ± 6.6	19.2±1.5	0.15
Pneumonia	40 (39%)	20 (51%)	20 (31%)	0.03
Onset of Pneumonia (Mean no. of days ± SEM)	8.9 ± 1.04	11.2 ± 1.8	6.3±0.6	0.02
At least 1 Pressure Ulcer	39 (42%)	39	N/A	
Use of Catheters	97 (93%)	38 (97%)	59 (91%)	0.18

Table 7. Univariate Logistic Regression of clinical and demographic factors (n=104).

Variables	*Individuals with at least 1 PU in acute care through inpatient rehabilitation N=39*	*Individuals with no PU in acute care through inpatient rehabilitation N=65*	*Sig (p)*
Age (mean)	38.12 y	42.50 y	0.22
Gender (N)	M= 32 F= 7	M= 51 F=14	0.79
ASIA (N)	A= 25 B= 3 C= 5 D= 5	A= 17 B=10 C= 19 D= 15	0.01*
Pneumonia (N)	Y= 21	Y= 19	0.01*
UTI (N)	Y= 24	Y= 29	0.09
Steroids (N)	Y=13	Y= 20	0.78
(N)	Y=7	Y= 8	0.43

*p < 0.05,

40
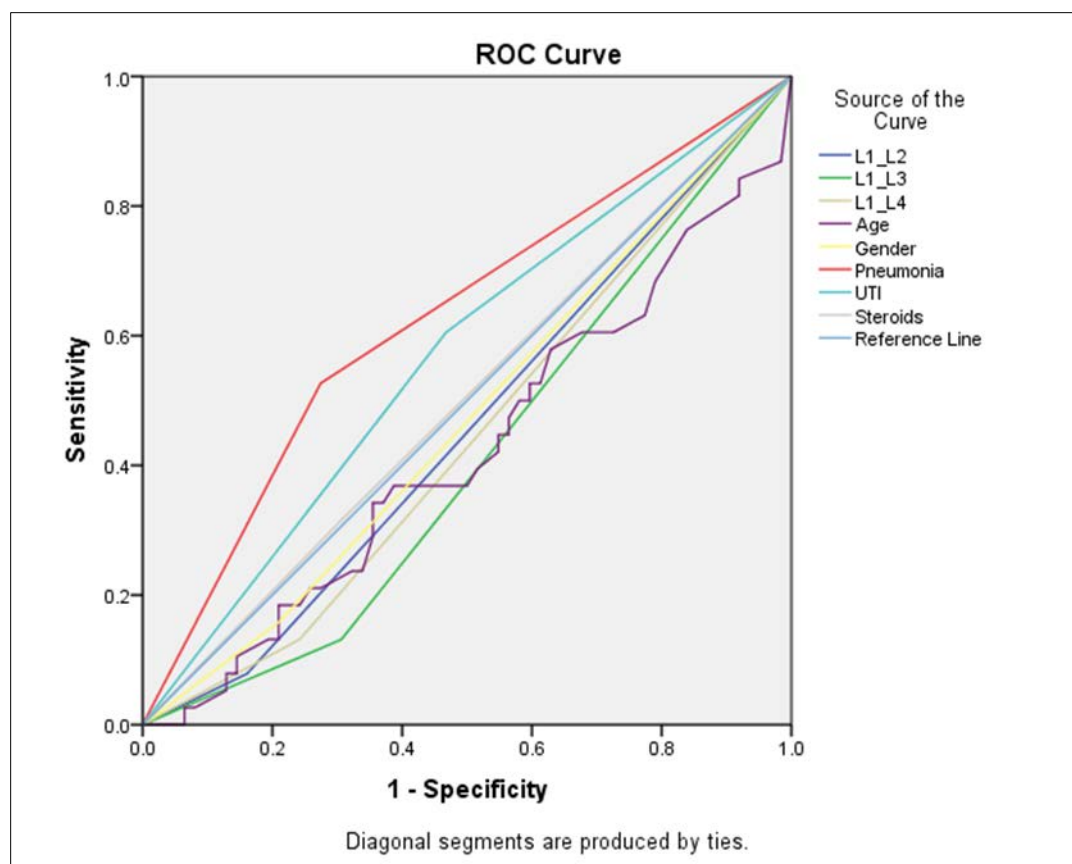
Figure 3. ROC curve for individual variables

Table 8. Area under the Curve for all Variables

Variables	Area	Std. Error	Sig(p)	Asymptotic 95% Confidence Interval	
				Upper Bound	Lower Bound
ASIA A – ASIA B	0.459	0.059	0.491	0.344	0.574
ASIA A – ASIA C	0.413	0.057	0.143	0.300	0.525
ASIA A – ASIA D	0.445	0.058	0.356	0.330	0.559
Age	0.423	0.059	0.197	0.306	0.540
Gender	0.474	0.059	0.665	0.358	0.590
Pneumonia	0.626	0.059	0.035*	0.511	0.741
UTI	0.569	0.059	0.250	0.453	0.685
Steroids	0.505	0.060	0.938	0.387	0.622

$*p<0.05$

Table 9. Multivariate Logistic Regression of Clinical and Demographic Factors, RERC on SCI

Independent Variables	B	SE	Wald	Sig (p)	Exp(B)
Age	-0.001	0.014	0.005	0.943	0.99
Gender	0.486	0.592	0.675	0.411	1.63
ASIA A – ASIA B	1.548	0.772	4.023	0.045*	4.7
ASIA A –ASIA C	1.701	0.658	6.678	0.01*	5.47
ASIA A – ASIA D	1.090	0.719	2.3	0.129	2.98
Pneumonia	-0.592	0.515	1.319	0.251	0.55
Urinary tract infection	-0.552	0.484	1.301	0.254	0.576
Steroids	-0.468	0.511	0.839	0.360	0.626
Constant	-3.482	1.809	3.703	0.054	0.031

Model χ^2 = 17.925, p < 0.05
Pseudo R^2 = 0.223
n = 104

Note: The dependent variable in this analysis is formation of pressure ulcer in acute care or inpatient rehabilitation, so that 0= did not have pressure ulcer and 1= had a formation of pressure ulcer
**p<0.01, *p< 0.05
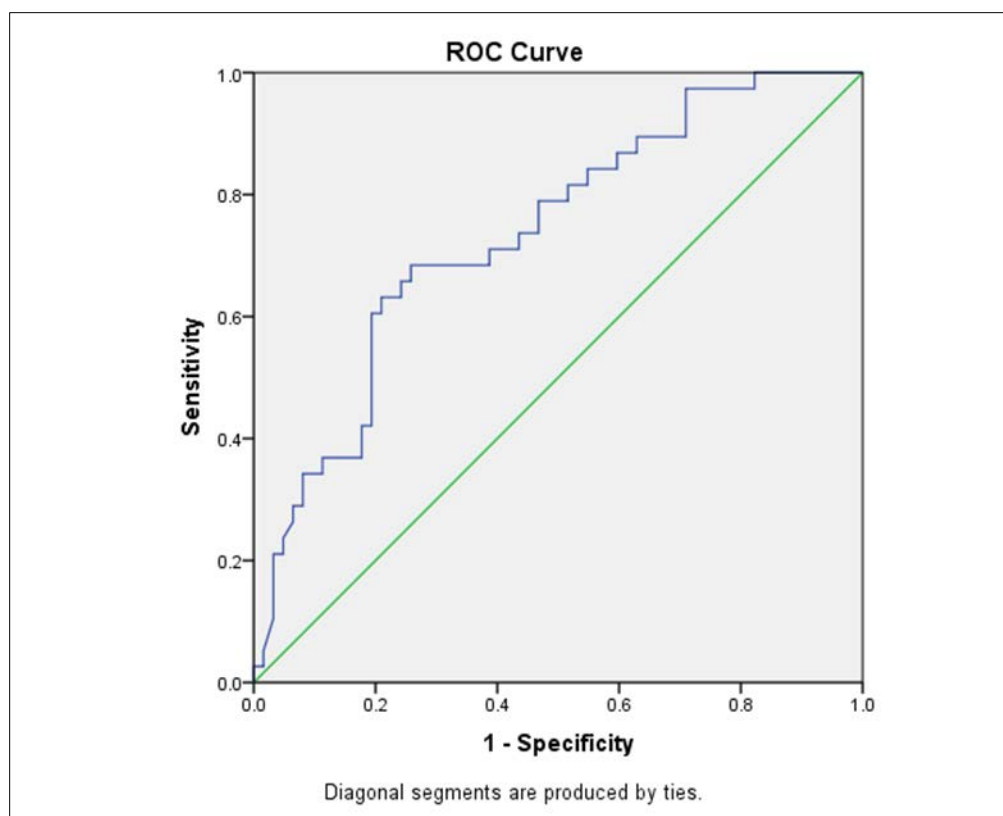
Figure 4. ROC curve for Multivariate Logistic Regression Model for clinical predictors in RERC on SCI population

Table 10. Area under the Curve for Multivariate Logistic Regression Model

Area	Std. Error	Sig	Asymptotic 95% Confidence Interval	
			Upper Bound	Lower Bound
0.735	0.051	0.000**	0.636	0.835

2.4 DISCUSSION

The results of this study investigated factors associated with the development of pressure ulcers (PUs) in acute care and inpatient rehabilitation in individuals with newly traumatic SCI (TSCI). Individuals with TSCI are at a risk for developing PUs and other secondary complications [17, 93]. This may be because of the clinical, epidemiological and functional changes following TSCI. Using the univariate and multivariate logistic regression model, severity of SCI (ASIA A) and pneumonia were identified to be associated with formation of first PU in this population.

The overall incidence rate of formation of at least one PU in the RERC on SCI population was 42%. Prior studies have found that approximately 47% of individuals with SCI develop at least one PU during the period of acute care hospitalization and rehabilitation [50]. Most of the PUs occur in the acute care as compared to the rehabilitation units [94, 95], these findings are comparable to this study. The incidence of pressure ulcers in the RERC on SCI population in the acute care setting and inpatient rehabilitation was 28% and 12% respectively. Thirty-nine percent of individuals in this study population developed at least one PU in acute care and inpatient rehabilitation, which is similar to 34% reported by the 2011 Model Spinal Cord Injury Care Systems [45].

A factor determined to predict first PU occurrence in this study was severity of injury measured by the ASIA scale score. In this study, individuals with ASIA A were more at risk to develop PUs than ASIA B, and C, similar to previous studies [96]. A prospective cohort study in individuals with SCI found completeness of injury a significant risk factor for the formation of PUs. The odds of forming a PU with AIS A were much higher than AIS C or D [97]. A complete motor and sensory lesion (ASIA A) severely limits mobility not allowing them perform the

activities of daily living and other activities that may require mobilizations. [15, 96, 98, 99]. Also, individuals with higher severity of injury (ASIA A) complete injury are usually on anesthesia and mechanical ventilation. This decreases their awareness to the pressure and shear forces on their bony prominences. Also, individuals with complete neurological injuries are more prone to urinary and or fecal incontinence that increases moisture and maceration around the skin [100]. This coupled with lack of sensory and motor supply increases their risk to develop pressure ulcers.

Pneumonia was another factor that predicted pressure ulcer outcome (from the results of the univariate analysis) in this study. Individuals in this study were diagnosed with pneumonia within eleven days (on average) following injury. The area under the receiver operator characteristic (ROC) determined that individuals with pneumonia have greater occurrence of PUs than individuals with no pneumonia. Hence, presence of pneumonia was significantly associated with the formation of PU in the RERC on SCI population, indicating a relationship between pulmonary disease and formation of PUs. Pneumonia was the only medical comorbidity that predicted occurrence of PU in the univariate logistic regression analysis, but was not significant in presence of other variables that were included in the multivariate logistic regression model. Increased severity of injury and complete loss of motor control predisposes them to pneumonia due to ventilatory incompliance. Hence, individuals with higher level and complete of SCI are dependent on mechanical ventilation. Lack of movement in individuals dependent on mechanical ventilators, may predispose them to form PUs. Correlation between pneumonia and the formation of PUs has been indicated in previous studies [11, 15, 19, 37]. The pathogenesis between the formation of PUs and presence of pneumonia is not known and has to be further explored.

46

Demographics such as age and gender were not significant predictors of first PU formation in this study. Although some studies have identified age [79] and gender [18] associated with PU formation, a recent study by Wilczweski and colleagues, determined demographics not to be a significant predictor for the occurrence of PUs [16]. In this study, all the individuals with SCI developed UTI within twenty-six days from injury, whereas individuals with PUs developed UTI within thirty-four days from the day of injury. Diagnosis with urinary tract infection (UTI) was not a significant predictor of PU development in this study. Studies have indicated urinary tract infections (UTIs), to be associated with the risk of development of PUs in acute care hospitals [21]. Most of the individuals (80%) in hospitals are predisposed to UTI's because if the use of catheters for bladder management and improper maintenance and insertion of catheters [101, 102]. In this study almost all the individuals (93% in acute care and 85% in inpatient rehabilitation) were on catheters for bladder management. This may be the reason why UTI was not a predictor of PU in the multivariate regression model. Diabetes is also known to be associated with the formation of PUs. [21, 103, 104]. Diabetes was not found to be significant predictor of PUs in the RERC on SCI population in the univariate analysis. This may have been due to the initial exclusion of people with diabetes in our study since we may have missed collecting data on individuals with diabetes who may have had the formation of pressure ulcers. The use of steroids was a not significant predictor of PU development in the univariate and multivariate analyses. There is not enough evidence regarding the use of steroids and formation of PUs. In a previous study the use of steroids was significant in the univariate analysis and not significant in the multivariate logistic regression model [16].

2.4.1 Limitations and Future Recommendations

There are potential limitations to this study. The sample size for this study was relatively small. Only one hundred and four individuals were recruited in this study. To have a good model fit and power for the study the general "rule of thumb" to have ten cases per predictor variable was considered [90]. Hence, only six variables were included in the final multivariate logistic regression model. Collaborative efforts are necessary to increase sample size and thus maximize power. A consensus must be established for the specific data collected to allow cross-comparison of findings.

Also, since the research design for this study was secondary analysis, we need to keep in mind the inherent disadvantages. Since the data in RERC on SCI was not collected keeping in mind the specific research question, other possible variables such as Braden scale score, depression score and pain scale at admission, could not be studied for association with the formation of PU. The timing of diagnosis of certain comorbidities such as cardiovascular heart disease, nutrition, sepsis, hypotension, and interventions such as dietary protein, and use of support surfaces were not specific. Hence these variables were not included in the final multivariate model. Only a limited number of factors were identified in this study for the occurrence of PU. Hence, it is essential to document additional medical factors from the electronic health records or medical records, if the research staff is not able to obtain information on risk factors or comorbidities at the time of data collection. It may be interesting to study additional risk associated with occurrence of PUs during hospitalization. Also, it may be interesting to explore the association between medical and rehabilitation interventions that these individuals were on and PU incidence.

2.5   CONCLUSION

Many risk factors have been associated with the formation of pressure ulcers. The individuals with SCI are at a higher risk to develop pressure ulcers during the time of hospitalization. This study confirmed that individuals with high injury severity are at increased risk to develop PUs. This study also suggests that medical co-morbidities such as pneumonia, is also associated with the formation of PUs. To further understand the association of presence of pneumonia and formation of PU, we investigated the association between the two conditions by noting the timing and sequence of formation of the two conditions (Chapter 5.0).

3.0 EARLY INFLAMMATORY BIOMARKERS ASSOCIATED WITH FUTURE PRESSURE ULCER DEVELOPMENT IN INDIVIDUALS WITH SPINAL CORD INJURY

3.1 INTRODUCTION

Spinal cord injury is a devastating neurologic disorder that has profound impact from physical, psychological and socioeconomic perspectives [44]. Increase in age and severity of injury are associated with rise in long-term complications after traumatic SCI [79]. The second leading cause of death in individuals with SCI is septicemia (88.6%); usually associated with urinary tract infections (UTI's), pneumonia, and presence of PUs [45]. Pressure ulcers (PUs) are the most frequent secondary complication in individuals with SCI from the time of acute hospitalization through community reintegration that affects their quality of life, length of stay during hospitalization and increases the mortality and morbidity in this population [1, 7, 12, 48, 49]. The 2011 annual statistical report for the Spinal Cord Injury Model Systems (SCIMS) state PUs to be the second most frequent complication and third leading cause of death after SCI [45]. The etiology of PUs is multifaceted. Many extrinsic and intrinsic factors such as moisture, shear, temperature, decreased blood flow and oxygenation to the tissues, decreased mobility and sensation after SCI, lack of nutrition are associated with the formation of PUs.

Activation of an inflammatory response by the immune system is inherent after traumatic SCI. After initial SCI mechanical damage to the axonal and neuronal cells, is followed by a complex secondary cascade of events [4, 23, 24]. The disruption of the blood spinal cord barrier (BSCB) after SCI increases the synthesis of the pro-inflammatory and anti-inflammatory cytokines and other inflammatory factors. This disrupted BSCB further increases the passage of these inflammatory markers into the circulation. The inflammatory response after SCI can be detrimental and predispose the individuals to secondary complications and tissue damage [25, 26]. On the other hand, inflammatory response after SCI also helps reduce injury by activating the process of wound healing, thus initiating repair [27]. Hence, inflammation after SCI is described as a "double-edged sword", and early inflammatory response may be detrimental whereas the late inflammatory response is likely beneficial [2]. In addition, a finely tuned balance between the pro-inflammatory and the anti-inflammatory response is essential for the beneficial role of the inflammatory response [28]. Previous studies have indicated that the activation of the cytokine and chemokine cascade may be related to the pathogenicity of the etiology of PUs [32, 105, 106].

The pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α increase immediately after injury until 5 hours post injury [29]. The inflammatory response can be explained by the early expression of the pro-inflammatory cytokines and chemokines from 24 hours until 4 days after injury [30]. The synthesis of pro-inflammatory cytokines such as TNF-α increase after subjecting mice to SCI in the lumbar region due to the disruption of the BSCB [24]. Increased serum concentrations of pro-inflammatory cytokines such as TNF-α, IL-2, and IL-1RA were found in individuals with SCI as compared to individuals without SCI [4]. The synthesis of anti-

inflammatory cytokines such as IL-10 suppresses the microglial activity and helps to decrease inflammation after SCI [27].

Previous studies measured the levels of the plasma mediators in individuals with chronic SCI having PUs. Out of the 70 individuals with long standing SCI, 19 of them had PUs. The inflammatory mediators in these individuals were compared to an able-bodied control group. The plasma concentrations of IL-6, IL_2, IL-2R and ICAM-1 were significantly elevated in individuals with SCI as compared to the control group. Individuals with slow healing PUs had highest increase in concentrations of these mediators when compared to able-bodied individuals or individuals with SCI and healing PUs [32]. The production of cytokines such as IL-1β, IL-6 and TNF-α was studied in elderly individuals with Stage III and Stage IV PUs. This study reported poor nutritional status and increase in the serum concentrations of IL-6 in these individuals when compared to control group who were not at risk to develop PUs. Thus the elevation of cytokines in serum could have aggravate the malnutrition in individuals with PUs [107].

Although inflammatory mediators in plasma and its association with disease, infection and trauma is well established, there are relatively fewer studies describing the contribution of these mediators in urine. Studies investigating the inflammatory mediators in various diseases, are gradually utilizing urine assays to analyze these mediators. Inflammatory mediators such as eosinophil's in urine was shown to be associated with asthma, [108]. Urine samples can be sampled non-invasively, and studies have shown association of urine inflammatory predictors in congestive heart failure[109], renal disease[110], lung disease[111]. The urine inflammatory mediators have not been explored in individuals with SCI.

The purpose of this study was to determine the pattern of inflammatory mediators in urine (and plasma) within four days following acute traumatic SCI, which would help predict a future pressure ulcer development in individuals during acute care and inpatient rehabilitation. We hypothesized that the concentrations of pro-inflammatory mediators such as TNF-α, IL-1β, IL-2, IL-6, and anti-inflammatory mediators such as IL-10 and IL-1RA measured within four days after traumatic SCI will indicate increased risk for formation of PUs. We also hypothesized that the inflammatory mediators in urine that predict occurrence of PUs will be same to those in plasma.

3.2   METHODS

3.2.1   Research Design

The research design for this study is a secondary analysis, since the existing database was used to examine the variables and to explore the relationship between presence of pneumonia and PU outcome [80].

3.2.2   Inclusion and Exclusion criteria

The inclusion and exclusion criteria of RERC on SCI are mentioned in Chapter 2, Section 2.2.2. For this study, individuals who had plasma and urine samples collected within four days after traumatic SCI were included. 54 individuals had plasma samples collected and assayed within

four days after TSCI and 53 individuals had urine samples collected and assayed within four days after TSCI.

3.2.3 Data Collection

The data collected for the RERC on SCI are mentioned in Chapter 2, Section 2.2.3.

3.2.4 Processing of Urine and Plasma Samples

Plasma and urine samples were collected in the RERC on SCI study thrice a week when the subjects are in acute care, and weekly in inpatient rehabilitation. The human inflammatory MILLIPLEX ™ MAP Human Cytokine/Chemokine Panel-Premixed 26 Plex (Millipore Corporation, Billerica, MA) and Luminex xMAP (Luminex, Austin, TX) were used to measure plasma and urine cytokine levels. The Luminex™ system was used in accordance to manufacturer's instructions. Standard curves were established based on various dilutions. NO_2^-/NO_3^- was measured using the nitrate reductase/Griess assay (Cayman Chemical Co., Ann Arbor, MI). The plasma and urine samples were centrifuged, and plasma and urine aliquots were stored in cryoprecipitate tubes at -80°C for subsequent analysis of cytokine levels. Multiplex analysis for plasma cytokines and chemokines levels was performed. All data were stored in de-identified fashion in an Oracle™ database. Twenty-three inflammatory mediators in plasma and urine were assayed in this study. The inflammatory mediators include Eotaxin, GM-CSF, IFN-γ, IFN-α, IL-

1RA, IL-1β, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-17, IP-10, MCP-1, MIP-1α, and MIP-1β, TNF-α, MIG and NO3-/NO2-.

3.2.5 Procedure

Individuals in acute care and inpatient rehabilitation from the RERC on SCI were included for this study. The earliest time point of the collection of plasma and urine samples, within the four days of SCI, was included to identify inflammatory mediators associated with future development of first pressure ulcer.

3.2.6 Normalization of data

The outliers (extreme data points) were identified (>3SDs) and were adjusted in the data for each inflammatory mediator in both plasma and urine. The data for each inflammatory mediator were ranked in descending order. The highest extreme values (>3SDs) were replaced with the highest value for that inflammatory mediator.

The ranges and scales for the concentrations of inflammatory mediators varied among the different substances making assessments of their effects difficult using the absolute values. For this reason, these adjusted responses for the plasma and urine concentrations of cytokines were transformed into percentages of the range for each cytokine. This normalization technique allowed comparison between multiple inflammatory mediators on a common scale.

3.2.7 Data Analysis

Two separate analyses were performed to identify the significant cytokines in plasma and urine. All data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistics using means, frequencies, standard deviations and standard error of mean was performed for individuals included in the study to identify plasma and urine inflammatory mediators.

Mann-Whitney U test

The demographics of the two groups of subjects (those who developed PUs and those who did not develop PU) were compared using a two-tailed Mann-Whitney U test (two-tailed); with continuous-level variables such as age and ISS[88].

Mann-Whitney U statistic is for the two groups are

$$U_{PU} = n_{PU}n_{NO\,PU} + \frac{n_{PU}\,(n_{PU}+1)}{2} - R_{PU}$$

- U= Mann-Whitney U statistic

- n_{PU} is sample size of group 1 (individuals with PU)

- $n_{NO\,PU}$ is sample size of group 1 (individuals with no PU)

- R_{PU} is sum of the ranks for age (or ISS) in PU group

The Mann-Whitney U statistic (U) is the smaller of the two values calculated for U_{PU} and $U_{NO\,PU}$.

Mann-Whitney U follows a z distribution.

$$z = \frac{U - \dfrac{n_{PU}n_{PU}}{2}}{\dfrac{n_{PU}n_{PU}(n_{PU}+n_{PU}+1)}{12}}$$

Our hypotheses are:

H₀ (Null Hypothesis): The mean ranks of age (and ISS) between the two groups (individuals with PUs and individuals with no PUs) are expected to be the same. (or) Calculated z is between -1.96 and 1.96

H₁ (Alternate Hypothesis): There is a difference between the ranks of age (and ISS) in the two groups (individuals with PUs and individuals with no PUs). (or) z < -1.96 or z > 1.96.

The significance level was set at α = 0.05.

Chi-Square Test

A nonparametric test was performed on the nominal (gender male/female) and ordinal (ASIA scale score, A, B, C, D and E) data. The Chi-square statistic (two-tailed) was performed to test differences between the level of injury (and gender) in the two groups (those who developed PUs and those who did not develop PU). The chi-square statistic is[80, 88]:

$$\chi^2 = \sum \frac{(f_0 - f_e)^2}{f_e}$$

- f_o is observed frequency of ASIA (or gender)
- f_e is expected frequency (or gender)

Our hypotheses are:

H₀ (Null Hypothesis): The severity of injury measured by ASIA scale score (or gender) and individuals who developed PUs are not related.

H₁ (Alternate Hypothesis): The severity of injury measured by ASIA scale score (or gender) and individuals who developed PUs are related.

The significance level was set at α =0.05.

57

3.2.7.1 Logistic Regression

Univariate Logistic Regression

A univariate logistic regression analysis was conducted to assess each inflammatory mediator's ability to predict the probability of the outcome (PU, yes/no). The model is[88]:

$$\text{logit } (Y) = \frac{1}{1 + e^{-(\beta_0 + \beta_1 X_1)}} = \beta_0 + \beta_1 X_1$$

- β_0 is a constant

- β_1 is the coefficient of the individual predictor variable.

- X_1 is the inflammatory mediator (predictor) in question

Our hypotheses are:

H₀ (Null Hypothesis): $\beta_1 = 0$

H₁ (Alternate Hypothesis): $\beta_1 \neq 0$

The significance level was set at $\alpha = 0.05$.

Multivariate Logistic Regression

The stepwise backward elimination logistic regression technique was used to identify inflammatory mediators to predict the outcome (PU yes/no). The model started with all 22 plasma inflammatory mediators. In this full model the likelihood-ratio test for each mediator was examined as if it was the last one to enter given that other mediators are in the model. The process, by which the variables were included for the elimination of the model, was the likelihood-ratio test. A predictor with lowest likelihood-ratio test statistic was deleted from the model one at a time at each step since it won't contribute to the outcome after other predictors are included and the equation is recomputed. The sequence is stopped when there are no more

predictors that are not significant on the statistic. The probability to remove a variable was set at 0.10. In this model $\alpha = 0.05$ was selected to be the significance at each step, assuming that any $\alpha > 0.05$ would contribute to noise rather than the predictive ability of the model. This means variables with $\alpha > 0.05$ were neither significant nor a confounding factor. The backward stepwise regression model was built similarly for the urine inflammatory mediators. Two-tailed significance was set for $\alpha = 0.05$.

A multivariate logistic regression was then conducted with the significant plasma inflammatory mediators obtained from the stepwise backward elimination logistic regression technique. The outcome for the model was occurrence first pressure ulcer (yes/no). The model is[88]:

$$\textbf{logit (Y)} = \frac{\textbf{1}}{\textbf{1} + e^{-(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots \beta_k X_k)}} = \boldsymbol{\beta_0} + \boldsymbol{\beta_1 X_1} + \boldsymbol{\beta_2 X_2} + \cdots \ldots + \boldsymbol{\beta_k X_k}$$

- $\beta_1, \beta_{2\ldots} \beta_k$ are the regression coefficients for the independent variables of the regression equation.

- $X_1, X_2 \ldots X_k$ are the independent variables (risk factors in question).

Our hypotheses are:

H_0 (Null Hypothesis): $\beta_1 = \beta_2 = \ldots = \beta_k$ (or) $\beta_i = 0$

H_1 (Alternate Hypothesis): $\beta_1 \neq 0$ (or) $\beta_i \neq 0$

The significance level was set at $\alpha = 0.05$ (Two-tailed).

The logistic regression coefficients, Wald statistic and the interpretation of these are described in Section 2.2.5.2 and 2.2.5.3.

3.2.7.2 Hosmer-Lemeshow goodness of fit

The Hosmer-Lemeshow goodness of fit was computed. The Hosmer-Lemeshow test statistic is explained in is explained in Section 2.2.5.4.

3.2.7.3 Area under the Receiver Operating Characteristic (ROC) curve

The area under the ROC curve was computed and plotted. The area under the ROC curve and classification is explained in Section 2.2.5.5.The inflammatory mediators in urine were analyzed similarly.

3.3   RESULTS

3.3.1   Plasma Analysis

3.3.1.1 Time course of plasma inflammatory mediators (n = 54)
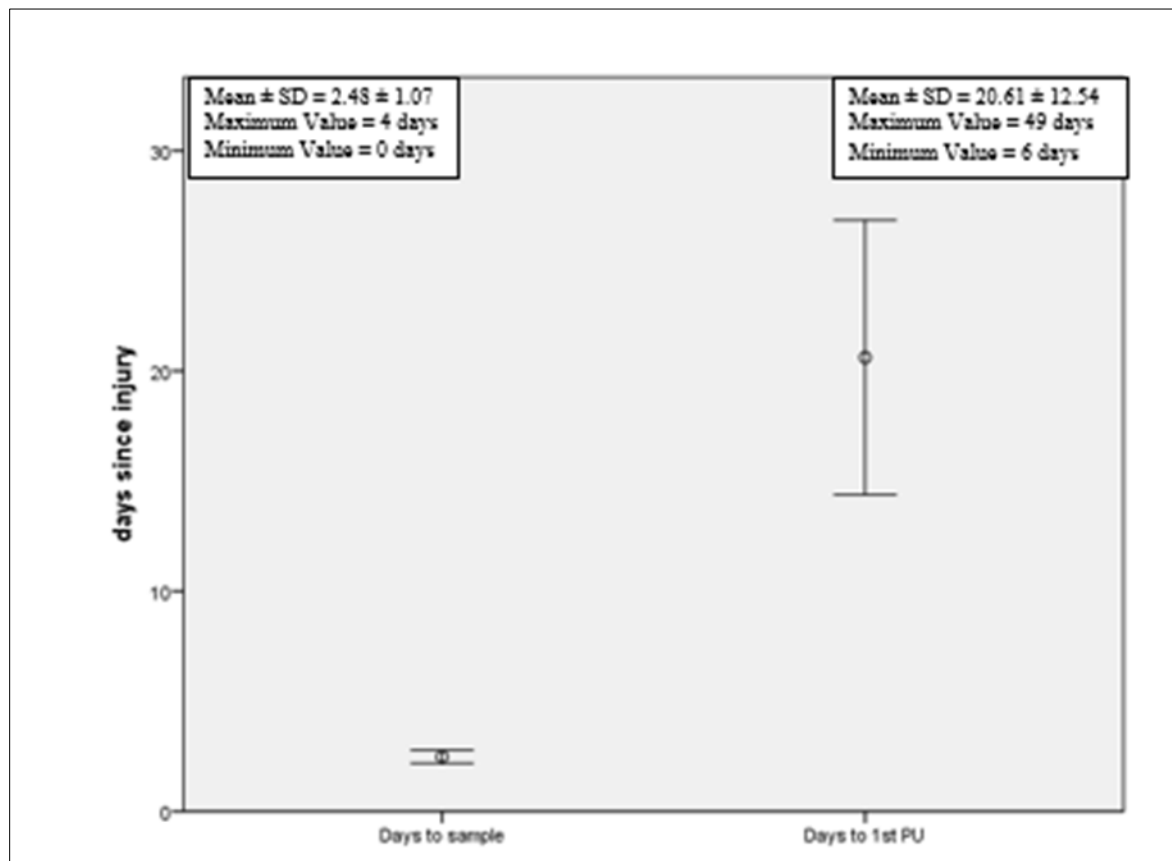
Figure 5. Time from SCI to measure the plasma mediator and first PU.

3.3.1.2 Demographics

The demographics and characteristics of individuals with PU and no PU in acute care through inpatient rehabilitation are listed in Table 11.

3.3.1.3 Age and Gender

The individuals with no formation of PU were older (45 years) than the individuals with PU (37 years). The Mann-Whitney U test produced no significant difference in ages between the two groups. A majority of individuals in both the groups were males. The chi-square test produced no significant differences in gender distribution between the two groups.

3.3.1.4 Injury Severity

The severity of injury for the two groups is listed in Table 11. The chi-square test showed significant differences in the severity of injury ASIA and ISS between the two groups, p=0.002 and p=0.002, respectively. Individuals with PU were mostly ASIA A's (72%).

