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## SERUM CARTILAGE OLIGOMERIC MATRIX PROTEIN: A BIOMARKER FOR ACUTE ARTICULAR CARTILAGE DAMAGE

Johanna M. Hoch

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SERUM CARTILAGE OLIGOMERIC MATRIX PROTEIN: A BIOMARKER FOR  
ACUTE ARTICULAR CARTILAGE DAMAGE

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DISSERTATION

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A dissertation submitted in partial fulfillment of the requirements  
for the degree of Doctor of Philosophy in the College of Health Sciences  
at the University of Kentucky

By  
Johanna M. Hoch

Lexington, Kentucky

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and Dr. Lori S. Gonzalez, Full Professor of Communication Sciences & Disorders

Lexington, Kentucky

2012

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## ABSTRACT OF DISSERTATION

### SERUM CARTILAGE OLIGOMERIC MATRIX PROTEIN: A BIOMARKER FOR ACUTE ARTICULAR CARTILAGE DAMAGE

Bone bruise lesions (BBL) are documented on MRIs diagnosing acute knee ligament injury (AKLI). Recent evidence has indicated that a majority of patients that sustain an AKLI, especially anterior cruciate ligament (ACL) knee injury, will develop post-traumatic osteoarthritis (PTOA) 10-20 years following injury. It has been proposed that the initial damage sustained to the articular cartilage overlying BBL causes a cascade of events that may result in PTOA.

Researchers have proposed a modification to treatment protocols for more severe BBL, or have stressed the need for the development of protective therapies to protect the articular cartilage. However, there are limited tools available to evaluate the clinical outcome of articular cartilage overlying BBL. Furthermore, damage to the cartilage overlying BBL may be different according to differing BBL severities. Therefore, the use of a cartilage degradation biomarker, serum cartilage oligomeric matrix protein (sCOMP) and the use of a BBL severity classification system may be useful to determine if differences exist between patients with and without BBL, and with differing BBL severities.

The purpose of this dissertation was to investigate the utility of sCOMP as a biomarker for acute articular cartilage damage. The purposes of these studies were to determine the inter and intraday reliability of this marker, to document sCOMP longitudinally in collegiate athletes and following AKLI, and to determine if differences in sCOMP and self-reported pain and function exist for patients with and without BBL, and differing BBL following AKLI.

The results of these studies indicated sCOMP measures had strong inter and intraday reliability. Additionally, exercise does seem to influence sCOMP levels; however, these elevations may not be clinically meaningful. Furthermore, sCOMP levels were not different between patients with BBL and without, and between differing

BBL severities. The results of these studies support the use of a BBL severity classification for future research studies in order to further elucidate the outcomes of these lesions.

Keywords: Articular cartilage, Biomarkers, Bone Bruise Lesions, Acute Knee Ligament Injury, Patient Reported Outcome

Johanna M. Hoch  
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April 13, 2012  
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SERUM CARTILAGE OLIGOMERIC MATRIX PROTEIN: A BIOMARKER FOR  
ACUTE ARTICULAR CARTILAGE DAMAGE

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## Chapter One: Introduction

### Background

It is estimated that approximately 80,000 to 250,000 anterior cruciate ligament (ACL) injuries occur each year.<sup>29</sup> Regardless of whether the ligament is reconstructed; these patients have a 50% likelihood of developing posttraumatic osteoarthritis (PTOA) 10-20 years after joint injury.<sup>54, 57, 91, 99</sup> Numerous investigations have been conducted with the primary aim of identifying contributing risk factors for PTOA development following ACL injury.<sup>52, 64, 74, 77, 87, 88</sup> However, the underlying mechanism associated with the development of PTOA following ACL injury is not clear. Numerous investigators have turned their attention to the damage sustained by the articular cartilage that overlies concomitant bone bruise lesions (BBL) associated with ACL injury, and the role the damage to the articular cartilage and underlying subchondral bone may play in the development of PTOA.<sup>13, 24, 71, 79</sup> The purpose of this review is to provide an overview of the severity classification systems, and volumetric measuring techniques for acute BBL associated with acute knee ligament injury (AKLI). The second purpose of this review is to provide evidence from patients studied at the time of ACL reconstruction (ACLR), documenting damage to articular cartilage overlying BBL, and to review articular cartilage scoring systems that have been used to evaluate the damage sustained to the articular cartilage overlying BBL arthroscopically. In addition, this review will summarize the current evidence provided by studies investigating cartilage oligomeric matrix protein (COMP) associated with AKLI and the role this biomarker may play in elucidating the development of PTOA following ACL injury and acute articular cartilage damage. Finally, this review will summarize the current evidence regarding differences in

self-reported pain and function for patients with AKLI, with and without concomitant BBL.

### Bone bruise lesions

Bone bruise lesions (BBL) are defined as traumatically involved, geographic, and non-linear areas of signal loss involving the subcortical bone on T-1 weighted magnetic resonance images (MRI) and areas of increased signal intensity on T-2 weighted images, and are not evident on plain radiographs.<sup>66, 94</sup> Following ACL injury, these lesions are most often located in the lateral compartment of the tibiofemoral joint,<sup>7, 18, 59, 76, 84, 98</sup> and are present on an estimated 80% of MRIs diagnosing ACL injury.<sup>59, 84, 90, 94</sup> These lesions have also been documented on MRIs used to diagnose injuries to the posterior cruciate ligament (PCL),<sup>61</sup> medial collateral ligament (MCL)<sup>65</sup> and posterolateral corner (PLC) complex.<sup>28, 94</sup> In addition to documenting the commonality and location of these injuries in association with ligament injuries, studies have also classified these lesions into differing categories,<sup>94</sup> severity classifications<sup>18, 59</sup> and have developed methods to measure the volume of the lesions.<sup>20, 78, 81</sup>

### Bone bruise classification systems

Bone bruise lesions have been classified into five categories: reticular, geographic, linear, impaction and osteochondral fractures.<sup>94</sup> Reticular lesions were defined as “regions of reticular, serpiginous stranding of diminished signal intensity on T1-weighted images within the epiphyseal and metaphyseal marrow” (page 272).<sup>94</sup> These lesions were distant from the cortical bone of the subjacent articular surface and were often the result of focal cortical impaction (page 272).<sup>94</sup>

The term geographic was characterized by the “contiguity to the subjacent cortical bone, which may demonstrate focal cortical impaction” (page 273).<sup>94</sup> Geographic occult lesions were further classified into Type I and Type II geographic lesions. Type I geographic lesions were defined as “coalescent with the exception of their periphery, which may demonstrate evidence of reticulation of the adjacent, contrasting, high-signal-intensity epiphyseal fat” (page 273).<sup>94</sup> Type II geographic lesions were defined as “coalescent, crescent like lesions demarcating a circumscribed central zone of marrow fat abutting the subjacent cortical bone” (page 273).<sup>94</sup>

Linear occult subcortical fractures were defined as “discrete linear regions of diminished signal intensity on T1-weighted images” (page 273).<sup>94</sup> Impaction fractures were fractures that “occurred in conjunction with the geographic or reticular fractures and showed variable degrees of depression of the osteochondral surface” (age 273).<sup>94</sup> Osteochondral fractures were described as “either occult or overt lesions identified as discrete adherent or distracted cortical fractures that contain an intact cartilage surface” (page 273).<sup>94</sup>

Following a prospective investigation of the prevalence of occult subcortical fractures for patients with acute knee joint effusions, findings yielded a total of 70% of the occult lesions documented on MRI were reticular, and 22% of the lesions were geographic Type I and Type II lesions, 5% were impaction fractures, 0.6% were linear fractures and 0.6% were osteochondral fractures.<sup>94</sup> In addition, 81% of the lesions were documented on the lateral compartment of the tibiofemoral joint.<sup>94</sup> Finally, this study confirmed occult fractures are present for patients with injury to the ACL, PCL, MCL and the lateral collateral ligament (LCL).<sup>94</sup>

### Bone bruise severity classification systems

There are few published classification systems used to classify BBL severity (Table 1.1).<sup>18, 59</sup> Lynch et al. classified BBL into three distinct categories: Type I, Type II and Type III lesions (Table 1.1).<sup>59</sup> Following a retrospective review of 434 MRIs, 87 patients had BBL visible on MRI for a total of 142 lesions.<sup>59</sup> Of the 142 lesions, 99 were Type I, 19 were Type II and 24 were Type III lesions.<sup>59</sup> In addition, arthroscopic documentation of the overlying articular cartilage was available for a portion of the patients, indicating 28% of the Type I and Type II lesions and 75% of the Type III lesions had associated changes to the overlying articular cartilage.<sup>59</sup>

In addition to the Lynch et al.<sup>59</sup> classification system, Costa-Paz (Figure 1.1) classified BBL into three distinct categories: Type I, Type II and Type III lesions (Table 1.1).<sup>18</sup> The results of the pre-operative MRI review revealed a total of 29 BBL with 13 Type I, 11 Type II and 5 Type III lesions.<sup>18</sup>

It is of interest to note that Type I lesions for each of the severity classifications were identified the most frequently in each of their studies. Also, Lynch and associates<sup>59</sup> reported more Type III lesions than Type II lesions while Costa-Paz<sup>18</sup> reported very few Type III lesions. One of the major differences in these classification systems is the definition of the Type III lesion. Lynch et al.<sup>59</sup> describes the Type III lesion as having no cortical disruption as Costa-Paz<sup>18</sup> describes the Type III lesion as having disruption or depression of the cortical surface.

### Volumetric measurements of bone bruise lesions

Volumetric measuring techniques have also been developed to measure the volume of BBL utilizing images on MRI viewing software. Roemer and Bohndorf

developed a method to quantify the volume of BBL using a three dimensional method (Figure 1.2).<sup>81</sup> The volume of each of the BBL was determined by following three steps while using the largest amount of signal change in the coronal images.<sup>81</sup> First, a straight line was drawn from the most peripheral margins of the BBL (line a), second, a bisecting line was drawn (line b), and in the sagittal plane a straight line was drawn to connect the peripheral margins of the BBL (line c).<sup>81</sup> Volume was then calculated using the formula  $\text{volume} = a \times b \times c$ .<sup>81</sup> The results of their study indicated that the average size of the BBL was  $14.75\text{cm}^3$ .<sup>81</sup>

A different volumetric technique was described by Paakkala et al. to measure the volume of BBL associated with acute, traumatic patellar dislocations.<sup>78</sup> To measure bone bruise volume, the BBL area ( $\text{mm}^2$ ) was traced for each image slice (slice thickness was 3mm gap between slices was 0.3mm).<sup>78</sup> The bone bruise volume was then calculated using the following formula:  $\text{volume} = \text{mm}^2 \times 3.3\text{mm}$ .<sup>78</sup> The results of their study indicated the median volume for BBL on the femur were  $25,831\text{mm}^3$  and the lesions on the patella had a median volume of  $2,832\text{mm}^3$ .<sup>78</sup>

Davies and associates also measured BBL volume for patients who suffered acute knee injury.<sup>20</sup> For the purposes of their investigation, BBL volume was calculated by measuring the background noise for each of the patients and drawing a region of interest around the tibial plateau and femoral condyles.<sup>20</sup> The total cross-sectional area of the BBL was calculated and multiplied by the slice thickness (4mm in the case of this study) and summed for a total volume of each bone bruise.<sup>20</sup> The results of their study indicated that patients who suffered ACL injury had a median BBL volume of  $40.5\text{cm}^3$  and patients who suffered a collateral ligament injury had a median BBL volume of  $38.4\text{cm}^3$ .<sup>20</sup>

While these methods of measuring volume are slightly different, it appears the final outcomes are similar. When converting the  $\text{mm}^3$  to  $\text{cm}^3$ , it appears the average BBL volume ranges from  $14.75\text{cm}^3$ <sup>81</sup> to  $40.5\text{cm}^3$ .<sup>20</sup> While the data for specific compartments were not available (lateral femoral condyle (LFC), medial femoral condyle (MFC), lateral tibial plateau (LTP) and medial tibial plateau (MTP)), it would be of interest to determine if differences in volume exist between each of the compartments.

#### Acute articular cartilage injury following ACL injury

Damage to the articular cartilage overlying BBL is not frequently visible on MRI,<sup>49, 66</sup> however, there have been reports of visible damage ranging from chondral softening to chondral defect during arthroscopy.<sup>41, 59, 76, 91</sup> Arthroscopic inspection of the articular cartilage overlying BBL in 10 patients undergoing ACLR revealed chondral softening when probed (n=4), visible fissuring or fibrillation (n=5) and 1 patient with a chondral defect (Table 1.2).<sup>41</sup> Additionally, another report noted that 94% of ACLR patients had visible articular cartilage damage in the LFC and 80% had visible articular cartilage damage overlying the BBL in the LTP.<sup>76</sup> Of the 34 patients with articular cartilage injuries (included patients with BBL and without BBL) to the LTP, 18 were classified as ICRS Grade I and 11 cases were classified as ICRS Grade 2 (Table 1.2).<sup>76</sup> In a third report, 46% of the patients undergoing ACLR had visible fractures and cracks in the articular cartilage overlying BBL.<sup>91</sup> The results of these studies indicate that damage occurs to the articular cartilage overlying BBL following ACL injury. Understanding the extent of damage to the articular cartilage matrix and chondrocytes is the next step in further understanding the clinical outcome of the damage to the articular cartilage that overlies BBL.

Recent basic science evidence has indicated that the articular cartilage overlying BBL suffers substantial damage that may not be repairable and this may serve as the first stage in the PTOA process.<sup>24, 41</sup> Following biopsies of the articular cartilage overlying BBL, Johnson et al.<sup>41</sup> reported 4 of 10 patients had evidence of chondrocyte death and matrix degeneration and 2 of the patients had chondrocyte death and severe matrix degeneration throughout the depth of the articular cartilage. Furthermore, loss of the proteoglycan component in the extracellular matrix was also identified in patients with BBL.<sup>24</sup> This evidence confirms that the impact associated with BBL during acute ACL injury also causes damage to the overlying articular cartilage and that this damage can be severe. Damage to the articular cartilage may start the clinical cascade of degenerative changes, as the integrity of the matrix may be unable to overcome such severe damage.<sup>24, 71</sup> However, at this time there are no cost-effective minimally invasive objective tools with which to prospectively measure the extent of articular cartilage damage and the clinical course associated with this injury.

#### Serum cartilage oligomeric matrix protein

The relationship between ACL injury and PTOA development could be better elucidated with an improved ability to identify the extent of the articular cartilage damage, to monitor it longitudinally and to understand its long term ramifications.<sup>24, 47, 58</sup> Serum or synovial fluid biomarkers, specifically cartilage degradation markers, may be useful objective tools with which to prospectively measure the extent and course of increased turnover, or chondrocyte death and loss of extracellular matrix following ACL rupture<sup>5, 24, 47</sup> and provide the means for prospective monitoring.



