Early Detection of Pressure Ulcer Development Following Traumatic Spinal Cord Injury Using Inflammatory Mediators

Shilpa Krishnan, PT, PhD,a Patricia E. Karg, MS,a Michael L. Boninger, MD,a,b,c,d,e Yoram Vodovotz, PhD,d,f Greg Constantine, PhD,d,g Gwendolyn A. Sowa, MD, PhD,b,h David M. Brienza, PhD,a,c,g

From the aDepartment of Rehabilitation Science and Technology, School of Health and Rehabilitation Science, University of Pittsburgh, Pittsburgh PA; bDepartment of Physical Medicine and Rehabilitation, University of Pittsburgh, School of Medicine, Pittsburgh, PA; cHuman Engineering Research Laboratories, Veterans Affairs Pittsburgh Healthcare System, Pittsburgh, PA; dMcGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA; Departments of eBioengineering, fSurgery, and gMathematics, University of Pittsburgh, Pittsburgh, PA; and hFerguson Laboratory for Orthopaedic Research, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Current affiliation for Krishnan, Division of Rehabilitation Sciences, University of Texas Medical Branch, Galveston, TX.

Abstract

Objective: To identify changes in concentrations of inflammatory mediators in plasma and urine after traumatic spinal cord injury (SCI) and before the occurrence of a first pressure ulcer.

Design: Retrospective; secondary analysis of existing data.

Setting: Acute hospitalization and inpatient rehabilitation sites at a university medical center.

Participants: Individuals with a pressure ulcer and plasma samples (n = 17) and individuals with a pressure ulcer and urine samples (n = 15) were matched by age and plasma/urine sample days to individuals with SCI and no pressure ulcer (N = 35).

Interventions: Not applicable.

Main Outcome Measures: Plasma and urine samples were assayed in patients with SCI, capturing samples within 4 days after the SCI to a week before the formation of the first pressure ulcer. The Wilcoxon signed-rank test was performed to identify changes in the inflammatory mediators between the 2 time points.

Results: An increase in concentration of the chemokine interferon-γ—induced protein of 10kd/CXCL10 in plasma (P < .01) and a decrease in concentration of the cytokine interferon-α in urine (P = .01) were observed before occurrence of a first pressure ulcer (~4d) compared with matched controls.

Conclusions: Altered levels of inflammatory mediators in plasma and urine may be associated with pressure ulcer development after traumatic SCI. These inflammatory mediators should be explored as possible biomarkers for identifying individuals at risk for pressure ulcer formation.

Archives of Physical Medicine and Rehabilitation 2016; - - - - - -

ª 2016 by the American Congress of Rehabilitation Medicine

Pressure ulcers (PUs) are one of the most frequent comorbidities after spinal cord injury (SCI) and can decrease quality of life and life expectancy. Approximately 47% of individuals develop at least 1 PU during the period of acute care hospitalization and rehabilitation.1 Loss of independence, altered body image, and being a burden to others are some of the concerns expressed by individuals with SCI, resulting in anxiety, emotional and psychological complications.2,3 Increased pain and decreased
mobility in these individuals interfere with their rehabilitation care. It is unknown why certain individuals with SCI develop PUs and others do not, despite having similar risk factors. This risk may be attributable to the differences in the intrinsic inflammatory state in the individual. An inflammatory response characterized by neutrophil infiltration at the site of injury is apparent after traumatic SCI. The disrupted blood–spinal cord barrier after SCI allows passage of inflammatory mediators, such as interleukin (IL)-1α, IL-1β, interleukin-1 receptor antagonist (IL-1RA), IL-6, IL-10, and tumor necrosis factor-α. Increases in human histologic expressions of proinflammatory cytokines soon after injury have been reported.

An impaired or excessive inflammatory response post-SCI can lead to an array of secondary complications such as tissue damage and skin breakdown, and decreased functional outcomes. Part of these complications can be attributed to inflammation causing a decreased production of growth factors essential for healing and repair. Further, the anti-inflammatory mediators suppress microglial activity, thus attenuating injury-induced damage and dysfunction. Anti-inflammatory mediators such as IL-10 can also drive neuroprotective effects after SCI. In rats, increases in serum and urine concentrations of inflammatory biomarkers occurred concomitantly with secondary complications and deep tissue injury. An in vitro study revealed augmented synthesis and proliferation of inflammatory cytokines, such as IL-1α, after mechanically loading tissue-engineered epidermal equivalents, even before tissue damage was discernible. Imbalances in plasma concentrations of proinflammatory mediators have been observed in individuals with SCI having PUs or slow-healing PUs as compared with subjects without SCI or subjects with SCI and no PUs.