Table 11. Demographics comparison of PU and no PU groups

Demographics	All subjects n=54	Subjects with PUs n= 18	Subjects with no PU n=36	P value
Age (years) Mean ± SEM	42.17±2.39	37±4.19	44.75±2.88	0.088
Gender	M=42(77.8%) F=12(22.2%)	M=12(66.7%) F=6(33.3%)	M=30(83.3%) F=6(16.7%)	0.165
ASIA	A=19(35.2%) B=9(16.7%) C=16(29.6%) D=8(14.8%) U=2(3.7%)	A=13(72.2%) B=1(5.6%) C=2(11.1%) D=1(5.6%) U=1(5.6%)	A=6(16.7%) B=8(22.2%) C=14(38.9%) D=7(19.4%) U=1(2.8%)	0.002**
Injury Severity Score (ISS)	28.1±2.35	24.09±2.54	36.63±4.4	0.002*

Table 12. Means and SEM of Plasma and Urine inflammatory mediators after adjusting for outliers

Inflammatory mediators (pg/ml)	Subjects with PU Mean ± SEM Plasma	Subjects with no PU Mean ± SEM Plasma	Subjects with PU Mean ± SEM Urine	Subjects with no PU Mean ± SEM Urine
IL-1β	5.03±2	6.8±1.9	4.04±3	1.09±0.5
IL-1RA	60.51±40.1	78.89±28.4	804.75±236.4	778.6±312.7
IL-2	7.63±2.7	8.79±1.3	1.39±1.1	1.75±0.5
IL-6	107.98±35.6	79.62±20.1	30.43±14	11.74±4.4
IL-10	52.28±16.5	52.75±19.3	31.72±13.7	31.41±9.9
TNF- α	10.34±4.5	7.34±2	1.49±0.6	5.69±2.9
Eotaxin	33.82±4.8	44.5±5.4	21.56±11.7	4.3±1.4
GM-CSF	6.85±4.6	5.47±2.5	26.71±13.9	16.29±4.8
IFN-α	35.3±20.4	41.49±14.3	36.31±11.5	30.54±7.6
IFN-γ	30.57±16.6	25.92±8.8	4.72±2.6	8.06±2.7
IL-4	11±9.2	14.1±6.1	35.68±14.2	33.84±10.9
IL-5	6.93±4	6.99±2.7	2.87±2.1	5.61±1.6
IL-7	8.06±3.4	10.04±3.6	0.39±0.2	2.94±1.4
IL-8	33.82±9.8	23.63±5	108.79±32.1	3.93±17.9
IL-13	5.97±3	9.01±2.9	0.74±0.5	7.78±2.3
IL-15	15.12±3.7	11.9±2	30.24±14.1	36.94±10.4
IL-17	5.8±3.2	7.67±2.5	5.65±5.4	13.21±4.7
IP-10	503.86±146.9	426.47±80.1	228.79±88.8	112.42±32.7
MCP-1	515.32±155.6	411.24±76.5	1406.74±478.8	1075.08±289.5
MIP-1α	60.67±22.6	34.83±7.5	51.15±21.1	38.26±12.3
MIP-1β	46.15±14.7	47.99±8.4	22.47±5.9	20.16±4.5
MIG	980.33±337.9	875.86±203	411.07±216.2	90.61±48.7
NO2-/NO3-	22.54±4.9	19.85±2.4	281.96±43.1	317.62±29.1

3.3.1.5 Univariate Logistic Regression

The means and SEM of the plasma and urine inflammatory mediators for individuals with and without PUs, after adjusting for the outliers is listed in Table 12. Table 13 shows the univariate logistic regression analysis for the 23 inflammatory mediators in plasma. The baseline concentrations of the individual plasma inflammatory mediators could not significantly predict the formation of first PU.

Table 13. Univariate Logistic Regression for Plasma and Urine Biomarkers within 4 days after SCI

Inflammatory mediators	p value Plasma	p value Urine
IL-1β	0.537	0.271
IL-1RA	0.556	0.958
IL-2	0.666	0.756
IL-6	0.289	0.132
IL-10	0.987	0.985
TNF- α	0.977	0.516
Eotaxin	0.221	0.237
GM-CSF	0.364	0.285
IFN-α	0.704	0.916
IFN-γ	0.783	0.469
IL-4	0.481	0.922
IL-5	0.990	0.357
IL-7	0.723	0.421
IL-8	0.222	0.774
IL-13	0.514	0.160
IL-15	0.291	0.713
IL-17	0.377	0.361
IP-10	0.612	0.265
MCP-1	0.296	0.643
MIP-1α	0.203	0.576
MIP-1β	0.791	0.766
MIG	0.335	0.211
NO2-/NO3-	0.585	0.977

3.3.1.6 Multivariate Stepwise Logistic Regression

A multivariate logistic regression was performed with pressure ulcer as outcome (PU present and no PU) and eleven predictors obtained from the stepwise backward logistic regression. The PU outcome was dichotomized into 2 levels. Table 14 shows the results of the multivariate logistic regression. All assumptions were met. There was a significant prediction of PU outcome by inflammatory mediators in plasma included in the final model, $\chi 2$ (11) = 24.237, p=0.012.

There was significant prediction of PU outcome by of plasma inflammatory mediators IL-1RA (p=0.02), GM-CSF (p=0.03), IFN-γ (p=0.04), IL-5 (p=0.02), IL-17 (p=0.03), MIP-1α (p=0.01), MIP-1β (p=0.03) and MIG (p=0.04). The odds to develop PU increases by 1.3 and 1.2 times increase in the plasma concentrations of MIP-1α and GMCSF, respectively. There was no significant prediction of pressure ulcer occurrence by IL-6 (p=0.08), IL-10 (p=0.2), and IL-8 (p=0.09).

3.3.1.7 Hosmer-Lemeshow test statistic

There was no significant difference between the observed and predicted group membership, Hosmer-Lemeshow $\chi 2$ (8) =6.987, p= 0.538. This means that there is a good overall fit of the multivariate logistic regression model and that there was no misspecification of the predictors.

3.3.1.8 Area under the Receiver Operating Characteristic (ROC) curve

The area under the curve for the plasma inflammatory mediators included in the multivariate logistic regression model is 0.864 (Figure 6) with 95% confidence interval (0.77, 0.959). Also, the area under the curve is significantly different from 0.5 since p value is < 0.001 (Table 15).

This means that the plasma factors in the multivariate logistic regression model accurately classify individuals with formation of PU in the RERC on SCI population.

3.3.1.9 Adjusted Multivariate Logistic Regression

A multivariate logistic regression was performed with pressure ulcer as outcome (PU present and no PU) and eleven predictors obtained from the stepwise backward logistic regression. This model was adjusted for level of injury measured by ASIA scale score. The PU outcome was dichotomized into 2 levels. Table 16 shows the results of the multivariate logistic regression. All assumptions were met. There was a significant prediction of PU outcome by inflammatory mediators in plasma included in the final model, $\chi2$ (14) = 9.77, p = 0.02. There was no significant prediction of pressure ulcer occurrence by the plasma mediators and the level of injury.

Table 14. Multivariate Logistic Regression of the plasma predictors within 4 days of SCI

Independent Variables	B	SE	Wald	Sig (p)	Exp(B)
IL-1RA	-0.091	0.040	5.218	0.022*	0.913
IL-6	0.086	0.050	3.042	0.081	1.09
IL-10	-0.098	0.068	2.095	0.148	0.907
GM-CSF	0.221	0.101	4.801	0.028*	1.247
IFN-γ	-0.414	0.198	4.381	0.036*	0.661
IL-5	0.338	0.147	5.334	0.021*	1.403
IL-8	0.063	0.037	2.921	0.087	1.065
IL-17	-0.157	0.071	4.894	0.027*	0.855
MIP-1α	0.266	0.107	6.138	0.013*	1.305
MIP-1β	-0.167	0.075	4.976	0.026*	0.846
MIG	-0.061	0.029	4.451	0.035*	0.941
Constant	0.145	0.704	0.42	0.837	1.156

Model χ^2 = 24.237, p < 0.05
Pseudo R^2 = 0.502
n = 54

Note: The dependent variable in this analysis is formation of pressure ulcer in acute care or inpatient rehabilitation, so that 0= did not have pressure ulcer and 1= had a formation of pressure ulcer
**p<0.01, *p< 0.05
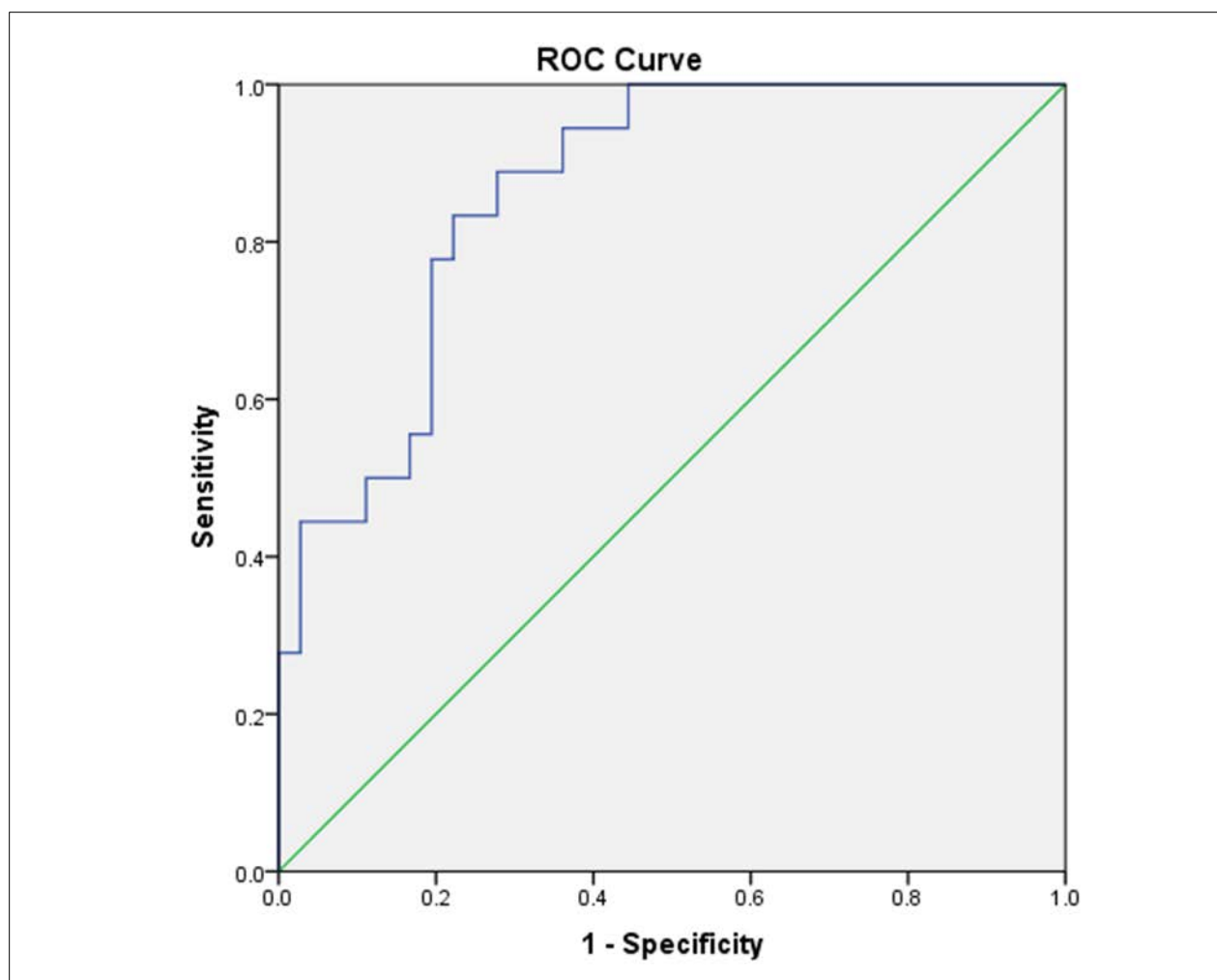
Figure 6. ROC Curve for Multivariate Logistic Regression Model for plasma biomarkers in RERC on SCI population

Table 15. Area under the Curve for Multivariate Logistic Regression Model for plasma predictors

Area	Std. Error	Sig	Asymptotic 95% Confidence Interval	
			Upper Bound	Lower Bound
0.864	0.048	0.000**	0.770	0.959

Test Variable (s): Predicted probability   **p<0.01

Table 16. Multivariate Logistic Regression - Plasma predictors within 4 days of SCI adjusted with level of injury

Independent Variables	B	SE	Wald	Sig (p)	Exp(B)
ASIA			6.46	0.09	
ASIA A – ASIA B	2.2	1.37	2.61	0.1	9.08
ASIA A– ASIA C	-18.86	12.3^3	0.00	0.99	0.00
ASIA A– ASIA D	-0.51	1.51	0.11	0.73	0.6
IL-1RA	-0.07	0.05	1.96	0.16	0.93
IL-6	0.02	0.07	0.11	0.73	1.03
IL-10	-0.05	0.07	0.48	0.48	0.95
GM-CSF	0.15	0.1	2.03	0.15	1.16
IFN- γ	-0.28	0.21	1.86	0.17	0.75
IL-5	0.22	0.18	1.43	0.23	1.24
IL-8	0.03	0.04	0.45	0.5	1.03
IL-17	-0.09	0.1	0.79	0.37	0.91
MIP-1α	0.18	0.12	2.35	0.12	1.2
MIP-1β	-0.11	0.06	2.86	0.08	0.9
MIG	-0.02	0.03	0.39	0.53	0.98
Constant	-0.67	1.33	0.25	0.62	0.51

Model χ^2 = 9.77, p < 0.05
Pseudo R^2 = 0.66
n   = 54

Note: The dependent variable in this analysis is formation of pressure ulcer in acute care or inpatient rehabilitation, so that 0= did not have pressure ulcer and 1= had a formation of pressure ulcer

3.3.1.10   Correlational Analysis

In order to see if injury severity or any other demographic variables were related to the concentrations of the inflammatory mediators in plasma, a correlational analysis was performed. A correlational analysis was performed between the plasma mediators and demographics such as age (Spearman's rank correlation), gender (Point-biserial correlation) and injury severity measured by ASIA scale (spearman's rank correlation). Significant correlations are listed in Table 17. The Pearson's correlational analysis between the plasma inflammatory mediators that were included in the multivariate model is listed Table 18.

Table 17. Correlations – Plasma Mediators and Demographics

Demographics	Inflammatory mediators	Sample Size	(Correlation Coefficient)	Sig. (2-tailed)
Severity of Injury (ASIA)	IL-6	52	-.361**	0.009
	IL-10	52	-.380**	0.005
	IL-8	52	-.304*	0.029
	MCP-1	52	-.412**	0.002
Gender	IL-1β	54	.270*	0.048
Age	IL-6	54	-.290*	0.034

*p<0.05, **p<0.01

Table 18. Correlations between plasma inflammatory mediators

Mediators		IL-1RA	IL-6	IL-10	GM-CSF	IFN-γ	IL-5	IL-8	IL-17	MIP-1α	MIP-1β	MIG
IL-1RA		1	0.29*	0.07	0.36**	.38**	0.53**	0.24	0.32*	-0.05	0.08	-0.13
IL-6		0.29*	1	0.27*	0	0.20	0.18	0.56**	0.34*	0.28*	0.57**	0.13
IL-10		0.07	0.27*	1	0.02	0.03	-0.03	0.26*	0.02	0.15	-0.03	-0.08
GM-CSF	Pearson Correlation	0.36**	0	0.02	1	0.76**	0.61**	-0.04	0.64**	0.5**	0.39**	-0.18
IFN-γ		0.38**	0.20	0.03	.76**	1	0.84**	0.01	0.82**	0.67**	0.51**	-0.24
IL-5		0.53**	0.18	-0.03	.61**	0.84**	1	0.07	0.79**	0.29*	0.36**	-0.21
IL-8		0.24	0.56**	0.26*	-0.04	0.01	0.07	1	-0.00	0.07	0.22	0.41**
IL-17		0.32*	0.34*	0.02	0.64**	0.82**	0.79**	-0.01	1	0.52**	0.58**	-0.22
MIP-1α		-0.05	0.28*	0.15	0.50**	0.6**	0.29*	0.07	0.52**	1	0.63**	0.01
MIP-1β		0.08	0.57**	-0.03	0.39**	0.51**	0.36**	0.22	0.58**	0.63**	1	-0.00
MIG		-0.13	0.13	-0.08	-0.18	-0.24	-0.2	0.40**	-0.22	0.01	-0.0	1

*p<0.05, **p<0.01

3.3.2 Urine Analysis

3.3.2.1 Time course of the data (n = 53)

Mean ± SD = 2.58 ± 1.06
Maximum Value = 4 days
Minimum Value = 0 days

Mean ± SD = 19.88± 12.78
Maximum Value = 49 days
Minimum Value = 6 days

days since TSCI

Days to sample

Days to 1st PU
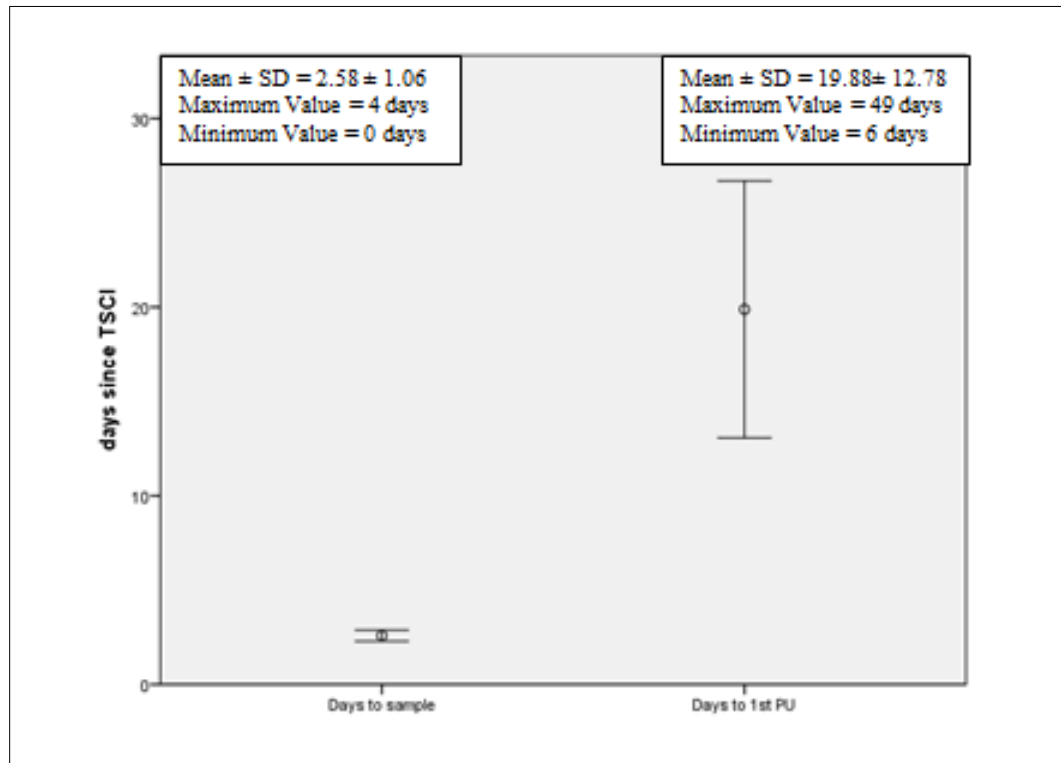
Figure 7. Time from SCI to measure the urine mediator and first PU.

3.3.2.2 Demographics

The demographics and characteristics of individuals with PU and no PU in acute care through inpatient rehabilitation are listed in Table 19.

3.3.2.3 Age and Gender

The individuals with no formation of PU were older (45 years) than the individuals with PU (38 years). The Mann-Whitney U test produced no significant difference in ages between the two groups. The Chi-square test produced no significant differences in gender distribution between the two groups.

3.3.2.4 Injury Severity

The severity of injury for the individuals with PUs and individuals with no PU is listed in Table 19. There were significant differences in the severity of injury ASIA and ISS between the two groups, $p < 0.001$ and $p < 0.01$ respectively. Individuals with PUs were mostly ASIA A's (81%).

Table 19. Comparison of Demographics in individuals with and without PUs

Demographics	All subjects N=53	Subjects with PUs n=16	Subjects with no PU n=37	p value
Age (years) Mean ± SEM	43.15±2.41	38.31±4.41	45.24±2.85	0.157
Gender	M=43(81.1%) F=10(18.9%)	M=12(75%) F=4(25%)	M=31(16.2%) F=6(83.8%)	0.453
ASIA	A=19(35.8%) B=8(15.1%) C=17(32.1%) D=8(15.1%) U=1(1.9%)	A=13(81.2%) B=0(0%) C=2(12.5%) D=1(6.2%) U=0(0%)	A=6(16.2%) B=8(21.6%) C=15(40.5%) D=7(18.9%) U=1(2.7%)	<0.001**
Injury Severity Score (ISS)	26.68±2.15	33.27±3.85	23.86±2.47	0.004**

3.3.2.5 Univariate Logistic Regression

Table 13 lists the results of the univariate logistic regression analysis for the 23 inflammatory mediators in urine. No individual inflammatory urine mediators were found significant.

3.3.2.6 Multivariate Logistic Regression for Urine Biomarkers

Because of differences in scaling of the urine inflammatory mediators, they were standardized to their percentage score. A multivariate logistic regression was performed with pressure ulcer as outcome (PU present and no PU) and twelve predictors obtained from the stepwise backward logistic regression: The PU outcome was dichotomized into 2 levels. Table 20 shows the results of the multivariate logistic regression. All assumptions were met. There was a significant prediction of PU outcome by all inflammatory mediators in urine included in the final model, χ^2 (12) = 34.650, p=0.001. There was significant prediction for the incidence of pressure ulcer by

urine inflammatory mediators IFN-γ (p=0.01), IL-5(p=0.02), IL-6 (p=0.04), IL-8(p=0.02), IL-13(p=0.01), IL-17(p=0.02), MCP-1(p=0.04), TNF-α (p=0.02), and MIG (p=0.00). The odds to develop PU increases by 6.8 times with increased urine concentrations of IL-5, by 1.6 times with increased urine concentrations of IFN-γ and by 1.2 times with increase in the urine concentrations of MIG, immediately following SCI. There was no significant prediction of pressure ulcer occurrence by GMCSF (p=0.1), IP-10 (p=0.08), and NO_2^-/NO_3^- (p=0.06)

3.3.2.7   Hosmer-Lemeshow goodness of fit

The Hosmer-Lemeshow in this study was χ2 (8) =10.2, p=0.251. This means that there was a good overall fit of the multivariate logistic regression model with no misspecification of the predictors.

3.3.2.8   Area under the Receiver Operating Characteristic (ROC) curve

The area under the curve for the urine inflammatory mediators included in the multivariate logistic regression model is 0.932 (Figure 8) with 95% confidence interval (0.852, 1.000). Also, the area under the curve is significantly different from 0.5 since p value is < 0.001 (Table 21).

3.3.2.9   Adjusted Multivariate Logistic Regression

A multivariate logistic regression was performed with pressure ulcer as outcome (PU present and no PU) and twelve predictors obtained from the stepwise backward logistic regression. This model was adjusted for level of injury measured by ASIA scale score. The PU outcome was dichotomized into 2 levels. Table 22 shows the results of the multivariate logistic regression. All assumptions were met. There was a significant prediction of PU outcome by inflammatory

mediators in plasma included in the final model, $\chi2$ (15) 15.9, p <0.001. There was no significant

prediction of pressure ulcer occurrence by the urine mediators and the level of injury.

Table 20. Multivariate Logistic Regression of the urine factors with 4 days of SCI

| Independent Variables	B	SE	Wald	Sig (p)	Exp(B)
GM-CSF	-0.182	0.118	2.366	0.124	0.834
IFN-γ	0.48	0.178	7.247	0.007**	1.616
IL-5	1.92	0.827	5.391	0.02*	6.819
IL-6	0.139	0.068	4.218	0.04*	1.149
IL-8	-0.127	0.056	5.118	0.024*	0.880
IL-13	-0.863	0.347	6.175	0.013*	0.422
IL-17	-1.946	0.802	5.891	0.015*	0.143
IP-10	0.193	0.108	3.168	0.075*	1.213
MCP-1	-0.126	0.060	4.371	0.037*	0.882
TNF-α	-1.797	0.751	5.719	0.017*	0.166
MIG	0.25	0.087	8.150	0.004**	1.283
NO_2^-/NO_3^-	-0.069	0.036	3.727	0.054	0.933
Constant	0.195	1.443	0.018	0.892	1.216

Model χ^2 = 34.65, p < 0.05*
Pseudo R^2 = 0.68
n = 53

Note: The dependent variable in this analysis is formation of pressure ulcer in acute care or inpatient rehabilitation, so that 0= did not have pressure ulcer and 1= had a formation of pressure ulcer
*p< 0.05, **p<0.01
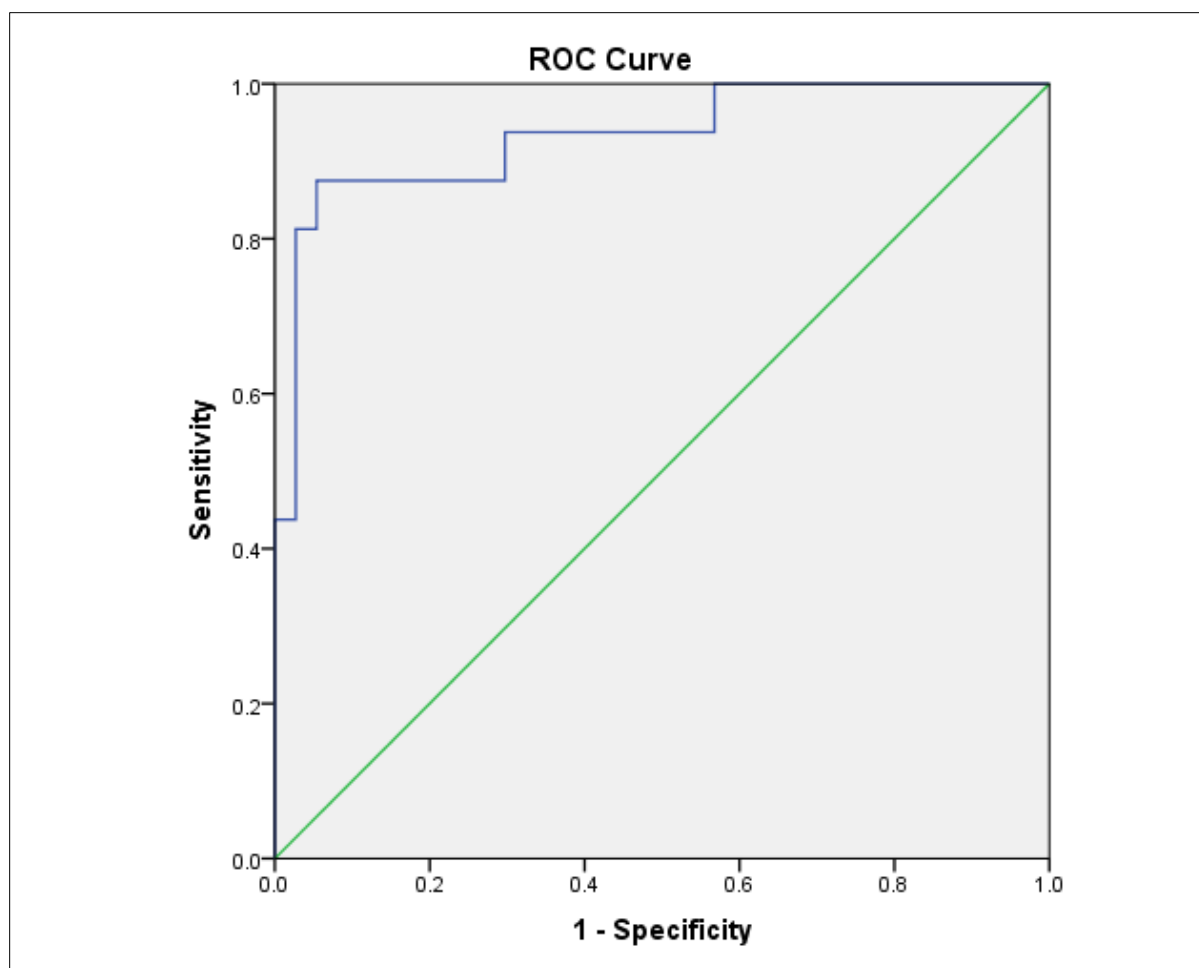
Figure 8. ROC Curve for Multivariate Logistic Regression Model for urine biomarkers in RERC on SCI population

Table 21. Area under the Curve for Multivariate Logistic Regression Model for urine predictors

Area	*Std. Error*	*Sig*	*Asymptotic 95% Confidence Interval*	
			Upper Bound	**Lower Bound**
0.932	0.041	0.000**	0.852	1.000

**p<0.01

Table 22. Multivariate Logistic Regression - Plasma predictors within 4 days of SCI adjusted with level of injury

Independent Variables	B	SE	Wald	Sig (p)	Exp(B)
ASIA			3.85	0.27	
ASIA A – ASIA B	19.07	15.84	1.43	0.23	17.9×10^7
ASIA A – ASIA C	-42.1	4.7×10^3	0.00	0.99	0.00
ASIA A – ASIA D	14.18	14.17	1.00	0.31	14.5×10^5
GM-CSF	0.02	0.49	0.00	0.96	1.02
IL-13	-1.24	0.86	2.09	0.14	0.28
IL-6	-0.06	0.13	0.2	0.64	0.93
IFN-γ	1.19	0.79	2.29	0.13	3.3
IL-5	1.42	0.18	1.43	0.23	4.14
IL-8	-0.07	0.13	0.29	0.58	0.93
IL-17	-2.13	1.62	1.74	0.18	0.11
IP-10	-0.62	0.37	2.71	0.1	1.86
MCP-1	0.15	0.2	0.53	0.46	1.16
TNF-α	-1.45	1.21	1.44	0.23	0.23
MIG	0.12	0.1	1.3	0.25	1.13
NO_2^-/ NO_3^-	-0.28	0.19	2.17	0.14	0.75
Constant	-12.43	13.27	0.87	0.34	0.00

Model χ^2 = 15.9, p < 0.01**
Pseudo R^2 = 0.87
n = 53

Note: The dependent variable in this analysis is formation of pressure ulcer in acute care or inpatient rehabilitation, so that 0= did not have pressure ulcer and 1= had a formation of pressure ulcer
*p< 0.05, **p<0.01

3.3.2.10   Correlational Analysis

A correlational analysis was performed between the urine mediators and demographics such as age (spearman's rank correlation), gender and (Point-biserial correlation) and injury severity measured by ASIA scale (spearman's rank correlation). Significant correlations are listed in. Table 23. The Pearson's correlational analysis between the plasma inflammatory mediators that were included in the multivariate model is listed in Table 24.

Table 23. Correlations- Urine Mediators and Demographics

	Inflammatory mediators	*Sample Size*	*Correlation Coefficient*	*Sig. (2-tailed)*
Severity of Injury (ASIA)	GM-CSF	52	-.278*	0.046
	IL-1Ra	52	-.355**	0.01
	IL-1b	52	-.391**	0.004
	MIP-1b	52	-.301*	0.03

*p<0.05, **p<0.01

Table 24. Correlations between urine inflammatory mediators

Mediators		GM-CSF	IFN-γ	IL-5	IL-6	IL-8	IL-13	IL-17	IP-10	MCP-1	TNF-α	MIG	NO_2^-/NO_3^-
GM-CSF		1	0.19	0.47**	0.59**	.44**	0.22	0.39**	0.5**	-0.04	0.17	-0.07	0.02
IFN-γ		0.19	1	.81**	0.08	0.07	0.49**	0.88**	-0.14	-0.14	0.32*	-0.08	-0.18
IL-5		0.47**	0.81**	1	0.14	0.21	0.62**	0.90**	-0.05	-0.11	0.24	-0.11	-0.23
IL-6		0.59**	0.08	0.14	1	0.56**	0.07	0.17	0.37**	0.10	-0.02	0.09	0.09
IL-8	Pearson Correlation	0.44**	0.07	0.21	0.56**	1	0.22	0.13	0.52**	0.29*	0.05	0.41**	-0.15
IL-13		0.22	0.49**	0.62**	0.07	0.22	1	0.65**	-0.14	-0.02	-0.06	-0.09	-0.22
IL-17		0.39**	0.88**	0.9**	0.17	0.13	0.65**	1	-0.16	-0.15	0.24	-0.12	-0.23
IP-10		0.50**	-0.14	-0.05	.37**	0.52**	-0.14	-0.16	1	0.27*	0.25	0.29*	0.07
MCP-1		-0.04	-0.14	-0.11	0.10	0.29*	-0.02	-0.15	0.27*	1	-0.09	0.66**	0.06
TNF-α		0.17	0.32*	0.24	-0.02	0.05	-0.06	0.24	0.25	-0.09	1	-0.00	-0.03
MIG		-0.07	-0.08	-0.11	0.09	0.41**	-0.09	-0.12	0.02*	0.66**	-0.00	1	0.02
NO_2^-/NO_3^-		0.02	-0.18	-0.23	0.09	-0.15	-0.22	-0.23	0.07	0.06	-0.03	0.02	1

*p<0.05, **p<0.01

3.4   DISCUSSION

This is the first study to investigate the inflammatory mediators in both plasma and urine biofluids immediately after SCI associated with the formation of the first PU. This study identified the plasma and urine inflammatory predictors by using the stepwise backward elimination technique [112]. The goal of this study was to explore relationships in the plasma (and urine concentrations) of inflammatory mediators associated with the formation of first PU following TSCI. The inflammatory mediators associated with formation of first PU in both plasma and urine biofluids were IFN-γ, IL-5, IL-17 and MIG. Decreased plasma and urine concentrations of IL-17 and increased plasma and urine concentrations of IL-5, increased urine concentrations and decreased plasma concentrations of IFN-γ and MIG were associated with the formation of first PU immediately after traumatic spinal cord injury. Increased plasma concentrations of GM-CSF and MIP-1α, and decreased plasma concentrations of IL-1RA and MIP-1β, but no changes in the urine concentrations; and increased urine concentrations of IL-6 and IP-10; and decreased urine concentrations of IL-8, IL-13, MCP-1 and TNF-α, but no changes in plasma concentrations were shown to be associated with formation of first PU in this study. The concentrations of some inflammatory mediators in urine, immediately after SCI that predict formation of the first PU, were not similar as compared to the plasma concentrations. Previous studies comparing the inflammatory cytokines in both plasma and urine after exercise [113] and renal disease [110], also reported different plasma and urine mediators associated with exercise and renal disease.

The inflammatory mediators in plasma and urine, which correlated with severity of injury, were not similar to the mediators that were associated with the formation of first PU. Hence, the inflammatory mediators that were significant were specific to the formation of PUs.