Data on biomarkers that were studied specifically in patients with ACL injury and articular cartilage injury is sparse. However, one biomarker that has been studied in patients with AKLI is cartilage oligomeric matrix protein (COMP). COMP is a cartilage degradation biomarker that is primarily identified in articular cartilage and plays an important role in the maintenance of the extracellular matrix.<sup>22, 47, 93</sup> Increases in synovial fluid and serum COMP levels have been reported for patients following acute knee injury.<sup>19, 47, 58</sup> In a cross-sectional study, patients with an ACL injury had significant increases in synovial fluid COMP levels compared to healthy controls.<sup>58</sup> However, there were no differences in serum COMP levels between the healthy and injured groups.<sup>58</sup> Furthermore, the authors reported that the largest differences in synovial fluid COMP levels for the ACL or meniscus group were measured when the samples were taken as close to the time of injury as possible.<sup>58</sup> In addition, increases in synovial fluid COMP levels were still present 6-12 months after ACL or meniscus injury, compared to a healthy reference group.<sup>58</sup> In summary, the results of Lohmander et al.<sup>58</sup> indicate that synovial fluid COMP levels are increased following ACL injury compared to healthy controls and these levels are highest when the sample is collected as close to the time of injury as possible. In addition, their results indicate there are no differences in serum COMP levels between patients with ACL injury compared to controls.<sup>58</sup>

Synovial fluid COMP differences for patients with acute knee injury were also reported in a cross-sectional study investigating increases in synovial fluid COMP levels following acute knee injury.<sup>19</sup> Patients with acute knee injury (type of injuries ranged from collateral and cruciate ligament injury, meniscus or isolated cartilage injury) were divided into three groups; acute (samples collected less than 4 weeks from time of

injury), sub acute (samples collected 4-52 weeks from injury) and chronic (samples collected more than one year after injury).<sup>19</sup> Patients in the acute and chronic group had increased synovial fluid COMP levels compared to healthy controls.<sup>19</sup> In addition, it was reported that synovial fluid COMP levels in the uninjured knee for the acute and chronic group were increased when compared to the healthy control synovial fluid COMP levels.<sup>19</sup> Interestingly there were no differences in synovial fluid COMP levels in this study between the injured and uninjured knees for patients with acute knee injury.<sup>19</sup> However, it must be noted that while the authors report only patients without radiographic osteoarthritis (OA) were included, only the injured knee was viewed arthroscopically and radiographically. Synovial fluid COMP levels may have been similar between the injured and uninjured knees because the uninjured knee had radiographic signs of OA that were not discovered. The results of this study further corroborate the evidence that there are increases in COMP levels following acute knee injury.<sup>19</sup>

Serum COMP levels have also been studied longitudinally following AKLI and subsequent arthroscopy in a group of athletes.<sup>47</sup> For patients with cruciate ligament and or meniscus injury, serum COMP at the time of surgery was significantly increased compared to the healthy controls.<sup>47</sup> It was also noted that there was a trend for serum COMP levels to increase the first year following surgery and there was a decrease in serum COMP at the two years following surgery for most subjects.<sup>47</sup> However, a subgroup of patients had an increase in serum COMP two years post surgery and it was concluded that these patients may be at a higher risk of developing PTOA.<sup>47</sup> Interestingly, these and the previous results indicate a significant difference in serum

COMP levels, while Lohmander et al.<sup>58</sup> reported no differences in serum COMP levels between injured patients and healthy controls. While the time from injury to serum collection was similar for both studies, it could be hypothesized that the patients in the Kuhne et al.<sup>47</sup> study had more severe articular cartilage damage compared to the patients in the Lohmander et al. study.<sup>58</sup> Therefore, it appears that it is important to present data regarding concomitant injuries, such as articular cartilage damage or BBL identified either by MRI or arthroscopy, as these data may elucidate why differences in serum or synovial fluid exist for patients with AKLI when compared to healthy controls.

A recent investigation documented COMP levels following acute ACL injury and described changes from time of injury to ACLR.<sup>13</sup> A total of 11 patients with acute ACL injury and concomitant injuries such as BBL, meniscus tears and chondral defects had serum and synovial fluid COMP levels measured acutely after injury (15±12 days) and at the time of ACLR (48±12 days after injury).<sup>13</sup> Interestingly, the authors reported a trend of decreasing synovial fluid COMP levels from time of injury to time of surgery.<sup>13</sup> These results indicate that this marker is elicited immediately following acute articular cartilage injury and supports the previous recommendations that serum or synovial fluid samples should be collected as close to the time of injury as possible<sup>58</sup> as it may provide a means to quantifying the extent of damage.

Histology and immunostaining for COMP has also been performed on articular cartilage biopsies for patients who suffered an ACL injury and had concomitant BBL.<sup>24</sup> A total of 13 patients with ACL injury and concomitant BBL had synovial fluid samples and biopsies of the articular cartilage collected at the time of arthroscopy.<sup>24</sup> The immunohistochemical staining revealed a distribution of COMP in the articular cartilage

that is similar to the COMP distribution that would be found for patients with OA.<sup>24</sup> In addition, Western blot analysis indicated there was a large amount of COMP fragments in the synovial fluid from the injured knee.<sup>24</sup> This report indicated synovial fluid COMP levels were higher in the injured knee compared to the uninjured knee.<sup>24</sup> Based on these results it appears that COMP is a promising marker for future research studies that investigate the damage to articular cartilage following acute knee injury and the PTOA development process.

Biomarkers can be used to diagnose disease, monitor disease progression, or determine the efficacy of therapeutic interventions.<sup>55</sup> As summarized here, there are differences in cartilage degradation biomarkers detected between patients with AKLI when compared to healthy controls.<sup>19, 47, 58</sup> It has been suggested that the damage sustained by the articular cartilage during acute ACL injury is irreversible and immediate treatment to slow the degradative process might be warranted.<sup>13</sup> Given that BBL are present on an estimated 80% or higher of MRIs diagnosing ACL injury<sup>59, 84, 90, 94</sup> and that damage to the articular cartilage overlying these lesions has been well documented,<sup>24, 41, 76, 91</sup> it is rational to presume that biomarkers of cartilage metabolism are tools that might provide insights into the degree of articular cartilage damage and its deleterious effect on overall joint health.

In order for a biomarker to be effective for diagnosing disease or monitoring disease progression, it must be sensitive to differences between healthy individuals and those with cartilage damage and among varying degrees of severity.<sup>3, 14, 15</sup> One limitation to many of the studies investigating changes in serum or synovial fluid COMP for patients following ALKI<sup>13, 19, 47, 58</sup> is the lack of reporting of concomitant injuries to the

articular cartilage. It is reasonable to hypothesize that a majority of these patients had a concomitant BBL and damage to the articular cartilage overlying this area. Therefore, data documenting the extent of concomitant BBL and articular cartilage injuries are needed to further investigate if differences in serum or synovial fluid cartilage degradation biomarker levels exist for patients following AKLI when compared to healthy controls.

Cartilage degradation biomarkers must also be sensitive to varying degrees of severity of acute articular cartilage damage in order to be used for future research studies.<sup>3, 14, 15</sup> A recent systematic review and meta-analysis revealed larger effect sizes for more patients with more severe OA when compared to healthy controls, indicating that sCOMP is sensitive to OA severity.<sup>35</sup> While sCOMP is sensitive to OA severity, a chronic articular cartilage disease,<sup>15, 35</sup> there is no evidence to suggest COMP is sensitive to differing severities of acute articular cartilage damage. Therefore, incorporating a method of measuring the severity of articular cartilage damage at time of arthroscopy for patients following acute knee injury in order to assess the sensitivity of these biomarkers to the severity of articular cartilage damage is warranted.

#### Effects of bone bruise lesions on pain and function pre-operatively

There is inconsistent evidence investigating the short term effects of BBL on pain and function in patients with AKLI with concomitant BBL. It has been reported that patients with acute ACL injury and concomitant BBL had greater levels of self-reported pain and loss of function compared to patients without BBL.<sup>42</sup> Furthermore, it was reported patients with BBL had longer lasting effusions and needed a longer period of time to achieve normal gait compared to patients without BBL.<sup>42</sup> In addition, patients

with intra-articular knee pathology who also had BBL had poorer function measured by the Noyes scale.<sup>97</sup> However there was no difference in function, as measured by the Noyes scale, six months post injury when compared to patients without BBL.<sup>97</sup>

In contrast, no significant differences in self-reported pain scores between acute knee injured patients with and without BBL documented on MRI has been reported.<sup>6</sup> Patients who had an acute knee injury completed a numeric pain scale at the time of their MRI and patients who had a documented BBL on MRI reported a baseline pain score of 4.15 compared to the no BBL group that reported an average pain score of 3.88 (p=0.45).<sup>6</sup> At follow-up visits patients with BBL reported more pain, however, the differences were not statistically significant when compared to the no BBL group (p=0.16).<sup>6</sup> Additionally, a second report found no difference in pain and symptoms for patients with ACL injury and concomitant BBL compared to patients with ACL injury without BBL.<sup>23</sup> Patients were given three patient reported outcome instruments (PROs): the Knee Injury and Osteoarthritis Outcome Scores (KOOS) pain subscale, the KOOS symptom subscale and the Short-Form (SF)- 36 scale; and the results did not show higher scores on the PROS for patients with BBL.<sup>23</sup>

The results of these studies indicate there is inconsistent evidence regarding the effects of BBL on pain and function for patients with AKLI. Inconsistencies may exist as the effects of BBL on pain and function may be relative to the severity of the BBL. The benefits of employing a BBL severity classification system may provide insights to determine if there are in fact differences in pain and function pre-operatively for patients with AKLI.

### The Problem

The consequences of BBL and the suspected articular cartilage injury overlying these lesions require careful consideration when considering the management of AKLI. For example, numerous researchers have proposed changes in pre- and post-operative care, such as a delay in surgical intervention and/or weight bearing status prior to surgical intervention in order to allow the BBL and associated articular cartilage damage to resolve and heal.<sup>16, 24, 49, 63, 71, 84</sup> A delay in weight bearing status has been recommended for patients with large or severe BBL in order to allow the bone and articular cartilage to heal, preventing further damage such as the development of PTOA.<sup>63, 71, 84</sup> However, at this time these recommendations are based on hypothetical claims and prospective studies and randomized control trials to elucidate these phenomena have not been performed. Therefore, the identification of a biomarker that can differentiate between patients with and without BBL and between differing BBL severities is necessary to begin longitudinal investigations for these hypothetical claims. Additionally, the inclusion of the severity classification system may elucidate differences in self-reported pain and function for patients with AKLI and associated BBL.

### Purpose

There were 4 purposes of this dissertation. The first purpose was to determine the inter and intraday reliability of sCOMP in a physically active cohort. The second purpose was to document the stability of sCOMP in collegiate athletes during athletic participation and following AKLI. The third purpose was to determine if differences in sCOMP levels exist for patients following AKLI with and without BBL, and when compared according to BBL severities. The fourth purpose was to determine if

differences in self-reported pain and function exist for patients following AKLI with and without BBL, and when compared according to differing BBL severities. These studies were designed to address the following aims:

1. To determine the inter and intraday reliability of sCOMP in a non-elite, physically active cohort.
2. To document the stability of sCOMP over the duration of an athletic season and to determine if differences are present following AKLI.
3. To determine if differences in sCOMP levels exist between patients with BBL and without BBL following AKLI.
4. To determine if differences in sCOMP levels exist when patients are compared according to differing BBL severities following AKLI.
5. To determine if differences in self-reported pain and function exist between patients with BBL and without BBL.
6. To determine if differences in self-reported pain and function exist when patients are compared according to differing BBL severities following AKLI.

#### Overview

The methods, results, discussion, limitations and conclusions for each of the six aims are presented in the following sequence. Chapter 2 summarizes the inter and intraday reliability of sCOMP in a non-elite, physically active cohort. Chapter 3 summarizes the stability of sCOMP in an elite group of athletes over the duration of a soccer season and following AKLI. Chapter 4 summarizes the differences in sCOMP in patients with and without BBL, and for differing BBL severities. In addition, Chapter 4 summarizes the differences in self-reported pain and function for patients with and



without BBL, and for differing BBL severities. Finally, Chapter 5 summarizes the findings from each of the studies and future research implications.

#### Operational Definitions

For the purposes of these studies, the following definitions will be used:

1. Acute knee ligament injury (AKLI): An injury where one or more of the intra-, or extra-articular knee ligaments are sprained and the injury is documented via MRI.
2. Bone bruise lesion (BBL): Increased signal intensities on T2-weighted images.
3. Patient reported outcome instruments (PROs): Survey instruments used to assess patient oriented outcomes such as pain, function and health related quality of life following ALKI.
4. Lower extremity surgery: Any type of invasive surgical technique that was performed to the articular joints of the lower extremity.
5. History of surgery: Any type of invasive surgical technique that was performed to the upper or lower extremity articular joints, or any other musculoskeletal surgery.
6. Skeletal maturity: Complete closure of the epiphyseal plates of the femur and the tibia assessed using standard anterior/posterior and lateral radiographs confirmed by an orthopaedic surgeon.

#### Assumptions

The primary assumptions for this dissertation were the following:

For Chapter 2:

1. The subjects recalled their medical history correctly and had no history of lower extremity surgery.

For Chapter 3:

1. Subjects participated in the Spring 2010 soccer season.
2. Subjects answered the PROs to the best of their abilities.
3. The medical records that were reviewed were up to date and contained the correct previous medical history.

For Chapter 4:

1. Subjects recalled their medical history correctly and had no history of surgery or severe knee ligament injury.
2. Subjects recalled their date of injury correctly.
3. Subjects answered the PROs to the best of their abilities.
4. Subjects did not have rheumatoid arthritis.
5. The surgeons viewing the articular cartilage overlying the BBL understood the classification system that was employed.

#### Delimitations

The primary delimitations of this dissertation are the following:

For Chapter 2:

1. Subjects were males and females between the ages of 18 -50.
2. Subjects had no history of severe knee ligament injury.
3. Subjects had no history of lower extremity surgery.
4. Subjects were considered physically active as qualified by a 6 or higher on the NASA physical activity scale.

For Chapter 3:

1. Subjects were males and females between the ages of 18-30.

2. Subjects were considered physically active as qualified by participating in NCAA Division I soccer.

For Chapter 4:

1. Subjects were males and females between the ages of 13-50.
2. Subjects had no history of severe knee ligament injury.
3. Subjects had no history of surgery.
4. Subjects were skeletally mature.
5. Subjects had an MRI and had visited the physician within four weeks of their injury.
6. Subjects were considered physically active as qualified by a score of 8 or higher on the Tegner Activity Scale.

### Limitations

For Chapter 2:

1. Subjects were included if they had a history of upper extremity surgery.

For Chapter 3:

1. Subjects were included if they had a history of injury or surgery.
2. Data regarding the musculoskeletal injuries that were treated prior to the Spring 2010 soccer season were retrospectively collected.
3. Pertinent medical history data were retrospectively collected.

For Chapter 4:

1. A single radiologist measured the volume of the BBL and determined the severity of each lesion.
2. Multiple surgeons viewed and scored the articular cartilage overlying the BBL.

3. Injuries to the ACL, PCL and MCL were included in this research study.
4. Multiple MRI machines were used during this research study.

Table 1.1: Bone Bruise Severity Classification Systems.

