Human serum mast cell tryptase levels in elbow fractures or dislocations and its association with injury severity

Crystal S. Liu1 | Ayoola Ademola1,2 | Mei Zhang1,2 | Alexandra Garven1 | Michaela Kopka1,3 | Paul T. Salo1,2 | David A. Hart1,2,4 | A. Dean Befus5 | Kevin A. Hildebrand1,2

1Department of Surgery, University of Calgary, Calgary, Alberta, Canada
2McCaig Institute for Bone and Joint Health, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
3Banff Sport Medicine, Banff, Alberta, Canada
4Bone and Joint Health Strategic Clinic Network, Edmonton, Alberta, Canada
5Department of Medicine, Alberta Respiratory Centre, University of Alberta, Edmonton, Alberta, Canada

Correspondence
Crystal Liu, University of Calgary, 3330 Hospital Dr NW Room 1450, Calgary, AB T2N 4N1, Canada.
Email: crystal.liu@ucalgary.ca

Funding information
University of Calgary Surgical Research Development Fund; University of Calgary Orthopaedic Research Fund; Canadian Institutes of Health Research, Grant/Award Number: 123790

Abstract
Mast cells contain an abundance of tryptase, and preclinical models have shown elevated serum mast cell tryptase (SMCT) in the setting of posttraumatic joint contractures. Therefore, SMCT emerged as a potential biomarker to help recognize patients with more severe injuries and a higher likelihood of developing contractures. The objective of this study is to assess SMCT levels in participants with varying severity of elbow fractures and/or dislocations. A prospective cohort including 13 participants with more severe injuries that required an operation and 28 participants with less severe injuries managed nonoperatively were evaluated. A control group of eight individuals without elbow injuries was also evaluated. The SMCT levels were measured using an enzyme-linked immunosorbent assay kit specific for human mast cell tryptase. A one-way analysis of variance and Tukey’s Honest Significance test was used to assess for statistical significance among and between the three groups. The average time from injury to the collection of the blood samples was 4 ± 2 days. Highly significant differences were identified between the operative, nonoperative, and control groups (P = .0005). In the operative group, SMCT levels were significantly higher than the nonoperative group (P = .0005) and the control group (P = .009), suggesting a correlation between SMCT levels and injury severity. There was no statistically significant difference in SMCT levels between the nonoperative and control groups. The SMCT levels were elevated in participants with acute elbow injuries requiring operative intervention, suggesting that SMCT levels were higher in injuries regarded as more severe.

KEYWORDS
elbow, fracture, injury severity, mast cell, tryptase

1 | INTRODUCTION

Traumatic elbow injuries make up approximately 15% of emergency department visits for upper-extremity musculoskeletal injuries annually.1 Following a traumatic elbow injury, patients are at risk for developing a contracture, which decreases the elbow range of motion, leading to considerable disability. Currently, injury management decisions are made by taking into account a combination of factors including clinical assessment, radiographs, and patient preference.2 However, it is difficult to predict the likelihood of a patient...
developing posttraumatic joint contractures based on these parameters alone. Since the severity of the initial injury is linked with the development of contractures, identifying a biomarker that reflects injury severity can significantly assist in management decisions.

Serum mast cell tryptase (SMCT) is a candidate biomarker as there is already evidence that it may play a role in the fibrotic changes in the joint capsule that underlie posttraumatic joint contractures. In our preclinical studies involving a rabbit model of joint injury, SMCT correlated with the severity of subsequent joint contracture, implying an increased activity of mast cells in affected joint capsules. During the acute phase of fracture healing, mast cells have been implicated in joint capsule fibrosis through the release of growth factors. Other advantages to SMCT as a biomarker include its specificity and abundance in mast cells, longer half-life, and ease of sample collection. It is detectable and relatively constant several hours to days after the initial trauma, while many other immunologic mediators, such as histamine and histamine metabolites, return to normal approximately 20 to 60 minutes after the mast cell stimulation. For these reasons, SMCT has emerged as a potential biomarker for injury severity.

This prospective study was designed to test the hypothesis that individuals with elbow fractures or dislocations will have higher levels of SMCT levels compared to healthy individuals without elbow injuries. Furthermore, high serum tryptase levels will identify individuals with injuries severe enough to require operative intervention.

2 | METHODS

A prospective cohort study (level II evidence) embedded within a randomized clinical trial comparing Ketotifen to placebo in the prevention of contractures following elbow fractures and dislocations was conducted (ClinicalTrials.gov Identifier NCT01902017). Participants were recruited at the Foothills Medical Center and the Peter Lougheed Centre (Calgary, AB, Canada) from 2013 to 2015. Data were collected from the first 41 participants of the randomized control trial. In the study, all participants were over the age of 17 and had sustained a fracture or dislocation about the elbow. Participants were excluded from the study if they had injuries that were more than 7 days old, were unable to move their elbow within 14 days of injury, experienced polytrauma, or were unable to provide informed consent. Participants with pre-existing conditions, including elbow contracture, osteoarthritis, inflammatory arthritis, gout, or monoarticular arthritis of the ipsilateral elbow were also excluded. For safety reasons, individuals who were pregnant, breastfeeding, on oral hypoglycemic therapy, or had a seizure disorder were excluded from the trial. Institutional ethics approval was gained through the University of Calgary Conjoint Health Research Ethics Board (protocol REB15-0081). Informed consent was obtained from participants to store data for research purposes. This study was performed in accordance with the tenets of the Declaration of Helsinki.