3.4.1   Interferon-gamma (IFN-γ)

We found increased plasma concentrations and decreased urine concentrations of IFN-γ following initial injury associated with later development of first pressure ulcer in individuals with traumatic SCI. IFN-γ is a cytokine that belongs to type II interferon family [114]. It is activated by natural killer cells and T lymphocytes and is known to stimulate macrophages and immune cells. The macrophages at the site of wound tissue in turn stimulate the synthesis of chemical mediators such as the IFN-γ that participates in the fibroblast proliferation and migration. [115, 116]. It has both anti-tumor and anti-proliferative activities [117]. It is a chemoattractant and increases during infections [118] and bronchial asthma [119]. Therapeutically it is used as a treatment protocol for idiopathic pulmonary fibrosis [120] and systemic sclerosis [121]. IFN-γ plays an essential role in innate immunity by participating in the first line of defense against infection and also contributes towards the activation of adaptive immunity [122]. IFN-γ thus regulates the immune system and participates in the process of wound healing [116]. It is known to increase during the late phase of inflammation [123]. Although IFN-γ is known to have an important role in initial stages of infection and participates in the process of wound healing it can also predispose the body to a pathological state [122]. This can explain the imbalance in IFN-γ in urine and plasma that is associated with development of PUs in this population. It is still unclear as to why there were differences in concentrations of

IFN-γ in the urine and plasma bio fluids associated with the formation of first PU in this population.

3.4.2 Interleukin-5 (IL-5)

We found increased plasma and urine concentrations of IL-5 following initial injury associated with later development of first pressure ulcer in individuals with traumatic SCI. IL-5 is a cytokine that belongs to the hematopoietic family produced by mast cells [124]. It stimulates macrophages and immune cells [115]. Imbalance in the concentrations of IL-5 is known to be associated with allergic conditions such as asthma [125], eosinophilia due to increased concentrations of eosinophils in blood, bone marrow and spleen [126, 127]. Eosinophils in turn plays a role in tissue damage [126]. Administration with anti-IL-5 decreased the development of eosinophilia [126], infections [128] and asthma [129]. Increased synthesis of IL-5 is associated with delay in the process of wound healing since it enhances the inflammatory process and inhibits the process of re-epithelialization [130]. The increase in the plasma and urine concentrations of IL-5 increases the overall pro-inflammatory response that may be associated with the development of first PU in this population.

3.4.3 Interleukin-17 (IL-17)

We found decreased plasma and urine concentrations of IL-17 following initial injury associated with later development of first pressure ulcer in individuals with traumatic SCI. IL-17 is a pro-inflammatory cytokine [131]. During the initial stages of immune response, IL-17 signals the

neutrophils to accumulate at the site of inflammation [123]. IL-17 stimulates the production of other cytokines and chemokines such as GM-CSF, IL-8, IL-1RA, TNF-α, IL-6 and IL-10; and thus up regulates the immune system [123, 132]. The synthesis of IL-17 increases in a variety of inflammatory conditions such as psoriasis [133], multiple sclerosis [134], arthritis [135], amyotrophic lateral sclerosis [136] and neuropathic pain [137]. IL-17 further directs the neutrophils and macrophages to the site of injury. The protein and mRNA expression levels of IL-17 were increased in mice with pressure ulcers as compared to the no PUs. This indicates the association of IL-17 in formation and development of PUs [123]. Imbalance in the inflammatory response due to decreased secretions of IL-17 may be associated with the formation of PU in this population.

3.4.4 Monokine induced by gamma interferon (MIG)

We found increased urine concentrations and decreased plasma concentrations of MIG following initial injury associated with later development of first pressure ulcer in individuals with traumatic SCI. MIG belongs to the CXC chemokine family is chemoattractant induced by IFN-γ is also called CXCL9. It is functionally related to IP-10 and is involved in recruitment of lymphocytes [138]. It is elevated during ocular sarcoidosis [139], meliodosis [140], rheumatoid arthritis [141] and is known to have anti-tumor activity [142]. It is one of the dermal mediators expressed during skin inflammation, various skin diseases [143] and venous leg ulcers [144]. The imbalance in concentrations of MIG in urine and plasma may be attributed to PU formation in this population. It is unclear as to why there are differences in concentrations of MIG in the urine and plasma bio fluids associated with the formation of first PU in this population.

3.4.5 Granulocyte-macrophage colony-stimulating factor (GM-CSF)

We found increased plasma concentrations of GM-CSF following initial injury associated with later development of first pressure ulcer in individuals with SCI. GM-CSF is a cytokine that is stimulated by T cells, fibroblasts and endothelial cells [145]. Although the mechanism of GM-CSF is not well understood during inflammation, it is known to function systemically [146]. Studies have shown increased concentrations of GM-CSF in inflammatory and autoimmune diseases and host protection [146, 147] such as rheumatoid arthritis [148], renal and lung disorders [149] suggesting its pro-inflammatory response. Some studies also suggested its anti-inflammatory response playing a beneficial role in pulmonary fibrosis [150]. Studies have indicated difficulty in detecting the GM-CSF levels in human serum in individuals with sepsis [151]. GM-CSF is known to increase the secretion of other pro-inflammatory cytokines such as TNF-α, interleukin-1 (IL-1) and interleukin-8 (IL-8) [152]. Since GM-CSF leads to synthesis of the pro-inflammatory mediators, blockage of GM-CSF is therapeutic since it decreases the adverse inflammatory effects caused by this cytokine [153]. Local injections of GM-CSF are known to heal sacral PUs. It also promotes the formation of granulation tissue by enhancing tissue proliferation and thus increases the fibroblast activation [154, 155]. Increase in GM-CSF activity was found in wound fluids and tissues in individuals with chronic leg venous ulcers. This indicates the role of this cytokine in the regulation of the immune system during the process of wound healing [156]. Studies have shown that the production of GM-CSF is essential for communication between the local tissue cells during an inflammatory reaction that takes place after SCI [146]. In this study the increase in plasma concentrations of GM-CSF immediately

after SCI associated with for development of PUs can be attributed to its increased pro-inflammatory activity.

3.4.6   Macrophage inflammatory protein-1α (MIP-1α)

We found increased plasma concentrations of MIP-1α following initial injury associated with later development of first pressure ulcer in individuals with SCI. MIP-1α also called CCL3, is responsible for the synthesis and secretion of many pro-inflammatory cytokines such as IL-6 and TNF-α as it induces the recruitment of lymphocytes at the site of inflammation [157]. MIP-1α is known to have variety of pro-inflammatory properties, and there is evidence of increased MIP-1α in subjects with rheumatoid arthritis, and osteoarthritis [158], acute lung injury [159], early after spinal cord injury at the level of lesion and is further elevated as the days since injury increases [160]. Previous studies have shown increase in synthesis of MIP-1α in the injured spinal cord immediately after SCI and eventually decreases days after the injury [161, 162]. MIP-1α alters the overall process of wound healing by decreasing fibrosis [163]. Deficiency in synthesis of MIP-1α in the brain of mice decreased the pro-inflammatory responses [164]. Previous studies have shown the importance of the inflammatory process in the prevention of PUs [72]. In this study the increase in plasma concentrations of MIP-1α immediately after SCI was associated with development of PUs. We hypothesize that the increase in plasma concentrations of MIP-1α immediately after SCI may decrease the overall anti-inflammatory response essential for the repair and regeneration process to prevent secondary complications after SCI such as PUs.

3.4.7 Interleukin-1 receptor antagonist (IL-1RA)

We found decreased plasma concentrations of IL1-RA following initial injury associated with later development of first pressure ulcer in individuals with SCI. IL-1RA belongs to the interleukin 1 cytokine family, secreted by neutrophils is involved in wound healing and inflammation[115]. It is an anti-inflammatory cytokine and inhibits the synthesis of IL-1 [165]. Increased plasma and/or urine levels of IL-1RA were associated with sepsis [166], rheumatic diseases and osteoporotic fractures [167, 168], and schizophrenia [169]. IL-1RA is an acute phase protein and previous literature shows increase in the plasma concentrations of IL-1RA when individuals were subjected to trauma and during infections [165]. IL-1RA had been used as a therapeutic intervention is individuals, cancer [170] and neuropathy [171]. IL-1RA was identified as one of the early markers for mechanically induced epidermal damage along with IL-8 and TNF- α [71]. Although the role of IL-1RA in individuals with SCI and PUs needs to be clarified, it is known to maintain homeostasis throughout the acute inflammatory response after SCI, by maintaining a balance between IL-1 and IL-1RA, thus influencing the host immune response to the traumatic event [172].

3.4.8 Macrophage inflammatory protein-1β (MIP-1β)

We found decreased plasma concentrations of MIP-1β following initial injury associated with later development of first pressure ulcer in individuals with SCI. MIP-1β also called CCL4 is a chemoattractant especially leukocyte attractant. Although this can be beneficial since it accumulates leukocytes to inhibit infection and contributes to repair, excess accumulation of

leukocytes due to the synthesis of MIP-1β could be pathological [173]. Its secretion increases in multiple sclerosis [174], osteoarthritis [175, 176], myositis [177], bronchitis [178] and rheumatoid arthritis [176]. The expression of MIP-1β was increased in ulcerative tissues as compared to healthy individuals [179]. Imbalance in the MIP-1β activity was found in wound fluids and tissues in individuals with chronic leg venous ulcers. This indicates the role of this cytokine in the regulation of the immune system during the process of wound healing [156]. The lack of leucocyte infiltration to inhibit the infection, due to decreased synthesis of MIP-1β could contribute to the development of PUs in this population.

3.4.9   Interleukin-6 (IL-6)

We found increased urine concentrations of IL-6 following initial injury associated with later development of first pressure ulcer in individuals with SCI. IL-6 is a pro-inflammatory cytokine and takes part in anti-microbial function and tissue repair. It is stimulated by macrophages, mast cells, lymphocytes and immune cells [115]. It is associated with diseases such as rheumatoid arthritis [180], cancer [181], diabetes [182], renal disease [183], immunosuppression [184], cardiovascular disease [185] and psoriasis [186]. Increase in IL-6 activity was found in wound fluids, tissues in individuals with chronic leg venous ulcers [156] and cutaneous ischemic reperfusion cutaneous injury [187]. There were increased concentrations of IL-6 observed in both human and rodents with SCI [162]. Previous studies indicated increased plasma concentrations of IL-6 associated with individuals with SCI, and their concentrations was further increased in individuals with SCI having PUs as compared to able-bodied individuals [32]. The blood levels of IL-6 were increased in individuals with malnutrition and aggravated the catabolic

activity in these individuals predisposing them with PUs [107]. The levels of IL-6 remain low during physiological conditions, but a slight increase in stress, injury or infection increased the levels of IL-6 since their source is not only the immune cells but also the muscles, keratinocytes, hepatocytes, adipose tissue and fibroblasts [184]. Although the urine concentrations of IL-6 increases after SCI in this study is associated with the incidence of first pressure ulcer it may not be a true marker of formation of PUs given a varied number of co-morbidities among individuals with SCI and IL-6 increases in slight stress and inflammation.

3.4.10 Interferon gamma induced protein (IP-10)

We found increased urine concentrations of IP-10 following initial injury associated with later development of first pressure ulcer in individuals with SCI. Previous studies have shown the increased synthesis of IP-10 in pulmonary disease, tuberculosis, diabetes mellitus, autoimmune and thyroid diseases [188-195]. IP-10 was one of the macrophage/monocyte-associated inflammatory mediators associated with non-healing of diabetic foot ulcers in both wound fluid and plasma samples [196, 197]. This connects the presence of systemic inflammatory biomarkers in diabetic foot ulcers manifested with infection. Nevertheless, the role of IP-10 in pressure ulcers in individuals with SCI remains obscure. This is the first study to find significant increase in the urine concentrations of IP-10 in individuals immediately after SCI (within four days) in the RERC on SCI population. IP-10 is a chemokine secreted by the CXCL10 gene. As a chemoattractant it activates the T cells towards sites of tissue inflammation [118, 198]. IP-10 is known to have both pro-inflammatory and anti-angiogenic properties. As a pro-inflammatory chemokine, IP-10 inhibits and limits the fibroblast recruitment and motility in individuals with

chronic wounds [199]. During physiologic conditions the process of angiogenesis is finely regulated associated with formation of new blood vessels that supply oxygen and nutrients to the tissue and aid in the formation of granulation tissue. The process of angiogenesis is important for the wound healing process [200]. IP-10, as an angiogenic inhibitor prevents the growth of the new blood vessels from pre-existing vessels [201]. This probably explains the increase in the urine levels of IP-10 immediately after SCI in this study since there is no growth of new vessels to participate in tissue regeneration process. This increase in the pro-inflammatory cells precipitates a complex inflammatory cascade of events that predisposes the individuals to form PU. Intervention with an antibody to neutralize the inflammatory effects of IP-10 in mice subjected to SCI reduced tissue injury by neutralizing the inflammation and thus improving functional recovery by angiogenesis [202, 203]. This increased urine secretion of IP-10 following SCI during the process of regeneration and repair may spill over in systemic circulation thus causing secondary complications such as PUs. This clarifies the role of IP-10 in the pathogenesis of PUs in individuals with SCI.

3.4.11 Interleukin-8 (IL-8)

We found decreased urine concentrations of IL-8 following initial injury associated with later development of first pressure ulcer in individuals with SCI. IL-8 is a pro-inflammatory cytokine that is stimulated by macrophages, lymphocytes and mast cells [115]. It promotes angiogenesis and signals the feed-forward mechanisms that in turn increases the secretion of IL-8 from neutrophils [115]. During physiologic conditions the process of angiogenesis is finely regulated associated with formation of new blood vessels that supply oxygen and nutrients to the tissue and

aid in the formation of granulation tissue. The process of angiogenesis is important for the wound healing process [115, 200]. IL-8 is associated with diseases such as rheumatoid arthritis [204] and psoriasis [205]. Imbalance in the IL-8 activity was found in wound fluids and tissues in individuals with chronic leg venous ulcers [156]. There were increased concentrations of IL-8 observed in both human and rodents with SCI [162]. IL-8 was identified as one of the early markers for mechanically induced epidermal damage along with IL-1RA and TNF- α [71]. In this study the urine concentrations of IL-8 decreases after SCI that is associated with the incidence of first pressure ulcer. Increased levels of IL-8 is seen in healing wounds since it participates in regeneration and repair process [206]. Lack of angiogenesis due to decreased IL-8 synthesis on this study after SCI can probably explain the association to the formation of pressure ulcer.

3.4.12 Interleukin-13 (IL-13)

We found decreased urine concentrations of IL-13 following initial injury associated with later development of first pressure ulcer in individuals with SCI.IL-13 has both pro-inflammatory and anti-inflammatory properties [28]. The synthesis of IL-13 increases in when there is allergic inflammation [207], lung disease and asthma [208], pulmonary fibrosis [209] and sclerosis [210]. There were increased concentrations of IL-13 observed in rodents with SCI [162]. IL-13 also induces tissue fibrosis, as it stimulates fibroblast proliferation and collagen synthesis [209]. It affects the process of wound healing and lung function [211]. It mainly has anti-inflammatory and suppresses the production and synthesis of pro-inflammatory cytokines such as IL-6, IL-1, IL-10, IL-12, TNF-α and GM-CSF; and chemokines such as IL-8, and MIP [207]. In this study

the urine concentrations of IL-13 decreases after traumatic SCI that is associated with the incidence of first pressure ulcer. The decrease in the anti-inflammatory activity due to decrease in the urine excretions of IL-13 may not be sufficient to inhibit or control the inflammatory response after SCI predisposing the individuals to PUs.

3.4.13 Monocyte chemotactic protein-1 (MCP-1)

We found decreased urine concentrations of MCP-1 following initial injury associated with later development of first pressure ulcer in individuals with SCI. MCP-1 is a chemokine and attracts monocytes, participates is host defense and inflammatory response. Macrophages, lymphocytes, endothelial cells and fibroblasts secrete MCP-1. It contributes towards ischemic reperfusion injury in myocardium and kidney [212]. The concentration of MCP-1 increases in individuals with coronary artery disease since it is a chemoattractant and activates monocytes and macrophages to the site of inflammation [213]. Its synthesis increases during inflammatory diseases such as psoriasis [214], arthrosclerosis [215], rheumatoid arthritis [216], and during neurological conditions such as epilepsy [217], ischemia [218], and traumatic brain injury [219]. Previous studies have shown that the plasma concentrations of MCP-1 also increase in individuals with diabetes [220] and diabetic foot ulcers [212]. In this study the urine excretions of MCP-1 decreases immediately after SCI that is associated with the development of first pressure ulcer. There were increased concentrations of MCP-1 observed in both human and rodents with SCI [161, 162]. The protein levels of MCP-1 increase during the compressed phase of ischemic reperfusion cycles. Also, the healing of the wounds was significantly more during MCP-1 deficiency since it reduces the ischemia reperfusion injury. Also, there is reduction in

number of skin infiltrating cells such as macrophages, and the activation of pro-inflammatory mediators such as iNOS and TNF-α were inhibited, following IR cycles in MCP-1 deficient mice [187]. In this study the urine concentrations of MCP-1 decreases after traumatic SCI that is associated with the incidence of first pressure ulcer. Insufficient inflammatory response may contribute towards formation of the PU in this population.

3.4.14 Tumor necrosis factor-alpha (TNF-α)

We found decreased urine concentrations of TNF-α following initial injury associated with later development of first pressure ulcer in individuals with SCI. In this study the urine excretions of TNF-α decreases immediately after SCI that is associated with the incidence of first pressure ulcer. TNF-α, a pro-inflammatory cytokine is synthetized after activation of neutrophils. It is stimulated by macrophages and neutrophils and thus participates in the process of wound healing and inflammation [115]. It is a chemoattractant and increased synthesis of TNF-α cause infection such as fever and granulocytosis. It also induces angiogenesis [115]. During physiological state TNF-α is not detected in human serum and tissue [221]. Its concentration increases during inflammatory conditions such as asthma [119], rheumatoid arthritis, inflammatory lung diseases and traumatic brain injury[221]. During physiologic conditions the process of angiogenesis is finely regulated associated with formation of new blood vessels that supply oxygen and nutrients to the tissue and aid in the formation of granulation tissue. The process of angiogenesis is important for the wound healing process [200]. TNF- α was identified as one of the early markers for mechanically induced epidermal damage along with IL-8 and IL-1RA [71]. It was increased during cutaneous ischemic reperfusion cutaneous injury [187]. There are increased

proportions of TNF-α in the wound fluids in individuals with chronic venous ulcers. Also, increased serum proportions of TNF-α were related to non-healing ulcers as compared to healing ulcers [222]. TNF-α is also known to stimulate the synthesis of collagen and collagenase[115]. Collagenase helps in prevention and treatment of pressure ulcers[223]. This can explain the decreased urine concentrations of TNF-α associated with development of PU in this study.

TNF-α, and IFN-γ to contribute towards the inflammatory process during the process of wound healing by augmenting the synthesis of nitric oxide (NO) [224]. This affects the infiltration and proliferation of the fibroblasts and the collagen gels at the site of the wounds thus helps in enhancing overall wound healing process [115]. In this study there was significant decrease in the excretion of TNF-α, and IFN-γ in urine that is associated with the formation of first pressure ulcer. The decrease production of these cytokines may be associated with the damage to the tissue in these individuals. Previous studies have shown elevation of IP-10, IL-6, IL-8 and MCP-1 in the CSF levels of individuals with SCI [225]. In this study there were increased urine concentrations of IP-10, and IL-6; and decreased urine concentrations of IL-8 and MCP-1. Hence, the underlying inflammatory factors due to TSCI could contribute towards the imbalance in inflammatory mediators associated with development of pressure ulcers in this population.

Adjusting for the Level of Injury

To identify the baseline (within 4 days after SCI) concentrations in plasma (and urine), to predict first pressure ulcer; the severity of injury (ASIA scale score) was included with the significant mediators obtained from the initial analysis of Aim 2. Including ASIA scale score in the final model, did decrease the overall degree significance for the individual mediators (plasma and

urine) as compared to the initial analysis (since the power decreased by adding more predictors in the multivariate logistic regression model) and the ASIA scale score did not turn up to be significant. Adding the ASIA scale score does not add much to the original analysis. This is not surprising, since the mediators (in plasma and urine) that were significant in original analysis in Aim 2 were not correlated with ASIA scale score. The impact of cardiovascular, pulmonary and other comorbidities, and medications and other interventions with the inflammatory markers were not examined. Future studies should be conducted adjusting for these conditions.

Additional Analysis

In an attempt to build a parsimonious model with few inflammatory mediators in urine and plasma immediately after SCI, that can explain the occurrence of PU, the all possible regressions was performed as an alternate analysis. The model was built to identify the most important plasma and urine predictors that can explain the occurrence of pressure ulcer. The all possible regressions model helps to predict well in a new sample and thus is more reliable and is a well-known data exploration technique. The model was built with 10 independent variables (plasma mediators) and 12 independent variables (urine mediators) obtained from the backward logistic regression. Since the models are not nested within each other, an AIC criterion was used to compare the model equations with the 10 plasma and 12 urine predictors obtained from the stepwise backward logistic regression technique. A restricted model was thus obtained with fewer predictors. The best subset model consisted of 9 plasma inflammatory predictors. The overall model significantly predicted the pressure ulcer outcome. $\chi 2$ (9) = 17.67, p=0.039. Decreased plasma concentrations of IL-1RA (p=0.05), and increased plasma concentrations of IL-6 (p=0.03), IL-5 (p=0.05) and MIP-1α (p=0.05) were identified as the most important

predictors that can explain the occurrence of PU in this population. IFN-γ (0.13), GM-CSF (0.07) and MIG (0.16) were not significant. The best subset model consisted of 8 urine inflammatory predictors. The overall model significantly predicted the pressure ulcer outcome. $\chi2$ (8) = 24.7, p=0.002. Decreased urine concentrations of IL-13 (0.01), and increased plasma concentrations of IL-6 (p=0.04), MIG (p=0.03), MIP-1β (p=0.07) and IFN-γ (p=0.02) were identified as the most important predictors that can explain the occurrence of PU in this population. TNF-α (p=0.2), IL-8 (p=0.06), IL-17 (p=0.06) and NO2-/NO3- (p=0.07).

The significant predictors obtained from the parsimonious model were the best subset of predictors that were obtained from the original analysis. This means the parsimonious model helps with the significant predictors helps to explain the formation of first PU in individuals with SCI using fewer parameters.

3.4.15 Limitations and Future Work

The data in this study were analyzed using stepwise logistic regression technique. This method has its potential problems while selecting significant predictors since multiple comparisons are made [226]. Also, this technique was performed in a small sample size, 54 individuals having plasma biomarkers and 53 individuals' urine biomarkers. The variable selection for the stepwise regression may be unstable because of the limited power to select significant variables, in small datasets [226]. Although some plasma (and urine) predictors were correlated to one another, there was not a high degree of multicollinearity found in the logistic regression model. Given the limited sample size, the data was not cross validated in this study. The significant predictors obtained in this study should be verified in a different sample population. The final model

retained for significant plasma and urine predictors should be cross validated in future studies. Validating the model may be essential to make conclusions of the significant inflammatory predictors in the population [227]. The interaction between the inflammatory mediators in plasma (and urine) has not been taken into account in this study, and it will be interesting to test the interactions in forthcoming studies. In order to study the temporal relationship of the inflammatory mediator's sophisticated techniques such as principal component analysis and factor analysis may be useful.

Although the data were collected prospectively, the timing for the measurements of the blood and urine samples for inflammatory mediator analysis was not uniform. Hence, for the baseline measure the first time point available within four days after injury was considered. The inflammatory mediators that could have contributed for the formation of first PU within hours after SCI may have been missed. This study analyzed the mediators at a single time point. We may have missed an optimal time point when a specific mediator was associated with formation of PU. Forthcoming studies, should investigate a temporal pattern of these mediators. The amount of spot urine samples may differ from person to person given the variability in the waste products voided. Prior studies adjusted for their creatinine levels, in urine to avoid this variability [228]. Future studies should control for this variation before assaying the inflammatory mediators in urine.

3.5   CONCLUSION

The concentrations of inflammatory mediators in urine and plasma immediately following TSCI is associated with the occurrence of pressure ulcers. A large number of predictors may be associated with the formation of pressure ulcer that were either previously identified or may have some pathophysiological association with formation of pressure ulcers [4, 24, 27]. The stepwise logistic regression using the backward elimination technique is a well-established data reduction technique that helps to explore the relationship between the variables associated with the formation of pressure ulcer. Inflammatory response after a traumatic injury is essential for the repair and regenerative process, but imbalance in the synthesis of the inflammatory mediatory predisposes individuals with TSCI to secondary complications such as PUs. Of all the significant inflammatory predictors in plasma and urine obtained in this study, prior evidence suggests also suggests IL-5, IL-6, IFN- γ and GM-CSF play an active role in cell proliferation and differentiation [115]. This may be the reason why imbalance in these inflammatory mediators is associated with the formation of pressure ulcers. Further research is essential to investigate possible intervention strategies and develop an objective risk prediction tool to identify individuals who may be at risk to develop pressure ulcers. The findings of this study imply that a complex inflammatory cascade is involved with development of PUs following SCI. This study thus can contribute towards hypothesis testing in the future to identify individuals at risk for pressure ulcer formation following TSCI.

4.0   INFLAMMATORY BIOMARKERS THAT PREDICT IMMINENT PRESSURE ULCER DEVELOPMENT IN INDIVIDUALS WITH SPINAL CORD INJURY

4.1   INTRODUCTION

Spinal cord injury (SCI) has profound impact from physical, psychological and socioeconomic perspectives [44]. The second leading cause of death in individuals with SCI is septicemia (88.6%); usually associated with presence of pressure ulcers [45]. Pressure ulcers (PUs) are the most frequent secondary complication in individuals with SCI from the time of acute hospitalization throughout life that affects their quality of life, length of stay during hospitalization and increases the mortality and morbidity [1, 7, 12, 48, 49]. According to the 2011 annual statistical report for the Spinal Cord Injury Model Systems (SCIMS), PUs are the second most frequent complication and third leading cause of death after SCI [45].

In individuals who sustain traumatic SCI, the production of cytokines and chemokines and other inflammatory mediators result in an inflammatory response characterized by neutrophil infiltration (the first inflammatory cells to arrive) at the site of injury followed by macrophages [23]. The resident macrophages also known as microglia are diffusely spread in the central nervous system (CNS). These macrophages are activated post-SCI and result in secretion of the cytotoxic substances and pro-inflammatory mediators such as TNF-α, IL-1, IL-6, nitric oxide, reactive free radicles and oxygen free radicals. Post-traumatic SCI the inflammatory response

further deteriorates the functional outcome and predisposes the host to secondary complications such as tissue damage and other infections [25, 26]. Thus, these cytotoxic substances lead to secondary complications by damaging the production of growth factors essential for tissue repair. Anti-inflammatory mediator such as IL-10 is known to suppress most of the microglial activity and helps in attenuating damage and dysfunction. The beneficial role of this inflammatory process can be attributed to the dual nature of the inflammatory process [27]. After SCI, due to the disrupted blood spinal cord barrier, most of the inflammatory mediators such as IL-1α, IL-1β, IL-1RA, IL-6, and TNF-α pass freely through the blood brain barrier (BBB) [24]. Increase in the pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α was noted immediately after injury until 5 hours post injury, and from the second day post injury the expression of these cytokines declines to baseline [29].

Clinically it is still unknown why certain individuals with SCI develop PUs and some do not despite having similar risk factors that predispose them to develop PUs. However, this could be attributed to the differences in the intrinsic inflammatory state in the individual, since evidence supports an increase in serum concentrations of pro-inflammatory cytokines in individuals with PUs [34]. A multidisciplinary expert panel sponsored by SCI Quality Enhancement Research Initiative (SCI QUERI) identified "research on biomarkers" for the development of PUs in individuals with SCI as "highest priority risk factor research" [34]. There are conflicting hypotheses regarding the role of inflammatory mediators in wounds and pressure ulcers. Blood and urine samples were collected at baseline and periodically (hours) after DTI induced by pressure in a chronic SCI rat model. Increase in serum and urine concentrations of biomarkers associated with muscle injury such as myoglobin (MB), heart type fatty acid binding protein (H-FABP), were observed in rats with deep tissue injury (DTI) [35]. *In vitro* studies have

shown increased synthesis and proliferation of inflammatory cytokines such as IL-1α after prolonged mechanical loading the tissue engineered epidermal equivalents, before the tissue damage is barely visible. The elevated levels of IL-1α was an indicator of tissue damage due to inflammation before it was visible on the skin [31]. Increase in the plasma concentrations of pro-inflammatory mediators such as IL-2R), andICAM-1 were noted in individuals with SCI having PUs or slow healing PUs as compared to able-bodied subjects or subjects with SCI having no PUs [32]. The severity of PUs is thought to be related to the disturbance or imbalance in these pro-inflammatory markers. The continued inflammatory stimulus in individuals with pressure ulcers could be as a result of the damage to the tissue due to ischemia. Fluid analysis at the site of the pressure ulcers have shown elevation of IL-1β and TNF-α [5]. The alterations in serum proteins such as C reactive protein, and erythrocyte sedimentation rate, were observed in individuals with PUs when compared to individuals with no PUs. These serum alterations disappeared with the healing of the PU [36]. IL-8, an acute inflammatory chemokine was observed to be elevated in the tissue biopsies of individuals with non-healing thermal wounds. The elevated concentration of IL-8 impaired the process of regeneration and repair [33]. Systemic evidence of inflammation (TNF-α level) was observed in individuals with long term SCI and pressure ulcers when compared to control group [229]. Elevated concentrations of pro-inflammatory mediators have been observed in individuals with SCI with and without pressure ulcers. The serum profile of IL-1β, IL-1RA, IL-6 and TNF-α were compared in individuals with SCI without infections, pain and PUs, and in individuals with SCI having these secondary complications. These secondary complications after SCI were attributed to the increase in the serum concentrations of TNF-α and IL-2 [4].

The inflammatory markers seem to be promising indicators of infection in individuals with SCI having PUs. Also, there are no objective tools to measure the risk assessment for PUs and it is essential to identify the potential inflammatory markers and discover new markers that may increase before the tissue damage is visible that may help prevent formation of PUs. Analyzing the concentrations of these mediators in serum just before the clinical diagnosis can possibly help identify the intrinsic factors associated with the formation of PUs. Hence, the aim of this study was to explore the change in the inflammatory biomarkers in the plasma (and) urine from within a week before the development of PU that can predict imminent pressure ulceration as compared to immediately after SCI, in individuals with PU and control group (individuals with no PU), during acute care and inpatient rehabilitation in this population. We hypothesized there will be a change in the concentrations of inflammatory mediators such as TNF-α, IL-1β, IL-8, and IL-6 and GM-CSF measured just before clinical diagnosis of PU as compared to immediately after SCI for those who developed pressure ulcers when compared to the control group. We also hypothesized that the inflammatory markers in urine that predict imminent occurrence of PUs will be same to those in plasma. Assaying the inflammatory markers from plasma and urine is less invasive than tissue biopsies and assaying at the site of pressure ulcers.

4.2   METHODS

4.2.1   Research Design

The research design for this study is a secondary analysis, since the existing database was used to examine the variables and to explore the relationship between presence of pneumonia and PU outcome [80].

4.2.2   Inclusion and Exclusion Criteria

The inclusion and exclusion criteria of RERC on SCI are mentioned in Chapter 2, Section 2.2.2.

For this particular study the inclusion criteria were:

1. Individuals with SCI recruited for the RERC on SCI population until August 2012 from acute care through inpatient rehabilitation.

2. Having inflammatory mediators collected within four days after SCI.

3. For individuals with PUs, who have inflammatory mediators collected within a week before formation of PU.

4. For individuals with no PUs (in the control group), who have inflammatory mediators assayed in plasma (or urine) matched to the plasma (or urine) samples by ±1day in the PU group.

4.2.3 Data Collection and Processing of Urine and Plasma Samples

The processing of urine and plasma samples and inflammatory mediator measurement are mentioned in Chapter 3, Section 3.2.4.

4.2.4 Procedure

The timing (in days) for the formation of the first PU diagnosis was noted. The inflammatory mediators in plasma and urine within a week before the clinical diagnosis of the PU were analyzed. The time point closest to the clinical diagnosis (pre-PU time point) within a week before the clinical diagnosis of the PU was considered. The baseline time point was the earliest time point available and within 4 days after SCI. The difference in concentrations of the inflammatory mediators in plasma (and urine), between the two time points was analyzed for each mediator. The pre-PU time points of plasma and urine assay for individuals with PU were matched to the individuals who did not develop PU (control group).
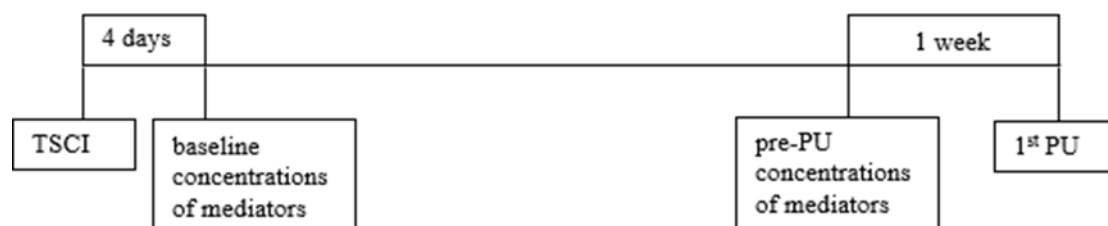
Figure 9. Time course for individuals with pressure ulcers
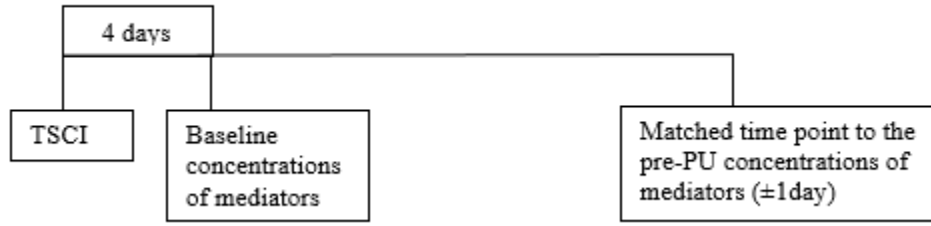
Figure 10. Time course for control group

4.2.5   Data Analysis

Two separate analyses were performed to identify the significant inflammatory mediators in plasma and urine. The same inflammatory mediators for individuals in the control group (individuals who did not develop PUs) and the individuals who developed PUs were analyzed. All data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Box plots and was plotted to subjectively explore the differences in the mediators between the two time points.