Classification System	Type I	Type II	Type III
Lynch et al. <sup>59</sup>	A loss of signal intensity on short TE images which are most often located within the medullary space of the bone, usually involving the epiphyseal and metaphyseal regions without interruption of the cortex.	A loss of signal intensity on short TE images which are associated with an interruption of the black cortical line.	A more profound signal loss than that of Type I or Type II lesions, which are often restricted to the region of bone immediately adjacent to the cortex without cortical interruption.
Costa-Paz et al. <sup>18</sup>	Diffuse signal with change in the medullary component, often reticular and distant from the subjacent articular cartilage.	Localized signal intensity with contiguity to the subjacent articular surface.	A disruption or depression of the normal contour of the cortical surface.

Definitions of the Lynch et al., and Costa-Paz et al. classification systems taken from their respective manuscripts.<sup>18, 59</sup>

Table 1.2: Articular Cartilage Injury Severity Classification Systems for Overlying Articular Cartilage Injury Associated with Bone Bruise Lesions.

Classification System	Grade 0	Grade I	Grade II	Grade III	Grade IV
Brittberg and Winalski <sup>10</sup>	Normal cartilage appearance.	Softening of the articular cartilage.	Fibrillation of the articular cartilage.	Fissuring of the articular cartilage.	Full-thickness defect with exposed subchondral bone.
Johnson et al. <sup>41</sup>	Normal cartilage appearance.	Normal appearance with softening on probing.	Fissuring or cracking along with softening.	Fibrillation and softening.	Overt chondral fracture or indentation.

Definitions of the Brittberg and Winalski and Johnson et al. classification systems were taken from their respective manuscripts.<sup>10,41</sup>

Figure 1.1: A figure depicting images of the individual Costa-Paz classifications<sup>18</sup> for bone bruise lesions.

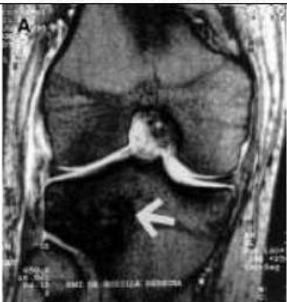

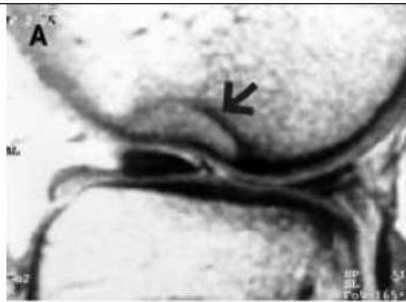
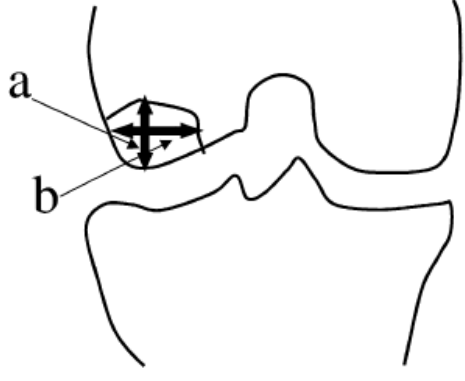
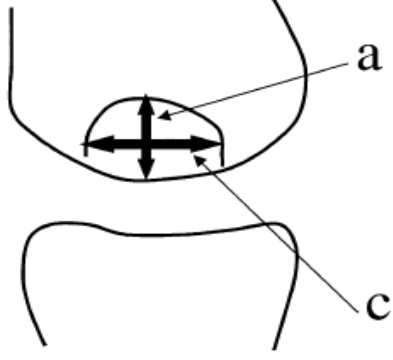
		
<p>Type I- Diffuse signal with change of the medullary component, often reticular and distant from the subadjacent articular surface.</p>	<p>Type II- Localized signal with contiguity to the subadjacent articular surface.</p>	<p>Type III- Disruption or depression of the normal contour of the cortical surface.</p>
<p>Images and definitions from: Costa-Paz M, Muscolo DL, et al. (2001). Magnetic resonance imaging follow-up study of bone bruises associated with anterior cruciate ligament ruptures. <i>Arthroscopy</i>, 17(5): 445-449.</p>		

Figure 1.2: A figure depicting the volumetric measuring technique of Roemer and Bohndorf.<sup>81</sup>

Step 1: Measuring plane (a) and (b)	Step 2: Measuring plane (c)
	
<p>Using the image with the largest amount of signal intensity in the coronal plane, a straight line (a) will be drawn from the most peripheral margins of the area. A second line (b) will be drawn bisecting line (a) to define the second plane.</p>	<p>Using the sagittal images, a straight line will be drawn to determine the third plane.</p>
<p>Images and definitions from: Roemer FW, Bohndorf K. Long-term osseous sequelae after acute trauma of the knee joint evaluated by MRI. <i>Skeletal Radiol.</i> 2002;31(11):615-623.</p>	



## Chapter Two: Determination of the Inter and Intraday Reliability of Serum Cartilage Oligomeric Matrix Protein in a Physically Active Population

### Introduction

Cartilage oligomeric matrix protein (COMP) is a protein primarily identified in articular cartilage.<sup>32, 68, 80</sup> Elevations of this biomarker have been identified in patients suffering from knee osteoarthritis (OA), a chronic articular cartilage degradation disease, when compared to healthy controls.<sup>14, 15, 25, 43, 70, 89, 100</sup> In addition, synovial fluid and serum elevations of this biomarker have been documented in patients who suffer acute knee ligament injury (AKLI), such as anterior cruciate ligament (ACL) rupture.<sup>19, 47, 58</sup> The basic assumption is that these elevations are associated with articular cartilage damage, not ligament injury. However, documentation of concomitant injuries such as bone bruise lesions (BBL) and associated articular cartilage injury was not reported in these studies.<sup>19, 47, 58</sup>

An estimated 80% of MRI confirmed ACL ruptures have documented concomitant BBL.<sup>59, 84, 90, 94</sup> Often these lesions are not associated with visible articular cartilage injury at the time of MRI and surgical intervention.<sup>49, 66</sup> However, biopsies of the overlying articular cartilage indicate degeneration of the chondrocytes and substantial damage to the superficial matrix.<sup>24, 41</sup> Therefore, COMP may be a useful biomarker to better understand the articular cartilage damage that is associated with BBL for patients who suffer AKLI.<sup>24</sup>

In order to further investigate the usefulness of COMP as a biomarker for acute articular cartilage damage, the reliability of this measure must be documented.

Determination of inter and intraday reliability measures is necessary to ensure that this

marker is clinically applicable for future research studies. The purpose of this study was to determine the inter and intraday reliability of serum (sCOMP) in a healthy, physically active cohort.

## Methods

### Design

A repeated measures reliability design was employed for this study. The independent variable was time (time 1= T1, time 2= T2, time 3=T3) and the dependent variable was sCOMP.

### Population

A volunteer sample of 23 physically active subjects (4 males and 19 females; age:  $27.2 \pm 3.8$  years; height:  $168.6 \pm 6.3$  cm; weight:  $150.6 \pm 31.4$  kgs) volunteered to participate. Subjects were included if they had no history of lower extremity surgery and no history of lower extremity injury within the last 3 months. Subjects were considered physically active if they scored a 6 or higher on the NASA Physical Activity Status Scale.<sup>102</sup> A score of 6 represents heavy aerobic exercise in the form of either running 6 to 10 miles a week or walking 7 to 13 miles per week.<sup>102</sup> Subjects maintained their usual daily activities while participating and hourly documentation logs were collected while the subjects were enrolled. This study was approved by the University of Kentucky Institutional Review Board and approved informed consent was obtained from the subjects prior to participation in our study.

### Procedure

A total of three serum collections were performed on two separate days. To determine the interday (between day) reliability, subjects reported to the laboratory once

in the morning (T1) and approximately 7 hours later on the same day (T2). To determine intraday reliability (within day), subjects reported to the laboratory the morning after (24 hours) the first draw (T3).

Upon arrival, each subject was asked to remain seated at a table for 30 minutes prior to data collection based on the recommendations of Andersson et al.<sup>2</sup> and Mundermann et al.<sup>69</sup> Following 30 minutes of rest, a maximum of 10ccs of blood was collected from the antecubital vein in a red top safety tube following standard operating procedures for venipuncture.

#### Enzyme-linked immunosorbent assay

Immediately after serum collection, the blood was placed on ice and transported for separation. After clotting for 30 minutes at room temperature, sera were separated in a centrifuge at 4°C at 2000g for 15 minutes, placed in labeled aliquots and stored in a -80°C freezer. Once all samples were collected, sCOMP concentrations were determined using a commercially available, competitive enzyme-linked immunosorbent assay (ELISA) kit (IBL Euro-Diagnostica, Malmo, Sweden). Serum COMP values are expressed as ng/mL. The average intraassay coefficient of variance (CV) of all controls was 2.8%, the average interassay CV of all controls was 2.1% and the average CV of all samples was 2.5%.

#### Statistical Analysis

Descriptive statistics (means  $\pm$  standard deviations) for each test session were calculated. Inter and intraday reliability were estimated using intra-class correlation coefficients (ICC<sub>2,1</sub>). A total of 20 participants' data were used to calculate interday reliability (T1 vs. T3). A total of 23 participants' data were used to calculate intraday reliability (T1 vs. T2). Standard error of measurement (SEM) was also calculated for

each correlation. ICCs were interpreted as weak if they were less than 0.40, moderate if between 0.41 and 0.69 and values of more than 0.70 were interpreted as strong.<sup>17</sup> Within day and between day minimal detectable change (MDC) values were also calculated using the inter and intraday reliability and SEM data collected during each session. MDC values were calculated with a 95% level of confidence using the formula  $SEM * 1.96 * \sqrt{2}$ .<sup>4, 104</sup> All statistical analyses were performed using PWAS software, version 18.0 (Somers. NY).

### Results

The mean sCOMP value ( $\pm$ standard deviation) for T1 was 1287.44 ( $\pm$ 205.70) ng/mL, for T2 was 1292.90 ( $\pm$ 228.00) ng/mL and for T3 was 1270.15 ( $\pm$ 213.60). The ICC for both the interday (0.76) and intraday (0.74) were both interpreted as strong. The interday 95% MDC value (SEM 105.3) was 292 ng/mL and the intraday 95% MDC (SEM 154) was 320 ng/mL.

### Discussion

To our knowledge we were the first to document the inter and intraday reliability of sCOMP in a healthy, physically active cohort with no history of lower extremity surgery or acute lower extremity injury within the last three months. The results of this investigation indicate strong inter and intraday reliability for sCOMP in a young, physically active cohort. To provide a more comprehensive interpretation of our reliability values, comparisons of these findings to those that have been previously published are made.

Several studies have reported temporal patterns for sCOMP. For example, Vilim et al.<sup>95</sup> conducted a study to verify the ELISA they used was sensitive to changes

overtime in sCOMP in multiple populations. As a subcomponent of their larger study, the authors reported that intersession variability was insignificant between days.<sup>95</sup>

Specifically, the authors collected sCOMP over a period of six days in five volunteers with no obvious joint pathology.<sup>95</sup> However, the specific variability of sCOMP was not reported as the data were presented in a line graph figure. Our interpretation of their graph was that the variability of the five volunteers did not differ more than 1 µg/mL. Notably, no documentation of participant demographics (age, gender, past medical history) were provided.<sup>95</sup> These previous results in addition to the results within this study indicate sCOMP levels are stable and reliable between days in healthy populations.

The timing of data collection for sCOMP can be influenced by food intake. In addition, Vilim et al.<sup>95</sup> also reported significant differences in time point one (morning fasting sample) and time point two (2 hours after lunch) in four of the 20 subjects that participated in their research study. For the remaining 16 subjects in their research study, no variation in the serum samples between the time points was identified.<sup>95</sup> From the results of their investigation, a fasting, morning serum sample was recommended for collection in clinical research studies as it was concluded that the differences noted for the 4 individuals was related to food consumption.<sup>95</sup> While this is a good recommendation it should be noted that the results of this study indicate strong intraday reliability, where the subjects were allowed to eat a non-regimented meal prior to each of the testing sessions (breakfast and lunch).

Diurnal variation of sCOMP has been documented in patients with OA and rheumatoid arthritis during a 24 hour observational study.<sup>1, 46</sup> The results of a 24 hour observational study where patients with OA and rheumatoid arthritis had serum collected

every four hours indicated no significant changes in sCOMP levels throughout the waking hours.<sup>1</sup> Patients that participated in both groups had regulated exercise regimens, scheduled meals and scheduled bed times.<sup>1</sup> For patients with OA, the results of the investigation revealed no significant changes in sCOMP between 09:00 and 21:00.<sup>1</sup> However, the results of the study did reveal a significant decrease in sCOMP levels at 05:00 ( $p<0.03$ ).<sup>1</sup> For patients with rheumatoid arthritis, no significant changes were observed between 08:00 and 20:00.<sup>1</sup> In addition, a significant decrease in sCOMP was noted at 04:00 ( $p<0.001$ ).<sup>1</sup> The results of this study indicate sCOMP levels remain consistent during the waking hours, indicating sCOMP levels can be collected throughout the day for either clinical and or research purposes.<sup>1</sup> Therefore, based on the results of this study and our study, the variation in sCOMP across the day is insubstantial.

A study conducted by Kong et al.<sup>46</sup> reported diurnal variation in sCOMP in a cohort of patients with OA. The results of their clinical laboratory investigation revealed significant differences in sCOMP levels measured between the time before arising from bed (T0) and 1 hour after rising from bed (T1,  $p<0.001$ ); between T0 and 4 hours after arising from bed (T2,  $p<0.001$ ); and between T0 and 12 hours of daily activity (T3,  $p<0.01$ ).<sup>46</sup> It must be noted the authors reported they did not allow the participants to remain seated for more than 30 minutes at a time during study participation; the collection at T0 was obtained while the patients remained supine after awakening from the previous night's rest and subjects were encouraged to engage in activities of daily living throughout the study duration.<sup>46</sup> The significant differences in sCOMP levels between each of the time points may be due to the fact that the T0 sample was collected following rest and the other samples were collected following activity.<sup>46</sup> Based on the

recommendations of Andersson et al.<sup>2</sup> and Mundermann et al.<sup>69</sup>, 30 minutes of rest prior to serum collection is necessary to ensure elevations in sCOMP levels are not due to any moderate exercise, such as walking to the data collection site. However, it must be noted that the results reported by Kong et al.<sup>46</sup> were published at the same time as Mundermann et al.<sup>69</sup> and Andersson et al.<sup>2</sup>; therefore, they did not include this recommendation in their methodology. Based on these previous recommendations, the subjects were required to remain seated for 30 minutes prior to serum collection. The addition of this resting time is necessary in order to provide the most valid results of sCOMP levels.