Blood samples were collected by venipuncture from 41 participants at the time of enrollment and before any study medication was administered. The need for operative intervention (fracture fixation and/or soft-tissue repair) was not randomized and it was determined by the surgeon and participant based on injury characteristics and participant goals. The orthopedic surgeons were fellowship-trained in upper extremity or trauma surgery. In total, 13 participants required an operation and 28 participants were treated nonoperatively for their elbow injuries. Control samples were obtained from eight volunteers with no elbow injuries.

The serum was isolated from the blood samples and stored at −80°C. SMCT levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit specific for human mast cell tryptase (no. MBS017369; MyBioSource, San Diego, CA). In this assay, the antigen was sandwiched between two types of antibodies: a monoclonal capture antibody raised in mice, which is immobilized to the plate, and a polyclonal detection antibody raised in rabbits, which is attached to keyhole limpet hemocyanin. Each sample was analyzed in triplicate following the company’s instructions and averaged to yield one measurement.

Fractures were classified using the Muller AO Classification of Fractures. Type A describes an extra-articular fracture, type B partial articular, and type C complete articular fracture. If participants had concomitant distal humerus and proximal radial/ulnar fractures, the more severe fracture classification was used in the analysis. Similarly, participants with fracture dislocations were placed in the fracture group.

2.1 | Statistical methods

Descriptive statistics were calculated for all variables of interest, and data are presented as mean and 95% confidence interval. A univariate two-tailed t test was used to compare the SMCT levels measured before and after surgery within the operative group. A one-way analysis of variance (ANOVA) with Tukey’s Honest Significant Difference (HSD) was used to compare the three groups: operative, nonoperative, and control. A P value of less than .05 was used to determine significance.

3 | RESULTS

All three groups (operative, nonoperative, and control) were similar with respect to age and sex ratio while the operative and nonoperative groups had a similar body mass index (Table 1). All dislocations without fractures were in the nonoperative group and the most common fracture was type B in both injury groups (Table 2). Both operative and nonoperative groups had an average time from injury to sample collection of 4 ± 2 days (Table 1), although there was one protocol violation in which the blood sample was drawn 8 days after injury. Within the operative group, the serum was obtained before surgery in five subjects (6.7–119.8 ng/mL), and after surgery in eight subjects (30.4–79.9 ng/mL). There was no statistically significant difference comparing SMCT levels when obtained before or after
surgery. All samples in the three groups had detectable amounts of SMCT. The one-way ANOVA test showed that there was a significant difference (F = 9.07, P = .0005) between the three groups. The subsequent Tukey’s HSD test showed that the highest level of SMCT was measured in the operative group when compared with the nonoperative group (P = .0005) and the control group (P = .0089, Figure 1). There was no statistically significant difference in SMCT levels between the nonoperative group and the control group.

4 | DISCUSSION

Our study confirmed that the SMCT level was elevated in those with elbow injuries. A significant increase of SMCT was found in the operative group, compared with the nonoperative and control groups (Figure 1). Thus, those injuries deemed most severe by the surgeon and participants warranting an operative approach were also the injuries with the highest SMCT levels. Although SMCT levels had never been studied in the setting of musculoskeletal fractures in patients, acute elevation SMCT levels have been recorded in incidents of severe traumatic injury, measured within 6 days of the trauma. The elevation of SMCT levels can be explained by the local lysis of mast cells due to direct mechanical trauma. In addition, acute stress reaction after injury accompanied by the release of vasoactive substances may have triggered mast cell degranulation, resulting in an elevated SMCT level. The AO classification is used as a tool to standardize fracture descriptions. While it can infer injury severity based on whether fracture lines are extra-articular, partial articular, or complete articular with various amounts of comminution, we were unable to detect a correlation between SMCT levels and the AO classification at the level of types A, B, or C. The reason is unknown but we speculate that the AO classification does not account for the amount of fracture displacement and/or angulation. Displacement and angulation are critical factors in determining operative and nonoperative management (and by extension injury severity), and may explain the observation of SMCT levels varying with operative and nonoperative management.