Wilcoxon Signed-Ranks Test

The Wilcoxon Signed-Ranks Test is a version of the dependent samples t-Test that is performed on nonparametric data[88]. The two-tailed Wilcoxon signed-rank test was performed to identify the difference between the baseline measures (within four days of SCI) and the first time point from within a week before the formation of PUs, in subjects who had formation of PUs.

The Wilcoxon Signed-Ranks test statistic is [88]:

$$z = \frac{T - \frac{n(n+1)}{4}}{\sqrt{\frac{n(n+1)(2n+1)}{24}}}$$

106

- z = z score

- n= sample size of the group

- T= the lower value obtained from the summation of ranked scores (between positive and negative rank scores).

Our hypotheses were:

H_0 *(Null Hypothesis):* No difference between the concentrations of the inflammatory mediators between the two time points (immediately after SCI and just before formation of first PU), will be observed in individuals with PUs and control group, (or) Calculated z is between -1.96 and 1.96.

H_1 *(Alternate Hypothesis):* Difference between the concentrations of the inflammatory mediators will be observed between the two time points (immediately after SCI and just before formation of first PU) in individuals with PUs and control group.

Bonferroni correction was applied to the level of significance. The significance was set at $\alpha = 0.02$ for the plasma inflammatory mediators, (or) z < -1.96 or z > 1.96.

The inflammatory mediators in urine were analyzed similarly. Bonferroni correction was applied to the level of significance. The significance was set at $\alpha = 0.1$ for the urine inflammatory mediators.

4.3 RESULTS

4.3.1 Plasma Analysis

4.3.1.1 Demographics

A total of 17 individuals with PUs were included to analyze the plasma inflammatory mediators. The demographics of the individuals with PUs are given in Table 25. The mean age of individuals in this group was 38 years and most of the individuals were males. Individuals in this group had increased severity of SCI, with ASIA scale score A and ISS score of 36. The control group of 17 individuals was matched to the PU group by the day (±1day) when the plasma samples for the pre-PU time point were assayed. The time course for the plasma inflammatory mediators is for the two groups (individuals with PUs and control group) are given in Figure 11.

The chi-square test produced no significant differences in gender, and the Mann-Whitney U statistic produced no significant differences in ages between individuals with PUs and the control group. The ASIA scale score and ISS were significantly different between individuals with PUs and the control group.

Table 25. Demographics of individuals with PUs and Control group – Plasma analysis

Demographics	PU n= 17	Control Group (No PUs) n=17	P Value
Age (years) Mean ± SEM	38.12 ± 4.17	40.41±3.83	0.5
Gender	M=12(71%) F=5(29%)	M=14(82%) F=3(18%)	0.42
ASIA	A=13(76%) B=0 (0%) C=2 (1%) D=1 (6%) U=1 (6%)	A=6(35%) B=6(35%) C=3(18%) D=2(12%) U=0(0%)	0.04
Injury Severity Score (ISS)	36.44±4.42	27±4.4	0.03

4.3.1.2   Change in the inflammatory mediators in plasma

(a) Individuals with Pressure Ulcers

Since the data for the plasma inflammatory mediators were not normally distributed, box plots were plotted for differences between two time points (within four days after injury and just before the formation of PU) for each mediator. The change in inflammatory mediators between the two time-points was explored with the help of box plots of the differences in the plasma concentrations for all the inflammatory mediators between the two time points were plotted (Figures 12-14). In these figures, the circles are mild outliers (between inner and outer fence) and asterisks are extreme values (beyond outer fences). The mild outliers are values that lie from 1.5 to 3 interquartile range. Extreme values are those which lay more than 3 interquartile range.

After exploring the boxplots, IP-10 and MIG levels showed differences in the two time points subjectively.

Two-tailed paired t-test, Wilcoxon signed-rank test was performed between the two time points for IP-10 and MIP-1α that was identified by exploring the boxplots. Significance was α = 0.02 after the Bonferroni correction was applied. The test showed a significant increase in plasma concentration of IP-10 just before the formation of PU as compared to within four days after injury, z= -2.931, p=0.01. There was no statistically significant difference in concentration of MIP-1α between the two time points (just before the formation of PU as compared to within four days after injury), z= 2-.05, p=0.04 (Figure 15).

(b) Control Group (Individuals with no Pressure Ulcers)

For individuals who did not develop PUs, two-tailed paired t-test, Wilcoxon signed-rank test was performed between the two time points for IP-10, MIP-1α, GM-CSF and IL-6. There was no statistically significant difference in concentration of IP-10 (z = -1.25, p=0.21) and MIP-1α (z= -0.07, p=0.937), between the two time points (Figure 15).

4.3.1.3 Time course of plasma IP-10 levels in both groups

Figure 16 shows the time course of the concentrations of the plasma IP-10 levels in individuals with PUs and the control group. Day 0 indicates when the individuals had formation of PU in the PU group. Since the control group time points were matched to the PU groups, day 0 in the control group is the "expected" PU time point. The plasma inflammatory mediators were normalized for plotting.
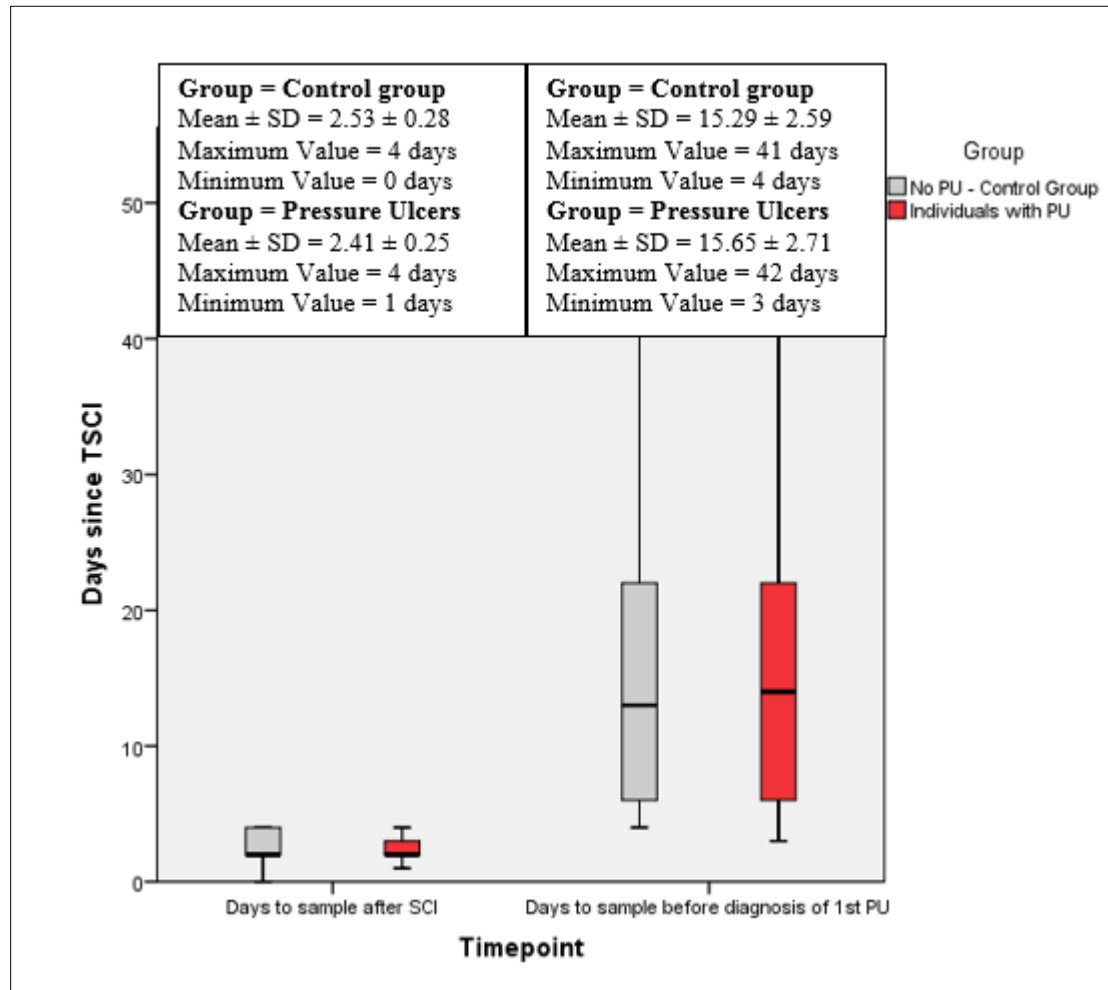
Figure 11. Time course of plasma inflammatory mediators in individuals with PUs and control group
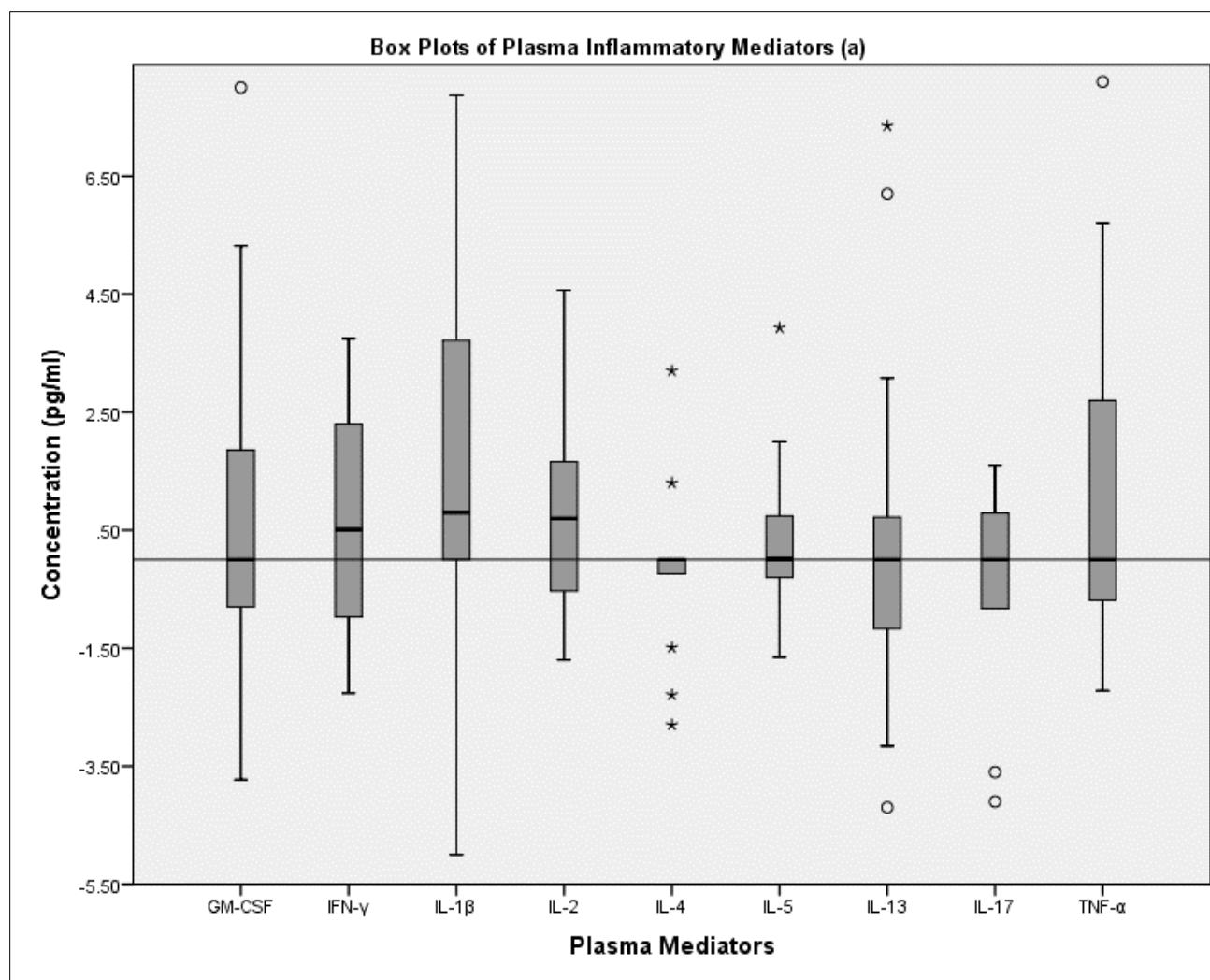
Figure 12. Box plots for individuals with Pus – differences in plasma inflammatory mediators' immediately after TSCI and just before formation of PU

(A)

112
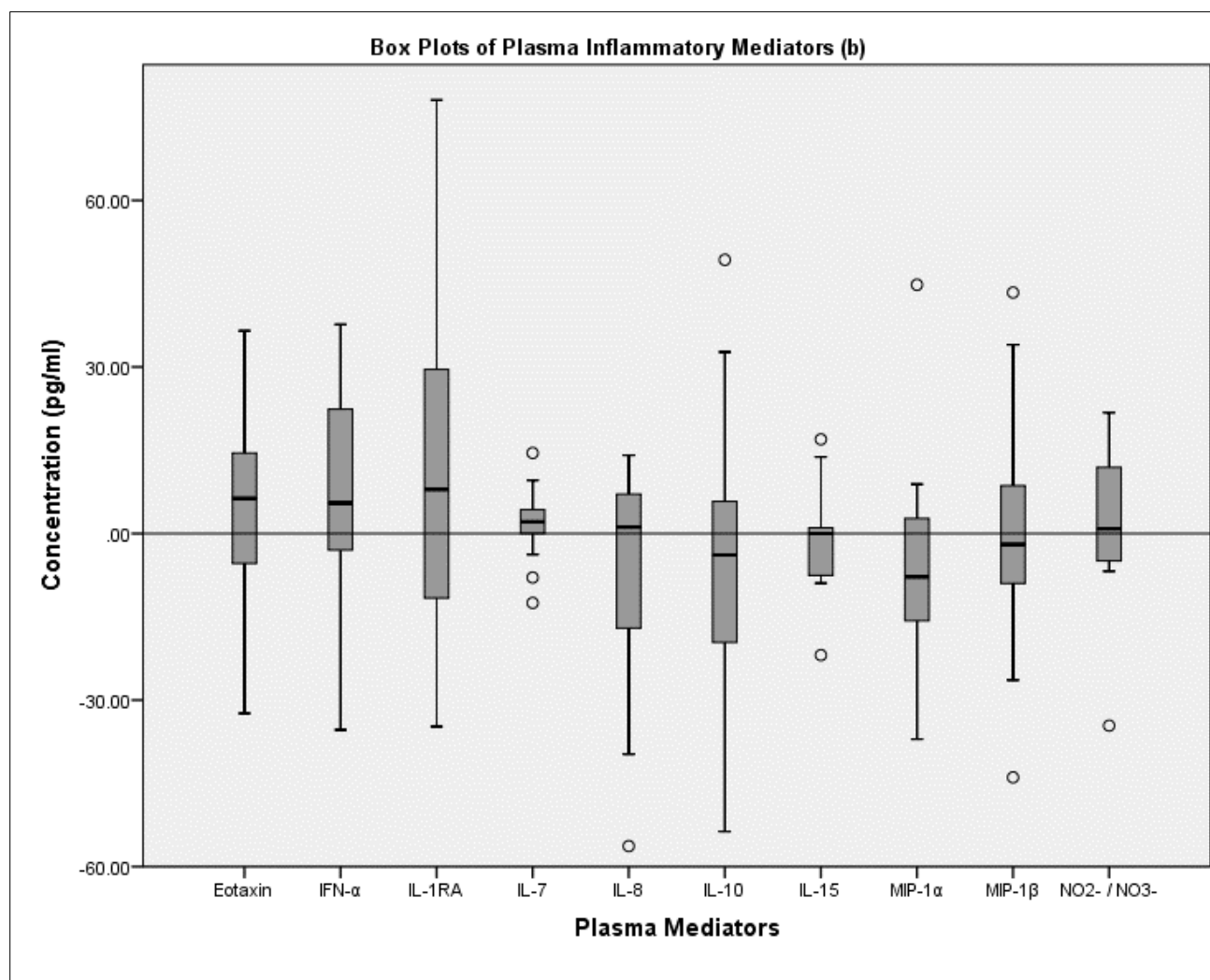
Figure 13. **Box plots for individuals with Pus – differences in plasma inflammatory mediators' immediately after TSCI and just before formation of PU (B)**

113
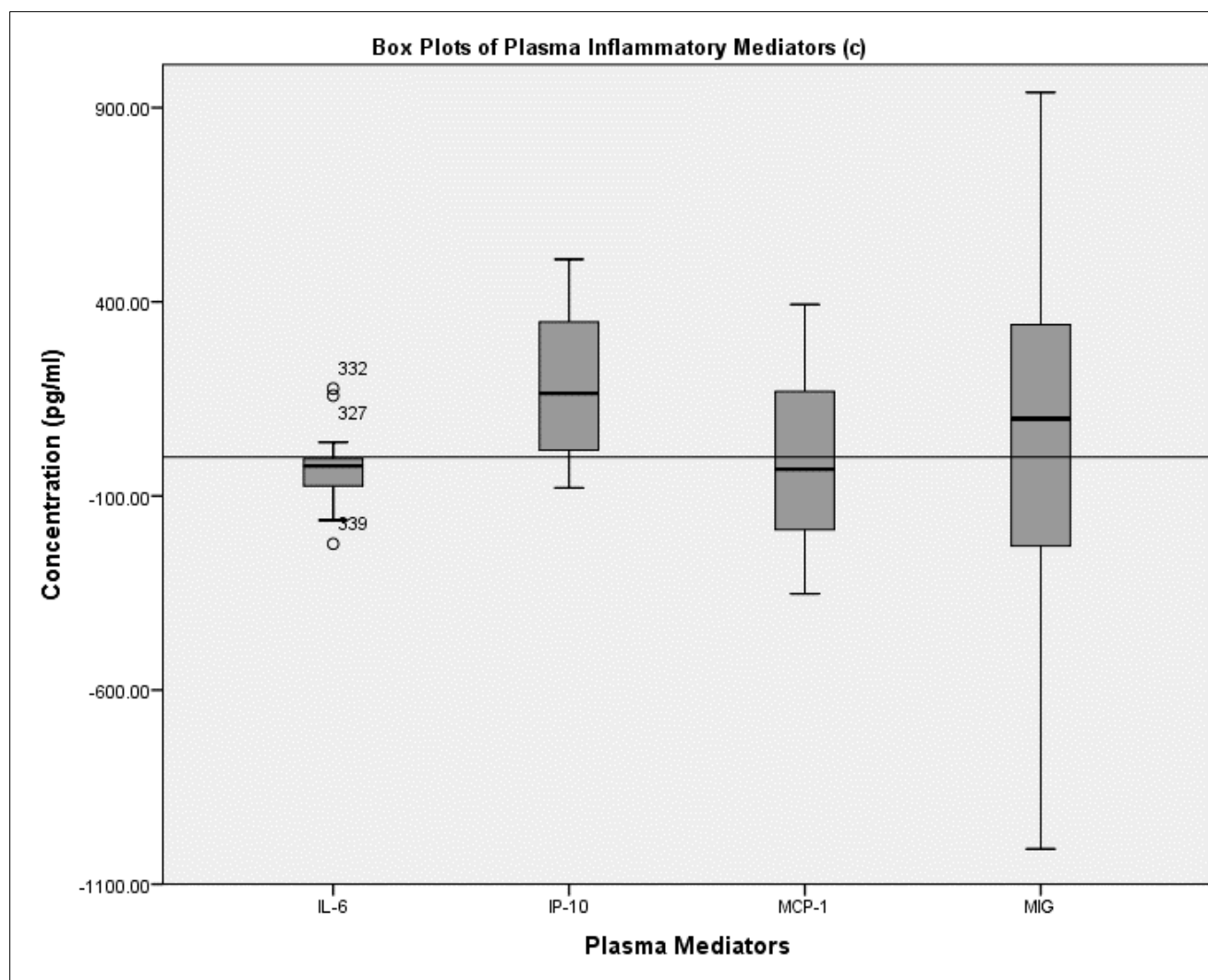
Figure 14. Box plots for individuals with Pus – differences in plasma inflammatory mediators' immediately after TSCI and just before formation of PU (C)

114
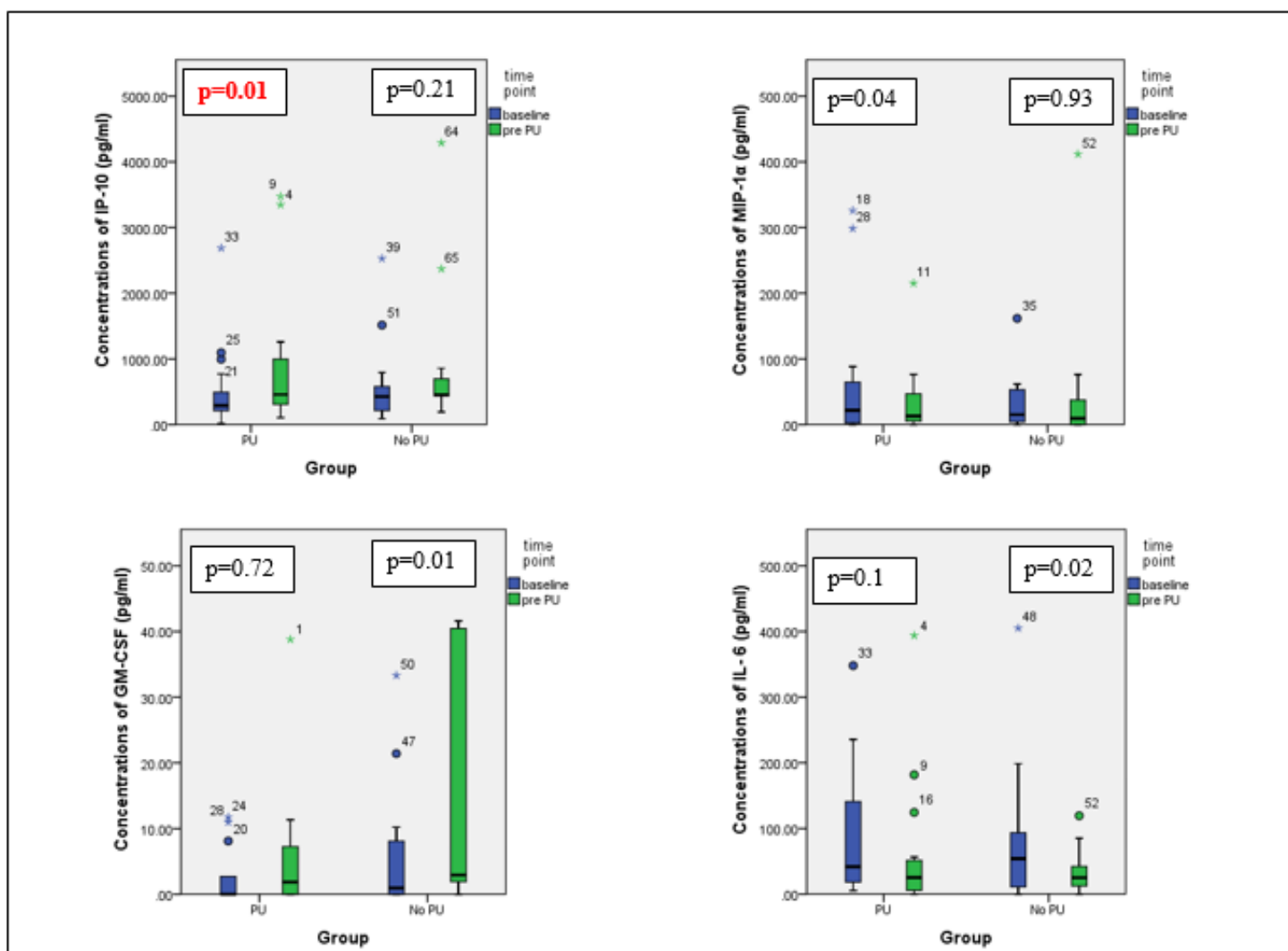
Figure 15. Box plots – Plasma concentrations of IP-10, MIP-1α, GM-CSF and IL-6 immediately after SCI and just before formation of first PU

115
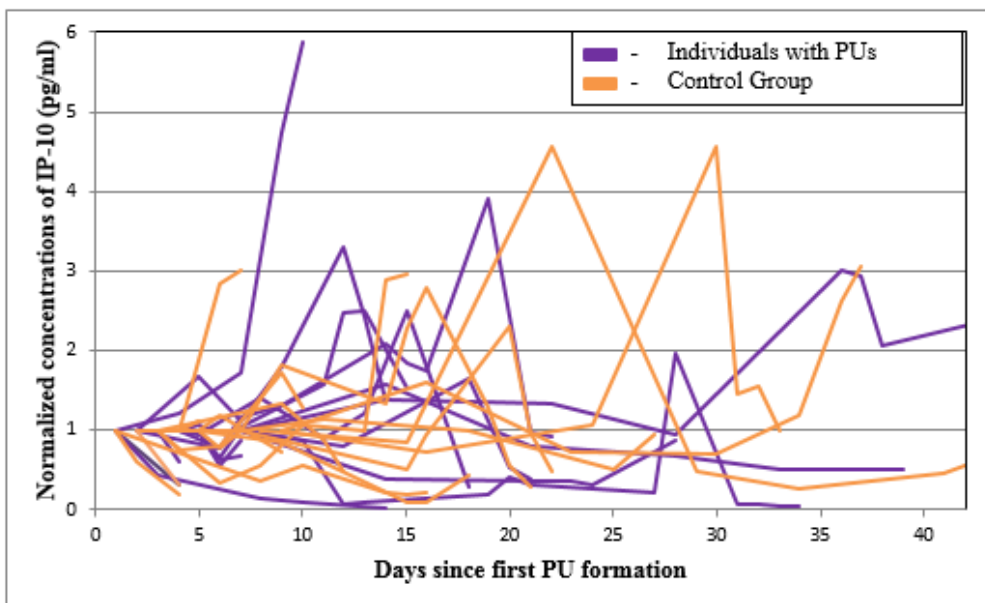
Figure 16. Time course of concentrations of IP-10 in plasma from the days since PU formation

116

4.3.2 Urine Analysis

A total of 15 individuals with PUs and control group (no PUs), having urine biomarkers were included to analyze the change in the urine inflammatory mediators. The control group of 17 individuals was matched to the PU group by the day (±1day) when the urine samples were assayed.

4.3.2.1 Demographics

The demographics of the individuals with PUs and the control group are listed in Table 26. The mean age of individuals with PUs was 40 years and most of the individuals were males. Individuals with PUs had increased severity of SCI, with AIS of A and ISS score of 34. The control group of 15 individuals without PUs was matched to the PU group by the day (±1day) when the urine samples for the pre-PU time point were assayed. The time course for the urine inflammatory mediators is for the two groups (individuals with PUs and control group) are given in Figure 17.

The chi-square test produced no significant differences in gender and ASIA scale score distribution between individuals with PUs and the control group. Also, the Mann-Whitney U statistic produced no significant differences in age and ISS score between individuals with PUs and the control group.

Table 26. Demographics of individuals with pressure ulcers and Control group – Urine analysis

Demographics	PU n= 15	Control Group (No PU) n=15	P value
Age (years) Mean ± SEM	39.53 ± 4.5	35.6 ± 3.61	0.72
Gender	M=11(73%) F=4(27%)	M=12(80%) F = 3(20%)	0.66
ASIA	A=12 (80%) B=0 (0%) C=1 (7%) D=0 (0%) U=0 (0%)	A=6(40%) B=6(40%) C=2(13%) D=1(7%)	0.46
Injury Severity Score (ISS)	33.57 ± 4.12	31.29 ± 5.26	0.39

4.3.2.2 Change in the inflammatory mediators in urine

(a) Individuals with Pressure Ulcers

Since the data for the urine inflammatory mediators for individuals with PUs were not normally distributed, box plots were plotted for differences between two time points (within four days after injury and just before the formation of PU) for each mediator. To explore the change in inflammatory mediators between the two time-points, box plots of the differences in the urine concentrations of the inflammatory mediators between the two time points were plotted for all the mediators (Figures 18-20). In these figures, the circles are mild outliers (between inner and outer fence) and asterisks are extreme values (beyond outer fences). The mild outliers are values that lie from 1.5 to 3 interquartile range. Extreme values are those which lay more than 3 interquartile range. After exploring the boxplots, IP-10 and MIG levels showed differences in the

two time points. After exploring the boxplots, IFN-α, IL-1RA, IL-1β MIP-1α levels showed differences in the two time points.

Two-tailed non-parametric paired test, Wilcoxon Signed Ranks test, between the two time points was performed for IFN-α, IL-1RA, IL-1β MIP-1α. Significance was α = 0.01 after the Bonferroni correction was applied. The test showed that there was a statistically significant decrease in concentration of IFN-α just before the formation of PU as compared to within four days after injury, z= -2.346, p=0.01. There was no statistically significant difference in concentration of IL-1RA, IL-1β, MIP-1α just before the formation of PU as compared to within four days after injury, z= -.402, p=0.68. z= -1.988, p=0.047 z= -2.197, p=0.028 and z= -1.992, p=0.046 respectively after Bonferroni correction (Figure 21).

(b) Control Group (Individuals with no Pressure Ulcers)

For individuals who did not develop PUs, two-tailed paired t-test, Wilcoxon signed-rank test was performed between the two time points for IFN-α, IL-1RA, IL-1β, MIP-1α. There was no statistically significant difference in concentration of IFN-α (z = -0.78, p=0.43), IL-1RA (z = -1.72, p=0.08), IL-1β (z = -0.5, p=0.61) and MIP-1α (z= - 0.07, p=0.937), just before the formation of PU as compared to within four days after injury (Figure 21).