The amount of physical activity each of the subjects engaged in while participating in this study was not controlled for. Also, the subject's meal times were not regulated, as the subjects were encouraged to engage in their normal activities of daily living while participating our research study. Therefore, these results indicate this biomarker is reliable even when the subjects are not in a research laboratory environment where exercise, meal times and sleeping habits are regulated.<sup>1,46</sup>

#### Limitations

This study is not without limitations. In order to be included in this research investigation, the subjects had to have no history of lower extremity surgery and no current acute lower extremity injury. Subjects were not given a physical examination or radiographic examination prior to subject inclusion. Therefore, we were unable to verify each of the subjects were free of joint damage, such as OA. However, these subjects were relatively young and reported no history of serious joint injury. Therefore, it is unlikely that substantial knee cartilage degradation had occurred in any of our participants.

### Conclusion

These results indicate strong inter and intraday reliability for sCOMP values in a healthy, physically active cohort with no history of lower extremity surgery. The strong reliability of this measure demonstrates serum samples can be collected throughout the day for future clinical research studies.<sup>1</sup> In addition, the stability of this marker further indicates increases in sCOMP levels following AKLI are due to injury and are not associated with diurnal variation for a population without disease history. Our 95% MDC values suggest a change in sCOMP levels of 290-320 ng/mL in an injured population represents a meaningful change which exceeds the variability associated with the measure. Future research studies are needed to further investigate the magnitude of change in this biomarker for patients with acute articular cartilage damage to determine its appropriateness for use in this population and for varying degrees of articular cartilage severity.

### Acknowledgements

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## Chapter Three: Longitudinal Documentation of Serum Cartilage Oligomeric Matrix Protein and Patient Reported Outcomes in Collegiate Soccer Athletes over the course of an Athletic Season and in the Occurrence of Injury

### Introduction

It has been theorized that continuous, intense physical activity can cause the development of primary osteoarthritis (OA) in elite athletes.<sup>12, 48</sup> Primary OA is characterized by irreversible joint destruction such as cartilage degradation, osteophyte formation and joint space narrowing,<sup>93</sup> and causes pain, loss of function, activity limitations and participation restrictions in the millions of individuals each year.<sup>2, 11, 25, 26, 96</sup> There has been speculation regarding how articular cartilage responds to increases in activity level and determination of the levels related to articular cartilage damage due to intense physical activity is unclear.<sup>30</sup> It has been hypothesized that sports that include rapid acceleration and deceleration moments, continuous training where the joints sustain high impacts and athletes that compete at elite levels for an extended period of time are at an increased risk of developing OA.<sup>12, 86</sup> However, it is unknown to what extent the amount, types and intensity of exercise is detrimental to the articular cartilage in the human joint.<sup>30, 86</sup> At this time there are limited tools available to prospectively investigate the relationship between the early stages of degenerative joint disease, such as articular cartilage degradation and athletic participation, and whether or not biomarkers can facilitate the understanding of these processes.

Described as an instrument to measure the progress of a disease or the effects of a treatment on disease progression, biomarkers may serve as a tool to elucidate the effects of exercise on cartilage degradation and the eventual development of primary OA.<sup>67</sup> A biomarker for cartilage degradation, known as serum cartilage oligomeric matrix protein

(sCOMP), is a non-collagenous protein identified in synovium, ligamentous tissue, tendon, meniscus and primarily articular cartilage.<sup>15, 21, 27, 32, 68, 73, 80, 85</sup> Serum COMP levels are elevated following intense exercise<sup>44</sup> and are elevated in subjects who participated in less strenuous forms of physical activity.<sup>70, 73, 75</sup>

Several studies have investigated changes in sCOMP during exercise. For example, in healthy subjects that participated in moderate walking exercises, marathons and ultra-marathons, there was an increase in sCOMP levels following activity.<sup>44,70, 73</sup> Serum COMP levels have been documented to return to baseline levels thirty minutes following a moderate walking exercise<sup>70</sup> and after a longer period of time for more intense exercise.<sup>44</sup> Following a marathon, sCOMP levels returned to baseline 24-48 hours following the race and for an ultra-marathon, sCOMP levels returned to baseline 6 days following the race.<sup>44, 73</sup> Therefore, elevated levels of sCOMP as a result of physical activity may indicate that this biomarker may be useful in better understanding the relationships between exercise and articular cartilage changes.

It has been reported that elite level athletes who participate excessively in high impact sports for an extended period of time have a higher risk for developing primary OA.<sup>86</sup> However, there are no prospective, longitudinal studies investigating the effects of continuous, intense physical activity over time on sCOMP. Therefore, to further investigate sCOMP as a biomarker for cartilage damage induced by exercise, it may be useful to prospectively study elite level athletes who participate in high impact sports. Documentation of the stability of this marker over the duration of an athletic season, rather than one bout of exercise, is needed for further investigation of the effects of exercise on articular cartilage. In addition, two patient reported outcome instruments

(PROS) to document self-reported function and knee related symptoms throughout the duration of the season were collected. These PROs were used to provide additional information that might explain changes in sCOMP levels such as decrease in function, or an increase in pain or knee related symptoms.

Elevated levels of COMP have also been reported following acute knee injury.<sup>19, 47, 58</sup> Serum and synovial fluid COMP levels in knee injured patients were elevated compared to controls, however, the documentation of associated concomitant injuries was not provided.<sup>19, 47, 56</sup> In addition to documenting the stability of this biomarker in elite soccer athletes, we also aim to document changes in sCOMP following AKLI with or without BBL. This investigation will be the first to prospectively document changes in sCOMP in an uninjured cohort that is subsequently injured following activity.

The primary purpose of this investigation was to document the stability of sCOMP in collegiate soccer athletes during a spring soccer season. In addition, we aimed to document patient reported outcomes assessing pain and function related to the knee joint, using two separate PROs. It was hypothesized that sCOMP levels and PRO scores would remain stable over the duration of the spring soccer season. The secondary purpose is to document changes in sCOMP levels following acute knee injury. It was hypothesized sCOMP levels would be increased following AKLI compared to baseline levels.

## Methods

### Design

A prospective, repeated-measures design was employed for the primary aim of this study.

### Population

A volunteer sample of 29 NCAA Division I soccer athletes (18 males, 11 females; age:  $19.6 \pm 1.2$  years; height:  $177.8 \pm 7.4$  cm; weight:  $73.8 \pm 10.2$  kgs) participated in this research study. Subjects were included if they were actively participating in the spring soccer season. Subjects were excluded if they were not participating in soccer related activities due to injury. Informed consent was obtained from the subjects participating in the study. This study was approved by the Institutional Review Board at the University of Kentucky.

### Procedure

Subjects reported for data collection at three time points (pre- (T<sub>1</sub>), mid- (T<sub>2</sub>) and post-season (T<sub>3</sub>)) over the duration of their spring soccer season (February 2010-May 2010). Upon arrival, each subject was asked to remain seated for 30 minutes. It is recommended that subjects remain seated prior to serum collection in order for serum levels to return to baseline following any moderate exercise such as walking to the data collection site.<sup>2, 69</sup> During the 30 minutes of rest, the subjects were asked to complete two PROs. (IKDC, Lysholm). Once the subject had been seated for 30 minutes and both PROs were completed, a maximum of 10ccs of blood was collected from the antecubital vein.

In the event a subject suffered an AKLI while participating in our research study, they were included in the secondary aim of our research investigation. Per our approved protocol, following AKLI, subjects were evaluated by an orthopaedic team physician within 72 hours. Serum was collected at the time of physician evaluation (Time of

Injury). If surgery was warranted, serum was collected immediately preceding surgical intervention (Time of surgery).

#### Enzyme-linked immunosorbent assay

The blood was immediately placed on ice and transported for separation. After clotting for 30 minutes at room temperature, sera was separated and stored in a -20° C freezer and eventually transported into a -80° C freezer until assayed. Once all samples were collected, sCOMP concentrations were determined using a commercial enzyme-linked immunosorbent assay (Euro-Diagnostica, ALPCO, Salem, NH). Serum COMP values are expressed as ng/mL. The average intra-assay coefficient of variance (CV) of all controls was 1%, the average intra-assay CV of all samples was 1% and the average inter-assay CV of all controls was 4%.

#### Patient reported outcomes

Subjects completed two PROs for this study, the IKDC and the Lysholm. These were used to assess function and symptoms related to the knee and were completed by each subject at each of the time points. The IKDC is a valid and reliable PRO that is used to measure knee symptoms, level of function and sports activity in patients following knee injuries.<sup>31, 40</sup> Higher scores represent patients with higher levels of function and fewer self-reported knee symptoms.<sup>31, 40</sup> A change of  $\pm 9$  points is required to indicate a clinically meaningful change in the patient's knee symptoms.<sup>40</sup> The IKDC has acceptable internal consistency and test-retest reliability.<sup>39</sup>

The Lysholm knee scale measures eight condition specific domains for the knee.<sup>60, 92</sup> These domains include: limping, use of supporting device, stair climbing, squatting, walking, locking, instability and pain.<sup>60, 92</sup> The Lysholm is scored from 0 to

100, where a score of 95 to 100 is excellent, 89 to 94 is good , 65 to 83 is fair and <65 is a poor.<sup>45</sup> A minimal detectable change (MDC) for the Lysholm is  $\pm 8.9$  points.<sup>8</sup> For patients with chondral and ACL injuries, the Lysholm has acceptable internal consistency and test-retest reliability.<sup>8,45</sup>

### Statistical Analysis

For the primary aim of this research study, descriptive statistics (Mean  $\pm$  Standard Deviation (SD)) for sCOMP and PROs for each time point were calculated. The independent variables were time and gender. The dependent variables were sCOMP values and scores on two PROs (Lysholm, International Knee Documentation Committee (IKDC)). Separate linear mixed models analyses were used to determine sex by time differences in each of the dependent variables (sCOMP, IKDC, Lysholm) for each of the time points (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>). Paired-sample t-tests were used to explain significant interactions or main effects. Hedge's *g* effect sizes<sup>33</sup> with 95% confidence intervals (CI) were calculated to examine the magnitude of change over time for each of the dependent variables. Calculated effect sizes were interpreted as weak if they were less than 0.40, moderate if between 0.41 and 0.69 and values  $\geq 0.70$  were interpreted as strong.<sup>17</sup> Alpha was set a priori at  $p \leq 0.05$ .

The secondary aim of our study was to document changes in sCOMP following acute knee injury. A cohort of nine uninjured females was used to provide reference sCOMP values for the T1 and T2 time points. For the uninjured cohort, 95% CI (SE\*1.96) were calculated for T1 and T2.

## Results

A total of eight subjects (3 females and 5 males) had a history of lower extremity surgery and none of the subjects had a history of upper extremity surgery; with the most recent surgical procedure occurring in the summer of 2009. Other pertinent medical history for participating subjects included patellar dislocation, patellar fracture, tibial stress fractures, wrist fracture, ankle fracture and clavicle fracture. Following a review of the medical records, seven athletes that participated in the research study were treated during the spring 2010 soccer season for common musculoskeletal injuries such as a quadriceps strain, Iliotibial tendonitis, ankle sprains, medial collateral ligament sprains, and spondylolisthesis. All subjects that participated with a history of surgery were medically cleared to participate in soccer activities as determined by an orthopaedic surgeon and a primary care physician. No ongoing or resolving musculoskeletal injuries were recorded in subjects that were enrolled into the study at the time of physical exam. All subjects that were treated for musculoskeletal injuries during the spring 2010 soccer season were included if they were actively participating in soccer activities during the data collection sessions.

Figure 3.1 depicts the total number of subjects that participated at each data collection session and the total number of sCOMP samples collected. Missing data points were attributed to: a) the inability of the phlebotomist to collect a sample from the subject, b) sample processing error, c) removal from the team, or d) severe knee injury at time of data collection. Figure 3.1 also depicts the time lapse between each of the data collection sessions. If sCOMP samples were not available for the analysis, the PRO scores for those subjects remained in the analysis for each of the time points.

### Serum COMP Values

Descriptive statistics for each of the time points can be found in Table 3.1. A graph of the course of sCOMP for each of time points can be found in Figure 3.2. There was no significant sex by time interaction for sCOMP ( $p=0.44$ ). There was no significant effect for sex ( $p=0.09$ ). There was a significant effect of time on sCOMP levels ( $p<0.001$ ), with significant increases between  $T_1$ - $T_2$  ( $p<0.001$ ),  $T_1$ - $T_3$  ( $p=0.002$ ), but not for  $T_2$ - $T_3$  ( $p=0.08$ ). Calculated Hedge's  $g$  effect sizes and 95% CIs for sCOMP were 1.00 (CI: 0.43, 1.57) for  $T_1$  vs  $T_2$  and 0.63 (CI: 0.02, 1.23) for  $T_1$  vs  $T_3$ . One of these effect sizes was interpreted as strong (1.0) and the second effect size was interpreted as moderate (0.63), and the associated 95% CI for both do not encompass zero.

### sCOMP Minimal Detectable Change

We calculated minimal detectable change (MDC) values using intersession reliability via an intraclass correlation coefficient (ICC) and standard error of measurement (SEM). A total of 18 subjects had sCOMP values for all three data time points and these values were used to calculate MDC scores. MDC scores were calculated with a 95% level of confidence using the formula  $SEM * 1.96 * \sqrt{3}$ .<sup>4, 104</sup> Intersession reliability over the three collection time points was  $ICC=0.61$  ( $SEM=136.9$  ng/mL). The MDC value for sCOMP was 464.6 ng/mL.

### Patient Reported Outcomes

Descriptive statistics for each of the PROs can be found in Table 3.1. There was no significant sex by time interaction for either the Lysholm ( $p=0.52$ ) or the IKDC ( $p=0.16$ ). There was no significant effect for sex for either the Lysholm ( $p=0.10$ ) or the IKDC ( $p=0.25$ ). A significant effect for time was noted for the Lysholm ( $p=0.04$ ), with



significant increases between T<sub>1</sub>-T<sub>3</sub> (p=0.03) and a trend for statistical difference between T<sub>2</sub>-T<sub>3</sub> (p=0.06). A significant effect for time was noted for the IKDC (p=0.02), with significant increases between T<sub>1</sub>-T<sub>3</sub> (p=0.02) and T<sub>2</sub>-T<sub>3</sub> (p=0.04).

For the Lysholm, the effect sizes were -0.09 (CI: -0.64, 0.46) for T<sub>1</sub> vs T<sub>2</sub> and 0.41 (CI: -0.15, 0.97) for T<sub>1</sub> vs T<sub>3</sub>, with one effect size interpreted as weak and one effect size interpreted as moderate. Calculated effect sizes for the IKDC were 0.10 (CI: -0.44, 0.64) for T<sub>1</sub> vs T<sub>2</sub> and 0.49 (CI: -0.08, 1.1) for T<sub>1</sub> vs T<sub>3</sub> with one effect size interpreted as weak and one effect size interpreted as moderate. However, caution must be used when interpreting these results as for each effect size, the 95% CIs did encompass zero.