A multidisciplinary expert panel sponsored by the SCI Quality Enhancement Research Initiative identified “research on biomarkers” for PUs as one of the areas of “highest priority.” Inflammation mediators in the circulatory system may provide an early indication of tissue damage and distinguish individuals at risk for PU development.

The aim of the present study was to explore changes in inflammatory mediators in plasma and urine after an acute SCI and a week before the occurrence of the first PU, and to compare the findings with those of a control group with no PUs. We postulated that we would observe differences in concentrations of inflammatory markers in plasma and urine between individuals with and without PUs. Interferon-γ–induced protein of 10kd (IP-10) drives IL-10 and downstream immunosuppression; hence, we specifically hypothesized that the changes in concentrations of IP-10 would be observed in individuals with SCI having PUs compared with individuals with no PUs.

### Methods

#### Study design

Secondary analyses were carried out using an existing database of the Rehabilitation Engineering Research Center on SCI to conduct this case-control study. Individuals were identified and recruited from the University of Pittsburgh Spinal Cord Injury Model Systems. The Rehabilitation Engineering Research Center on SCI protocol received approval from the appropriate institutional review board.

#### Inclusion and exclusion criteria

Individuals with new, acute traumatic SCI were recruited within 24 to 72 hours of admission. Plasma and urine samples, demographic and medical information were collected during their acute hospitalization through their rehabilitation stay until discharge. To be included in this study, the participants had to be 18 years or older, received acute care treatment and inpatient rehabilitation at the University of Pittsburgh Medical Center, and had to have plasma and urine inflammatory mediators collected within 4 days of SCI (baseline time point). In the original Rehabilitation Engineering Research Center on SCI study, individuals were followed up prospectively 3 times a week during acute hospitalization and once per week during inpatient rehabilitation. Clinical data, including skin assessments for PUs, along with plasma and urine samples, were collected during each follow-up. The severity of PUs was recorded according to the National Pressure Ulcer Advisory Panel staging guidelines. For the PU group, individuals had to have plasma and urine inflammatory mediators measured within the week before formation of the first PU (pre-PU time point). All stages of PU were included in this group. Individuals who did not have PUs were included in the control group. The control group was matched to the pre-PU group by timing of plasma and urine sample collection days ±1 day (matched time point), and age. The degree of impairment was measured by the American Spinal Injury Association Impairment Scale. Individuals excluded from the Rehabilitation Engineering Research Center study were those with pre-existing diseases (such as autoimmune or demyelinating diseases) affecting the inflammatory response to SCI, as well as those with previous SCI and other neurologic diseases that affected the motor and sensory function.

#### Data collection and processing of urine and plasma samples

The human inflammatory MILLIPLEX MAP Human Cytokine/Chemokine Panel—Premixed 26plex and Luminex xMAP were used to measure plasma and urine cytokine concentration levels. Standard curves were established based on various dilutions. Nitric oxide concentrations were measured by using the nitrate reductase/Griess assay following manufacturer instructions. Blood samples (4mL) were collected in ethylenediaminetetraacetic acid tubes and stored on ice for <2 hours before sample processing by centrifugation. Plasma and urine were aliquoted and frozen at −80°C until assayed. All data were de-identified. Twenty-three inflammatory mediators were assayed in plasma and urine including eotaxin, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (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, monocyte chemotactic protein-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, tumor necrosis factor-α, monokine induced by gamma interferon, and the nitric oxide reaction products nitric oxide2/3.

Statistical analyses

All data were analyzed using SPSS (version 22). The chi-square test was performed to test differences in sex and injury severity measured by the American Spinal Injury Association Impairment Scale, and the Mann-Whitney U statistic was performed to test differences in ages between individuals with PUs and no PUs. Box plots were used to explore differences in circulating levels of plasma and urine inflammatory mediators between the pre-PU time point and the baseline measurements. A 2-tailed Wilcoxon signed-rank test was performed to identify the difference between the 2 time points. After applying Bonferroni correction, the significance level was set at \( \alpha = .01 \). Separate analyses were performed for plasma and urine inflammatory mediators.