4.3.2.3 Time course of urine IFN-α levels in both groups

The time course of the urine concentrations of IFN-α in individuals with PUs and the control group is shown in Figure 22. Day 0 indicates when the individuals had formation of PU in the PU group. Since the control group samplings were matched to the time point of the IFN-α

119

collected in the PU group, the day 0 in the control group was the "expected" PU time point. The

urine IFN-α levels were normalized for plotting.
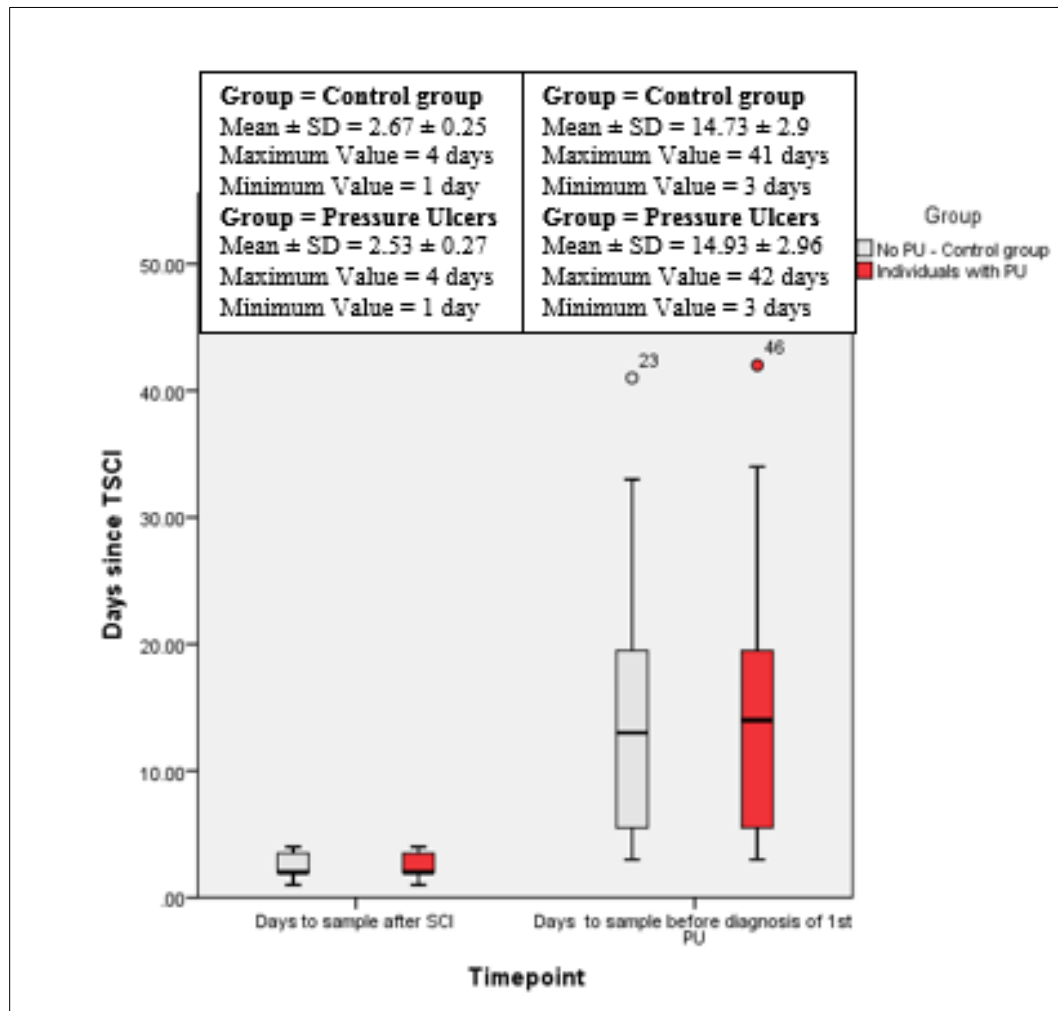
Figure 17. Time course of urine inflammatory mediators in individuals with PUs and control group
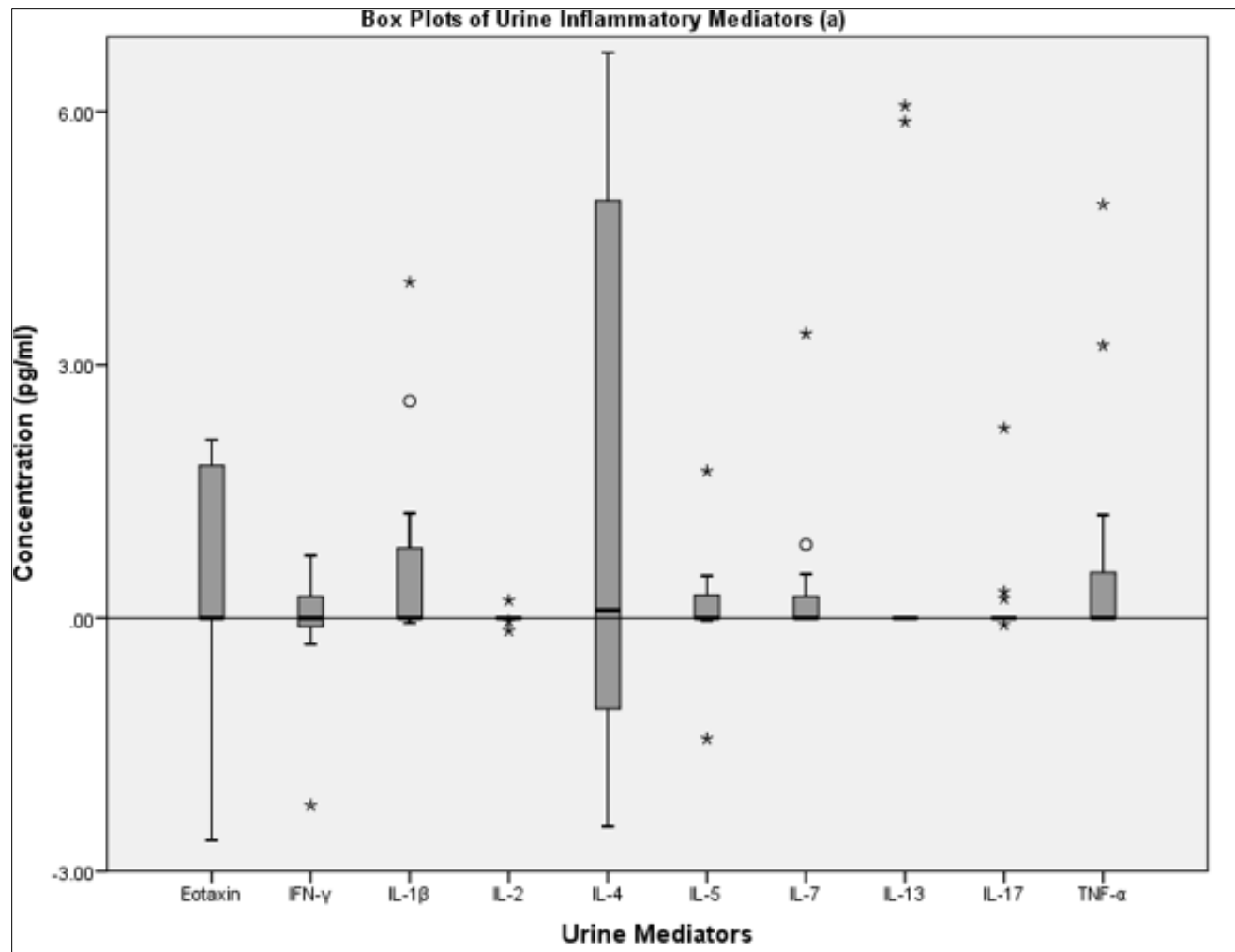
Figure 18. Box plots for individuals with PUs – differences in urine inflammatory mediators' immediately after TSCI and just before formation of PU (A)
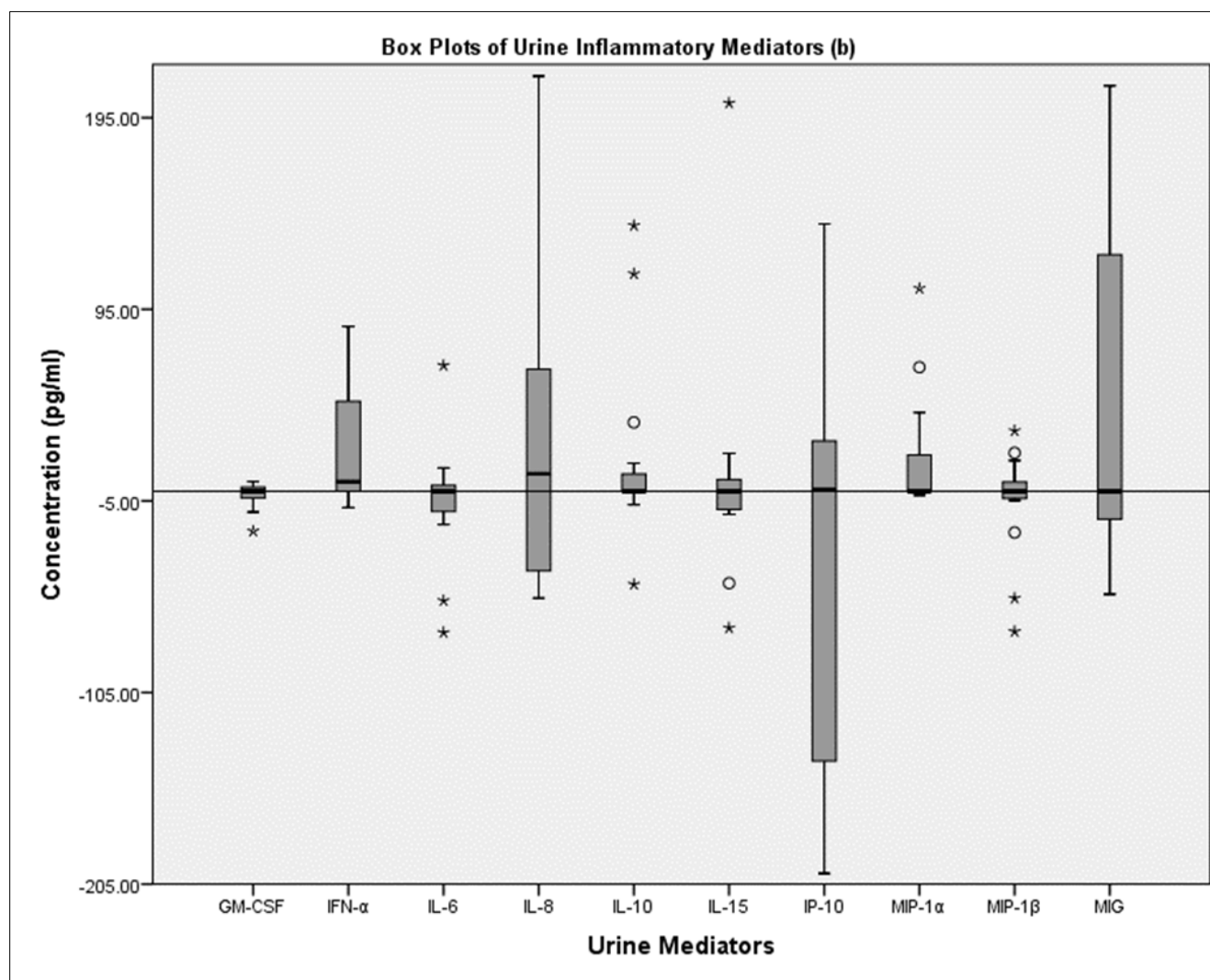
Figure 19. Box plots for individuals with PUs – differences in urine inflammatory mediators' immediately after TSCI and just before formation of PU (B)
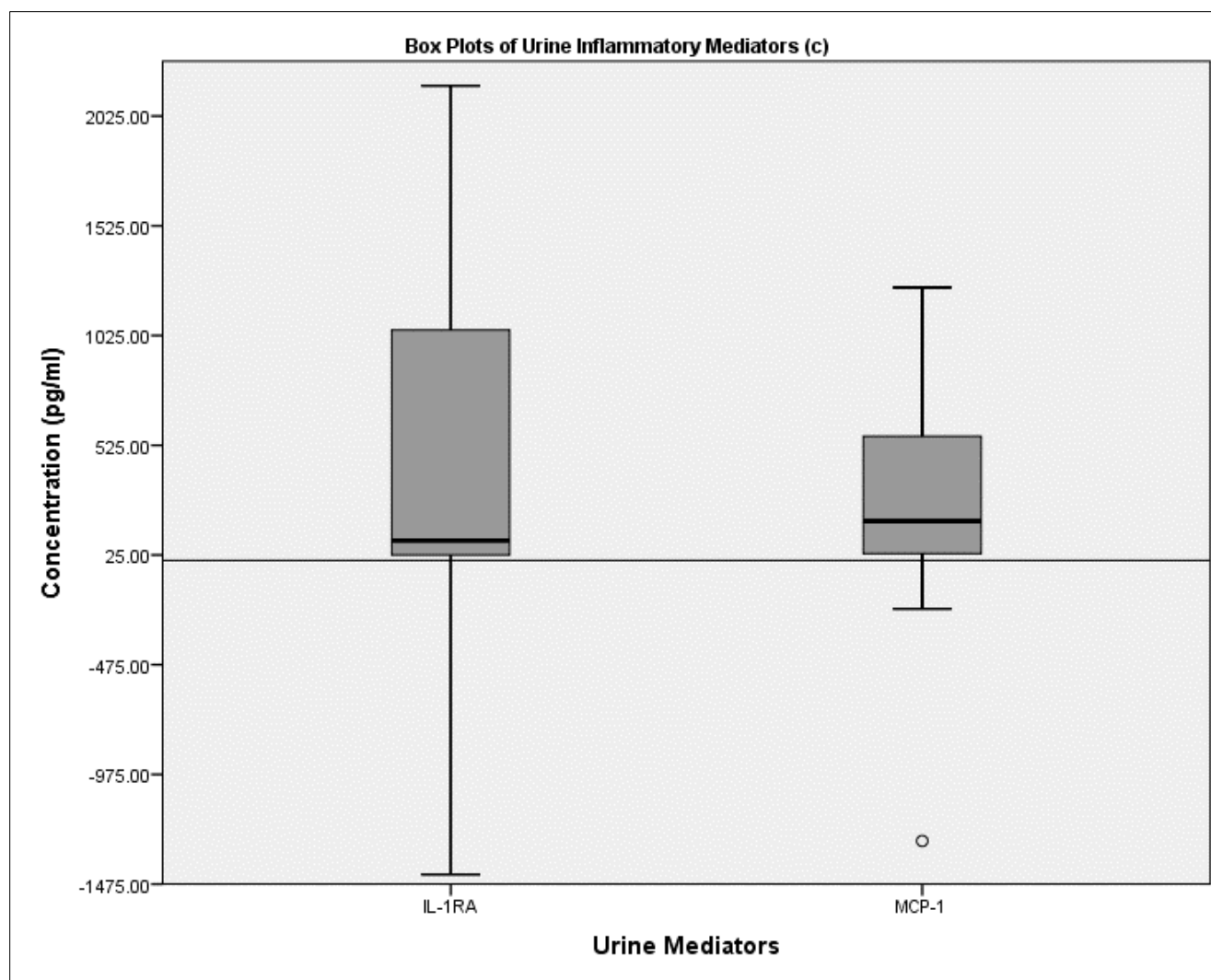
Figure 20. Box plots for individuals with PUs – differences in urine inflammatory mediators' immediately after TSCI and just before formation of PU (B)

123
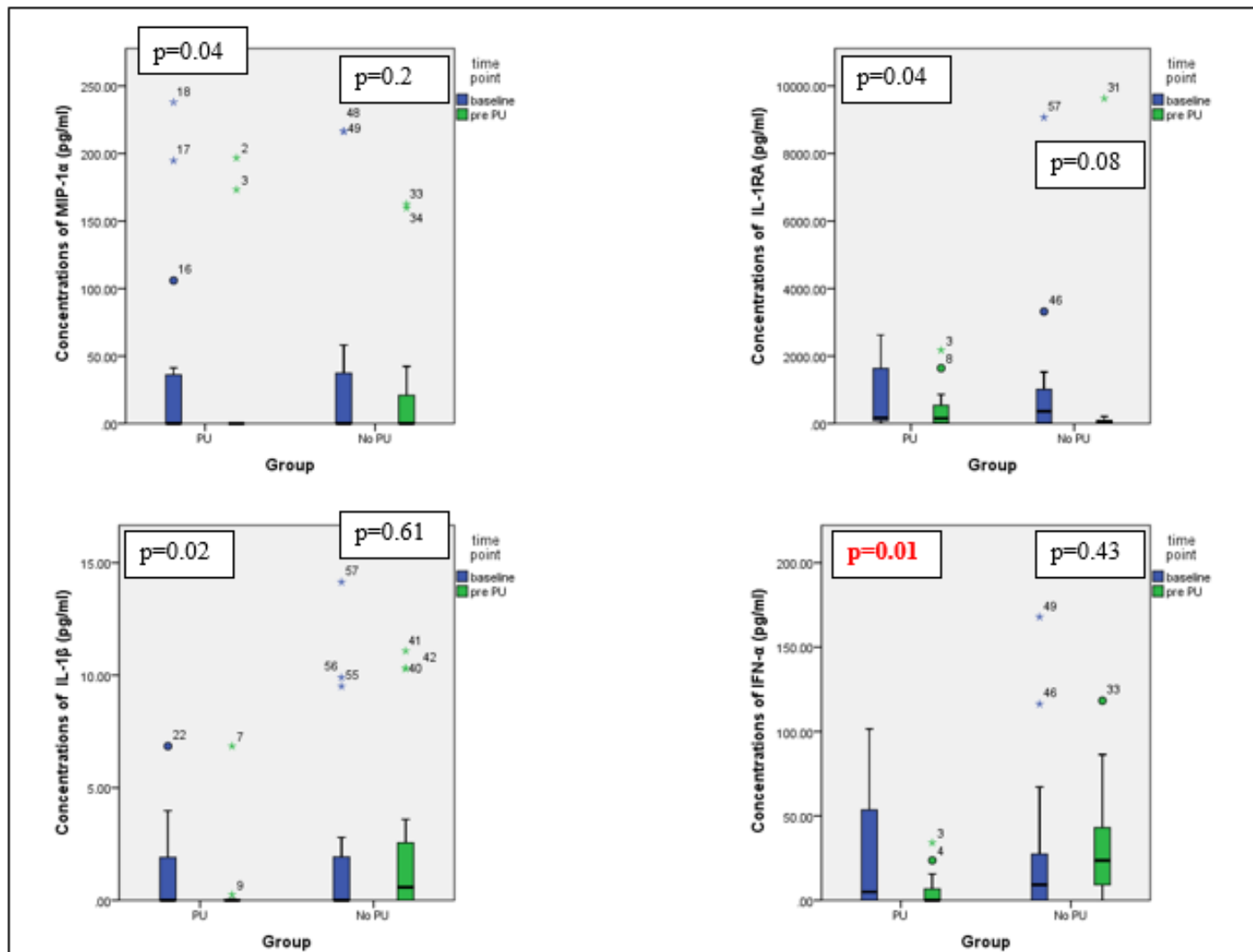
Figure 21. Box plots – Urine concentrations of MIP-1α, IL-1RA, IL-1β and IFN-α 6 immediately after SCI and just before formation of first PU

124
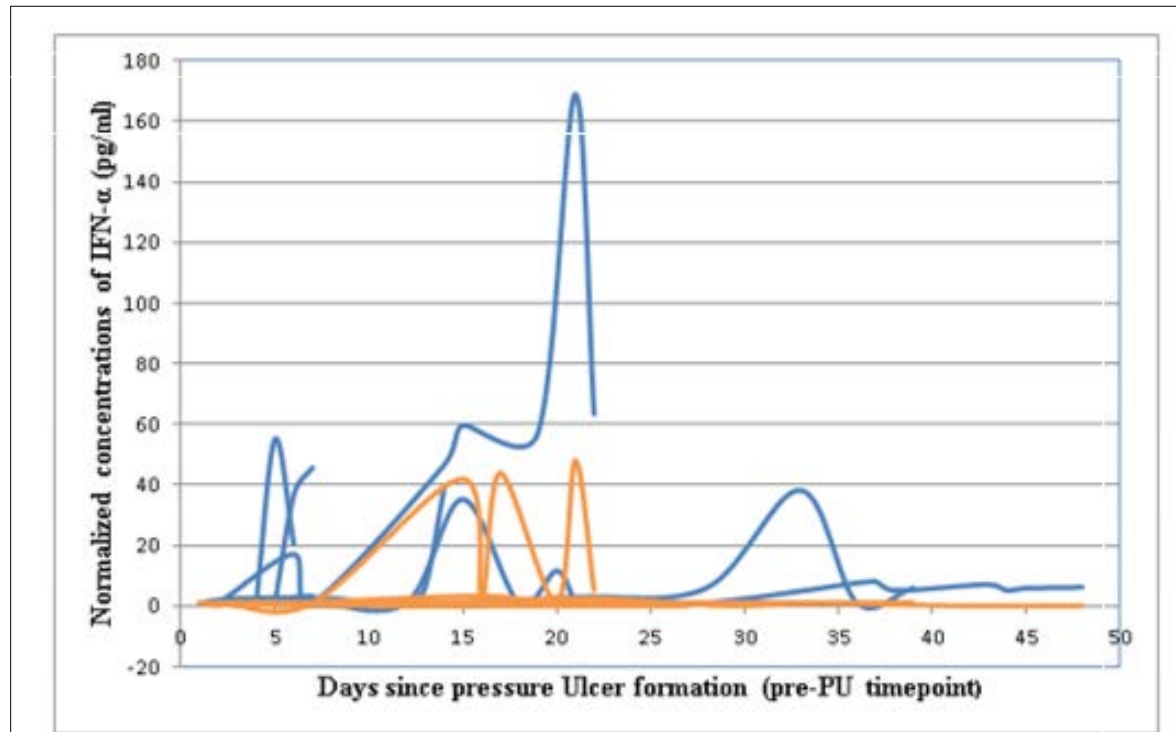
Figure 22. Time course of urine concentrations of IFN-α from the days since formation of first PU

4.4   DISCUSSION

Although pressure ulcer (PU) is a localized injury many systemic consequences such as deep vein thrombosis [230], urinary tract infections [231], rheumatoid arthritis [232], diabetes mellitus [104] have been associated with formation of PUs. In individuals with SCI these clinical manifestations are not only concomitant with formation of pressure ulcers but also associated with systemic manifestations such as increase in the inflammatory cells, excessive neutrophils, changes in the pro and anti-inflammatory cytokines and chemokines [233, 234]. The current study explored the change in the concentration of inflammatory mediators in urine and plasma biofluids within four days after SCI and just before the formation of PUs, in individuals with PUs.

Previous studies have shown the changes in synthesis circulating systemic biomarkers and tissue concentrations in individuals with SCI and PUs such as such as IL-1α, IL-1β, IL-2, IL-6, IL-8 and TNF-α [4, 24, 29, 31] indicating the possibility to characterize these individuals with PUs using the systemic biomarkers. This is the first study to report the changes in concentration between inflammatory mediators in both plasma and urine biofluids immediately after SCI and just before formation of the first PU. The changes in the urinary excretions for these inflammatory mediators from the baseline measure (immediately after SCI) to just before the formation of a PU, was not similar as compared to the plasma concentrations. Previous studies accounted for similar differences in the inflammatory cytokines in the plasma and urine after exercise [113] and renal disease [110].

4.4.1 Inflammatory biomarkers in plasma

In this study we demonstrated the alterations in the plasma concentrations of IP-10 and MIP-1α in individuals with SCI and PUs, in individuals with PUs.

4.4.1.1 Interferon gamma induced protein (IP-10)

Previous studies have shown the increased synthesis of interferon gamma induced protein (IP-10) in pulmonary disease, tuberculosis, diabetes mellitus, autoimmune and thyroid diseases [188-195]. IP-10 was one of the macrophage/monocyte-associated inflammatory mediators associated with non-healing of diabetic foot ulcers in both wound fluid and plasma samples [196, 197]. This connects the presence of systemic inflammatory biomarkers in diabetic foot ulcers manifested with infection. Nevertheless, the role of IP-10 in pressure ulcers in individuals with SCI remains obscure.

This is the first study to find significant increase in the plasma concentrations of IP-10 in individuals just before the formation of pressure ulcers as compared to their concentrations at baseline (within four days after SCI) in the SCI population. The plasma concentrations of IP-10 did not change significantly in the control group. IP-10 is a chemokine secreted by the CXCL10 gene. As a chemoattractant it activates the T cells towards sites of tissue inflammation [118, 198].

IP-10 is known to have both pro-inflammatory and anti-angiogenic properties. As a pro-inflammatory chemokine, IP-10 inhibits and limits the fibroblast recruitment and motility in individuals with chronic wounds [199]. During physiologic conditions the process of angiogenesis is finely regulated associated with formation of new blood vessels that supply

oxygen and nutrients to the tissue, and aids in the formation of granulation tissue. The process of angiogenesis is important for the wound healing process [200]. IP-10, as an angiogenic inhibitor prevents the growth of the new blood vessels from pre-existing vessels [201]. This probably explains the increase in the plasma levels of IP-10 just before formation of first pressure ulcer as compared to the baseline measure (within four days after SCI) in this study since there is no growth of new vessels to participate in tissue regeneration process. This increase in the pro-inflammatory cells precipitates a complex inflammatory cascade of events that predisposes the individuals to form PU. Intervention with an antibody to neutralize the inflammatory effects of IP-10 in mice subjected to SCI, reduced tissue injury by neutralizing the inflammation and thus improving functional recovery by angiogenesis [202, 203].

Interferon-γ (IFN-γ) is recognized to be an inducer of IP-10 [235, 236]. There were no significant changes in the plasma secretion of IFN-γ from the baseline measure as compared just before formation of PU in this study. This the increased secretion of IP-10 following SCI during the process of regeneration and repair may spill over in systemic circulation thus causing secondary complications such as PUs. This clarifies the role of IP-10 in the pathogenesis of PUs in individuals with SCI.

4.4.1.2 Macrophage inflammatory protein- 1 alpha (MIP-1α)

Although MIP-1α was not significant after application of Bonferroni correction due to multiple comparisons of the cytokines between the baseline measure and just before formation of pressure ulcer, there was modest decrease in the concentration of MIP-1α in plasma just before the formation of pressure ulcer as compared to the baseline measure (with four days after SCI). It is still interesting to recognize the systematic inflammatory process associated with the decrease in

the plasma levels of MIP-1α in this population. The plasma concentrations of MIP-1α did not change significantly in the control group.

MIP-1α also called CCL3, is responsible for the synthesis and secretion of many pro-inflammatory cytokines such as IL-6 and TNF-α as it induces the recruitment of lymphocytes at the site of inflammation [157]. MIP-1α is known to have variety of pro-inflammatory properties, and there is evidence of increased concentrations of MIP-1α in subjects with rheumatoid arthritis, and osteoarthritis [158], acute lung injury [159], early after spinal cord injury at the level of lesion and is further elevated as the days since injury increases [160]. Previous studies have shown increase in synthesis of MIP-1α in the injured spinal cord immediately after SCI and eventually decreases days after the injury [161].

MIP-1α alters the overall process of wound healing by decreasing fibrosis [163]. Deficiency in synthesis of MIP-1α in the brain of mice decreased the pro-inflammatory responses [164]. Previous studies have shown the importance of the inflammatory process in the prevention of PUs [72]. We hypothesize that the decrease in the plasma concentrations of MIP-1α just before the formation of PU in individuals with SCI and PUs may decrease the overall pro-inflammatory effect essential for the repair and regeneration process to prevent formation of PUs after SCI.

4.4.1.3   Granulocyte macrophage colony-stimulating factor (GM-CSF)

Although the concentrations of GM-CSF did not decrease significantly just before formation of pressure ulcer as compared to the baseline measurement in individuals with PUs; there was a significant increase in the plasma concentration of GM-CSF just before the pre-pressure ulcer time point as compared to the baseline measure (with four days after SCI) in the control group.

Evidence suggests use of human GM-CSF for the healing of wounds such as postsurgical wounds and chronic leg ulcers[237]. In this study the significant increase in plasma concentrations of GM-CSF in the control group could be a reason why they didn't develop a PU as compared to individuals with PUs who did not have a significant increase in the plasma concentrations just before the formation of the first PU.

4.4.1.4   Interleukin-6 (IL-6)

Although the concentrations of IL-6 did not significantly decrease just before formation of pressure ulcer as compared to the baseline measurement in individuals with PUs; there was a significant decrease in the plasma concentration of GM-CSF just before the pre-pressure ulcer time point as compared to the baseline measure (with four days after SCI) in the control group. Although there was a significant decrease in the plasma concentrations of IL-6 in the control group, this difference was not significant after Bonferroni corrections. It is still interesting to understand why there was no significant decrease in the plasma concentrations in individuals with PUs. Evidence suggests that individuals with SCI having PUs have significant increase in the plasma concentrations of IL-6 as compared to the able-bodied individuals [32]. In this study the significant decrease in the plasma concentrations of IL-6 in the control group could be a reason why they didn't develop a PU as compared to individuals with PUs who did not have a significant decrease in the plasma concentrations just before the formation of the first PU.

This study thus demonstrates the change in the plasma concentrations of IP-10 and MIP-1α that contributes to the pathogenesis of the first pressure ulcer formation in individuals with SCI. Hence, these inflammatory mediators can be considered to be potential diagnostic markers

for identifying individuals at risk for the formation of PUs in this population. It will be interesting to further clarify the functional implications of these differences in the expression of the cytokines and chemokines in plasma and their pathogenesis of PUs in individuals with PUs.

4.4.2   Inflammatory biomarkers in urine

In this study we observed the alterations in the IFN-α urine concentration of specific biomarker synthesis in individuals with SCI and PUs, in individuals with PUs. There were no differences in the concentrations of IFN-α in the control group.

4.4.2.1   Interferon alpha (IFN-α)

In this study the secretion of IFN-α significantly decreases just before the formation of PU as compared to immediately after SCI (with four days of SCI). IFN-α belongs to the Type 1 interferon group. This cytokine activates the immune cells during infection, by stimulating the T lymphocytes and macrophages. Interferons are a part of the innate immune system and participate in the first line of defense against the pathogens before the specific immune system responds. They mainly benefit the host by resisting infection and inflammation initiated after trauma. They are produced when the host is subjected to a viral infection or any other pathogen. They bind to the viral cell, and induce antiviral state. [238]. Interferon therapy is mainly used to stop or slow the growth of tumor cells, leukemia, melanoma, sarcoma, carcinoma [239], hepatitis [240], and thus protect the immune system.

During a physiological response, the timely interactions of the specific cytokine and the receptor help to control the inflammation and thus prevent secondary complications that may

occur after a traumatic SCI. Interferons also have an ability to up regulate the cytokine responses [238]. Although there is not much evidence about the role of IFN-α in individuals with SCI, type I interferons have been reported to contribute to promote functional activity after SCI as they inhibit the astrocytes that are responsible in the secondary cellular response after SCI [241]. Although the role of IFN-α in individuals with SCI having pressure ulcers is not elucidated, we hypothesize that the decrease in IFN-α secretion just before the formation of PU as compared to immediately after SCI indicates an imbalance in the inflammatory response in the immune system and thus predispose these individuals with formation of PUs.

4.4.2.2   Interleukin-1 beta (IL-1β)

Although the urine concentrations of IL-1β was not significant after application of Bonferroni correction due to multiple comparisons of the cytokines between the baseline measure and just before formation of pressure ulcer, there was modest decrease in the secretion of IL-1β just before the formation of pressure ulcer as compared to the baseline measure (with four days after SCI). There were no differences in the concentrations of IL-1β in the control group. It is thus interesting to recognize the systematic inflammatory process that is associated with the levels of IL-1β in this population.

IL-1β belongs to the IL-1 family. It is a pro-inflammatory cytokine produced by macrophages/monocytes and endothelial cells. The synthesis of IL-1β increases during acute or chronic infection [242]. Studies have shown that IL-1β inhibits the synthesis of proteoglycans [242]. Proteoglycans are a part of extracellular matrix (ECM) of skin. They are large hydrated molecules that help to cushion cells in the ECM and participate in the regulation of the wound

healing process by controlling angiogenesis, and granulation tissue formation by preventing scar tissue formation [243].

IL-1β also stimulates the production and synthesis of other cytokines and chemokines such as monocyte chemotactic peptide (MCP-1) and macrophage inflammatory protein (MIP-1α) [70]. Numerous studies have found the increase in the peak expression of IL-1β immediately after the injury and leads to the apoptotic cell death [244-246]. These increased levels of IL-1β may be associated with the formation of PUs that occurs in individuals with SCI. The amount of production of IL-1β at the site of lesion is responsible for either the regeneration of the tissues or deleterious effect after injury [247]. This study found decrease in the urine levels of IL-1β just before the formation of PU in individuals with SCI and we hypothesize the inflammatory process necessary for the prevention of tissue destruction may be inadequate.

4.4.2.3   Interleukin-1 receptor antagonist (IL-1RA)

Although IL-1RA was not significant after application of Bonferroni correction due to multiple comparisons of the cytokines between the baseline measure and just before formation of pressure ulcer, there was modest decrease in the secretion of IL-1RA just before the formation of pressure ulcer as compared to the baseline measure (with four days after SCI). There were no differences in the concentrations of IL-1RAin the control group. It is thus essential to recognize the systematic inflammatory process that is associated with the levels of IL-1RA in this population.

IL-1RA belongs to the interleukin 1 cytokine family, is an anti-inflammatory cytokine and inhibits the synthesis of IL-1 [165]. Increased plasma and/or urine levels of IL-1RA were associated with sepsis [166], rheumatic diseases and osteoporotic fractures [167, 168], and schizophrenia [169]. IL-1RA is an acute phase protein and previous literature shows increase in

the plasma concentrations of IL-1RA when individuals were subjected to trauma and during infections [165]. IL-1RA had been used as a therapeutic intervention is individuals, cancer [170] and neuropathy [171].

Although the role of IL-1RA in individuals with SCI and PUs needs to be clarified, it is known to maintain homeostasis during an acute inflammatory response after SCI, and provides a balance between IL-1 and IL-1RA, thus influencing the host immune response to the traumatic event [172]. In this study the urine concentrations of IL-1RA decreases just before formation of PU that may not be adequate IL-RA to maintain this homeostasis.

4.4.2.4   MIP-1α

Although MIP-1α was not significant after application of Bonferroni correction due to multiple comparisons of the cytokines between the baseline measure and just before formation of pressure ulcer, there was modest decrease in the secretion of MIP-1α just before the formation of pressure ulcer as compared to the baseline measure (with four days after SCI). There were no differences in the concentrations of IL-1RA in the control group. It is thus essential to recognize the systematic inflammatory process that is associated with the levels of MIP-1α in this population.

Studies have shown IL-1β induces the secretion of MIP-1α [242]. Hence the differences in the urine concentrations of MIP-1α may possibly be because of the differences found in the urine concentrations of IL-1β found in this study. Also, the plasma concentrations modestly decrease just before the formation of PU as compared to immediately after SCI. The plasma changes in the MIP-1α levels may partly explain the changes in the urinary concentrations of this marker. Previous studies have shown simultaneous changes in the plasma and urinary markers in patients with idiopathic hypercalciuria [248].

134

The immune dysregulation after SCI is associated with the secondary complications such as PUs. Previous studies similarly assayed blood and urine samples for ease in data collection in individuals [113, 171, 248]. We hypothesized TNF-α, IL-1β, IL-8, and IL-6 and GM-CSF will contribute to PU formation by reviewing similar studies [5, 31, 33]. Except for the concentrations of IL-1β, the inflammatory mediators detected were not similar to our hypothesis. This may be because of the difference in the timing of urine and plasma sample collection and assaying these samples during the time course after SCI. It is also essential to note that the plasma levels of these inflammatory mediators increases only during serious traumatic event because they have a relatively short half-life period, which would have contributed towards the variability between the individuals. Most of the prior studies found alterations in tissue concentrations of these cytokines in tissues or in vivo associated in formation of PUs. The severity of injury as recorded by the ISS and ASIA scale score was significantly different between the two groups (PU and control group). A lower degree of injury might be reasonably thought to be associated with a lower degree of inflammation; this might have been a confounding variable. The values of the plasma (and urine) inflammatory mediators in individuals with PUs and the control group had presence of outliers. This may be because the sample may not be representative of the population. The Wilcoxon signed rank test is resistant to the presence of outliers the test statistic may not be affected to a great extent. This test is known to perform well with paired difference with outliers [88].

4.4.3 Limitations and Future Work

This study is a secondary analysis and sample sizes were not estimated for the individual aims by using power analysis to obtain a desired power prior to data collection. The number of individuals with PUs for this study to analyze the blood and urine samples was relatively small. Only 17 individuals with PUs and plasma analysts and 15 individuals with PUs and urine analysts were obtained from the RERC on SCI study. Although the data were collected prospectively, the timing for the measurements of the blood and urine samples for cytokine analysis was not uniform. Hence, for the baseline measure the first time point available within four days after injury was considered and the first time point available a week before formation of PUs was noted. The inflammatory mediators that could have contributed for the formation of first PU within hours after SCI have not been analyzed for the lack of consistency. This study analyzed the mediators at a single time point and temporal time points were not studied. We may have missed an optimal time point when a specific mediator was associated with a disease

The amount of spot urine samples may differ from person to person given the variability in the void and the waste products that may be excreted. The urine samples used to assay these chemokines and cytokines were not adjusted for their creatinine levels, or osmolality [228]. This may not take into consideration the inter- and intra-individual variations of the excretions of the inflammatory mediators in urine. To successfully use the inflammatory mediator validation for the diagnosis of PU the timing for collecting the plasma and urine samples may be essential which was not taken into account in this study.

Further research is necessary to understand the mechanism of these cytokines that are cleared from the blood stream and are evident in the urine in individuals with SCI and PUs.

Understanding the mechanisms of the plasma synthesis and urine excretion of IP-10 and IFN-α may lead to new therapeutic interventions to modulate inflammation in PUs in individuals with SCI. It will be interesting to compare the inflammatory mediators and chemokines in healing versus non-healing ulcers. This will help us differentiate these inflammatory mediators in plasma and urine that may be beneficial and deleterious in this population. Blocking the chemokine or cytokine to prevent the incidence of pressure ulcer can be implemented as an effective intervention strategy as used in other inflammatory disease states such as infections related to HIV [249]. Future studies are required to understand the temporal relationship and interaction between these inflammatory responses for these inflammatory mediators from the days since SCI. The cross regulation and interaction between the mediators has not been taken into account in this study, and it will be interesting to see the primary regulator if this is taken into consideration.