#### Injured Data

One female subject (age 19 years) suffered an AKLI during data collection. Interestingly, she suffered an AKLI 24 hours after the T<sub>2</sub> data collection session and we were able to collect her time of injury serum one hour following her injury. Her sCOMP level T<sub>1</sub> was 1208 ng/mL, T<sub>2</sub> was 1844 ng/mL, at the time of injury was 1682 ng/mL, and time of surgery was 1068 ng/mL (Figure 3.3). Seven uninjured female subjects (age: 19.7±1.1 years, mass: 66.2±10.3 kgs, height: 173.1±8.6 cms) were included to provide reference sCOMP values for the injured cohort. The mean (±SE) sCOMP values for the uninjured cohort for the pre-season time point was 1269 (±64) ng/mL and for the mid-season time point was 1364 (±74) ng/mL (Figure 2.3). As seen in Figure 3.3, the value for the case subject is within the 95% CI at baseline (T<sub>1</sub>), however, her sCOMP value falls outside the 95% CI at the midseason time point (T<sub>2</sub>).

## Discussion

The purpose of this study was to document the stability of sCOMP levels and PRO scores in uninjured collegiate soccer athletes at three time points during a competitive athletic season. It was found that sCOMP levels and PRO scores increased over the duration of an athletic season. It was also demonstrated that an intersession MDC value of 464.6 ng/mL is necessary to identify significant changes that exceed the variability of the measure. In addition, this study documented sCOMP changes prospectively and following acute knee injury in one of the female soccer players. The injured subject's sCOMP levels were significantly elevated the day prior to her ACL injury and after her injury, compared to baseline levels.

### Stability of sCOMP during elite physical activity

There are few published studies that report baseline levels for young, healthy athletes.<sup>58, 85</sup> Previously reported levels of sCOMP for 6 healthy athletes with no history of joint disease or joint injury (ages 30±9) were 47µg/mL with a range from 10-109 µg/mL.<sup>58</sup> In addition, baseline levels have been reported using healthy blood donors ages 20-65 and healthy children ages 1-20, where sCOMP levels were reported to be 11.3µg/mL (1130 ng/mL) and 10.3µg/mL (1030 ng/mL) respectively.<sup>85</sup> When interpreting sCOMP results, it is important to realize inter-study variation may be due to several factors including standards used for the ELISA, age, BMI, ethnicity and previous surgical intervention.<sup>15, 43</sup> We believe it is imperative authors report these demographics to allow for proper interpretation of these data, particularly athletic populations who are likely to have had previous musculoskeletal injuries.

This is the first study to report intersession MDC values for sCOMP levels in a healthy, physically active population over the duration of an athletic season. These findings indicate an alteration in sCOMP levels of 464.6 ng/mL is required to identify changes that exceed the variability of the biomarker over a three month athletic season. In addition, we have recently reported inter and intraday MDC values for a physically active cohort with no history of lower extremity surgery.<sup>36</sup> These differences may be real, as subjects with a history of surgery were included in the present study but not in the prior Hoch et al. study.<sup>36</sup> Recent literature has hypothesized the use of sCOMP as a biomarker to elucidate the effects of acute articular cartilage injury and the development of preclinical posttraumatic osteoarthritis.<sup>24</sup> The reported MDC values may be beneficial in future investigations of this relationship, which will allow comparison to determine meaningful changes that are associated with this measure.

This is the first study to report changes in sCOMP levels over the duration of an athletic season in healthy subjects. Previous research investigations have reported changes in sCOMP levels following single bouts of activity.<sup>44, 69, 70, 73, 75</sup> The results of this study indicate a statistical difference in the increase in sCOMP levels between T<sub>1</sub> (1482.9±217.9 ng/mL) and T<sub>2</sub> (1723.5±257.9 ng/mL, p=0.001) and T<sub>1</sub> (1482.9±217.9 ng/mL) and T<sub>3</sub> (1624.7±231.6 ng/mL, p=0.002). In addition, there was a trend for a statistically significant decrease in sCOMP levels from T<sub>2</sub> (1723.5±257.9 ng/mL) and T<sub>3</sub> (1624.7±231.6 ng/mL, p=0.08). However, based on the calculated MDC value of 464.6 ng/mL, differences of 241 ng/mL for T<sub>1</sub>-T<sub>2</sub>, 142 ng/mL for T<sub>1</sub>-T<sub>3</sub> and 99 ng/mL for T<sub>2</sub>-T<sub>3</sub> are not clinically significant as they do not exceed the variability associated with the measure. At this time it is not exactly known whether or not changes following single

bouts of exercise are either indicative of an increase in cartilage matrix turnover or cartilage damage.<sup>2, 44, 73</sup> The current findings show, while not clinically significant, there are increases in sCOMP levels over the duration of an athletic season in young, healthy athletes. Even though we measured changes over the three month period, we are unable to speculate whether or not these fluctuations were related to an increase in cartilage matrix turnover or matrix degradation. In addition, we are unable to determine the longitudinal effects of these fluctuations on overall articular cartilage health. Finally, we hypothesize there was a trend for decreasing sCOMP levels from T<sub>2</sub>-T<sub>3</sub> due to a decrease of the amount of participation during the final four weeks of the season. Total time of participation data were not available to support this speculation; however, we do know that as the spring soccer season came to an end, the athletes participated in fewer practice sessions and games. Based on these results, future research studies are needed to investigate the influence participation on sCOMP levels in a physically active cohort.

The current findings indicate a statistical difference between time points for the IKDC and Lysholm. While these differences are important and documentation of change due to normal physical activity is important when employing PROs, it must be cautioned that these differences were not clinically significant based on previously reported MDC values. It has been reported a change of  $\pm 9$  points for the IKDC and  $\pm 8.9$  points for the Lysholm indicates a significant change in patient's knee symptoms.<sup>8, 40</sup> For the IKDC, a 5.1 point difference between T<sub>1</sub> (89.7 $\pm$ 12.4) and T<sub>3</sub> (94.8 $\pm$ 6.1) and a 4 point difference between T<sub>2</sub> (90.8 $\pm$ 8.8) and T<sub>3</sub> (94.8 $\pm$ 6.1) are not large enough to indicate a clinically significant change in a subject's knee symptoms. For the Lysholm, a 2.4 point difference between scores of T<sub>1</sub> (92.4 $\pm$ 8.4) and T<sub>3</sub> (96.2 $\pm$ 7.1) and a 4 point difference between T<sub>2</sub>

(92.3±10) and T<sub>3</sub> (96.3±7.3) are also not sufficiently large to indicate clinically significant changes in the subject's knee symptoms. Furthermore, recent literature has reported normative data for the Lysholm knee score in patients with normal knees.<sup>9</sup> The average Lysholm score was 94 (range 43-100) for uninjured, normal participants.<sup>9</sup> Our data are representative of this score, with scores ranging from 92 to 95 in our uninjured participants. The change in PRO scores did show that these athletes saw improvements in their knee joint health and function over the duration of the athletic season.

#### Course of sCOMP following acute knee injury

Serum COMP levels were elevated in the injured subject at her second data collection time point, which occurred the day prior to ACL injury. We were surprised sCOMP was elevated, and at this time it is unclear if this elevation could potentially be an indicator of high joint load, and whether this would have any implication for the injury she sustained.<sup>34</sup> One could speculate the increased levels of sCOMP at the mid-season data collection time point could signify an increased cumulative load on the knee joint over time; as the injured patient's sCOMP level at the second time point was greater than the upper limit of the uninjured cohort (Figure 3.3). However, additional data are required to better understand the relationship between cumulative load and elevated sCOMP values.

The injured female soccer player had a Costa-Paz<sup>18</sup> Type I concomitant bone bruise lesion documented on MRI. The location of the bone bruise on the femoral condyle was unique when compared to previous description.<sup>18</sup> Costa-Paz<sup>18</sup> has described tibial plateau lesions are more prevalent for Type I classifications and the prevalence of femoral condyle lesions are associated with Type II and III classifications. The

implication of the location of injury is not clear but it has been reported that Type I and Type II classification are more likely to resolve and are less likely to present as postoperative lesions.<sup>18</sup> Hence, the identification of biomarkers associated with subchondral lesions may provide additional information regarding damage prior to- and following injury.<sup>24</sup>

Our injured athlete's sCOMP levels returned to below baseline (pre-season) at the time of surgery. The return to below baseline values was likely due to her pre-surgical rehabilitation plan. The rehabilitation plan consisted of ambulation in a locked brace with the knee in extension, with partial weight bearing and exercises to restore ROM, increase strength and control edema. Therefore, there was a significant decrease in weight bearing, which may have reduced the compressive forces on the articular cartilage. Future prospective investigation of these biomarker levels to assess diagnostic and prognostic efficacy is warranted.

#### Limitations

The present study is not without limitations. First, subjects that participated in the study were not excluded based on prior medical history. For inclusion in our study, the subjects had to be participating in the 2010 spring soccer season and free of acute injury causing them to miss practice or games. Subjects with a history of injury, including anterior cruciate ligament knee reconstruction (ACLR) were included in our study. Since our aim was to determine the stability of sCOMP over time, we did not feel excluding subjects with past severe injury was necessary. Furthermore, both PROs we used were specific to the knee joint. Serum COMP is not specific to cartilage degradation at the knee joint. Therefore, the use of PROs for other joints, such as the Foot and Ankle

Ability Measurement (FAAM)<sup>62</sup> or the Disabilities of the Arm, Shoulder and Hand (DASH)<sup>37</sup> could have been employed to determine self-reported function for these joints. Finally, we did not control for participation in activities outside of soccer practice and soccer associated activities such as lifting and running.

### Conclusion

The present findings indicate that sCOMP levels do not remain stable in healthy, collegiate level soccer players over the duration of a soccer season. However, the difference between each of the separate time points was less than our calculated intersession MDC value of 464.6 ng/mL. These results imply that the effects of intense, continuous physical activity resulted in cartilage degradation as the result of microtraumatic injury or increased turnover, indentified as increased levels of sCOMP. In addition, change in PRO scores for the Lysholm and IKDC did not indicate any decrease in performance or function, or increases in pain. In contrast, the PROs indicated subjects were functioning at higher levels and were reporting near excellent knee joint health as the season progressed. Therefore, in our population sCOMP levels increased but potentially not at a level that is detrimental to articular cartilage health and not at a level that is clinically meaningful based on our MDC value. Future research investigations are warranted to further determine the relationship between these increased levels in sCOMP over the duration of an athletic season, cartilage degradation and a possible connection with primary OA development in athletes who participate in continuous, intense physical activity.

In addition, our study indicated sCOMP levels were elevated prior to injury, following injury and returned to baseline (pre-season) levels following a four week

period with limited weight bearing. Further study is required to better understand the relationship between sCOMP and cumulative load. Given sCOMP is a biomarker for cartilage degradation and our subject had a Costa-Paz<sup>18</sup> grade I bone bruise lesion visible by MRI, future research should also investigate subchondral injury and identifiable biomarkers.

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Table 3.1: Serum Cartilage Oligomeric Matrix Protein (sCOMP) Levels and Patient Reported Outcome Scores (Lysholm and International Knee Documentation Committee (IKDC)) for each of the Time Points (pre-season (T<sub>1</sub>), mid-season (T<sub>2</sub>) and post-season (T<sub>3</sub>)).

<b>Dependent Variable (Mean±SD)*</b>	<b>Pre-season (T<sub>1</sub>)</b>	<b>Mid-season (T<sub>2</sub>)</b>	<b>Post-season (T<sub>3</sub>)</b>
sCOMP (ng/mL)	1482.9±217.9 <sup>†,‡</sup>	1723.5±257.9	1624.7±231.6
Lysholm	93.1±8.1 <sup>†</sup>	92.3±10 <sup>†</sup>	96.3±7.3
IKDC	89.7±12.4 <sup>†</sup>	90.8±8.8 <sup>†</sup>	94.8±6.2

\*: Differences are significant at  $p \leq 0.05$ .

<sup>†</sup>: Significantly different from T<sub>3</sub>.

<sup>‡</sup>: Significantly different from T<sub>2</sub>.

Figure 3.1: Flow chart depicting subject participation, data loss, and time between data collection sessions.