Results

Participants

Of the 104 individuals enrolled in the Rehabilitation Engineering Research Center study, 39 individuals developed at least 1 PU. Of the 39 individuals who developed at least 1 PU, 17 individuals with PUs had plasma or urine samples at the 2 time points and were matched to a control group. Twenty-two individuals who developed PUs did not have the plasma and urine sample assayed for the 2 time points because of lack of availability of specimens, most often because of the logistics of obtaining the specimen. For the plasma analysis, 17 individuals with SCI and no PU development were matched by plasma sample collection days to 17 individuals who developed a PU. For the urine analysis, 15 individuals with SCI and no PUs were matched by urine sample collection days to 15 individuals who developed a PU. For individuals with PUs, 15 who were included in the urine analysis were also included in the plasma analysis. Two of the individuals with a PU included in the plasma analysis were not included in

![Graph](https://www.archives-pmr.org)
the urine analysis because they did not have the urine inflammatory mediators collected during the 2 time points. For individuals with no PUs, 14 who were included in the urine analysis were also included in the plasma analysis. Three of the individuals with no PUs were included in plasma analysis but not in the urine analysis, and 1 individual with no PUs was included in the urine analysis but not in the plasma analysis, as they did not have urine and plasma inflammatory mediators collected during the 2 time points.

### Plasma analysis

#### Demographics

There was no statistical difference in age and sex between individuals with PUs and no PUs, whereas a significant difference was found in injury severity measured by the American Spinal Injury Association Impairment Scale between the 2 groups (*P*<.05) (table 1).

#### Changes in plasma inflammatory mediators

The Wilcoxon signed-rank test with Bonferroni correction (*α*=.01) was performed for IP-10, GM-CSF, IL-6, and MIP-1α, since they depicted changes between the 2 time points visually in the box plots (increase in concentrations of GM-CSF and IP-10, and decrease in concentrations of IL-6 and MIP-1α). For individuals with PUs, the test showed a significant increase in plasma concentrations of IP-10 (*P*<.01), but no significant difference for GM-CSF, IL-6, and MIP-1α. For individuals with no PUs, the test showed a significant increase in plasma concentrations of GM-CSF at the matched time point (*P*=.01), whereas no significant differences were found in plasma concentrations for IP-10, IL-6, and MIP-1α (fig 1).

#### Urine analysis

#### Demographics

No statistical difference in age, sex, and injury severity measured by the American Spinal Injury Association Impairment Scale

---

**Table 2  Demographics of individuals with PUs and control group: urine analysis**

<table>
<thead>
<tr>
<th>Demographics</th>
<th>PUs (n=15)</th>
<th>Control Group: No PUs (n=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline time point† (d)</td>
<td>2.67±.25 (1–4)</td>
<td>2.53±.27 (1–4)</td>
<td>NA</td>
</tr>
<tr>
<td>Pre-PU time point‡ (d)</td>
<td>14.73±2.9 (3–41)</td>
<td>14.93±2.9 (3–42)</td>
<td>NA</td>
</tr>
<tr>
<td>Pre-PU time point and occurrence of 1st PU (d)</td>
<td>4.06±0.6 (1–7)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Age (y)</td>
<td>39.53±4.5</td>
<td>35.6±3.61</td>
<td>.72</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>.66</td>
</tr>
<tr>
<td>Male</td>
<td>11 (73)</td>
<td>12 (80)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>4 (27)</td>
<td>3 (20)</td>
<td></td>
</tr>
<tr>
<td>Injury severity (AIS)</td>
<td></td>
<td></td>
<td>.46</td>
</tr>
<tr>
<td>A</td>
<td>12 (80)</td>
<td>6 (40)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0 (0)</td>
<td>6 (40)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1 (7)</td>
<td>2 (13)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0 (0)</td>
<td>1 (7)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (13)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

*NOTE. Values are mean ± SEM (range), mean ± SEM, n (%), or as otherwise indicated.*

*Abbreviations: AIS, American Spinal Injury Association Impairment Scale; NA, not applicable.*

† Baseline time point is within 4 days of SCI.