4.5   CONCLUSION

In conclusion, the results of this study show that the localized injury to skin and/or underlying tissue in individuals with SCI can be associated with altered biomarker levels in systemic circulation and urine. The present study provides evidence that the plasma concentrations differ from the urine concentrations of the markers just before formation of PU in this population when compared within four days after injury. Changes in the concentrations of the inflammatory mediators such as increase in the plasma levels of IP-10, and decrease in urine excretion of IFN-α, just before the formation of PUs as compared to the baseline measure (within four days after SCI) was shown in this study. In the control group, a significant decrease in plasma concentrations of GM-CSF was observed. The ease in sampling of plasma and urine and the availability and reliability of the multiplex immunoassay technology with Luminex to detect different inflammatory mediators simultaneously in body fluids thus provides us with a new objective tool to identify the pressure ulcers in individuals with SCI.

5.0   ASSOCIATION OF PRESENCE OF PNEUMONIA AND INCIDENCE OF PRESSURE ULCERS IN INDIVIDUALS WITH TRAUMATIC SPINAL CORD INJURY

5.1   INTRODUCTION

Spinal cord injury (SCI) is a devastating neurologic disorder that has profound impact from physical, psychological and socioeconomic perspectives [44]. Increase in age and severity of injury are associated with rise in long-term complications after traumatic SCI [79]. The second leading cause of death in individuals with SCI is septicemia (88.6%); usually associated with urinary tract infections (UTI's), pneumonia, and/ or presence of PUs [45]. Pressure ulcers (PUs) are the most frequent secondary complication in individuals with SCI from the time of acute hospitalization through community reintegration PUs affect quality of life, length of stay during hospitalization and increases the mortality and morbidity [1, 7, 12, 48, 49]. The 2011 annual statistical report for the Spinal Cord Injury Model Systems (SCIMS) identify PUs as the second most frequent complication and third leading cause of death for people with SCI [45].

Many risk factors have been associated with the formation of pressure ulcers. Medical complications such as cardiac or renal disease were associated with the risk of developing PUs [8]. Individuals likely to develop PUs were those who have SCI of traumatic origin [17], males [18], have a history of smoking, alcohol or drug use [10], have medical comorbidities such as diabetes mellitus [19, 20], decreased oxygenation or hypotension [16], infections such as

139

pneumonia, urinary tract infections, osteomyelitis, and other bacterial infections [8, 21], those on mechanical ventilators [22] and use of steroids [16]. Moisture and/or urinary and fecal incontinence, hypo/hyperthermia, friction, shear, were reported to be the extrinsic risk factors for the formation of PUs in individuals in acute care and intensive care unit (ICU) [16, 19, 51-55]. Decreased nutrition or low serum albumin levels, decreased mobility and sensation, and impaired cognitive function contribute towards the intrinsic risk factors for the development of PUs in individuals with SCI [19, 20].

Pneumonia is an inflammatory condition of the lung, specifically inflammation of the alveoli (microscopic air sacs in the lungs). Although there are many causes of pneumonia, infection is the most common etiology. The infecting agents can be bacteria, viruses, fungi, or parasites [57, 58]. Pneumonia is the most frequent respiratory complication (66.9% cases) that occurs weeks after the SCI [45, 59, 60]. Around 30% of individuals with SCI develop pneumonia during the time of hospitalization [50]. Of all the secondary conditions following SCI, pneumonia is one of the most frequent causes of death [46, 47]. The diaphragm is the main inspiratory muscle of respiration and is supplied by the phrenic nerve that originates from third and extends to fifth cervical nerve (C3-C5). The muscles of respiration such as the diaphragm, abdominals and intercostal are affected with progressively higher level of SCIs, resulting in a higher incidence of pneumonia in individuals with tetraplegia than paraplegia [61]. Individuals with complete tetraplegia have altered lung volumes and ineffective cough, and present with a restrictive ventilatory pattern. The alteration in lung volumes decreases the lung compliance and increases the cost of energy, predisposing the individual to respiratory fatigue. Therefore, higher-level SCIs (tetraplegics at C3-C5) may produce complete respiratory paralysis [7, 37, 59, 62]. Overall 5% to 20% of individuals with SCI develop pneumonia during initial rehabilitation.

Pneumonia was found to be associated with atelectasis in these subjects that alters the pattern of respiration. Studies have shown that prior anesthesia increases the risk of developing pneumonia [61].

The SCI Quality Enhancement Research Initiative (QUERI) projects also points out the importance and challenge in identification of all the risk factors in individuals with SCI and implementing corresponding preventive techniques in the clinical practice [34]. A study investigated the association of semirecumbent v/s supine position in individuals on mechanical ventilation and pneumonia during their stay in the ICU. As a secondary endpoint the formation of PUs in both groups (supine and semirecumbent) was further investigated. An increased incidence of pneumonia and PUs in the heel and sacral regions were observed in individuals who were in the supine position when compared to the semirecumbent position [63]. The synthesis of the inflammatory biomarkers increases in individuals with the presence of PUs [5], and also in individuals having respiratory infections such as pneumonia [39]. However, an apparent association between them has not yet been explored. Hence, it is essential to identify the dependent and independent risk factors such as pneumonia associated with development of PUs in this population.

In order to look at the association between pneumonia and PU formation in individuals with SCI, a preliminary study in 86 subjects in the RERC on SCI population was performed. We hypothesized that individuals having pneumonia will have a higher risk to develop pressure ulcers than individuals who do not have pneumonia in acute care through inpatient rehabilitation following TSCI in the RERC on SCI population. The preliminary study in 86 subjects in the RERC on SCI population showed that the subjects were twice as likely to develop PUs when the PU was preceded by or coincided with the presence of pneumonia as compared to subjects who

did not have pneumonia Hence, the presence of pneumonia could indicate possible future pressure ulcer development in individuals with SCI during acute care and inpatient rehabilitation [38]. It was worthwhile to explore the data of the SCI Model Systems (SCIMS) to see if this association holds true in a larger sample size. Hence, this study investigated the association between the presence of pneumonia and pressure ulcers in the SCIMS database population. The objective of the proposed study was to confirm the RERC SCI study's results. We hypothesized that individuals with presence pneumonia will have a higher presence of pressure ulcers than individuals who do not have pneumonia in acute care hospitalization and inpatient rehabilitation in the SCIMS population.

5.2   RERC ON SCI POPULATION

5.2.1   Methods

5.2.1.1   Research Design

The RERC database was used in this study in a secondary analysis to explore and examine the relationship among the clinical and demographic variables and PU outcome [80].

5.2.1.2   Inclusion and Exclusion Criteria

The inclusion and exclusion criteria of RERC on SCI are mentioned in Chapter 2, 2.2.2. For this particular study the inclusion criteria included individuals with SCI recruited for the RERC on

SCI population until August 2012 from acute care through inpatient rehabilitation and subjects were excluded if they withdrew from the study in less than three weeks.

5.2.2 Data Collection

The data collected for the RERC on SCI are described in Chapter 2, Section 2.2.3.

5.2.2.1 Procedure

The presence of pneumonia and first PU were recorded. The subjects were grouped according to sequence and timing of presence of pneumonia and PU incidence into four groups. Three weeks was chosen to allow the PU ulcer to occur within the time course of resolution of pneumonia [250].

(1) Pneumonia and no PU within three weeks

(2) PU and no pneumonia preceding the ulcer

(3) Pneumonia coinciding with or preceding the ulcer within three weeks

(4) No PU and no pneumonia.

5.2.2.2 Data Analysis

The differences in the clinical and demographic data between the four groups of population were explored. The data were analyzed using SPSS version 20.0.

Kruskal-Wallis test

The Kruskal-Wallis is a version of independent measures (one-way) ANOVA that is performed on data that is not normally distributed. The two-tailed, Kruskal-Wallis one-way analysis of

variance was performed to test significant differences in ages between the four groups (only pneumonia, only PU, with pneumonia and PU; and no pneumonia and no PU). The Kruskal-Wallis statistic is[88]:

$$H = \frac{12}{N(N+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(N+1)$$

- N is total sample size

- k is total number of groups

- i is to denote the particular group

- R_i is sum of ranks for each group

- n_i is the sample size of that particular group

H$_o$ (Null Hypothesis) – The mean ranks from the populations in the four groups (only pneumonia, only PU, with pneumonia and PU; and no pneumonia and no PU) is expected to be the same.

H$_1$ (Alternate Hypothesis)- There is difference in the mean ranks of ages between the groups (only pneumonia, only PU, with pneumonia and PU; and no pneumonia and no PU).

The significance level was set at α = 0.05.

Chi-Square Test

It is a nonparametric test that is performed on nominal (gender male/female), and ordinal (ASIA scale score, A, B, C, D and E) data. The Chi-square statistic (two-tailed) was performed to test differences between the level of injury (and gender) in the two groups (those who developed PUs and those who did not develop PU). The chi-square statistic is[88]:

$$\chi^2 = \sum \frac{(f_0 - f_e)^2}{f_e}$$

- f_o is observed frequency of ASIA (or gender)

- f_e is expected frequency (or gender)

Our hypotheses are:

H₀ (Null Hypothesis): The severity of injury measured by ASIA scale score (or gender) and individuals who developed PUs are not related.

H₁ (Alternate Hypothesis): The severity of injury measured by ASIA scale score (or gender) and individuals who developed PUs are related.

The significance level was set at $\alpha = 0.05$.

A chi-square test was also performed to test the association between presence of pneumonia and occurrence of PU as compared to no pneumonia.

Hₒ (Null Hypothesis) – The presence of pneumonia and occurrence of PUs are not related. To reject the null hypothesis the observed frequency (of individuals with pneumonia having PUs) should be closer to the expected frequency.

H₁ (Alternate Hypothesis): The presence of pneumonia and occurrence of PUs are related.

The significance level was set at $\alpha = 0.05$.

Relative Risk Ratio

The relative risk ratio which is the ratio of the incidence of disease (PU) to the exposed subjects (individuals with pneumonia) to the incidence of disease (PU) to the unexposed (no pneumonia) was calculated for acute care population and inpatient population with the CI estimates. The

relative risk greater than 1.0 will indicate that pneumonia increases risk for the incidence of PU[80].

$$\text{Relative Risk} = \frac{CI_E}{CI_O} = \frac{a/(a + b)}{c/c + d}$$

- a is subjects who have PU and pneumonia
- b subjects who did not have PU but had presence of pneumonia
- c is subjects with PU and no pneumonia
- d is subjects with no pneumonia and no pressure ulcer

5.2.3   Results

Out of the 104 individuals recruited in RERC on SCI, data for 86 individuals were analyzed in acute care and inpatient rehabilitation. The rest of the individuals were excluded since they withdrew from the study in less than three weeks.

The demographics and characteristics for the total population included in this study and the individual groups are listed in (Table 27)

5.2.3.1   Age and Gender

The study population's mean age was 40 years. The Kruskal-Wallis test produced no significant difference in age among the four groups (p= 0.144). Most of the individuals were males (81%). A chi-square test revealed no significant differences in gender among the four groups (p=0.644).

5.2.3.2 Injury Severity

A majority of individuals (43%) in this population were classified as AISA A in the AIS scale. A chi-square test revealed significant differences in the severity of injury between all four groups in this population, p=0.01. Most of the subjects who had pneumonia and PU had ASIA A as their severity of injury as compared to subjects who did not have any of the two conditions

5.2.3.3 Pneumonia and Pressure Ulcer

The mean onset of pneumonia in the total population was 8.9 ± 1.1 days. There were significant differences in the mean onset of pneumonia between individuals who had PUs as compared to individuals with no PUs, p=0.02. Individuals with pneumonia and PU had a later onset of pneumonia (11.25 ± 1.8 days) as compared to those who had only pneumonia and no PU (6 ± 0.69 days). The mean onset of PU in the total population was 19.6 ± 1.8 days. There were no significant differences observed in the mean onset of PU between individuals who had pneumonia as compared to individuals with no pneumonia, p=0.4 (Table 27). Out of the 86 individuals in acute care through inpatient rehabilitation, 20 had pneumonia coinciding with or preceding the PUs, 16 had only pneumonia but no PUs, 15 had only PUs but no pneumonia and 35 individuals did not have PUs and pneumonia Table 28.

The chi-square test produced significant results for the presence of pneumonia coinciding with or preceding PU incidence in persons with SCI during acute care through inpatient rehabilitation χ^2 (1) =5.664, p=.017. The relative risk to develop PUs was found to be 1.9 in subjects diagnosed with pneumonia as compared to no pneumonia. The individuals who had pneumonia coinciding with or preceding the PU were more likely to be males (90%) with ASIA A's and their mean age is younger (32 years) as compared to the entire population (40 years).

Table 27. Demographics (Means and SEM) for the RERC on SCI population

Demographics	Total N=86	Pneumonia with PU n=20	PU no pneumonia n=15	Pneumonia no PU n= 16	No PU & no pneumonia n=35	P value
Age (years) Mean ± SEM	39.9 ± 1.9	32.1±3.1	42.4±5	39.9±4.5	43.4±3	0.144
Gender	M=70(81.4%) F=16(18.6%)	M=18(90%) F=2(10%)	M=11(73.3%) F=4(26.7%)	M=13(81.2%) F=3(18.8%)	M=28 F=7	0.644
ASIA	A=37(43%) B=12(14%) C=21(24.4%) D=13(15.1%) E=1(1.2%) U=2(2.3%)	A=15(75%) B=1(5%) C=3(15%) D=0(0%) E=0(0%) U=1(5%)	A=8(53.3%) B=2(13.3%) C=1(6.8%) D=4(26.7%) E=0(0%) U=0(0%)	A=8(50%) B=3(18.8%) C=4(19%) D=0(0%) E=0(0%) U=1(6.2%)	A=6(17.1%) B=6(17.1%) C=13(37.1%) D=9(25.7%) E=1(2.9%) U=0(0%)	0.01*
Onset of Pneumonia (days) Mean ± SEM	8.9±1.1	11.25±1.8	-	6±0.69	-	0.017*
Onset of PU (days) Mean ± SEM	19.6±1.8	20.4±2.1	18.5 ± 3.4	-	-	0.404
Injury Severity Score (ISS)	24.4 ± 1.9	39.7±4.1	29.5±5	26.8±4.2	20.7±2	<0.01**

Note: *p< 0.05, **p<0.01

Table 28. 2 X 2 Contingency table, Frequency distribution in the four groups for the RERC on SCI population

Risk	Pressure Ulcer	
	Present	**Absent**
Pneumonia	20	16
No Pneumonia	15	35

5.3   SCIMS POPULATION

The National Spinal Cord Injury Database (NSCID) has data comprising of individuals with SCI who are treated at the Model SCI Systems. The NSCID started in 1973 and has an estimated data of 13% of new cases in the US of individuals with SCI. The database has information on 28,711 as of May 2012. Two sets of data are collected in this System. Form I data are collected once for all individuals that includes demographic data and data and intervention outcomes in acute care and rehabilitation. Data on Form II is collected annually for a period of 5 years to reflect changes that occurred over the year and also the present status of the individual. The data collection for this project is an ongoing process. Data on presence of pneumonia and formation of PUs in individuals with spinal cord injury Model Systems (SCIMS) database in the acute care and rehabilitation are the variables of interest for this study. These variables were recorded during the 2000-2005 cycle in Form I. This project is funded by NIDRR and 16 federally funded Model SCI Care Systems centers contributed to the data collected during the 2000-2005 cycle. In order to get access to the Model Systems data, a proposal was submitted to the Model Systems directors via an internal pathway in November 2012. Data on presence of pneumonia and PUs in individuals in the SCIMS database in the acute care unit from Form I was requested. The project director in Pittsburgh, Dr. Michael Boninger requested the data in writing and the data were released to the investigators involved with this project. Analysis on the subjects in the RERC on SCI suggested that association exists between presence of pneumonia and incidence of PUs. The purpose of this study was to investigate an association between pneumonia and PUs in individuals with SCI in the SCIMS population.

5.3.1   Methods

5.3.1.1   Research Design

Retrospective cohort design

5.3.1.2   Inclusion and Exclusion Criteria

Individuals with SCI were eligible in the Model Systems study if they had the following inclusion criteria:

1. All persons must have a clinically discernible degree of neurologic (spinal cord) impairment following a traumatic event

2. All persons must receive System acute inpatient rehabilitation

3. All persons must be treated at a Model System within 1 year of injury

4. All persons must not have been previously treated at a Model System post-injury

5. A signed Informed Consent and HIPAA Authorization

6. All persons must reside in the geographic catchment area of the Model System at the time of the injury. Subjects may be injured outside the catchment area

7. Must be a citizen of the United States

5.3.1.3   Data Collection

Form I included data collected at acute care and acute inpatient rehabilitation. Form II includes data collected annually. Form I collected data on demographic information, complete neurologic exam at the time of initial admit to system, admit to the system inpatient rehabilitation and

during discharge, bladder management and mechanical ventilation during the system inpatient rehabilitation and discharge, location and stages of PUs, complication such as pneumonia.

For this study the following data was requested and retrieved from Form I during the term period between 2000 and 2005. Date of birth, age at injury, sex, date of first system admission, date of first system Inpatient rehab admission, number of days from injury to first system admission, number of days from injury first system Inpatient rehab admission, date of injury, date of discharge, number of days hospitalized in acute care and rehab care unit, traumatic etiology, external cause of injury, SCI nature of etiology, spinal cord nature of injury, ASIA impairment scale, ASIA motor index score, sensory and motor level, level of preserved neurological function, number of episodes of pneumonia, utilization of mechanical ventilation, number of PUs, location of PUs, grade of PUs, presence of pneumonia and incidence of PUs

5.3.1.4 Procedure

The subjects who had missing or no data on the presence of pneumonia or occurrence of pressure ulcer were excluded from the study. The presence of pneumonia and PUs was noted in acute care setting and inpatient rehabilitation. Subjects were grouped according to the presence of pneumonia and PU incidence as:

1. Pneumonia and PU

2. Pneumonia and no PU

3. PU and no Pneumonia

4. No PU and no Pneumonia

At the time of measurement and collection of data the severity of injury was changed from Frankel's Scale to AIS. The comparison between the scales is shown in Table 29. For this

151

study the individuals that have Frankel's grade was converted to AIS and the severity of injury was compared between the four groups. The AIS and Frankel's grade is similar, just that the AIS considers sacral sparing definition on incomplete as compared to Frankel's scale [251, 252]. Data for 24,763 for individuals with SCI was obtained in January 2013. After eliminating all the missing or no data collected on the presence of pneumonia and PUs, there were data for 3,887 subjects during the acute hospitalization and 11,022 individuals during inpatient rehabilitation.

Since the study is retrospective the sample sizes were not estimated by using power analysis to obtain a desired power prior to data collection. We estimated the number of subjects needed for each proposed aim with a significance level of $\alpha = 0.05$, a medium effect size and a power of 80%. Medium effect size was selected given the variability is observed in the clinical variables and there is no standardization in measuring these variables [253]. A sample size of 88 was estimated using Gpower3.1. The number of subjects for this study in the existing database was >1000. Hence, the sample size was sufficient for the desired power.

5.3.1.5 Data Analysis

All data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0.

Kruskal-Wallis test

The Kruskal-Wallis is a version of independent measures (one-way) ANOVA that is performed on data that is not normally distributed. The two-tailed, Kruskal-Wallis one-way analysis of variance was performed to test significant differences in ages between the four groups (only pneumonia, only PU, with pneumonia and PU; and no pneumonia and no PU)[80]. The Kruskal-Wallis statistic is:

$$H = \frac{12}{N(N+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(N+1)$$

- N is total sample size

- k is total number of groups

- i is to denote the particular group

- R_i is sum of ranks for each group

- n_i is the sample size of that particular group

H₀ (Null Hypothesis) – The mean ranks from the populations in the four groups (only pneumonia, only PU, with pneumonia and PU; and no pneumonia and no PU) is expected to be the same.

H₁ (Alternate Hypothesis)- There is difference in the mean ranks of ages between the groups (only pneumonia, only PU, with pneumonia and PU; and no pneumonia and no PU).

The significance level was set at α = 0.05.

Chi-Square Test

It is a nonparametric test that is performed on nominal (gender male/female), mechanical ventilation (yes/no) and ordinal (ASIA scale score, A, B, C, D and E) data. The Chi-square statistic (two-tailed) was performed to test differences between the level of injury (and gender, mechanical ventilation) in the two groups (those who developed PUs and those who did not develop PU). The chi-square statistic is[80, 88]:

$$\chi^2 = \sum \frac{(f_0 - f_e)^2}{f_e}$$

- f_0 is observed frequency of ASIA (or gender)

153

- f_e is expected frequency (or gender)

Our hypotheses are:

H₀ (Null Hypothesis): The severity of injury measured by ASIA scale score (or gender, mechanical ventilation) and individuals who developed PUs are not related.

H₁ (Alternate Hypothesis): The severity of injury measured by ASIA scale score (or gender, mechanical ventilation) and occurrence of PUs is related.

The significance level was set at $\alpha = 0.05$.

A chi-square test was also performed to test the association between presence of pneumonia and occurrence of PU as compared to no pneumonia across all ASIA scale score levels.

H₀ (Null Hypothesis) – The presence of pneumonia and occurrence of PUs are not related. To reject the null hypothesis the observed frequency (of individuals with pneumonia having PUs) should be closer to the expected frequency.

H₁ (Alternate Hypothesis): The presence of pneumonia and occurrence of PUs are related.

The significance level was set at $\alpha = 0.05$.

Cramer's V

To measure the degree of association a correlation coefficient, Cramer's V was calculated. Cramer's V measures strength of association between two nominal variables, and gives a value between ± 1[80]. The Cramer's V statistic is:

$$V = \sqrt{\frac{\chi^2}{N(q-1)}}$$

- Total number of subjects

- q is number of rows or columns

- χ^2 is the chi-square statistic obtained from the chi-square test.

H$_o$ (Null Hypothesis) – The presence of pneumonia and occurrence of PUs are not related.

H$_1$ (Alternate Hypothesis): The presence of pneumonia and occurrence of PUs are related.

The significance level was set at α = 0.05.

Relative Risk Ratio

For this cohort study the cumulative incidence estimates for exposed (CI$_E$) group that is the number of cases having PU among total number of subjects having pneumonia and unexposed groups (CI$_0$) that is the number of subjects having PU among the subjects who don't have pneumonia was calculated. The relative risk ratio which is the ratio of the incidence of disease (PU) to the exposed subjects (individuals with pneumonia) to the incidence of disease (PU) to the unexposed (no pneumonia) was calculated for acute care population and inpatient population with the CI estimates. The relative risk greater than 1.0 will indicate that pneumonia increases risk for the incidence of PU[80].

$$\text{Relative Risk} = \frac{CI_E}{CI_O} = \frac{a/(a+b)}{c/c+d}$$

- CI$_E$=Cumulative incidence estimate for exposed (pneumonia) group

- CI$_0$= Cumulative incidence estimate for unexposed (no pneumonia)group

- a is subjects who have PU and pneumonia

- b subjects who did not have PU but had presence of pneumonia

- c is subjects with PU and no pneumonia

- d is subjects with no pneumonia and no pressure ulcer

Table 29. Comparison between AIS and Frankel Scale Score [252].

Scale Grade	Frankel Scale	ASIA Impairment Scale (AIS)
A	Complete- All motor and sensory function is absent below the zone of partial preservation	Complete- No motor or sensory function is preserved in the sacral segments S4-S5.
B	Incomplete, preserved sensation only- preservation of any demonstrable, reproducible sensation excluding phantom sensation (must be present more than 3 levels below the injury). Voluntary motor functions are absent.	Incomplete- Sensory but not motor function is preserved below the neurologic level and includes the sacral segments S4-S5.
C	Incomplete, preserved motor nonfunctional- preservation of voluntary motor function that is minimal and performs no useful purpose. Minimal is defined as preserved voluntary motor ability below the level of injury where the majority of the key muscles test less than a grade of 3.	Incomplete- Motor function is preserved below the neurologic level, and at least half of key muscles below the neurologic level have muscle grade of 3 or more.
D	Incomplete, preserved motor functional- preservation of voluntary motor function which is useful functionally. This is defined as preserved voluntary motor ability below the level of injury, where the majority of key muscles test at least a grade of 3.	Incomplete-Motor function is preserved below the below the neurologic level, and at least half the key muscles below the neurologic level have muscle grade of 3 or more.
E	Complete return of all motor and sensory function, but one may still have abnormal reflexes.	Normal- Motor and sensory function is normal.

5.3.2 Results

The demographics and characteristics of individuals in the four groups in acute care setting and inpatient rehabilitation are listed in Table 30 and Table 33 respectively.

5.3.2.1 Age and Gender

The individuals in the acute care setting were older (38 years) than the individuals in the inpatient rehabilitation (34 years). The distribution of ages in the four groups in acute care inpatient setting is listed in Table 30 and Table 33 respectively. No significant difference in ages between the four groups in acute care setting that was produced by Kruskal-Wallis test. Kruskal-Wallis produced a significant difference in ages between the four groups in individuals in the inpatient setting (p=0.004). Majority of individuals in acute care setting and inpatient rehabilitation were males. The distribution of gender in the four groups in acute care setting and inpatient rehabilitation is listed in Table 30 and Table 33 respectively. Chi-square test produced significant differences in gender distribution between the four groups in acute care setting (p=0.017) and inpatient rehabilitation (p=.00).

5.3.2.1 Injury Severity

The severity of injury is for all the four groups in acute care and inpatient rehabilitation is listed in Table 30 and Table 33 respectively. Chi-square test detected significant differences in the severity of injury between the four groups during acute care (p<0.001) and inpatient rehabilitation (p<0.001). Individuals in acute care were mostly ASIA A's (43%). The severity of injury for most of the subjects in inpatient rehabilitation was unknown.

5.3.2.2 Mechanical Ventilation

Of the 11,022 individuals in inpatient rehabilitation, 18% of the individuals were on mechanical ventilation. Most of the individuals (55%) who had both pneumonia and pressure ulcer were on mechanical ventilation. Chi-square test revealed significant differences in all four groups during the inpatient rehabilitation with use of mechanical ventilation< 0.001 (Table 33).

5.3.2.3 Pressure Ulcer and Pneumonia in Acute Care

The location of PUs in inpatient rehabilitation is listed in Table 31. 1248 PUs were observed in individuals during acute care. Most of the PUs were present on sacral region (47%), followed by the heel, occiput and the ischium. Out of the 3887 individuals in acute care setting, 451 has presence of both pneumonia and PU, 361 had formation of PU and no pneumonia, 739 had presence of pneumonia but no PU and 2336 did not have any of the two comorbidities (Table 32). The chi-square test produced significant results for the presence of pneumonia and formation of PUs. χ^2 (1, n=3887) = 300.24, p=0.00. Cramer's V was 0.278, p<0.001. The cumulative incidence (CI) of PU for the group exposed to pneumonia is 0.38, and the CI of PU for the group not exposed to pneumonia was 0.13. The relative risk to develop PUs was 2.9 in individuals with presence of pneumonia as compared to no pneumonia. The individuals with pneumonia and PU in acute care were mostly males; ASIA A's and their mean age was 38 years (Table 30)

Out of the total individuals in acute care hospitalization, 1,671 had ASIA A scale score, 511 had ASIA B, 599 had ASIA C and 581 had ASIA D (Table 30). The Chi-Square tests were performed across all ASIA scale score levels for individuals in acute care hospitalization. The chi-square test produced significant results for the presence of pneumonia and formation of PUs;

in individuals with ASIA A levels, $\chi2$ (1) = 95.98, p<0.001, ASIA B levels $\chi2$ (1) = 31.35, p<0.001, ASIA C levels $\chi2$ (1) = 23.14. The relative risk to develop PUs was 2.2 in individuals with presence of pneumonia as compared to no pneumonia with ASIA scale score A. The relative risk to develop PUs was 2.6 in individuals with presence of pneumonia as compared to no pneumonia with ASIA scale score B and C. Although the chi-square statistic did not reveal significant association between the presence of pneumonia and formation of PU, in individuals with ASIA scale score D; the relative risk to develop PUs was 1.8 in individuals with presence of pneumonia as compared to no pneumonia in these individuals.

5.3.2.4 Pressure Ulcers and Pneumonia in Inpatient Rehabilitation

The location of PUs in inpatient rehabilitation is listed in Table 28. 4501 PUs were observed in individuals during inpatient rehabilitation. Most of the PUs were present on sacral region (37%), followed by the heel, ischium and the occiput.

Of the 11,022 individuals in inpatient rehabilitation, 875 has presence of both pneumonia and PU, 1987 had formation of PU and no pneumonia, 1169 had presence of pneumonia but no PU and 6991 did not have any of the two comorbidities (Table 34). The chi-square test produced significant results for the presence of pneumonia and formation of PUs. χ^2 (1, n=11022) = 370.261, p=.00. Cramer's V was 0.183 p<0.001. The cumulative incidence (CI) of PU for the group exposed to pneumonia is 0.43, and the CI of PU for the group not exposed to pneumonia was 0.22.The relative risk to develop PUs was 1.9 in individuals with presence of pneumonia as compared to no pneumonia. The individuals with pneumonia and PU in inpatient rehabilitation were males and their mean age was 35 years (Table 33). Individuals with pneumonia and PU and

individuals with pneumonia were older than the individuals who did not have presence of pneumonia in the inpatient rehabilitation.

Out of the total individuals in inpatient rehabilitation, 790 had ASIA A scale score, 260 had ASIA B, 322 had ASIA C and 408 had ASIA D (Table 33). The Chi-Square tests were performed across all ASIA scale score levels for individuals in inpatient rehabilitation. The chi-square test produced significant results for the presence of pneumonia and formation of PUs; in individuals with ASIA A levels, $\chi2$ (1) = 4.41, p = 0.03, ASIA B levels $\chi2$ (1) = 3.91, p = 0.048, ASIA C levels $\chi2$ (1) = 5.881, p = 0.01; and ASIA D levels $\chi2$ (1) = 6.12, p = 0.01. The relative risk to develop PUs in presence of pneumonia was 1.4 in individuals with ASIA scale score A, 2.1 in individuals with ASIA scale score B, 2.5 in individuals with ASIA scale score C and 7.8 in individuals with ASIA scale score D, when compared to no pneumonia.

Table 30. Demographics (Means and SEM) - SCIMS population- Acute Care

Demographics	Total N=3887(%)	Pneumonia with PU n=451(%)	PU no pneumonia n=361(%)	Pneumonia no PU n= 739(%)	No PU & no pneumonia n=2336(%)	p value
Age (years) Mean ± SEM	37.89±0.28	38.9±0.82	37.7±0.91	38.86±0.65	37.42±0.35	0.162
ASIA	A=1671(43%) B=511(13.1%) C=599(15.4%) D=581(14.9%) U=525(13.5%)	A=255(56.5%) B=52(11.5%) C=33(7.3%) D=7(1.6%) U=104(23.1%)	A=179(49.6%) B=50(13.9%) C=51(14.1%) D=29(8%) U=52(14.4%)	A=397(53.7%) B=94(12.4%) C=86(11.6%) D=59(8%) U=103(13.9%)	A=840 (36%) B=315 (13.5%) C=429 (18.4%) D=486 (20.8%) U=266(11.4%)	0.00*
Gender	M=3102(80%) F=785(20%)	M=382 F=69	M=289 F=72	M=599 F=140	M=1832 F=504	0.017*

Note: U=Unknown *p<0.05, **P<0.01

161

Table 31. Location of PUs in Acute Care and Inpatient Rehabilitation

PU Location	Acute Care 3,887 (%)	Inpatient Rehabilitation 11,022(%)
Occiput	82(6.5%)	154(3.4%)
L Scapula	33(2.6%)	104(2.3%)
R Scapula	23(1.8%)	103(2.2%)
L elbow	8(0.6%)	53(1.1%)
R elbow	8(0.6%)	49(1%)
L ribs	5(0.4%)	20(0.4%)
R ribs	4(0.3%)	23(0.5%)
Spine	13(1%)	85(1.8%)
L iliac crest	5(0.4%)	39(0.8%)
R iliac crest	5(0.4%)	32(0.7%)
Sacral	585(46.8%)	1659(36.8%)
L ischium	47(3.7%)	209(4.6%)
R ischium	47(3.7%)	232(5.1%)
L trochanter	11(0.8%)	70(1.5%)
R trochanter	5(0.4%)	62(1.3%)
Genital	13(1%)	147(3.2%)
L knee	11(0.8%)	34(0.7%)
R knee	8(0.6%)	44(0.9%)
L malleolus	8(0.6%)	66(1.4%)
R malleolus	12(0.9%)	52(1.1%)
L heel	98(7.8%)	398(8.8%)
R heel	92(7.3%)	350(7.7%)
L foot	34(2.7%)	121(2.6%)
R foot	29(2.3%)	125(2.7%)
L unclassified	16(1.2%)	102(2.2%)
R unclassified	21(1.6%)	78(1.7%)
C unclassified	25(2%)	90(1.9%)

Table 32. 2 X 2 Contingency table, Frequency distribution in the four groups - SCIMS population- Acute Care Setting, n=3887

Risk	*Pressure Ulcer*	
	Present	**Absent**
Pneumonia	451	739
No Pneumonia	361	2336

Table 33. Demographics (Means and SEM) - SCIMS population- Inpatient Rehabilitation

Demographics	*Total N=11022(%)*	*Pneumonia with PU n=875(%)*	*PU no pneumonia n=1987(%)*	*Pneumonia no PU n= 1169(%)*	*No PU & no pneumonia n=6991(%)*	*p value*
Age (years) Mean ± SEM	34.39±0.76	35.01± 0.54	33.58±0.36	35.25±0.49	34.4±0.19	0.004**
ASIA	A=790(7.2%) B=260(2.4%) C=322(3%) D=408(3.7%) U=9242(84%)	A=21(2.8%) B=5(0.6%) C=6(0.7%) D=1(0.1%) U=842(96.2%)	A=218(11%) B=57(2.9%) C=42(2.1%) D=26(1.3%) U=1644(82.7%)	A=27(2.3%) B=5(0.4%) C=11(0.9%) D=1(0.1%) U=1125(96.2%)	A=524(7.5%) B=193(2.8%) C=263(3.8%) D=380(5.4%) U=5631(80.5%)	<0.01**
Mechanical Ventilation	N=7632(69.2%) Y=1992(18.1%) U=1398(12.7%)	N= 311(35.5%) Y= 483(55.2%) U= 81(9.3%)	N=1366(68.7%) Y=360(18.1%) U=261(13.1%)	N=595(50.9%) Y=440(37.6%) U=134(11.5%)	N=5360(76.7%) Y=709(10.1%) U=922(13.2%)	<0.01**
Gender	M=8916(81%) F=2160(19%)	M=748 F=127	M=1692 F=295	M=928 F=241	M=5548 F=1443	<0.01**

Table 34. 2 X 2 Contingency table, Frequency distribution in the four groups - SCIMS population- Inpatient Rehabilitation (n=11,022)

Risk	*Pressure Ulcer*	
	Present	**Absent**
Pneumonia	875	1169
No Pneumonia	1987	6991

5.4 DISCUSSION

The results of these studies investigated the association between presence of pneumonia and formation of PUs in acute care through inpatient rehabilitation in RERC on SCI population as a preliminary analysis and confirmed the findings in a larger population, SCIMS; in both the acute care and inpatient setting. The hypothesis that there are significant differences in the formation of pressure ulcers in individuals with pneumonia as compared to individuals who do not have pneumonia is retained based on the results of these studies in both RERC on SCI and SCIMS population. The individuals with pneumonia have a moderate degree of association for the formation of PUs.