An unexpected result was that there was no significant difference between the SMCT levels in the nonoperative and the control groups. There are various reasons that could explain this observation: (a) the injury in the nonoperative group was not severe enough to result in a significant elevation in SMCT levels, (b) the control group had elevated SMCT levels due to other conditions, and (c) properties of the test itself. Further studies are required before a determination can be made about injury severity in nonoperative settings. While the control group was a healthy cohort, it was not ascertained whether they had environmental allergies or other episodic conditions that could have temporarily elevated SMCT levels. In terms of the properties of the SMCT tests, there are differences amongst various assays. The SMCT level in the control group (18.9–31.4 ng/mL) is higher than other quoted averages of 5 to 15 ng/mL. However, the control SMCT values fall within the upper range of the 95% confidence interval (11.4–25.9 ng/mL) from MyBioSource, the supplier of the ELISA assay. Because there were only eight participants in the control group, the limited sample size could also contribute to the control group having a value in the upper end of the normal range by chance. Furthermore, the SMCT levels may have fluctuated from the time of injury to the time of collection. However, previous studies involving a rabbit model have shown that the SMCT levels stay relatively constant for 21 days postinjury. Similarly, in a study involving human subjects, the majority (>50%) of endogenous tryptase remains in the serum 4 days after the triggering event. In subsequent studies, we hope to incorporate multiple measures of the SMCT levels over time to assess peak SMCT levels as it relates to injury severity.

Another potential value of SMCT lies in its correlation to contracture risk. Currently, a major challenge for physicians is to identify patients at risk of developing posttraumatic joint contractures. Early identification of patients at risk of developing

---

**TABLE 1** Select characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Operative (n = 13)</th>
<th>Nonoperative (n = 28)</th>
<th>Control (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age, year</td>
<td>48 ± 17</td>
<td>42 ± 14</td>
<td>38 ± 12</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.3 ± 9.2</td>
<td>25.7 ± 10.1</td>
<td>N/A*</td>
</tr>
<tr>
<td>Sex ratio (male:female)</td>
<td>6:7</td>
<td>15:13</td>
<td>4:4</td>
</tr>
<tr>
<td>Injury to sample collection, d</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>N/A*</td>
</tr>
<tr>
<td>Range of injury to sample collection, d</td>
<td>0-7</td>
<td>0-8</td>
<td>N/A*</td>
</tr>
</tbody>
</table>

Abbreviation: BMI, body mass index.

*No BMI data were collected for the control population.

**TABLE 2** Proportion of participants in each injury classification type, grouped by operative and nonoperative participants

<table>
<thead>
<tr>
<th>Injury Classification</th>
<th>Nonoperative, no. (%)</th>
<th>Operative, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dislocation only</td>
<td>5 (18%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>AO type A</td>
<td>4 (14%)</td>
<td>3 (23%)</td>
</tr>
<tr>
<td>AO type B</td>
<td>17 (61%)</td>
<td>8 (62%)</td>
</tr>
<tr>
<td>AO type C</td>
<td>2 (7%)</td>
<td>2 (15%)</td>
</tr>
</tbody>
</table>
contractures may allow them to benefit from timely medical intervention and decrease the need for invasive operative release of joint contractures. Therefore, it is important to identify a biomarker that correlates with injury severity and an increased likelihood of developing contractures. In the setting of joint injury, our previous rabbit model has shown that SMCT levels positively correlated with the severity of joint contracture. It follows that our current results also demonstrated an association between SMCT levels and the increased severity of the initial joint injury. In order to fully use SMCT as a biomarker to help predict the development of joint contractures, future studies directly linking injury severity and joint contractures would be required. Within patients that require operative management, the severity of the initial trauma has already been linked to a higher likelihood of developing contractures. To establish this connection, a subsequent study can be conducted to examine the relationship between elbow injury and its range of motion over time. Overall, larger sample size is needed to draw a stronger and more robust conclusion about SMCT as a biomarker for injury severity and risk for the development of contractures.

In summary, this study demonstrated that elevated SMCT levels can be detected after a traumatic elbow injury, suggesting an association between SMCT levels and injury severity. The SMCT levels were higher in participants with more severe elbow injuries requiring operative management. SMCT is a promising biomarker to assist physicians in distinguishing patients with more severe joint injuries and identify at-risk individuals who would benefit from novel methods to prevent the development of posttraumatic joint contractures.

ACKNOWLEDGMENT
This research was supported by grants from the University of Calgary Orthopaedic Research Fund, the University of Calgary Surgical Research Development Fund, and the Canadian Institutes of Health Research (Funding Reference Number 123790).

CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS
CL contributed to data analysis and interpretation, drafting, revising, and submitting the manuscript. AA contributed to data analysis and interpretation, drafting, and revising the manuscript. MZ contributed to research design, data acquisition and analysis, and revising manuscript. AG contributed to data acquisition and revising the manuscript. MK contributed to research design, data acquisition and interpretation, and revising manuscript. PS and DH contributed to data interpretation and revising the manuscript. DB contributed to research design, data interpretation, and revising manuscript. KH contributed to research design, data interpretation, drafting, and revising manuscript. All authors approved the final version of the manuscript.

INVESTIGATORS
Surgeons—Richard Buckley, Robert Korley, Paul Duffy, Ryan Martin, Shannon Puloski, Ian Le, David Longino, Jeremy LaMothe, and Aaron Bois. Coordinators/Staff—Meghan Deforest, Cynthia Chan, Kimberly Carcary, Melissa Lorenzo, and Georgia Carstensen.

ORCID
Crystal S. Liu http://orcid.org/0000-0002-8280-5405
Kevin A. Hildebrand http://orcid.org/0000-0001-8786-9021

REFERENCES