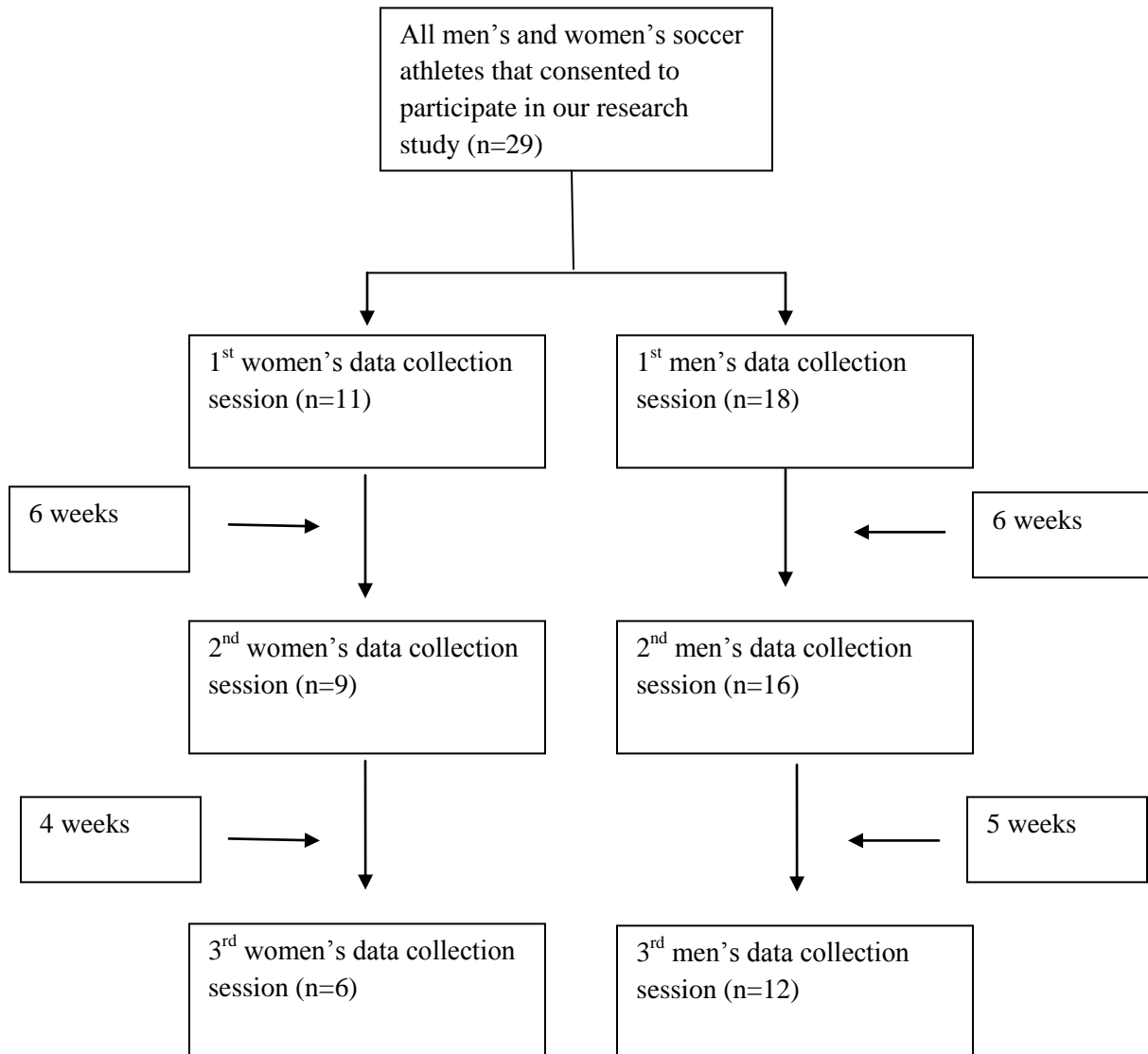
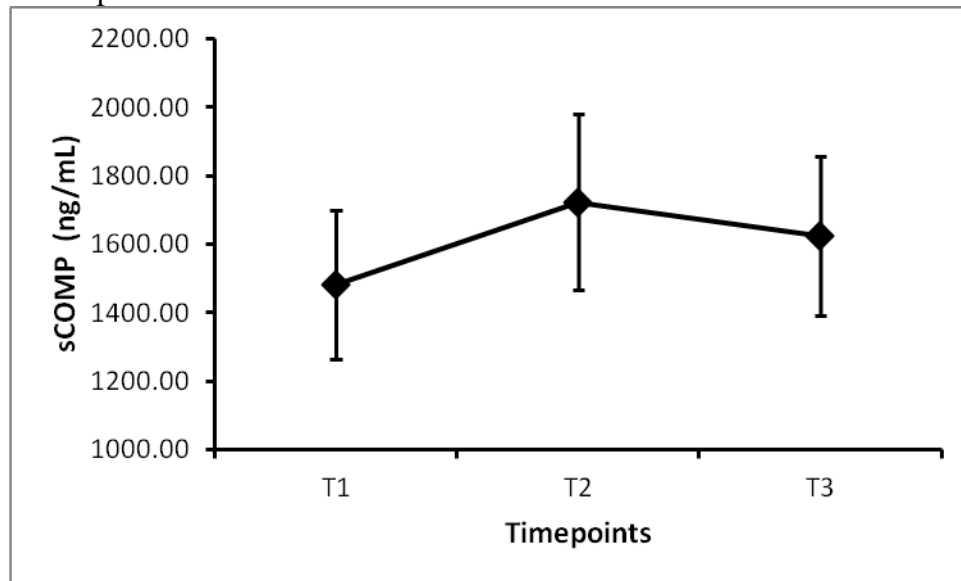
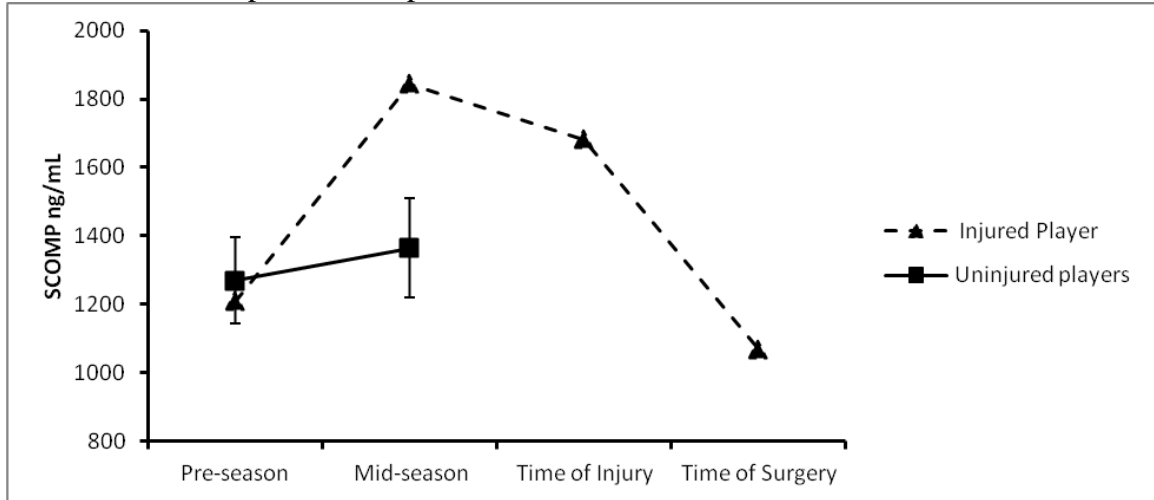


Figure 3.2. The course of serum cartilage oligomeric matrix protein (sCOMP) at three different time points over an athletic season.



Mean sCOMP values ( $\pm$ SD) at pre-season (T1), mid-season (T2), and post-season (T3) for the male and female soccer players.

Figure 3.3: Course of serum cartilage oligomeric matrix protein (sCOMP) in the injured female athlete compared to a representative female cohort.



Serum COMP at pre-season, mid-season, time of injury and time of surgery in a soccer player who suffered an acute ACL rupture and Costa-Paz type I bone bruise, compared to the uninjured female players (mean $\pm$ 95% CI).

## Chapter Four: Differences in articular cartilage damage, and self-reported pain and function for patients with acute knee ligament injury based on bone bruise lesion presence and severity

### Introduction

Bone bruise lesions (BBL) were first reported by Mink and Deutsch and were defined on T-1 weighted images as traumatically involved geographic and non-linear areas of signal loss involving the subcortical bone.<sup>66</sup> This investigation indicated that BBL were commonly associated with injuries to the anterior cruciate ligament (ACL), and that they are not visible on plain radiographic films.<sup>66</sup> Numerous investigations have since confirmed the commonality of these lesions as BBL are present on an estimated 80% of MRI confirmed ACL ruptures,<sup>84, 90, 94</sup> and have also been documented following injuries to the posterior cruciate ligament (PCL),<sup>61</sup> medial collateral ligament (MCL)<sup>65</sup> and posterolateral corner (PLC).<sup>28, 94</sup>

Numerous studies have proposed the modification of protected weight bearing to current rehabilitation protocols for patients with severe BBL prior to and following ligament reconstruction.<sup>16, 24, 50, 71</sup> It is hypothesized that delayed weight bearing for patients with more severe BBL will prevent irreversible damage from occurring to the articular cartilage by allowing time for the lesion and bone to heal.<sup>24, 71</sup> However, due to the lack of objective measuring tools available and the lack of use of severity classification systems, current rehabilitation protocols for patients suffering acute knee ligament injury (AKLI) do not account for severity of BBL nor account for the associated articular cartilage damage. The identification of a biomarker that can further elucidate the relationship between BBL severity and articular cartilage damage may be useful for understanding the effects of current rehabilitation protocols on these lesions,<sup>24</sup> and a more

cost-effective solution to understanding the outcomes of these lesions. For a biomarker to be effective for diagnosing disease or monitoring disease progression, it must be sensitive to differences between healthy individuals and those with cartilage damage and among varying degrees of severity.<sup>3, 14, 15</sup> For patients with AKLI, it has been hypothesized that serum cartilage oligomeric matrix protein (sCOMP) could be used as a biomarker to further investigate cartilage damage and metabolism.<sup>24, 47</sup>

Serum COMP, a cartilage degradation biomarker primarily identified in articular cartilage,<sup>32, 47, 68, 72, 80</sup> and has been investigated as a prognostic and diagnostic indicator for osteoarthritis (OA).<sup>22</sup> It has been proposed as a potential marker to monitor treatment effects following cartilage damage with the hope that cartilage protective therapies can delay further cartilage degeneration and prevent irreversible joint damage associated with OA.<sup>93</sup> The results of a recent systematic review and meta-analysis indicated sCOMP is able to differentiate between patients with and without OA, and between different levels of knee OA severity as measured by the Kellgren/Lawrence scale.<sup>35</sup> In addition, differences in synovial and sCOMP levels for patients with AKLI have been reported in the literature.<sup>19, 47, 58</sup> Serum COMP levels in knee injured patients were elevated compared to controls.<sup>47</sup> Furthermore, visible damage and histological changes of the overlying cartilage in patients with BBL have been reported.<sup>16, 24, 41, 91</sup> During arthroscopy varying degrees of cartilage damage have been visualized, ranging from pristine cartilage, “dimpling” of the area, to softening, fissuring, fibrillation and defect.<sup>16, 41, 50</sup> Biopsies of the articular cartilage overlying acute BBL indicated degeneration of the chondrocytes.<sup>41</sup> Furthermore, immunostaining for COMP of the articular cartilage overlying BBL demonstrated an increase of COMP in the superficial layer matrix,

indicating significant damage to the articular cartilage.<sup>24</sup> Based on this available evidence, sCOMP is thought to be a viable biomarker to investigate outcomes following AKLI and associated articular cartilage injury.<sup>24</sup> However, one limitation with the previous studies that investigated COMP levels following AKLI are that the documentation of associated concomitant injuries nor the severity of BBL were not provided.<sup>19, 47, 58</sup> Since damage to articular cartilage is not evident on plain radiographs and often not evident on MRI or during arthroscopy, we have chosen to use BBL presence and BBL severity as an indicator of an area of articular cartilage that has sustained a high impact injury. Therefore, we must first determine if this biomarker is able to differentiate between patients with and without the condition of interest (BBL), and between differing severities of BBL before it can be employed in prospective, longitudinal investigations.

Finally, there is inconsistent evidence investigating the short term effects of BBL on pain and function in patients with AKLI with concomitant BBL. It has been reported that patients with acute ACL injury and BBL had greater reported pain and loss of function compared to patients without BBL.<sup>42, 97</sup> In addition, these patients had an effusion for a longer period of time and took longer to achieve a nonantalgic gait compared to patients without BBL.<sup>42</sup> In contrast, no significant differences in self-reported pain scores between patients with and without BBL documented on MRI has been reported.<sup>6, 23</sup>

This evidence indicates that there are inconsistencies regarding the effects of BBL on pain and function for patients with AKLI.<sup>6, 23, 42, 97</sup> Inconsistencies may exist for several reasons and one reason may be that there is not a uniform measuring system to

assess the severity of these lesions. Hypothetically, the effects of BBL on pain and function may be relative to differing BBL severities. The benefits of a BBL severity classification system may provide insights to determine if there are in fact differences in pain and function for patients with AKLI and concomitant BBL.

Therefore, the purpose of this study was to determine if differences in articular cartilage damage, and self-reported pain and function exist for patients with AKLI, based on BBL presence and severity. The purposes are four fold and are presented below, along with the null (Ho:) and alternative (Ha:) hypotheses.

1. To determine if differences in sCOMP levels exist between patients with BBL and without BBL.

Ho: There will be no differences in sCOMP levels between patients with and without BBL.

Ha: Patients with BBL will have higher sCOMP levels when compared with patients without BBL.

2. To determine if differences in sCOMP levels exist when patients are compared according to differing BBL severities.

Ho: There will be no differences in sCOMP levels when patients are compared according to differing BBL severities.

Ha: Patients with more severe BBL will have higher sCOMP levels when compared to patients with less severe BBL.

3. To determine if differences in self-reported pain and function, as measured by patient reported outcomes (PROs), exists between patients with and without BBL.



Ho: There will be no differences in self-reported pain and function for patients with and without BBL.

Ha: Patients with BBL will have higher functional limitations and self-reported pain compared to those without BBL.

4. To determine if differences in self-reported pain and function, as measured by PROs, exists when patients are compared according to differing BBL severities.

Ho: There will be no differences in self-reported pain and function for patients with difference BBL severities.

Ha: Patients with more severe BBL will have more functional limitations and self-reported pain compared to patients with less severe BBL.

## Methods

### Design

A cross-sectional study design was employed to determine if: a) differences in serum cartilage oligomeric matrix (sCOMP) levels exist between patients with BBL and without BBL following AKLI; b) to determine if differences in sCOMP levels exist when patients are compared according to differing BBL severities following AKLI; c) to determine if differences in self-reported pain and function, as measured by PROs, exist between patients with and without BBL; and d) to determine if differences in self-reported pain and function, as measured by patient PROs, exist when patients are compared according to differing BBL severities following AKLI. The dependent variables were sCOMP values and scores on the PROs.

### Population

A volunteer sample of 59 patients (gender: 32 males and 27 females, age:  $21.4 \pm 8.7$  years, height:  $174.6 \pm 9.6$  cms, mass:  $80.0 \pm 20.3$  kgs, BMI:  $25.4 \pm 5.2$ ) were recruited at the University of Kentucky Orthopaedic and Sports Medicine Clinic. Patients were included if they had an AKLI (cruciate or collateral) confirmed on MRI. For the purposes of this study, the MRI, physician visit and data collection must have been obtained within four weeks from the date of injury. All patients were confirmed skeletally mature on plain radiographs, had no evidence of osteoarthritis development (Kellgren Lawrence score of 0), had no previous history of lower extremity surgery or severe knee ligament injury documented on MRI and were fluent in and able to read English. Patients were excluded if they were pregnant, a prisoner, or had diminished capacity to provide informed consent. Furthermore, patients were excluded if they had rheumatoid arthritis. This study was approved by the University of Kentucky Institutional Review Board (IRB). Informed consent was obtained from the participants prior to participating in the research study.

### Procedures

Following physician diagnosis and MRI confirmation of AKLI, patients were asked to participate in the research study. Once the patients reviewed and signed the informed consent document, they were asked to remain seated for 30 minutes prior to serum collection.<sup>2, 69</sup> During the 30 minutes of seated rest, patients were asked to complete three PROs (International Knee Documentation Committee (IKDC, Appendix A), The Knee Injury and Osteoarthritis Outcome Score (KOOS, Appendix B) and the Short Form-12 (SF-12, Appendix C)). Once the patients had remained seated for 30

minutes, and all PROs were complete, a maximum of 10cc of blood was collected from the antecubital fossa.

#### Processing of serum

Following serum collection, the blood was allowed to clot for 30 minutes at room temperature. The serum was then separated in a refrigerated centrifuge at 4°C, 2000g for 15 minutes, placed in labeled aliquots and placed on ice. The aliquots were then transported to a -80°C freezer for storage prior to the assays.

#### Bone bruise MRI assessment

All MRIs obtained for participating subjects were independently reviewed by one musculoskeletal radiologist in the University of Kentucky Division of Musculoskeletal Radiology. The radiologist documented: a) presence of BBL, b) number of BBL, c) location of each BBL, d) volume of each BBL and e) severity of each BBL. The radiologist was blinded to the patient's clinical examination to eliminate bias. Each patient was classified according to a) the presence of bone bruise (BBL present, BBL absent) and b) severity of bone bruise (Costa-Paz classification (CPC)-I, CPC- II CPC-III and no bone bruise). In the event the subject had more than one BBL, they were classified according to the most severe lesion for data analysis.

#### Articular cartilage assessment

For patients who underwent surgical reconstruction of their injured ligament(s), visualization of the articular cartilage overlying the BBL lesions was documented. During the surgical intervention, the orthopaedic surgeon visualized the overlying articular cartilage and documented the damage to the overlying articular cartilage: normal, softening, fibrillation, fissuring, or defect.