Eighty-six individuals in the RERC on SCI population were followed from the acute care hospitalization through inpatient rehabilitation. The individuals who had pneumonia had an association with the occurrence of PUs within three weeks after diagnosis of pneumonia than those who did not have pneumonia. The risk to develop PUs in individuals with pneumonia in this population was twice as compared to the individuals who did not have pneumonia.

To confirm the results of this preliminary analysis attained in the RERC on SCI population, the data were obtained for the same variables from the SCIMS database during the time period 2000 to 2005. The results of the SCIMS population in acute care and inpatient rehabilitation confirmed the results of the RERC on SCI population. Data analyzed for 3,887 individuals in the acute care SCIMS population showed significant association for the formation of PUs in individuals who had pneumonia as compared to individuals who did not have pneumonia. The risk of developing PUs for these individuals who had presence of pneumonia in SCIMS acute care population was thrice as compared to individuals who did not have

pneumonia. The analysis was repeated for 11,022 individuals in the SCIMS inpatient population and provided similar results. The risk of developing PUs for individuals who had presence of pneumonia in SCIMS inpatient population was twice as much as individuals who did not have pneumonia. In the SCIMS population, individuals in the acute care population were at a greater risk to develop pneumonia than the inpatient population.

The cervical or thoracic level of spinal cord injuries leads to respiratory compromise. This critical care management is imperative during the acute care immediately after spinal cord injury. The respiratory compromise and impaired airway secretion clearance predisposes the individual to pneumonia and/ or other pulmonary conditions. This airway compromise is managed with the help of mechanical ventilators to improve the pulmonary function. Studies have shown that the use of mechanical ventilators during acute care for more than 24 hours increases the risk to develop nosocomial infections. Hence individuals in acute care unit who are on mechanical ventilation were prone to ventilator associated pneumonia [254]. This can explain the increased risk to develop PUs in acute care as compared to inpatient SCIMS population. The data for mechanical ventilation for the acute care in SCIMS is was not collected. Majority of individuals in the inpatient SCIMS population who had presence of both PU and pneumonia were on mechanical ventilation. Literature shows the supine or semi-recumbent positioning of the individuals on ventilators increases the risk for the formation of PUs [63, 255]. The use of these external ventilators in individuals with pneumonia may limit mobility of the individuals, increase the pressure on their bony prominences and decrease the oxygenation [256, 257]. This could probably explain the increased association of PUs in individuals with pneumonia in acute care and inpatient rehabilitation in both the RERC on SCI and SCIMS population.

Literature that addresses risk factors for the development of PUs generally considers all the medical comorbidities as one factor while assessing the potential risks [257]. Pulmonary embolism, deep vein thrombosis, pneumonia or atelectasis and kidney stones were some of the clinical factors reported by previous studies to be associated with the formation of PUs [15, 56]. The finding in our study is supported by previous studies that indicated association of pulmonary disease with the formation of PUs [19, 56]. Among the pulmonary conditions one study showed association between chronic obstructive pulmonary disease and formation of PUs [232]. Although previous studies attributed pulmonary as one of the clinical factors towards developing a PUs, pneumonia was not found to be a significant risk factor [22, 258]. Even though studies have linked pulmonary disease as a possible risk factor to develop PU their pathology is not fully understood. An intrinsic response associated with the presence of pneumonia may leave individuals with SCI immunocompromised. This predisposes them to secondary complications such as infections, thus causing skin breakdown.

Ventilation perfusion ratio is the ratio of amount of air to the amount of blood reaching the alveoli. During the normal process of gaseous exchange, the lungs allow the oxygen from the inhaled air into the blood and transfer the carbon dioxide from the blood to the exhaled air [259]. Pneumonia is an inflammatory condition of the lung due to the infection in the alveoli. In individuals with pneumonia, the gas exchange functions of the lungs decline. This is initially localized to one lung during the early stage of the disease that causes an overall reduction in the alveolar ventilation. The impaired ventilation in these individuals causes an imbalance in the ventilation perfusion ratio. This leads to decrease in oxygen (hypoxia) and increased carbon dioxide (hypercapnia) in the blood. [260]. The oxygenated blood supplies oxygen and nutrients to the tissues. Impaired circulation due to decreased oxygen in the blood causes the tissues to

167

starve. Also, oxygen is essential for healing and repair and previous literature decreased oxygen in the tissue to be one of the causes of PUs [261, 262]. Studies have shown decreased oxygen to be a predictor for the formation of PUs [263]. This could probably explain why individuals with pneumonia have had increased formation of pressure ulcers as compared to individuals with no pneumonia. In individuals with SCI, it is essential prevent medical conditions such as pneumonia that affect the tissue oxygenation to implement precautionary measures that can help decrease the risk of occurrence of PUs.

5.4.1 Limitations

Given that this is an existing database, there are potential limitations in both the RERC on SCI and SCIMS studies. The research designs for the RERC on SCI study was secondary analysis and for the SCIMS study was retrospective. Hence the inherent limitations of the secondary data analysis should be kept in mind. In SCIMS study, the variables of interest were collected during the 2000-2005 time period. The longitudinal changes that have taken place in the patient-care, co-morbidities and patient demographics cannot be ignored. We hypothesize the association between presence of pneumonia and pressure ulcers in these individuals may be due to an intrinsic and not an extrinsic mechanism and hence this probably should not be a concern. Also, due to the changing focus of model systems database the measuring of the severity of injury was changed from Frankel's grade to AIS in 1993. To analyze the injury severity, the Frankel's grade was converted to AIS. The AIS uses sacral spring to define the incomplete injury as compared to Frankel's that uses preservation of sensation at least 3 levels below the injury level. There could have been potential misclassification in AISA A and B.

The time-points for the comorbidities were not defined in the SCIMS population. Hence we could only see if the individuals had presence or absence of the comorbidities (pneumonia and pressure ulcer) and were not able comment on their causal relationship. In the RERC on SCI population, the sequence and timing of pneumonia and PU was considered. Individuals who had formation of PU within three weeks of diagnosis of pneumonia were considered in the group of interest whereas in the SCIMS population any individual who had presence of both pneumonia and PU were considered in the group of interest. Also, the data analyzed for this study for the RERC on SCI population was on individuals who were flowed from acute care through inpatient rehabilitation. The data were analyzed separately for the acute care setting and impatient setting for the SCIMS population. Hence both the RERC and SCI and SCIMS populations were not completely homogenous.

5.5   CONCLUSION AND FUTURE WORK

The findings suggest an association between the presence of pneumonia and formation of pressure ulcer. The results indicate that a higher proportion of pressure ulcers occur in individuals with SCI having pneumonia. Thus, surveillance and prevention measures for pressure ulcers should be even more rigorous in those individuals. The association between presence of pneumonia and presence of PU could be because of restriction of mobility after pneumonia, decreased oxygenation or imbalance in the inflammatory response after presence of pneumonia. To investigate if the presence of pressure ulcers in individuals with pneumonia is linked through inflammation, a follow up study was performed (Chapter 6). Further studies should be conducted to test if the incidence of PU in individuals with pneumonia can be attributed to decreased oxygenation or mobility restriction.

6.0 INFLAMMATORY MEDIATORS ASSOCIATED WITH PRESSURE ULCER DEVELOPMENT IN INDIVIDUALS WITH TRAUMATIC SPINAL CORD INJURY AND PNEUMONIA

6.1 INTRODUCTION

Spinal cord injury is a devastating neurologic disorder that has profound impact from physical, psychological and socioeconomic perspectives [44]. Increase in age and severity of injury are associated with rise in long-term complications after traumatic SCI [79]. The second leading cause of death in individuals with SCI is septicemia (88.6%); usually associated with urinary tract infections (UTI's), pneumonia, and/or presence of pressure ulcers [45]. Pressure ulcers (PUs) are the most frequent secondary complication in individuals with SCI from the time of acute hospitalization through community reintegration. PUs affect quality of life, length of stay during hospitalization and increases the mortality and morbidity [1, 7, 12, 48, 49].

Around 200 risk factors are associated with the formation of PUs in individuals with SCI [8]. Many risk factors have been associated with the formation of pressure ulcers. Medical complications such as cardiac or renal disease were associated with the risk of developing PUs [8]. Individuals likely to develop PUs were those who have SCI of traumatic origin [17], males [18], have a history of smoking, alcohol or drug use [10], have medical comorbidities such as diabetes mellitus [19, 20], decreased oxygenation or hypotension [16], infections such as

171

pneumonia, urinary tract infections, osteomyelitis, and other bacterial infections [8, 21], those on mechanical ventilators[22] and use of steroids [16]. Moisture and/or urinary and fecal incontinence, hypo/hyperthermia, friction, shear, were reported to be the extrinsic risk factors associated with formation of PUs in individuals in acute care and intensive care unit (ICU) [16, 19, 51-55]. Decreased nutrition or low serum albumin levels, decreased mobility and sensation, and impaired cognitive function contribute towards the intrinsic risk factors for the development of PUs in individuals with SCI [19, 20]. The SCI Quality Enhancement Research Initiative (QUERI) projects points out the importance and challenge in identification of all the risk factors in individuals with SCI and implementing corresponding preventive techniques in the clinical practice [34].

Pneumonia is an inflammatory condition of the lung, specifically inflammation of the alveoli (microscopic air sacs in the lungs) or when the lungs are filled with fluid (called *consolidation* and *exudation*). There are many causes for the occurrence of pneumonia, infection being the most common. The infecting agents can be bacteria, viruses, fungi, or parasites [57, 58]. Pneumonia is the most frequent respiratory complication (66.9% cases) that occurs weeks after the SCI [45, 59, 60]. Around 30% of individuals with SCI develop pneumonia during the time of hospitalization [50]. Of all the secondary conditions after SCI, pneumonia is one of the most frequent causes of death [46, 47]. Overall 5% to 20% of individuals with SCI develop pneumonia during initial rehabilitation. Studies have shown pneumonia to be associated with atelectasis in these subjects that alters the pattern of respiration. Prior anesthesia increases the risk of developing pneumonia [61]. The incidence of ventilator associated pneumonia increases within days of intubation for individuals on mechanical ventilation [63].

Subjects with high-level traumatic SCI are usually dependent on mechanical ventilation, and report an increase in incidence of pneumonia within days of intubation [22, 63]. Significant correlations between individuals with SCI having PUs and pneumonia (or atelectasis) were observed after 1, 2 and 5 years of injury [37]. The diaphragm is the main inspiratory muscle of respiration and is supplied by the phrenic nerve that originates from third and extends to fifth cervical nerve (C3-C5). The muscles of respiration such as the diaphragm, abdominals and intercostal are affected with progressively higher level of SCIs, resulting in a higher incidence of pneumonia in individuals with tetraplegia than paraplegia [61]. Individuals with complete tetraplegia have altered lung volumes and ineffective cough, and these individuals present with a restrictive ventilatory pattern. The alteration in lung volumes decreases the lung compliance and increases the cost of energy, predisposing the individual to respiratory fatigue. Therefore, higher-level SCIs (tetraplegics at C3-C5) may produce complete respiratory paralysis [62].

The synthesis of the inflammatory biomarkers increases in individuals with the presence of PUs [5], and also in individuals having respiratory infections such as pneumonia [39]. However, an apparent association linked through inflammation has not yet been explored. Presence of pneumonia activates an acute inflammatory response to counteract the infection, which when not in balance may result in secondary complications such as PUs. TNF-α was one of the early mediators in the inflammatory process and induces activation of pro- and anti-inflammatory mediators and is responsible for the protective response in pneumonia. Cytokines such as IL-1β, IL-6, and IL-8 were correlated with TNF-α in subjects having pneumonia [40]. The inflammatory response in individuals with SCI may be different during the inception of pneumonia and days after the presence of pneumonia; as previous studies have shown temporal changes in the inflammatory response from onset of pneumonia [40]. Increased concentrations of

IL-1 and IL-10 were also found to be associated in individuals with pneumonia [3, 39]. These differences in the inflammatory response may help explain the formation of PUs in these individuals. The aim of this study was to explore and identify the change in the inflammatory mediators in urine and plasma, before and after the presence of pneumonia associated with formation of PUs in individuals with SCI during acute care hospitalization and inpatient rehabilitation. We hypothesized that differences in inflammatory mediators measured before and after the presence of pneumonia of TNF-α, IL-1β, IL-6, IL-8 and IL-10 will indicate increased risk for the formation of PUs.

6.2   METHODS

6.2.1   Research Design

The RERC database was used here in a secondary analysis to examine the variables and to explore the relationship among the clinical and demographic variables and PU outcome [80].

6.2.2   Inclusion and Exclusion criteria

The inclusion and exclusion for RERC on SCI are mentioned in Chapter 2, Section 2.2.2. For this particular study the inclusion criteria included:

1.   Individuals with SCI recruited from the RERC on SCI population from acute care through inpatient rehabilitation.

2.   For those who developed pressure ulcers, only those who developed pneumonia before or coinciding with the development of PU.

3.   Having inflammatory mediators collected within a week before and within a week after pneumonia.

6.2.1   Data Collection and Processing of Urine and Plasma Samples

The data collection for RERC on SCI is mentioned in Chapter 2, Section 2.2.3. The processing of urine and plasma samples and inflammatory mediator measurement are mentioned in Chapter 3, Section 3.2.4.

6.2.2   Procedure

The first PU was noted for the individuals with presence of pneumonia in acute care and inpatient rehabilitation. The time point closest to diagnosis of pneumonia; within a week before and after the presence of pneumonia, of the inflammatory mediators in plasma and urine was noted. Some inflammatory mediators such as TNF-α, IL-1β, IL-1, IL-6, IL-8 and IL-10 were previously identified in the literature in individuals with presence of pneumonia [3, 39, 40]. Additionally, predictors for analysis were included after reviewing the data collected for this study, and expert opinion. The outcome in this study was formation of first pressure ulcer. The differences of plasma (and urine) inflammatory mediators (t_1- t_2) between the first time point within a week before the onset of pneumonia (t_1) and the first time point within a week after the onset of pneumonia (t_2) were the independent variables for this study.

6.2.3   Data Analysis

Two separate analyses were performed to identify the significant inflammatory mediators in plasma and urine. All data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Descriptive statistics using means, frequencies, and standard error of mean was performed for individuals included in the study to identify plasma and urine inflammatory mediators.

Mann-Whitney U test

The demographics of the two groups of subjects (those who developed PUs and those who did not develop PU) were compared using a Mann-Whitney U test (two-tailed); with continuous-level variables such as age and ISS.

Mann-Whitney U statistic is for the two groups are[88]

$$U_{PU} = n_{PU}n_{NO\ PU} + \frac{n_{PU}\ (n_{PU} + 1)}{2} - R_{PU}$$

- U= Mann-Whitney U statistic

- n_{PU} is sample size of group 1 (individuals with PU)

- $n_{NO\ PU}$ is sample size of group 1 (individuals with no PU)

- R_{PU} is sum of the ranks for age (or ISS) in PU group

The Mann-Whitney U statistic (U) is the smaller of the two values calculated for U_{PU} and $U_{NO\ PU.}$

The Mann-Whitney U follows a z distribution[88].

$$z = \frac{U - \frac{n_{PU}n_{PU}}{2}}{\frac{n_{PU}n_{PU}(n_{PU} + n_{PU} + 1)}{12}}$$

Our hypotheses are:

H₀ (Null Hypothesis): The mean ranks of age (and ISS) between the two groups (individuals with PUs and individuals with no PUs) are expected to be the same, (or) Calculated z is between -1.96 and 1.96

H₁ (Alternate Hypothesis): There is a difference between the ranks of age (and ISS) in the two groups (individuals with PUs and individuals with no PUs), (or) z < -1.96 or z > 1.96.

The significance level was set at α = 0.05.

Chi-Square Test

It is a nonparametric test that is performed on nominal (gender male/female) and ordinal (ASIA scale score, A, B, C, D and E) data. The Chi-square statistic (two-tailed) was performed to test differences between the level of injury (and gender) in the two groups (those who developed PUs and those who did not develop PU). The chi-square statistic is[80]:

$$\chi^2 = \sum \frac{(f_0 - f_e)^2}{f_e}$$

- f_o is observed frequency of ASIA (or gender)
- f_e is expected frequency (or gender)

Our hypotheses are:

H₀ (Null Hypothesis): The severity of injury measured by ASIA scale score (or gender) and individuals who developed PUs are not related.

H₁ (Alternate Hypothesis): The severity of injury measured by ASIA scale score (or gender) and individuals who developed PUs are related.

The significance level was set at α = 0.05.

177

6.2.3.1 Normalization of data

The ranges and scales for the concentrations of inflammatory mediators varied among the different substances making assessments of their effects difficult using the absolute values. For this reason, the responses for the differences of plasma (and urine) inflammatory mediators between the two time points (t_1- t_2) were transformed into percentages of the range for all each cytokine. This normalization technique allowed comparison between multiple inflammatory mediators on a common scale.

6.2.3.2 Logistic Regression

The logistic regression coefficients and the interpretation of these are described in Section 2.2.5.2 and 2.2.5.3.

Univariate Logistic Regression

A univariate logistic regression analysis was conducted to assess individual risk factors ability to predict the probability of the outcome (PU, yes/no). The model is[80, 88]:

$$\textbf{logit (Y)} = \frac{\textbf{1}}{\textbf{1} + \boldsymbol{e}^{-(\beta_0+\beta_1 X_1)}} = \boldsymbol{\beta_0} + \boldsymbol{\beta_1 X_1}$$

- β_0 is a constant
- β_1 is the coefficient of the individual predictor variable.
- X_1 is the inflammatory mediator (predictor) in question

Our hypotheses are:

H_0 (Null Hypothesis): $\beta_1 = 0$

H_1 (Alternate Hypothesis): $\boldsymbol{\beta_1} \neq \boldsymbol{0}$

The significance level was set at $\alpha = 0.05$.

Multivariate Logistic Regression

The results from the univariate logistic regression analysis was used to identify important covariates that were moderately associated with the outcome (p<0.25) was used to build a multivariate logistic regression model [264]. One covariate at a time was included in the model and the importance of each variable was analyzed using Wald statistic. The variables that did not contribute to the importance of the model were deleted. Variables that were not initially included in the multivariate logistic regression model were added one by one to identify confounding variables. The outcome for the model was occurrence first pressure ulcer (yes/no). The model is[88]:

$$\textbf{logit (Y)} = \frac{\textbf{1}}{\textbf{1} + e^{-(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots \beta_k X_k)}} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots \ldots + \beta_k X_k$$

- $\beta_1, \beta_{2\ldots} \beta_k$ are the regression coefficients for the independent variables of the regression equation.

- $X_1, X_2 \ldots X_k$ are the independent variables (risk factors in question).

Our hypotheses are:

H_0 (Null Hypothesis): $\beta_1 = \beta_2 = \ldots = \beta_k$ (or) $\beta_i = 0$

H_1 (Alternate Hypothesis): $\beta_1 \neq 0$ (or) $\beta_i \neq 0$

The significance level was set at $\alpha = 0.05$.

6.2.3.3   Hosmer-Lemeshow goodness of fit

The Hosmer-Lemeshow test statistic was computed; this statistic is explained in Section 2.2.5.4.

6.2.3.4 Area under the Receiver Operating Characteristic (ROC) curve

The area under the ROC with the plasma inflammatory mediators will be computed and plotted. The area under the ROC curve and classification is explained in Section 2.2.5.5. The inflammatory mediators in urine were be analyzed similarly.

6.3 RESULTS

6.3.1 Plasma Analysis

6.3.1.1 Demographics

The demographics and characteristics of individuals with PU and no PU in acute care through inpatient rehabilitation are listed in Table 35.

6.3.1.2 Age and Gender

The Mann-Whitney U test detected no significant difference in ages between the two groups. Majority of individuals in both the groups were males. The Chi-square test detected no significant differences in gender distribution between the two groups.

(a) Injury Severity

The severity of injury is for the two groups in Table 35. There were no significant differences in the severity of injury ASIA and ISS between the two groups.

6.3.1.3 Univariate analysis

Table 36 shows the univariate logistic regression analysis for the 23 inflammatory mediators in plasma. The PU outcome was dichotomized into 2 levels (0= No PU and 1=PU). The individual plasma mediators were not significant.

6.3.1.4 Correlational Analysis

A correlational analysis performed between the inflammatory mediators in plasma and formation of PU produced no significant results. The normalized difference between the inflammatory mediators before and after the presence of pneumonia in plasma (t_1-t_2) was not correlated with the formation of first PU in this population (Table 37).

Table 35. Comparison of demographic population in subjects with PUs and subjects without PU

Demographics	All Subjects N=16	Subjects with PU n= 8	Subjects with no PU n=8	P value
Age (years) Mean ± SEM	37.6±4.4	35.6±6.2	39.6±6.5	0.598
Gender # of subjects (%)	M=12 F=4	M=6 F=2	M=6 F=2	1.00
ASIA # of subjects (%)	A=9 B=3 C=3 D=0 U=1	A=6 B=0 C=1 D=0 U=1	A=3 B=3 C=2 D=0 U=0	0.149
Injury Severity Score (ISS) Mean ± SEM	33.8±5	36.6±6.2	31±8	0.461

181

Table 36. Univariate Regression between PU and plasma and urine inflammatory mediators

Variables	p value plasma_normalized	p value urine_normalized
IL-β	0.846	0.967
IL-1RA	0.469	*0.173*
IL-2	0.669	0.905
IL-6	0.273	0.517
IL-10	0.579	0.573
TNF-α	0.118	0.437
Eotaxin	0.264	0.719
GM-CSF	0.720	0.616
IFN-α	*0.071*	0.733
IFN-γ	0.398	0.477
IL-4	0.577	0.445
IL-5	0.531	0.598
IL-7	0.685	0.603
IL-8	0.503	0.528
IL-13	0.863	0.908
IL-15	0.463	0.271
IL-17	0.425	0.999
IP-10	0.512	0.365
MCP-1	0.881	0.330
MIP1-α	0.425	0.738
MIP1-β	0.652	0.341
MIG	0.398	0.278
NO2-/NO3-	0.436	0.988

Table 37. Correlations between occurrence of first PU and plasma inflammatory mediators (N=16)

Outcome	Plasma Inflammatory Mediators	Point-biserial correlation coefficient	Sig. (2-tailed)
	Eotaxin	0.31	0.243
	GM-CSF	0.257	0.336
	IFN-α	0.298	0.262
	IFN-G	-0.222	0.409
	IL-1RA	-0.217	0.418
	IL-1β	0.049	0.858
	IL-2	0.109	0.688
	IL-4	0.145	0.593
	IL-5	-0.168	0.533
	IL-6	0.301	0.258
	IL-7	-0.102	0.706
PU	IL-8	-0.171	0.526
	IL-10	-0.14	0.604
	IL-13	0.043	0.874
	IL-15	0.186	0.49
	IL-17	0.256	0.338
	IP-10	0.172	0.524
	MCP-1	-0.037	0.89
	MIP-1α	-0.205	0.446
	MIP-1β	-0.114	0.675
	TNF-α	0.292	0.272
	MIG	-0.235	0.381
	NO2-/NO3-	-0.236	0.38

Note: The dependent variable in this analysis is formation of pressure ulcer
0= did not have pressure ulcer and 1= had occurrence of pressure ulcer

6.3.1.5   Multivariate Logistic Regression for Plasma Biomarkers- Model building strategy

A multivariate logistic regression was performed with pressure ulcer as outcome (PU present and no PU). The PU outcome was dichotomized into 2 levels (0= No PU and 1=PU). Table 38 shows the results of the best-fit multivariate logistic regression using the variables included in the model. All assumptions were met. There was a significant prediction of PU outcome by inflammatory mediators in plasma included in the final model, $\chi 2$ (3) =11.986, p=0.007. There was significant prediction of pressure ulcer occurrence by IFN-α (p= 0.049). The odds to develop PU increased 9.4 times, with a unit increase in the plasma concentration of IFN-α, after diagnosis with pneumonia. Although there was no significant prediction of PU outcome by IL-7 (p=0.2) and MIP-1α (p=0.2) they were included in the final multivariate model since they accounted to be confounding variables and the model fit increases with the inclusion of these variables.

6.3.1.6   Hosmer-Lemeshow test statistic

There was no significant differences between the observed and predicted group membership, Hosmer-Lemeshow $\chi 2$ (6) =8.081, p= 0.23. This means that there is a good overall fit of the multivariate logistic regression model and that there is no misspecification of the predictors.

6.3.1.7   Area under the Receiver Operating Characteristic (ROC) curve

The area under the curve for the plasma inflammatory mediators included in the multivariate logistic regression model is 0.938 (Figure 23) with 95% confidence interval (0.82, 1.000). Also, the area under the curve is significantly different from 0.5 since p <0.001 (Table 39).

Table 38. Multivariate Logistic Regression of the difference in plasma predictors before and after pneumonia

(t₁–t₂)

Independent Variables	B	SE	Wald	Sig (p)	Exp(B)
IFN- α	2.237	1.14	3.88	0.04*	9.36
IL-7	-0.06	0.04	1.88	0.17	0.94
MIP-1α	-0.54	0.05	1.38	0.24	0.94
Constant	-4.69	4.55	1.06	0.3	0.009

Model χ^2 = 11.99, p=0.007**
Pseudo R^2 = 0.703
n = 16

Note: The dependent variable in this analysis is formation of pressure ulcer in acute care or inpatient rehabilitation, so that 0= did not have pressure ulcer and 1= had a formation of pressure ulcer
*p< 0.05
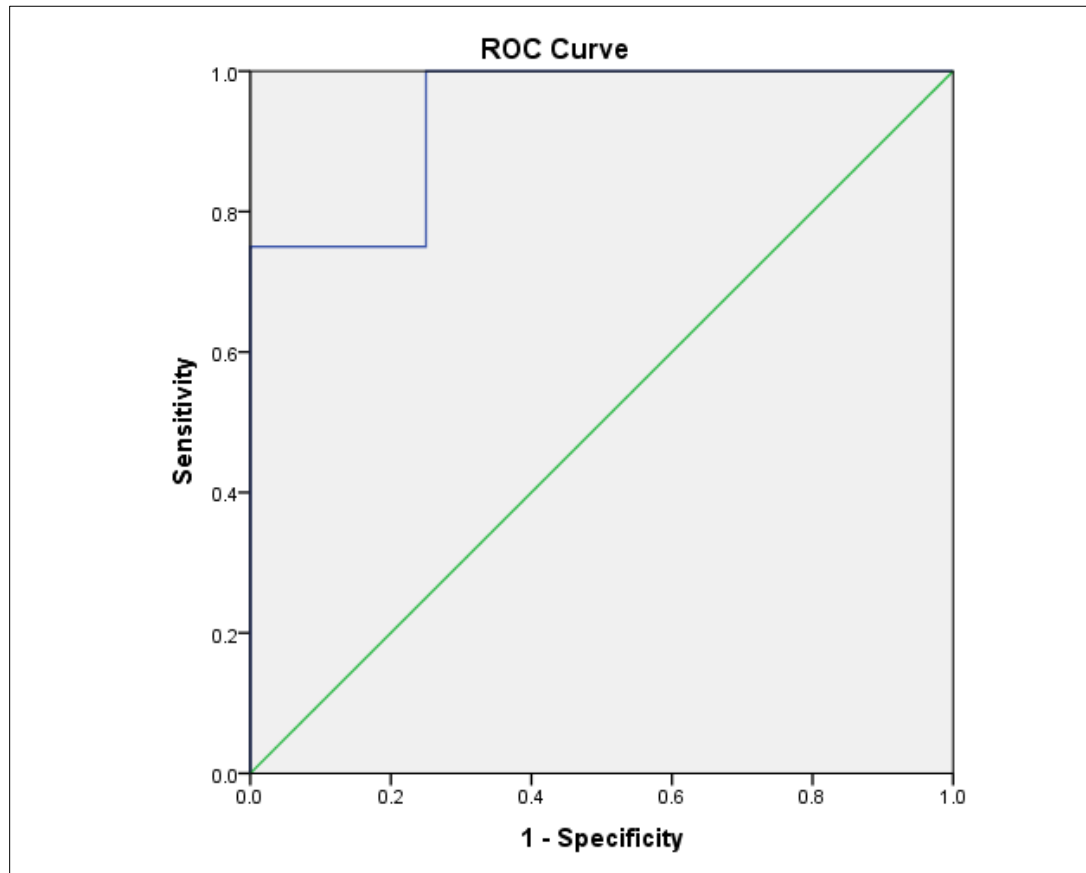
Figure 23. ROC Curve for Multivariate Logistic Regression Model for plasma biomarkers in individuals with pneumonia

Table 39. Area under the Curve for Multivariate Logistic Regression Model for plasma predictors in individuals with pneumonia

Area	*Std. Error*	*Sig*	*Asymptotic 95% Confidence Interval*	
			Upper Bound	**Lower Bound**
0.938	0.059	0.003**	0.882	1.000

Test Variable (s): Predicted probability   Note: *p< 0.05,

6.3.2 Urine Analysis

6.3.2.1 Demographics

The demographics and characteristics of individuals with PU and no PU in acute care through inpatient rehabilitation are listed in Table 40.

6.3.2.2 Age and Gender

The mean age for individuals with no formation of PU was 37 years and for the individuals with PU 36 years. Mann-Whitney U test detected no significant difference in ages between the two groups. Majority of individuals in both the groups were males. Chi-square test detected no significant differences in gender distribution between the two groups.

(a) Injury Severity

The severity of injury is for the two groups in Table 40. There were no significant differences in the severity of injury ASIA and ISS between the two groups.

6.3.2.3 Univariate Logistic Regression

Table 36 shows the univariate logistic regression analysis for the 23 inflammatory mediators in urine. The PU outcome was dichotomized into 2 levels (0= No PU and 1=PU). None of the individual urine inflammatory mediators were significant in the univariate analysis.

6.3.2.4 Correlational Analysis

A correlational analysis performed between the inflammatory mediators in urine and formation of PU produced no significant results. The difference between the inflammatory mediators before and after the presence of pneumonia in plasma was not correlated with the formation of first PU in this population (Table 41).

Table 40. Comparison of Demographic population in subjects with PU and subjects without PU

Demographics	All subjects N=15	Subjects with PU n=8	Subjects with no PU n=7	P value
Age (years) Mean ± SEM	36.4±4.5	35.6±6.2	37.2±7	0.816
Gender # of subjects (%)	M=11 F=4	M=6 F=2	M=5 F=2	0.876
ASIA # of subjects (%)	A=9 B=3 C=2 D=0 U=1	A=6 B=0 C=1 D=0 U=1	A=3 B=3 C=1 D=0 U=0	0.175
Injury Severity Score (ISS) Mean ± SEM	35.7±5	36.6±6.2	34.6±8.3	0.727

Table 41. Correlations between occurrence of first PU and urine inflammatory mediators (N=15)

Outcome	Urine Inflammatory Mediators	Point-biserial correlation coefficient	Sig. (2-tailed)
PU	Eotaxin	0.094	0.739
	GM-CSF	0.131	0.641
	IFN-α	0.089	0.753
	IFN-G	0.276	0.319
	IL-1RA	0.325	0.237
	IL-1β	0.011	0.97
	IL-2	-0.031	0.913
	IL-4	-0.264	0.341
	IL-5	-0.143	0.611
	IL-6	0.359	0.189
	IL-7	-0.137	0.627
	IL-8	-0.171	0.542
	IL-10	-0.29	0.295
	IL-13	-0.03	0.915
	IL-15	0.353	0.197
	IL-17	0.372	0.172
	IP-10	0.252	0.364
	MCP-1	0.289	0.296
	MIP-1α	0.087	0.758
	MIP-1β	0.263	0.343
	TNF-α	0.268	0.335
	MIG	0.446	0.096
	NO2-/NO3-	0.004	0.989

6.3.2.5 Multivariate Logistic Regression for Urine Biomarkers- Model building strategy

Table 42 shows the results of the multivariate logistic regression using the variables included in the model. Multivariate logistic regression was performed with pressure ulcer as outcome (PU present and no PU). The PU outcome was dichotomized into 2 levels (0= No PU and 1=PU) and three predictors: IL-1RA, IL-2 and IFN-α. All assumptions for logistic regression were met. There was a significant prediction of PU outcome by all inflammatory mediators in urine included in the final model, $\chi2$ (7) = 13.87, p=0.003. There was some prediction of pressure ulcer occurrence by IL-1RA (p= 0.073). The risk to develop PU increased 9.9 times with increased urine concentrations of IL-1RA after pneumonia. There was no significant prediction of pressure ulcer occurrence by IL-2 (p=0.1), and IFN-α (p=0.1).