‡ Pre-PU time point is within a week before formation of PU for PU group and matched urine sample days for control group.

---

**Fig 2**  Box plots showing differences in urine inflammatory mediators between 2 time points for individuals with PUs and no PUs. Note the differences in scale on y-axis for the concentrations of mediators. Abbreviation: Sig, significant.
between individuals with PUs and individuals with no PUs was found (table 2).

**Changes in urine inflammatory mediators**

The Wilcoxon signed-rank test with Bonferroni correction ($\alpha = .01$) was performed for IFN-\(\gamma\), IL-1RA, IL-1\(\beta\), and MIP-1\(z\), since they depicted changes between the 2 time points visually in the box plots (decrease in concentrations of IFN-\(\gamma\), IL-1RA, IL-1\(\beta\), and MIP-1\(z\)). For individuals with PUs, the test showed a significant decrease in IFN-\(\gamma\) concentrations before PU formation ($P = .01$), but no significant difference for IL-1RA, IL-1\(\beta\), and MIP-1\(z\) (fig 2). For individuals with no PUs, significant differences between the 2 time points were not found in urine concentrations for IFN-\(\gamma\), IL-1RA, IL-1\(\beta\), and MIP-1\(z\).

**Discussion**

This is the first study to report changes in concentrations of inflammatory mediators in plasma and in urine after a new traumatic SCI and before the occurrence of the first PU. In this study, changes were found in concentrations of IP-10 in plasma and in concentrations of IFN-\(\gamma\) in urine between time points shortly after a traumatic SCI and before the occurrence of a PU. Although a PU is a localized injury, many systemic consequences such as deep vein thrombosis, diabetes mellitus, rheumatoid arthritis, and urinary tract infections have been associated with PU formation. Immune dysregulation after SCI is associated with the secondary complications such as PUs. Systemic manifestations such as increases in inflammatory cells and excessive neutrophils, as well as changes in proinflammatory and anti-inflammatory cytokines and chemokines, are found to have been associated with PU formation.24,25

**Inflammatory biomarkers in plasma**

As a chemoattractant, IP-10 targets T cells to sites of tissue inflammation.26,27 Previous studies28,29 have demonstrated increased synthesis of IP-10 associated with nonhealing diabetic foot ulcers in both wound fluid and plasma samples. The role of IP-10 in individuals with SCI having PUs remains unknown. IP-10 is known to have both proinflammatory and antiangiogenic properties. As a proinflammatory chemokine, IP-10 inhibits and limits the fibroblast recruitment and motility in chronic wounds.30 In the course of physiological conditions, the angiogenesis process is finely regulated by the formation of new blood vessels that supply oxygen and nutrients to the tissue that aid in the formation of granulation tissue during wound healing. The antiangiogenic properties of IP-10 prevent the growth of the new blood vessels from pre-existing vessels.31 Tissue injury has been shown to be reduced in mice after SCI by neutralizing the inflammatory effects of IP-10, which decreased inflammation.32 With the use of dynamic network analyses, IP-10 was suggested to drive systemic IL-10 and subsequent immunodepression in individuals with SCI.33 Studies suggest that lack of IL-10 increases secondary complications such as impaired limb function after SCI.12 Although not directly demonstrated in this study, the increase in the proinflammatory concentrations of IP-10 before the formation of the first PU may precipitate a complex inflammatory cascade of events and possibly predispose the individual to form PUs. The concentrations of IP-10 were high in both the PU and control group; hence, future research must be conducted in a larger sample to determine whether this mediator can identify risk to develop PUs in this population.

Although the concentrations of GM-CSF did not change significantly before PU formation compared with the baseline measurement in individuals with PUs, a significant increase in the plasma concentrations of GM-CSF was found at the matched time point compared with the baseline measure in individuals with no PUs. GM-CSF is a cytokine that prevents apoptotic cell death and cell adhesion, and causes chemotaxis of inflammatory cells. GM-CSF induces the generation and maturation of neutrophils, which in turn could inhibit infections.33 It also has the ability to activate macrophages, promote neovascularization, and initiate repair and regeneration during Wallerian degeneration by inducing activation of secondary cytokines.34,35 Lack of the GM-CSF gene has been shown to be associated with impaired wound healing and decreased vascularization.36 Evidence also suggests the use of human GM-CSF for the healing of wounds such as postsurgical wounds and chronic leg ulcers.37 Although not proven in this study, the increased plasma concentrations of GM-CSF in individuals with no PUs may have triggered a protective response by enhanced vascularization, and this may explain why they did not have formation of PUs compared with individuals who had PUs.