## Instrumentation

### Patient reported outcomes

#### *International Knee Documentation Committee (IKDC)*

The IKDC is a valid and reliable tool that consists of questions that measure symptoms, level of function and sports activity in patients who suffer knee injuries.<sup>40</sup> The patients are asked to check boxes that represent their ability to perform certain tasks or symptoms that best represent their given their knee injury. A higher score represents a greater level of function and lower level self-reported knee symptoms.<sup>31, 40</sup> A minimal detectable change (MDC) of  $\pm 9$  points must be calculated to demonstrate a clinically meaningful change in knee symptoms.<sup>40</sup> The IKDC has high internal consistency (ICC=0.92) and high test-retest reliability (ICC=0.94).<sup>40</sup> For the purposes of this study, the IKDC current pain score (0-10) was used to assess pain for this study. In addition, the IKDC current function score (0-10) and the overall IKDC score were used to assess function for this study.

#### *The Knee Injury and Osteoarthritis Outcome Score (KOOS)*

The KOOS tool is comprised of 5 domains which assess: pain (KOOS-P), symptoms (KOOS-S), function related to activities of daily living (KOOS-ADL), function related to sports and recreation (KOOS-SP) and quality of life (KOOS-QOL) related to the knee following joint injury.<sup>83</sup> Each domain has a set of questions with five possible choices for the answer such as: never-rarely-sometimes-often-always. A score of 0 represents severe knee impairments and a score of 100 represents no knee impairments.<sup>83</sup> Test-retest reliability ICCs were 0.85, 0.93, 0.75, 0.81 and 0.86 respectively for each of the measured domains.<sup>83</sup> A change of  $\pm 8$  points is needed to

demonstrate a clinically meaningful change in patients who undergo ACL reconstruction.<sup>82</sup> For the purposes of this study, the KOOS-P was used to assess pain for each of the patients. In addition, the KOOS-ADL and the KOOS-SP were used to assess function for each of the patients.

#### *12-Item Short-Form Health Survey (SF-12)*

The SF-12 is a general measure of quality of life that was developed as a condensed version of the Medical Outcomes Study 36-Item Short Form (SF-36).<sup>101</sup> This PRO consists of two domains, the physical component summary (PCS) and the mental component summary (MCS) which are scored separately.<sup>101</sup> The SF-12 component scores are derived from a scoring algorithm that uses item weights to determine the score relative to the representative score of the general population.<sup>101</sup> The test-retest reliability of the PCS-12 was ICC=0.89 and the MSC-12 was ICC=0.76.<sup>101</sup> For the purposes of this study, the SF-12 PCS score will be used as a measure of function.

#### Enzyme-linked immunosorbent assay

Serum COMP concentrations were determined using a commercial enzyme-linked immunosorbent assay (ELISA, ALPCO, Euro-Diagnostica, Malmo, Sweden, COMP-200). This ELISA kit utilized a rabbit polyclonal antiserum directed to human COMP and the standard curve was determined using native human articular cartilage COMP (Wieslab hCOMP quantitative kit, IBL Euro-Diagnostica, Malmo, Sweden). Serum COMP values are expressed as ng/mL. The intra-assay coefficient of variation (CV) of the controls for each of the assays was <2.5%. The average intra-assay CV of the samples for each of the assays was <3.0%.

### Bone bruise volumetric measurement

The Roemer and Bohndorf technique was employed to determine volumetric measurements for each BBL.<sup>81</sup> This volumetric measurement method was performed using the following steps: 1) Using the coronal images: the image with the largest amount of signal change was selected, and a straight line from the most peripheral margins of the zone of hypertensity were drawn (A); 2) A bisecting orthogonal line (B) was drawn to define the second plane; 3) In the sagittal plane, a straight line (C) was drawn to connect the peripheral images. The volume was then be calculated using the formula: Volume= A x B x C.<sup>81</sup>

Intrarater reliability for the musculoskeletal radiologist was determined using the intraclass correlation coefficient (ICC)<sub>3,1</sub>. A random number generator was used to randomly select 15 subjects from the data set. The radiologist then re-measured the volume for each BBL for each of the 15 subjects. If a patient included in the reliability set did not have a BBL, they were excluded from analyses. If a patient had only one lesion, only that lesion was included in the analysis. Finally, if a patient had 2, 3, or 4 lesions, a random number generator was then used to select which BBL would be included in the analysis using the following code: 1=LFC, 2=MFC, 3=LTP and 4=MTP.

### Costa Paz classification system

The Costa-Paz classification (CPC) system was employed to determine the severity of BBL.<sup>18</sup> Type I lesions were defined as “a diffuse signal with change of the medullary component, often reticular and distant from the subjacent articular surface”; Type II defined as: “a localized signal with contiguity to the subjacent articular surface

and are usually crescent lesions”; and Type III was defined as: “a disruption or depression of the normal contour of the cortical surface” (p.446).<sup>18</sup>

Intrarater reliability for the musculoskeletal radiologist was determined using the kappa statistic ( $\kappa$ ). A random number generator was used to randomly select 15 subjects from the data set. The radiologist then re-assigned the CPC grade for each BBL for each of the 15 subjects. If a patient included in the reliability set did not have a BBL, they were excluded from the analysis. If a patient had only one BBL, only that lesion was included in the analysis. Finally, if a patient had 2, 3, or 4 lesions, a random number generator was then used to select which BBL would be included in the analysis using the following code: 1=LFC, 2=MFC, 3=LTP and 4=MTP.

#### Classification system for articular cartilage injury

The severity of the articular cartilage overlying the bone bruise lesion was documented during surgical intervention. The classification normal was given to articular cartilage with normal appearance, softening classified articular cartilage that was soft when probed but normal on appearance, fissuring classified articular cartilage that had visible fissuring or cracks, fibrillation classified articular cartilage that had visible fibrillation and defect classified articular cartilage that had visible chondral fractures or indentations.<sup>10, 41</sup> In the event the patient had more than one BBL with visible damage to the overlying articular cartilage, the patients were classified according to the more severe articular cartilage damage visualized.

#### Data reduction

To determine if differences in sCOMP levels, or self-reported pain and function exist between patients with BBL and without BBL following AKLI the independent

variable was group (BBL present, BBL absent). Subjects included in the BBL group had a CPC I, CPC-II, or CPC-III lesion documented on MRI. Subjects were included in the BBL absent group if there were no visible BBL documented on MRI. The dependent variable was sCOMP levels (ng/mL). The dependent variables for pain were the scores on the IKDC-Pain and the KOOS-P. The dependent variables for function were the scores on the IKDC, IKDC-Function, KOOS-ADL, KOOS-SP and SF-Physical.

To determine if differences in sCOMP levels and self-reported pain and function exist when patients are compared according to differing BBL severities following AKLI, the independent variable was BBL severity (as defined by the CPC system). There were 3 levels of the independent variable. Patients were classified into one of 3 severities (CPC-I, CPC-II and CPC-III). If patients had more than one BBL documented on MRI, they were classified according to their most severe lesion. The dependent variable was sCOMP levels (ng/mL). The dependent variables for pain were the scores on the IKDC-Pain and the KOOS-P. The dependent variables for function are the scores on the IKDC, IKDC current function score (0-10), KOOS-ADL, KOOS-SP and SF-Physical.

### Statistical Analysis

All statistical computations were performed using SAS version 9.3 (SAS Institute, Cary, NC) or IBM SPSS version 19 (IBM SPSS, Chicago, IL).

#### Demographic descriptive statistics

Descriptive statistics (means (SD), counts (percentage)) were calculated for the demographic variables measured. Comparisons of demographic variables measured between the BBL present and BBL absent groups were performed using an Independent samples t-test. In the event the test for equal variance was significant, the unequal



variance p-value was reported. Comparisons of demographic variables measured between the differing BBL severities (CPC-II and CPC-III) were performed using an Independent Samples t-test. There was only one patient in the CPC-I group and this patient's data were removed from the comparisons of demographic variables of interest between the different BBL severities.

#### Bone bruise lesion descriptive statistics

Descriptive statistics (count (percentage)) were calculated for the side (medial or lateral), location (femur or tibia), compartment (LFC, MFC, LTP, MTP) and for CPC severity (CPC-I, CPC-II, CPC-III) for the total number of BBL in this cohort.

Additionally, volumetric measurements (mean (SD)) for each of the locations and classifications were calculated for all BBL in this cohort.

#### Reliability

Intrarater reliability of the musculoskeletal radiologist's ability to determine BBL volume was estimated using ICC<sub>3,1</sub>. ICC's were interpreted as weak if they were less than 0.40, moderate if between 0.41 and 0.69 and values of more than 0.70 were interpreted as strong.<sup>17</sup> A kappa statistic ( $\kappa$ ) was used when determining reliability of the musculoskeletal radiologist's ability to assign CPC grades (CPC-I, CPC-II, CPC-III) because these were ordinal data. Intrarater reliability was considered excellent if  $\kappa \geq 0.80$ , substantial if  $\kappa 0.79-0.60$ , moderate if  $\kappa 0.59-0.40$  and poor if  $\kappa < 0.39$ .<sup>51</sup>

#### Dependent variable descriptive statistics

Descriptive statistics (means (SD), counts (percentage)) were calculated for the dependent variables collected. Independent samples t-tests were used to determine if differences in sCOMP levels, and self-reported pain and function scores (IKDC, IKDC-

Function, IKDC-Pain, KOOS-P, KOOS-ADL, KOOS-SP and SF-Physical) exist between patients with BBL and without BBL following AKLI. In the event the test for equal variance was significant, the unequal variance p-value was reported. Independent samples t-tests were employed to determine if differences in sCOMP levels or self-reported pain and function scores (IKDC, IKDC-Function, IKDC-Pain, KOOS-P, KOOS-ADL, KOOS-SP and SF-Physical) exist when patients were compared according to BBL severity (CPC-II and CPC- III lesions). There was only one patient in the CPC-I group and this patient's data were removed from the comparisons of demographic variables of interest between the different BBL severities. Additionally, descriptive statistics (means (SD)) and differences in scores on the KOOS-S, KOOS-QOL and SF-Mental were calculated and presented.

#### Confounding variable selection

Confounding variables were determined based on several factors. These included trends identified in the literature, clinical experience, or the results of correlation analyses. Furthermore, when determining if differences in BBL severity exist, number of lesions and total volume were included as additional confounding variables associated with BBL severity.

#### Specific aim #1

A one-way ANCOVA was employed to determine if differences in sCOMP levels exist between patients with BBL and without BBL using age, BMI and time elapsed from injury to data collection as covariates. Alpha was set *a priori* at 0.05 for all statistical tests. If significant differences were detected, the adjusted means (95% CI) are reported for each group.

### Specific aim #2

A one-way ANCOVA was employed to determine if differences in sCOMP levels exist between differing BBL severities (CPC-II and CPC-III) using age, BMI, total number of BBL, total BBL volume and time elapsed from injury to data collection as covariates. Alpha was set *a priori* at 0.05 for all statistical tests. If significant differences were detected, the adjusted means (95% CI) are reported for each group.

### Specific aim #3

A one-way ANCOVA was employed to determine if differences in self-reported pain (IKDC-Pain, KOOS-P), function (IKDC-function, KOOS-ADL, KOOS-SP, SF-Physical) and other dependent PRO variables collected (KOOS-S, KOOS-QOL and SF-Mental) exist when those with and without a BBL were compared while using age, BMI and time elapsed from injury to data collection as covariates. Alpha was set *a priori* at 0.05 for all statistical tests. If significant differences were detected, the adjusted means (95% CI) are reported for each group.

### Specific aim #4

An ANCOVA was employed to determine if differences in self-reported pain (IKDC-Pain, KOOS-P), function (IKDC-Function, KOOS-ADL, KOOS-S and SF-Physical) and other dependent PRO variables collected (KOOS-S, KOOS-QOL and SF-Mental) when compared according to BBL severity using age, BMI, total number BBL, total BBL volume and time elapsed from injury to data collection as covariates. Alpha was set *a priori* at 0.05 for all statistical tests. If significant differences were detected, the adjusted means (95% CI) are reported for each group.

### Exploratory regression analysis

An exploratory logistic regression analysis was performed to determine a predicative model for BBL presence and BBL severity using the significant regressors from the one-way ANCOVAs. The area under the curve (AUC) from the receiver operator characteristic curve was used to determine how well the significant regressors predict the outcome of interest (BBL presence or BBL CPC-III). First, univariate logistic regression was performed to determine the AUC for each regressor. Finally, a bivariate logistic regression was performed to determine the AUC for all significant regressors.

### Results

A total of 59 patients consented to participate in this research study. One patient did not complete any of the PRO forms; therefore data for 58 patients were available for these dependent variables. Additionally, serum data were available for 51 patients. Reasons for missing serum data were due to the inability to collect serum from the patient or the patient refused collection. Finally, 47 of the 52 patients with BBL had data available regarding damage to the overlying articular cartilage. Missing articular cartilage scores were either due to the patient not undergoing surgery, or the articular cartilage score was unavailable following surgery.

### Demographic Descriptive Statistics

Descriptive statistics for the demographic characteristics for each of the BBL group can be found in Table 4.1. There were no significant differences in demographic characteristics between the BBL absent and BBL present groups ( $p > 0.05$ ). Descriptive statistics for the demographic characteristics for each of the severity groups (CPC-I, CPC-II and CPC-III) can be found in Table 4.2. There were no significant differences in

demographic characteristics between the severity groups. However, it is of interest to note that there was a significant difference in time elapsed from injury to data collection ( $p=0.04$ ), with patients in the CPC-III group having more time elapsed between time of injury to data collection compared to the patients in the CPC-II group.

#### Bone Bruise Lesion Descriptive Statistics

A total of 52 patients had BBL documented on MRI. Of these patients, 7(13.5%) had 1 BBL, 15 (28.8%) had 2 BBL, 16 (30.8%) had 3 BBL and 14 (26.9%) had 4 BBL documented on MRI.

A total of 143 BBL were documented on MRI for the entire cohort. The average volume for all 143 BBL was 4.4 (8.1)  $\text{cm}^3$ . A majority of the lesions were located on the lateral side and the lateral sided lesions had larger volume when compared to the medial side (Table 4.3). The LFC lesions had the largest volume, followed by the lesions on the LTP. A majority of the lesions in this cohort were CPC-II (77.8%), while the least amount of BBL in this cohort were CPC-I (5.6%). Finally, CPC-III lesions appear to have the largest volume when compared to the CPC-I and CPC-II lesions. Counts (percentages) and volume means (SD) for each of the locations, sides, compartments and CPC severity for all of the BBL can be found in Table 4.3.

There was articular cartilage damage at time of arthroscopy data available for 47 patients. Descriptive statistics for the visible articular cartilage for patients with BBL and for differing BBL severity groups can be found in Table 4.4 and 4.5 respectively. The results of our study indicate that a majority of the articular cartilage overlying BBL is normal in appearance. However our results indicate that 11 BBL had visual articular cartilage damage overlying the LFC (6 softening, 4 fissuring, 1 defect); 9 BBL had

visualized articular cartilage damage in the LTP (7 softening and 2 fissuring); 4 BBL had visualized damage in the articular cartilage overlying the MTP (3 softening and 1 fissuring) and 3 BBL had visualized damage to the articular cartilage in the MFC (2 softening and 1 fissuring). Type II and Type III lesions had more visible changes to the overlying articular cartilage as: 10 Type II lesions were soft to probe, 6 Type II lesions had visible fissuring and 1 Type II lesion had visible defect. In contrast, 4 Type III lesions were soft to probe and 2 Type III lesions had visible fissuring while only 4 Type I lesions had softening when probed.