6.3.2.6 Hosmer-Lemeshow test statistic

There was no significant differences between the observed and predicted group membership, Hosmer-Lemeshow $\chi2$ (6) =3.109, p= 0.795. This means that there is a good overall fit of the multivariate logistic regression model and that there is no misspecification of the predictors.

6.3.2.7 Area under the Receiver Operating Characteristic (ROC) curve

The area under the curve for the urine inflammatory mediators included in the multivariate logistic regression model is 0.946 (Figure 24) with 95% confidence interval (0.83, 1.000). Also, the area under the curve is significantly different from 0.5 since p value is <0.001 (Table 43).

Table 42. Multivariate Logistic Regression of the difference in urine predictors before and after pneumonia ($t_1.t_2$)

INDEPENDENT VARIABLES	B	SE	WALD	SIG (P)	EXP(B)
IL-1RA	2.297	1.279	3.225	*0.07*	9.94
IL-2	-0.479	0.327	2.147	0.14	0.62
IFN-α	-0.39	0.251	2.424	0.12	0.67
Constant	24.14	19.85	1.479	0.22	3.04^{10}

Model χ^2 = 13.87, p= 0.003
Pseudo R^2 = 0.806
n = 15

Note: The dependent variable in this analysis is formation of pressure ulcer in acute care or inpatient rehabilitation, so that 0= did not have pressure ulcer and 1= had a formation of pressure ulcer
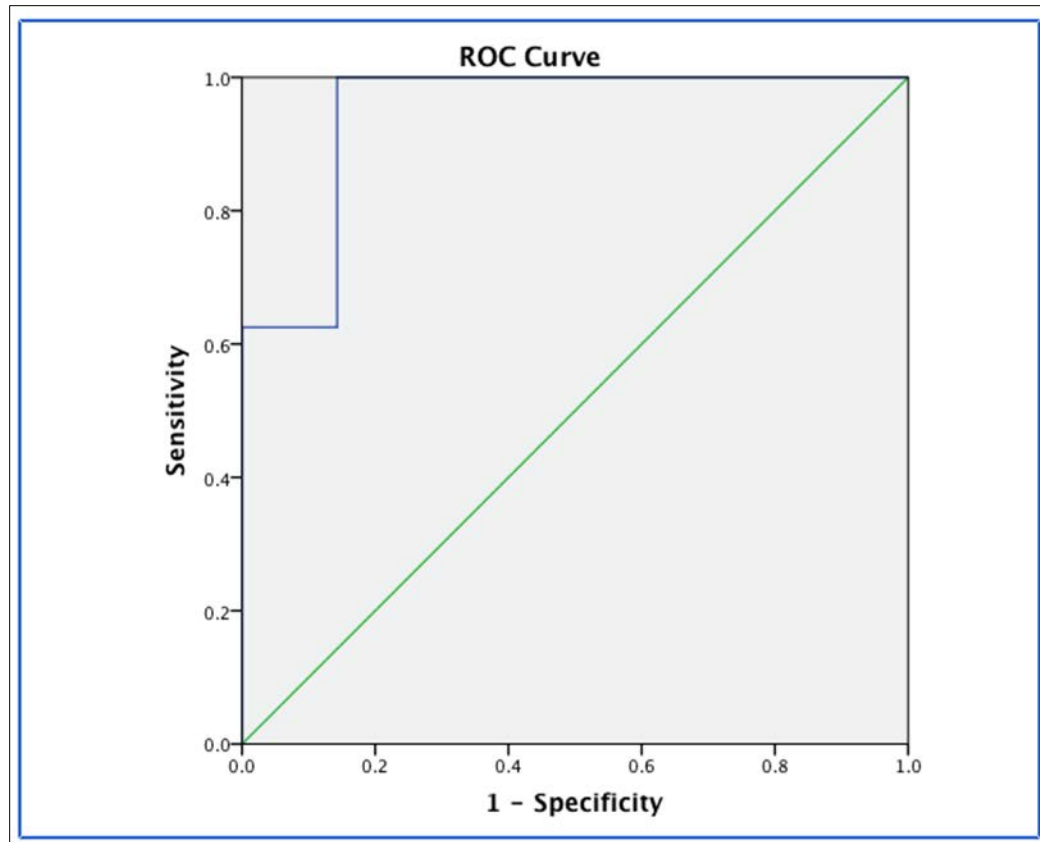
Figure 24. ROC Curve for Multivariate Logistic Regression Model for urine biomarkers in individuals with pneumonia

Table 43. Area under the Curve for Multivariate Logistic Regression Model for urine predictors in individuals with pneumonia

Area	*Std. Error*	*Sig*	*Asymptotic 95% Confidence Interval*	
			Upper Bound	Lower Bound
0.946	0.06	0.004**	0.829	1.000

Test Variable (s): Predicted probability   Note: *p< 0.05,

192

6.4   DISCUSSION

The aim of the current study was to investigate if in individuals with pneumonia the formation of PUs could be linked through inflammation. We hypothesized that the formation of PU in these individuals would be associated with differential presence of the inflammatory mediators. The findings of this study showed increase in the plasma synthesis of IFN-α and urine synthesis of IL-1RA associated with the formation of first PU in individuals with SCI and pneumonia. An association between presence of pneumonia and formation of PU in both RERC on SCI population and SCIMS populations was shown in Chapter 5.0 . Prior studies indicate that plasma inflammatory mediators to play an important role in the pneumonia [265-269] and pressure ulcer [4, 32, 74]. This is a first study to investigate the inflammatory mediators in plasma associated with formation of PUs in individuals with PUs after formation of pneumonia.

In this study, the odds of developing PUs in individuals with SCI and pneumonia increase by nine times with a unit increase plasma concentrations of IFN-α. IFN-α belongs to the Type 1 interferon group. IFN-α activates the immune cells during infection, by stimulating the T lymphocytes and macrophages. Interferons are a part of the innate immune system and known to participate in the first line of defense against the pathogens before the specific immune system responds. They benefit the host by resisting infection and inflammation initiated after trauma. They are produced when the host is subjected to a viral infection or any other pathogen binding to the viral cell to induce an antiviral state. [238]. Interferon therapy is used to stop or slow the growth of tumor cells, leukemia, melanoma, sarcoma, carcinoma [239], hepatitis [240], and thus protect the immune system. During a physiological response, the timely interactions of the specific cytokine and the receptor help to control the inflammation and thus prevent secondary

complications that may occur after a traumatic SCI. Interferons also have an ability to up regulate other cytokine responses [238]. Although there is not much evidence about the role of IFN-α in individuals with SCI, type I interferons have been reported to contribute to promote functional activity after SCI as they inhibit the astrocytes that are responsible in the secondary cellular response after SCI [241]. Increased concentrations of IFN-α has been also been shown to be associated development and exacerbation of pneumonia [270-272]. While the role of IFN-α in individuals with SCI having pressure ulcers remains not elucidated, we hypothesize that the increase in plasma concentrations of IFN-α after pneumonia generates an imbalance in the inflammatory response in the immune system and thus predispose these individuals with formation of PUs.

Although urine is a useful portal for measuring the excretion of inflammatory mediators, not much evidence is reported on imbalance in the urine synthesis of inflammatory mediators in the literature in subjects with pneumonia and PU. Increased concentrations of urinary leukotriene were associated with pneumonia [273]. This is a first study to explore the inflammatory mediators in urine associated with formation of PUs in individuals with PUs after formation of pneumonia.

The odds to develop PU in individuals with SCI and pneumonia increase by ten times with a percent increase in the urine concentrations of IL-1RA. IL-1RA belongs to the interleukin-1 cytokine family, secreted by neutrophils is involved in wound healing and inflammation[115]. It is an anti-inflammatory cytokine and inhibits the synthesis of IL-1 [165]. Increased plasma and/or urine levels of IL-1RA were associated with sepsis [166], rheumatic diseases and osteoporotic fractures [167, 168], and schizophrenia [169]. IL-1RA had been used as a therapeutic intervention is individuals, cancer [170] and neuropathy [171]. Increase in the

plasma concentrations of IL-1RA were noted in individuals subjected to trauma and during infections [165]. IL-1RA was identified as one of the early markers for mechanically induced epidermal damage [71]. Although IL-1RA is used to treat individuals with pneumonia by blocking IL-1 and other pro-inflammatory mediators, studies show increase in the IL-1RA blockage increases mortality[274]. Studies also indicate elevation of anti-inflammatory cytokines such as IL-1RA in plasma at the time of admission in individuals with community associated pneumonia[275]. In this study the increased urine concentrations of IL-1RA were associated with the formation of first PU after the presence of pneumonia in individuals with TSCI. IL-1RA maintains homeostasis throughout the acute inflammatory response after SCI, by maintaining a balance between the pro and anti-inflammatory mediators (IL-1 and IL-1RA); it thus influences the host immune response to the traumatic event [172].

The model building strategy in this study was developed using the results from the univariate analysis since the predictor variables were highly collinear with one another. This model building technique avoided large standard errors, effects of multicollinearity, and error messages from the fitting program as compared to the stepwise logistic regression technique because of multicollinearity and redundancy of the predictor variables.

Additional large scale studies are necessary to confirm, explore and further clarify the role of these inflammatory mediators in individuals with SCI and pneumonia in the pathogenesis of pressure ulceration.

6.4.1   Limitations

The analysis for this study was performed from an existing database hence, potential limitations needs to be addressed. The sample size in this study was relatively small. The number of subjects for this proposed aim in the existing database is 16 for the plasma inflammatory predictors and 15 for the urine inflammatory predictors. Given the small number of observations and large number of predictor variables the collinearity between the predictor variables was considerably large. Adding multiple predictors increased the chances of Type II error; hence univariate analysis was used in the model building strategy. Given the small sample size, and large number of independent variables the one-way interactions between the variables were not included in the model building procedure. It would be interesting to study the one-way interactions between the predictor variables along with the main effects.

This study analyzed the concentrations of the mediators between the two time points (difference between the mediators before and after pneumonia). Since temporal time points were not studied we may have failed to capture some of the inflammatory mediators. Given the limited sample size we included individuals with presence of pneumonia coinciding and preceding with the first pressure ulceration. In this process the inflammatory pattern right before the formation of pneumonia may have been missed.

6.5   CONCLUSION

This study explored the relationship between inflammation and pressure ulcers in individuals with SCI diagnosed with pneumonia. Increased plasma concentrations of IFN-α and increased urine concentrations of IL-1RA were shown to be associated with PU formation in individuals with SCI and pneumonia. The results of this study suggest that inflammation may play a role in formation of PUs in individuals with SCI and pneumonia. Future studies are required to investigate the further potential of these mediators in the pathogenesis of PUs in this population. Although further investigation is essential, this study suggests that urine and plasma biological fluids can aid the diagnosis of pressure ulceration in individuals with SCI and pneumonia.

7.0   SUMMARY AND OUTLOOK

An increased incidence of pressure ulcers (PUs) is observed in individuals with spinal cord injury (SCI) during hospitalization. Although many clinical factors are associated with the formation of PUs, very few studies have investigated the associated risk factors during acute care hospitalization and inpatient rehabilitation. Traumatic SCI induces an increased synthesis of pro-inflammatory and anti-inflammatory mediators at the site of injury. These mediators spill out into blood circulation due to the disrupted blood-spinal cord barrier. Any imbalance in secretion and concentrations of these inflammatory markers predisposes the individual to secondary complications such as pneumonia, urinary tract infections (UTIs) and other infections. Individuals with SCI, especially higher-level injuries, dependent on mechanical ventilation are predisposed to acquire pneumonia. The body's immune system counteracts these infections by synthesizing pro-inflammatory cytokines such as IL-1 and TNF-α, and anti-inflammatory cytokines such as IL-10 and IL-1RA to reduce injury. Decreased or excessive inflammatory response to counteract the effects after SCI results in inadequate or maladaptive response, which predisposes the body to secondary injury and dysfunction (Figure 25).

In this dissertation the demographic, medical comorbidities and inflammatory factors associated with the risk of formation of PUs in individuals with newly traumatic SCI were studied. This research study also explored the relationship between presence of pneumonia and

development of PU, linked through inflammation. A secondary analysis was performed on the data collected for the RERC on SCI Center from acute care hospitalization through inpatient rehabilitation. The following specific aims were examined and corresponding hypotheses tested:
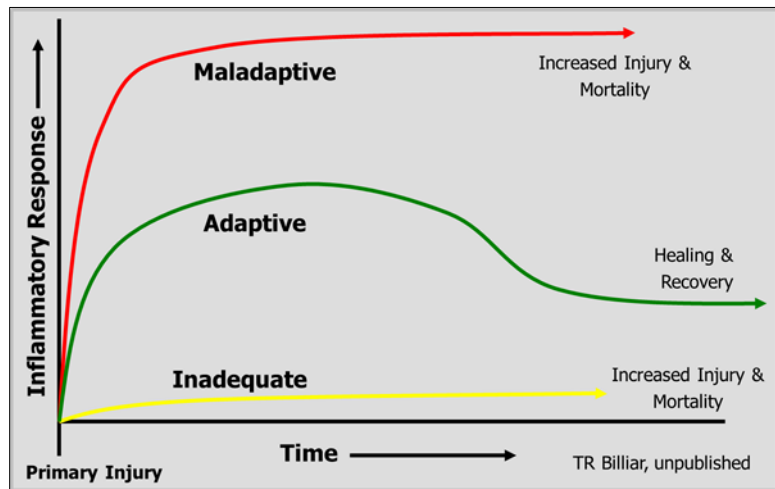
Figure 25. Inflammatory responses post trauma (TR Billar, unpublished)

Specific aim 1 (Chapter 2):

The aim was to identify the demographic factors and medical comorbidities associated with formation of the first pressure ulcer. The factors to be included were identified through a literature review, expert opinion and by reviewing the data collected on medical and demographic information for individuals enrolled in the RERC on the SCI. A total of 104 individuals with traumatic SCI were followed in acute care hospitalization and inpatient rehabilitation. Thirty-eight percent of individuals developed at least one PU in these settings. After performing a univariate logistic regression analysis; age, gender, severity of spinal cord injury graded by the ASIA impairment scale (AIS), and medical comorbidities viz., pneumonia, UTI, steroids and diabetes were included in a multivariate logistic regression model. The outcome measure for the study was formation of the first pressure ulcer. There was a significant

199

prediction of PU outcome by the clinical and demographic factors included in the multivariate model. Increased severity of spinal cord injury was associated with formation of first PU. Subjects with ASIA A were five times more likely to develop a pressure ulcer as compared to subjects with ASIA B, and Subjects with ASIA A were six times more likely to develop a pressure ulcer as compared to subjects with ASIA C. Although pneumonia was not significant in the multivariate the model, this was the only medical comorbidity that predicted occurrence of PU in the univariate analysis.

Specific Aim 2 (Chapter 3):

Inflammatory response following SCI may be detrimental and can predispose the individual to secondary complications and comorbidities. The inflammatory mediators in plasma and urine immediately (within four days) after SCI were explored that can aid in predicting the formation of pressure ulcers. In this study, twenty-three inflammatory mediators in plasma and urine biofluids were assayed using Luminex. Two separate analyses were performed to identify the inflammatory mediators in plasma (54 observations) and urine (53 observations). A backward stepwise logistic regression model was built to explore the mediators. Decreased plasma concentrations of IL-17, IFN-γ IL-1RA, MIP-1β and MIG; and increased plasma concentrations of IL-5, GM-CSF and MIP-1α were associated with later development of pressure ulcer. The odds to develop PU increased by 1.3 and 1.2 times with a unit increase in the plasma concentrations of MIP-1α and GM-CSF, respectively. Decreased urine concentrations of IL-17, IL-8, IL-13, MCP-1 and TNF-α; and increased urine concentrations of IL-5, IFN-*γ,* MIG, IL-6 and IP-10 were associated with later development of PU. The odds to develop PU increases by 1.6 and 1.2 times, respectively, with a unit increase in urine concentrations of IFN-*γ* and MIG

within four days after injury. The findings of this study imply the high degree of immunoactivation and complex inflammatory cascade, following traumatic SCI, may predispose these individual to PUs.

Specific Aim 3 (Chapter 4):

The aim was to identify significant changes in the concentrations of inflammatory mediators in plasma and urine within four days after injury as compared to just before the formation of the first PU. Wilcoxon signed rank test between the baseline measures (within four days of SCI) and the earliest time point from within a week before the formation of PUs were performed on inflammatory mediators in urine and plasma for individuals with recorded formation of PUs. An increase in the plasma concentrations of IP-10 and a decrease in the urine concentrations of IFN-α were observed just before formation of the first pressure ulcer in individuals with PUs. Thereby suggesting these mediators in plasma and urine could help detect imminent pressure ulceration.

Specific aim 4 (A) - (1) (Chapter 5):

The aim was to identify the dependent and pneumonia associated with development of PUs in this population. It was motivated by the lack of available literature focusing on the apparent association between pneumonia and pressure ulcer formation. *Phase 1* of this chapter was a preliminary study involving eighty-six subjects in the RERC on SCI population devoted to investigate any association between the presence of pneumonia and the formation of PU. The individuals were grouped according to sequence and timing of presence of pneumonia and PU incidence into four groups: (1) Pneumonia and no PU within three weeks (2) PU and no

pneumonia preceding the ulcer (3) Pneumonia coinciding with or preceding the ulcer within three weeks and (4) No PU and no pneumonia. Three weeks was chosen to allow the PU ulcer to occur within the time course of resolution of pneumonia. Chi-squared test revealed a significant association between pneumonia and the development of PUs as compared to no pneumonia. The risk to develop PUs in individuals diagnosed with pneumonia was twice as compared to the individuals who did not have pneumonia.

Specific aim 4 (A) - (2) (Chapter 5):

The aim of *Phase 2* was to explore the data in a larger population obtained from the SCI Model Systems (SCIMS), through the National Spinal Cord Injury Database, to confirm the results obtained in the *Phase 1* of the study. The chi-squared analysis performed in the SCIMS population of 3,887 individuals in acute care hospitalization and 11,022 individuals in the inpatient rehabilitation confirmed the association between presence of pneumonia and formation of PUs. The risk of developing PUs for these individuals with presence of pneumonia in acute care population and inpatient rehabilitation was thrice and twice respectively as compared to individuals who did not have pneumonia. Increased severity of SCI results in airway compromise and/or pneumonia, which are managed by mechanical ventilation. Decreased movement during acute care hospitalization and mechanical ventilation could compromise these individuals with SCI to develop PUs.

Specific Aim 4 (B) (Chapter 6):

These results of Aim 4(A) and 4(B) study thus lead to the development of a mechanistic study on the relationship between the two conditions. Inflammatory mediators were known to be

associated with the presence of pneumonia. Hence, the aim of *Chapter 6* was to investigate if the association between pneumonia and PU shown in *Chapter 5* could be associated with differential presence of the inflammatory mediators in plasma and urine. Twenty-three inflammatory mediators in plasma and urine biofluids were assayed using Luminex. The changes in the concentrations of the inflammatory mediators before and after diagnosis of pneumonia (t_1-t_2) were posited to predict the formation of first PU. Two separate analyses were performed to identify the mediators in plasma (16 observations) and in urine (15 observations). The data on the difference between the mediators before and after pneumonia (t_1-t_2) were normalized. A multivariate logistic regression model was built for both plasma and urine predictors from the results obtained from the univariate logistic regression analysis. There was a significant prediction of PU outcome by the inflammatory mediators in plasma and urine that were included in the multivariate model. The results of this study showed that the increased plasma concentrations of IFN-α and urine concentrations of IL-1RA after diagnosis with pneumonia were associated with the formation of the initial PU in individuals with SCI and pneumonia. Increase in a unit of plasma concentration of IFN-α increased the odds of developing PU by nine times. Increase in a unit of urine concentration of IL-1RA increased the odds of developing PU by ten times. These findings suggest an association between the presence of pneumonia and formation of PU could be linked through inflammation.

Conclusions and Future Research Implications:

Pneumonia and increased severity of spinal cord injury were associated with the formation of first PU following traumatic SCI. Although the concentrations of plasma inflammatory mediators associated with disease and injury is well established, not many studies have explored the urine concentrations of these mediators. The results shown in this study strengthen the association of the plasma inflammatory mediators with occurrence of PUs in individuals with traumatic SCI. The systemic effects of these inflammatory mediators can potentially be diagnostic markers and supplement in early detection of pressure ulcers. The study also showed concentrations of urine inflammatory mediators associated with formation of PUs.

Near Future Priorities

The results of this study should be cross-validated in a larger population. The inflammatory responses after traumatic SCI are multifaceted; it would therefore be of interest to further investigate the temporal evolution associated with the inflammatory mediators via principal component analyses and/or factor analyses, to identify the inflammatory patterns associated with occurrence of PUs. We also hope to model the effect of time evolution of inflammatory mediators on PU and pneumonia outcomes. We also seek to find a multivariate logistic regression model that involves relatively 2 (perhaps 3-4 mediators) and their two factor interactions that will be useful to predict the pressure ulcers. This would help confirm the pathogenesis of PUs. We also will pursue analyzing the association of inflammatory mediators with UTI outcomes.

Long term Priorities

From the results obtained in this study the next step would be to explore the mediators' specific to pressure ulceration in different patient cohorts. It will be interesting to study other risk factors such as the Braden Scale score at admission, pain and Patient Health Questionnaire-9 (depression scale) at admission. An immune response is usually activated immediately following SCI that increases the synthesis of pro-inflammatory and anti-inflammatory mediators. Future studies capturing the inflammatory mediators activated hours after SCI should be investigated. It is postulated that the time of collection of plasma and urine samples plays a critical role and as such it is imperative that forthcoming studies should control for time variations in data collection between subjects. The pattern of these mediators should be compared in healing and non-healing PUs. Further research is essential to investigate possible intervention strategies and develop an objective risk prediction tool to identify individuals at risk to develop pressure ulcers. Additional mediators such as the chromatin- associated protein high-mobility group box 1 (HMGB1), which is a damage-associated molecular pattern molecules (DAMPs) could be analyzed in these plasma and urine samples.

APPENDIX

RERC DATA COLLECTION FORMS

Rehabilitation Engineering Research Center on Spinal Cord Injury							
RERC-SCI Injury							
Information							

Study ID: _____

Date of Interview: _____

Info Obtained from: _____

Injury Information				
Cause or injury (circle one) MVA Violence Sports Fall Other				
If "Other"				
Associated injuries				
Level of injury: BiLateral Cervical Thoracic Lumbar Sacral Coccyx no data				BiLateral vertebra
Level of injury: Right Cervical Thoracic Lumbar Sacral Coccyx no data				Right vertebra
Level of injury: Left Cervical Thoracic Lumbar Sacral Coccyx no data				Left vertebra
Complete or Incomplete	ASIA score		Lower extremity	ISS

206

Rehabilitation Engineering Research Center on Spinal Cord Injury

RERC-SCI

Personal Data

Study ID: _____	Date of Interview: _____	Enrolled? Y / N

Declined enrollment (circle one)

Fear of testing   no interest   unable to obtain consent

Excluded (circle one)

Age   Not acute SCI   Motor score >30   Pre-existing disease   Previous SCI/neuro disease

Admission date: _____	Consent date _____	w/in 72 hours? Y / N

Date Of Injury: _____   Age at injury : _____

Gender: (circle one)

Male   Female

Marital Status: (circle one)

Married   Divorced   Single   Widowed

Education level: (circle one)

in high school   high school graduate   tech/2 yr. degree   4 yr. college   post-graduate

Ethnicity (circle one)

African American/black   American Indian   Asian-American   Caucasian/white

Hispanic   Other   No answer

Height and Weight	Inches and pounds: Height: Weight:	Meters and kilograms: Height: Weight:
PMH Alcohol: Y / N	PMH Smoking: Y / N	

Rehabilitation Engineering Research Center on Spinal Cord Injury

RERC-SCI Medical

Information

Study ID: _____ Discharge: Y / N. If Yes Date of Discharge: _____

Discontinue: Y N Reason for discontinuing____

Phase: Acute _____ Inpatient _____ Outpatient: _____

AC: _____ In Pt.: _____ Outpatient: _____

Date of Interview: _____ Date when blood sample is taken: _____

MRN _____ Info Obtained from: _____

General Information			
Alcohol Use: Yes No		How Often	Number of drinks/week
Tobacco Use: Yes No			
Amount/how often	Duration		Stopped When
Musculoskeletal Pain: Yes No			
Site	Character		Duration
Pain Scale Score (1- 10)	Pain Score Comments		

Pain Intensity Scale

0   1   2   3   4   5   6   7   8   9   10

no pain   moderate pain   worst pain

Patient Health Questionnaire (PHQ-9)				
In the past 2 weeks, how often have you been bothered by any of the following problems?				
	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself---or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed. Or the opposite---being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead, or of hurting yourself in some way	0	1	2	3
Add columns		_____ +	_____ +	_____
TOTAL		_____		
10. If you checked off any problems, how difficult have these problems made itt for you to do your work, take care of things at home, or get along with other people?	Not difficult at all _ Somewhat difficult _____ Very difficult _____ Extremely difficult _____			

Medical Co Morbidities	circle Yes or No If yes, add comments		
Cardiovascular Yes No	comments		
Hematopoietic Yes No	comments		
Respiratory Yes No	comments		
ENT Yes No	comments		
Gastrointestinal Yes No	comments		
Liver Yes No	comments		
Renal Yes No	comments		
Genitourinary Yes No	comments		
Rheumatologic Yes No	comments		
Musculoskeletal Yes No	comments		
Skin Integrity Yes No	comments		
Neurological Yes No	comments		
Endocrine/Metab. olic Yes No	comments		
Immunological Yes No	comments		
Psychiatric: Yes No	comments	Hospitalized Yes No	Treated Yes No
Malignancy Yes No	comments		
Substance Abuse Yes No	comments		
Body Weight Yes No	comments		
Other (specify) Yes No	comments		
Incidence of UTI Yes No	comments		
Bowel Management Yes No	comments		
Urodynamics Yes No	comments		

210

Integumentary (skin) Yes No	comments
Braden Score	

Method of Bladder Management

A ---none

B ---Indwelling Urethral Catheter

C ---Indwelling Catheter after Augmentation or Continent Diversion

D ---Catheter Free with External Collector, No Sphincterotomy

E ---Catheter Free with External Collector and Sphincterotomy

F ---Catheter Free with External Collector, Sphincterotomy unknown

G ---Catheter Free without External Collector (includes stimulation/pressure)

H ---Intermittent Catheterization Only

 I ---Intermittent Catheterization with External Collector

J ---Intermittent Catheterization after Augmentation or Continent Diversion

K ---Intermittent Catheterization, External Collector, Augmentation, or Continent
     Diversion Unknown

Medications				
NSAID Yes No				
list meds	start date	end date	dosage	frequency
Antibiotics Yes No				
list meds	start date	end date	dosage	frequency
Steroids Yes No				
list meds	start date	end date	dosage	frequency

Medications continued				
Pain meds Yes No				
list meds	start date	end date	dosage	frequency
Other meds Yes No				
list meds	start date	end date	dosage	frequency

Updated monthly for first 6 months post injury, then every 3 months

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AMBULATION

<u>TO BE COMPLETED AT TIME OF INPATIENT DISCHARGE AND OUTPATIENT PHASES ONLY</u>

Ambulation 1= non-ambulation 2 = therapeutic 3 = household 4 = community (circle one)			
Are you able to walk for 150 feet in your home?	0 = no	1 = yes	9 = unknown
Are you able to walk for one street block outside?	0 = no	1 = yes	9 = unknown
Are you able walk up one flight of steps?	0 = no	1 = yes	9 = unknown
Which of the following mobility aids do you currently use most often? Circle one. 1=straight cane 2=quad cane 3=walker 4=crutches 5=AFO 6=KAFO 7=Other 8=N/A or not ambulatory			
Do you use a wheelchair or scooter over 40 hours a week?	0 = no	1 = yes	9 = unknown
What type of wheelchair or scooter do you use most often? Circle one 1=Manual 2=Power 4=Scooter 7=Other 8=N/A			

Rehabilitation Engineering Research Center on Spinal Cord Injury
RERC-SCI Pressure Ulcer
Information

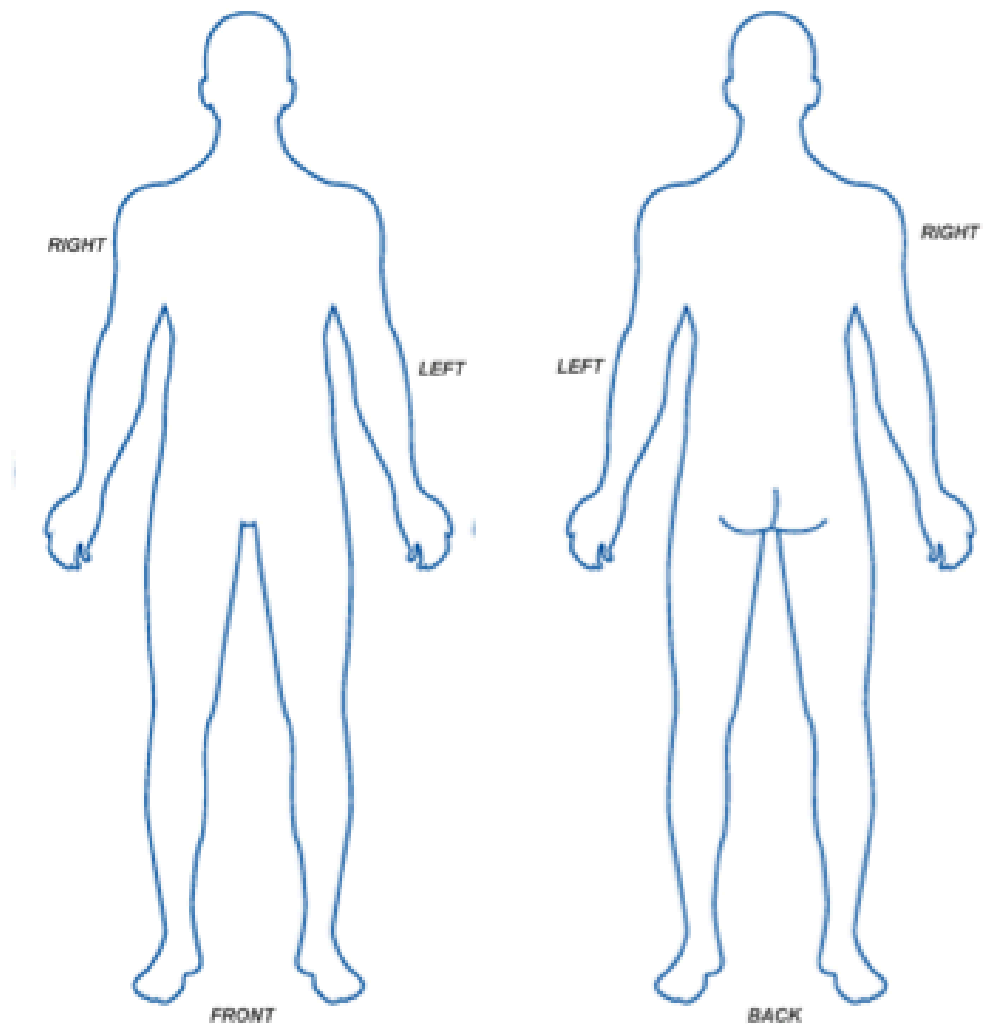Study ID: _____   Date of Interview: _____
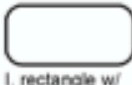
Phase: ____Acute ____Inpatient ____Outpatient   **Pressure Ulcer #** _____

AC_____ In Pt. _____ Out Pt. _____

Pressure Ulcer Healed/Resolved	Yes / No
Pressure Ulcer Merged	Yes / No With P.U # _____

RIGHT

LEFT

LEFT

RIGHT

FRONT

BACK

Pressure Ulcer Site   to be completed for each pressure ulcer present at interview phases

Ischial's (circle one)	Right	Left	Both

Sacrum
Coccyx
Trochanter

Heel (circle one)	Right	Left	Both

If above sites are not applicable please note ulcer location:

Stage (circle one):   1   2   3   4   unstageable   deep tissue injury

Size (cm)	Length:		Width:		Depth:
V&V (cm)	V&V Length:		V&V Width:		V&V Depth:

Shape –

A. rectangle   B. square   C. parallelogram   D. cross   E. star   F. lightening bolt
G. hexagon   H. triangle   I. rectangle w/ rounded corners   J. circle   K. starburst   L. hourglass
M. pentagon   N. elliptical   O. crescent   P. diamond   Q. bucket   R. ribbon

General Appearance – circle best ONE description in each category

   A. Necrotic tissue type:
      1   not visible
      2   white/grey and/or non adherent yellow slough
      3   loosely adherent yellow slough
      4   yellow/brown/black eschar
      5   firmly adherent eschar

   B. Necrotic tissue amount:
      1   not visible
      2   less than 25%
      3   25% to 50%
      4   >50% < 75%
      5   75% to 100%

   C. Wound base
      1   skin intact or partial thickness wound
      2   bright, beefy red
      3   pink or dull dusky red
      4   fibrin with granulation buds
      5   no granulation

D. Ulcer edges
    1   intact
    2   indistinct
    3   distinct, even with wound base
    4   well defined detached from wound base
    5   well defined rolled under, thickened
    6   well defined, fibrotic, scarred

E. Drainage
    1   red
    2   light red to pink
    3   clear, light color
    4   cloudy yellow to tan
    5   yellow, tan, green, thick
    6   no drainage

Appearance Comments:

(include)Wound base: Fibrin

       Fibrin + granulation buds

Surrounding tissue description – circle one
    1   erythema
    2   warmth
    3   swelling
    4   induration
    5   none
    6   other

Surrounding tissue Comments:

Additional Comments – circle one
    1. undermining
    2. tunneling
    3. odor
    4. pain
    5. none

Ulcer Comments:

Treatment:

Steroid Protocol (circle one):   Yes   No

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