The severity of injury, recorded by using American Spinal Injury Association Impairment Scale scores, was significantly different between the PU and the control group in the plasma analysis, and with the limited sample size we were unable to control for this group difference. The increased plasma concentrations of IP-10 found in this study could be related to the injury severity or any other unmeasured factor that differed between the 2 groups.

**Inflammatory biomarkers in urine**

IFN-\(\gamma\) belongs to the type 1 interferon group, a part of the innate immune system that participates in the first line of defense against pathogens before the specific immune system responds. The increased concentrations of IFN-\(\gamma\) after trauma benefit the host by stimulating inflammation and resistance to infection.38 Although there is limited information available about the role of IFN-\(\gamma\) in individuals with SCI, type I interferons have been reported to promote functional activity after SCI by inhibiting the astrocytes responsible in the secondary cellular response.39 Although not proven in this study, the decreased urine concentrations of IFN-\(\gamma\) before PU formation compared with baseline concentrations may represent an underlying imbalance in the inflammatory response.

**Study limitations**

This study is a secondary analysis, and thus sample sizes were not estimated before data collection. To increase our confidence in the generalizability of this study, the sample sizes must be higher. Although the data were collected prospectively, the timing for measuring and assaying the plasma and urine samples for cytokine analyses was not uniform. The inflammatory mediators that may have contributed to the formation of the first PU within hours after traumatic SCI have not been analyzed for a lack of consistency of the data collection time points. Hence, we considered the first time point available within 4 days after injury as the baseline measure of plasma and urine samples, and the first time point available from within a week before formation of PUs as the pre-PU time point. Individuals who had PUs but did not have their plasma or urine samples assayed during the 2 time points were by necessity excluded from the analysis, which may have potentially biased the results of this study. Hence, the results of this study must be confirmed in a larger sample. Although we analyzed the changes
between the mediators at 2 time points, we may have missed an optimal time point when a specific mediator was associated with PU formation. Thus, it may be of value to study the temporal patterns of these mediators. It may be important in future studies to account for secondary complications, such as pneumonia and urinary tract infections, that may fluctuate the concentrations of the inflammatory mediators.

Future work

Further research is necessary to understand the mechanism by which the mediators are cleared from the bloodstream and become evident in the urine of these individuals. Clarifying the functional implications of these differences in the expression of the inflammatory mediators in plasma and urine and their pathogenesis involved in PU formation in this population is important. Further understanding of the mechanism of the plasma concentrations of IP-10 and urine concentrations of IFN-α may help in developing potential diagnostic tests in individuals with SCI that indicate the risk for PUs. Also, comparing the inflammatory mediators and chemokines in healing versus nonhealing ulcers will be interesting. This can help differentiate between beneficial and deleterious inflammatory mediators in plasma and urine in this population.

Conclusions

An increase in plasma concentrations of IP-10 and a decrease in urine concentrations of IFN-α were found within the week before the formation of the first PU when compared with concentrations determined within 4 days after SCI. Decreases in plasma concentrations of GM-CSF were also observed in individuals with no PUs. The results of this study suggest that in individuals with SCI, the localized injury to skin, underlying tissue, or both, may be associated with altered concentrations of inflammatory mediators in the plasma and urine. These inflammatory mediators should be explored in future studies as possible diagnostic markers for identifying individuals at risk for PU formation.

Suppliers

a. MILLIPLEX MAP Human Cytokine/Chemokine Panel—Premixed 26 Plex; Millipore Corp.
b. Luminex xMAP; Luminex Corp.
c. Nitrate reductase/Griess assay; Cayman Chemical Co.
d. SPSS for Macintosh version 22; IBM Corp.

Keywords

Biomarkers; Early diagnosis; Pressure ulcer; Rehabilitation; Risk factors; Spinal cord injuries

Corresponding author

Shilpa Krishnan, PT, PhD, Postdoctoral Fellow, Division of Rehabilitation Sciences, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555-1137. E-mail address: shikrish@utmb.edu.

References

Inflammation and pressure ulcers