### Reliability

The results of the ICC<sub>3,1</sub> indicated the musculoskeletal radiologist had excellent reliability when measuring BBL volume, ICC=0.99. Furthermore, the musculoskeletal radiologist had substantial agreement when assigning CPC grades ( $\kappa=0.76$ ).

### Dependent Variable Descriptive Statistics

Means (SD) and counts (percentage) for the dependent variables measured for each of the BBL group can be found in Table 4.4. The results of the Independent samples t-test revealed there were significant differences on two PROs between the groups, with the BBL absent group having significantly higher scores on the KOOS-S ( $p=0.01$ ) and KOOS-QOL ( $p=0.04$ ) when compared to the BBL present group. In addition, means (SD) and counts (percentage) for the dependent variables measured for each of the severity groups (CPC-I, CPC-II and CPC-III) can be found in Table 4.5. The results of the Independent samples t-test employed to determine differences between the severity groups (CPC-II and CPC-III) revealed no significant differences. However, there was a

trend noted for total BBL volume, with patients in the CPC-III BBL group have larger volume when compared to the CPC-II ( $p=0.08$ ).

#### Specific Aim #1

For specific aim #1, we aimed to determine if differences in sCOMP levels exist between patients with BBL and without BBL. While controlling for confounding variables (age, BMI, time elapsed from injury to serum collection) the results of the ANCOVA revealed there are no significant differences in sCOMP levels between patients with and without BBL (Omnibus F-test,  $p=0.08$ ).

#### Specific Aim #2

For specific aim #2, we aimed to determine if differences in sCOMP levels exist when patients were compared according to differing BBL severities (CPC-II and CPC-III). While controlling for potential confounding variables (age, BMI, time elapsed from injury to serum collection, total BBL volume and total number of BBL) the results of the ANCOVA revealed there are no significant differences in sCOMP levels between BBL severities (Omnibus F-test,  $p=0.35$ ).

#### Specific Aim #3

For specific aim #3, we aimed to determine if differences in self-reported pain and function exist between patients with BBL and without BBL. While controlling for confounding variables (age, BMI, time elapsed from injury to data collection) the results indicated patients with BBL reported more knee related symptoms when performing ADLs (60.9, 95% CI: 56.1-65.8), as measured by the KOOS-ADL, when compared to patients without BBL (75.0, 95% CI: 61.9-88.2,  $p=0.05$ ). There were no other significant differences between the other variables.

#### Specific Aim #4

For specific aim #4, we aimed to determine if differences in self-reported pain and function exist when patients were compared according to differing BBL severities (CPC-II and CPC-III). While controlling for potential confounding variables (age, BMI, time elapsed from injury to data collection, total BBL volume and total number of BBL) for each of the severity groups, the results indicated patients with CPC-III BBL reported more knee related symptoms when performing sport related activities (9.5, 95% CI: 1.7-17.3), as measured by the KOOS-SP, when compared to patients with CPC-II BBL (21.2, 95% CI: 14.7-27.8,  $p=0.03$ ).

For the remaining dependent variables that were collected, significant differences between the BBL presence and BBL absent groups were revealed for the KOOS-QOL. Based on the results of the one-way ANCOVA, when controlling for confounding variables, patients without BBL reported higher levels on the KOOS-QOL (46.1, 95% CI: 33.9-58.4) when compared to patients with BBL (30.1, 95% CI: 25.6-34.6,  $p=0.02$ ). Additionally, when patients are compared according to differing BBL severity, patients with less severe lesions (CPC-II) report higher levels on the KOOS-QOL (35.2, 95% CI: 29.4-41.1) when compared to patients with CPC-III lesions (24.2, 95% CI: 17.2-31.2,  $p=0.03$ ).

#### Exploratory Regression Analysis

##### BBL presence

A univariate logistic regression was employed to predict BBL presence and it was found that the AUC for the KOOS-QOL was 0.73 and the AUC for the KOOS-ADL was 0.70. Using a bivariate logistic regression for both regressors, the AUC was 0.76.



Therefore, based on these results it appears the combination of these two PROs will predict BBL presence roughly 76% of the time. However it must be cautioned that this is a preliminary analysis and more subjects are needed to properly interpret these results.

#### BBL severity

The results of the univariate logistic regression predicting the most severe BBL (CPC-III) revealed the AUC for the KOOS-SP was 0.59 and the AUC for the KOOS-QOL was 0.58. Using a bivariate logistic regression for both regressors the AUC was 0.60. Therefore, based on these results it appears a combination of these two PROs will predict the most severe BBL (CPC-III) approximately 60% of the time. However it must be cautioned that this is a preliminary analysis and more subjects are needed to properly interpret these results.

#### Discussion

The study was the first to document if differences in sCOMP levels, and self-reported pain and function scores exist for patients with and without BBL and for patients with differing BBL severities following AKLI. The results of this study revealed there are no differences in sCOMP levels between patients with and without BBL or between differing BBL severities. In addition, we were able to determine that patients with BBL report more functional deficits, as measured by the KOOS-ADL than patients without BBL pre-operatively. Furthermore, patients with more severe BBL (CPC-III) reported more functional deficits, as measured by the KOOS-SP, than patients with less severe BBL (CPC-II). Finally, it was determined that patients with BBL and with more severe BBL report lower levels on the KOOS-QOL compared to patients without BBL and with less severe lesions (CPC-II).

Numerous studies have proposed the modification of protected weight bearing to current rehabilitation protocols for patients with severe BBL prior to and following ligament reconstruction.<sup>16, 24, 50, 71, 84, 91, 103</sup> However, when reviewing the BBL literature there is inconsistent use of a severity measurement for these lesions. Few severity classification systems for BBL exist in the literature.<sup>18, 59</sup> While the definitions of the CPC-I and CPC-II lesions are quite similar to Type I and Type II lesions in the classification of proposed by Lynch et al.<sup>59</sup> for the purposes of this study, we chose the CPC system.<sup>18</sup> The advantage of the CPC systems is CPC-III grade lesions involve damage to the cortical surface and it was our hypothesis that the involvement of the cortical surface would represent more damage to the overlying articular cartilage,<sup>18</sup> compared to the Type III classification described by Lynch that specifically states there is no cortical disruption.<sup>59</sup>

Few studies have used these severity classifications for BBL.<sup>18, 59, 84</sup> The results of the Costa-Paz study<sup>18</sup> revealed 13 (44.8%) CPC-I lesions, 11 (37.9%) CPC-II lesions and 5 (17.2%) CPC-III lesions. Additionally, the results of the Lynch et al. study revealed 99 (69.7%) Type I lesions, 19 (13.4%) Type II lesions and 24 (16.9%) Type III lesions.<sup>59</sup> Additionally, Rosen et al.<sup>84</sup> used a modified Lynch et al.<sup>59</sup> classification where Type I was located in the medullary bone without cortical disruption and Type II lesions had an interruption of the cortex. This report indicated 76 of the 84 BBL were Type I and 8 were Type II.<sup>84</sup> While our study had substantially more BBL compared to the Costa-Paz study,<sup>18</sup> and comparable numbers to that of Lynch et al.,<sup>59</sup> and Rosen et al.,<sup>84</sup> our results indicated a majority of the lesions were CPC-II (112, 78%). In fact, the fewest BBL documented in our patients were CPC-I (8, 5.5%), which was the majority of the lesions

documented for each of the aforementioned investigations.<sup>18, 59, 84</sup> However, we must use caution when comparing our results to the Lynch et al.<sup>59</sup> study as their study was retrospective and patients were included if they had osteoarthritis.<sup>59</sup> Additionally, our results support the findings of Costa-Paz<sup>18</sup> in that the most of the CPC-I lesions are located in the LTP (3/8, 37.5%) but we also found an equal number in the MFC (3/8, 37.5%) and a majority of the CPC-III lesions are located in the LFC (18/23, 78.3%).<sup>18</sup> However, our results indicate that more of the CPC-II lesions occur in the LTP (41/112, 36.7%) as opposed to the LFC proposed by Costa-Paz.<sup>18</sup> Regardless of the classification system used, it does appear that there are trends for more severe lesions to be located in the lateral compartment. While lateral compartment BBL are commonly associated with AKLI, severity classification systems should be used to further understand the outcome of these lesions.<sup>7, 18</sup>

While this study was cross-sectional in nature, previous researchers have reassessed patients using repeat MRI an average of 34 months after injury.<sup>18</sup> The results of the repeat MRI revealed that 1 (9%) of the CPC-II lesions and all 5 (100%) of the CPC-III lesions remained visible and the 5 CPC-III BBL patients had associated articular damage on MRI.<sup>18</sup> Of our 52 patients with documented BBL, 30 had at least 1 CPC-II lesion and 21 had at least 1 CPC-III lesion documented on MRI. If we apply the results of Costa-Paz to our patients, it is estimated roughly 40-45% of the patients included in this study will have articular cartilage changes visible on MRI approximately 2-3 years after injury.<sup>18</sup> The mean age of our patients with BBL is approximately 21 years; if they are likely to have articular changes within a 2-4 year period, it would indicate that 40% of our patients will have articular changes prior to 30 years of age. Regardless of whether

the ACL is reconstructed or not, it is a concern of many physician and scientists that articular cartilage changes occur very rapidly and these patients will develop visible PTOA 10-20 years after injury.<sup>54, 57, 91, 99</sup> While alterations in current treatment rehabilitation protocols for patients with severe BBL is not supported by evidence, it remains quite clear that severe BBL should be acknowledged and further research regarding their relationship with articular cartilage changes are warranted.

We also measured BBL volume as an additional measure to support BBL severity. The volumetric technique selected for the purposes of this study was that described by Roemer and Bohndorf.<sup>81</sup> This technique was chosen as it was believed this technique could be accurately applied to all MRIs, given that our study did not exclude patients based on where the MRI was obtained. The average BBL volume for the Roemer and Bohndorf cohort was 14.75cm<sup>3</sup>. The average volumetric measurement of the BBL in this study was 4.4cm<sup>3</sup>, which appears to be quite different than that of Roemer and Bohndorf.<sup>81</sup> These differences may exist due to differing imaging techniques as the Roemer and Bohndorf<sup>81</sup> study utilized STIR images, and all of the MRIs in our study did not have STIR images. However, of even more interest is the follow-up MRIs of 49 patients from the original study.<sup>81</sup> A total of 49 patients were re-evaluated approximately 2 years after injury and 6 BBL remained visible at follow-up, with an average volume of 2.26cm<sup>3</sup>.<sup>81</sup> The authors did not report the index volumetric measurements of the BBL that were visible on the follow-up MRI, therefore we cannot conclude whether the largest lesions were those that remained visible at follow-up. However, the authors concluded the remaining BBL had visible articular cartilage damage on MRI and this may be the beginning signs of PTOA changes.<sup>81</sup> While the long term outcome of BBL is poorly

understood, it appears that the use of a severity classification system or a volumetric measurement technique is important to identify if these lesions are related to the development of early degenerative changes in longitudinal studies.

The identification of articular cartilage injury overlying BBL at the time of arthroscopy has been inconsistent in the literature. For the purposes of this study, we asked the orthopaedic surgeon to visualize the articular cartilage overlying the BBL and report either: normal, softening, fissuring, fibrillation, or defect. For the patients for which we had these data (Table 4.4), the results indicated that the most severe damage to the articular cartilage overlying BBL was softening (17%), followed by fissuring (11%) and chondral defect (2.2%). Furthermore, we were able to compare the visualization of the articular cartilage overlying the BBL between the differing CPC severities. The results of a smaller study (n=10) revealed damage to the overlying articular cartilage for all patients with BBL.<sup>41</sup> The damage in this cohort ranged from softening to chondral defect, with the most common damage being softening of the area.<sup>41</sup> A second report that investigated articular cartilage changes in the LFC and LTP revealed 14.7% of the LFC BBL had visual articular cartilage damage and 34.4% of the LTP BBL had visual articular cartilage damage.<sup>76</sup> Data concerning the actual damage sustained to the area were only presented for the LTP and the results indicated that 18 were classified as ICRS grade I (softening) and 11 were ICRS grade II (fibrillation).<sup>76</sup> It appears that visualization of the damage to the articular cartilage overlying the BBL may not represent the extent of the damage to these areas. In order to truly quantify the amount of damage that has occurred to these overlying areas, a more specific measurement of articular

cartilage damage, such as biopsy for further immunohistochemical staining may be warranted.

Recent evidence has shown sCOMP levels were significantly increased in patients with AKLI compared to healthy controls.<sup>47</sup> Given that 80% of MRIs diagnosing AKLI have documented BBL, we hypothesized patients with BBL would have damage to the articular cartilage that would be measurable using sCOMP. Furthermore, we hypothesized patients with more severe BBL would have higher levels of sCOMP, indicating more articular cartilage damage compared to the less severe lesions. However, our results indicated there are no differences in sCOMP levels between patients with BBL ( $1362.5 \pm 310.0$ ) and without BBL ( $1509.1 \pm 384.6$ ), and between patients with CPC-II lesions ( $1407.9 \pm 322.5$ ) and patients with CPC-III lesions ( $1315.2 \pm 296.5$ ). Because the ELISA that was utilized for this study was not used in previous research, it is hard to make direct comparisons to the literature that has measured sCOMP in patients with AKLI.<sup>13, 19, 47, 58</sup> However, we can speculate why these differences might not have been detected amongst these groups. First, the patients in our study could have suffered an AKLI up to four weeks prior to inclusion in our study. Numerous researchers have reported that serum or synovial levels of COMP are highest immediately after injury.<sup>19, 58</sup> Potentially the time elapsed from injury to serum collection was too long to detect measurable differences for the patients in our study. However, one must take into consideration the utility of this marker, if there is only a short window of opportunity to measure the levels at their highest, this marker may not be clinically useful given that the clinical population may not be accessible relatively soon after injury. Second, we collected sCOMP as opposed to synovial fluid levels of COMP, and potentially serum

levels of COMP are not specific enough to detect these differences. While one study has supported differences in sCOMP levels between patients with AKLI and healthy controls,<sup>47</sup> another study did not find differences.<sup>58</sup> It could be speculated that differences in the Kuhne et al.<sup>47</sup> study were detected as the patients included had more severe damage to the articular cartilage following AKLI, however, these data were not presented. However, studies have reported differences in the synovial levels of this biomarker when patients are compared to healthy controls.<sup>19, 58</sup> Therefore, synovial levels of this biomarker may be a better indicator of the damage to articular cartilage as the release of COMP in the synovial fluid may be specific enough to represent the damage associated with BBL. However, not all patients that report to the physician's office will have an effusion to warrant removal of the fluid for assessment. Additionally, while these studies report differences in synovial levels for patients with AKLI compared to healthy controls, synovial levels are the highest when sampled closer to the time of injury.<sup>19, 58</sup> While justification of collecting synovial samples at each clinic visit may be difficult, one could justify taking synovial samples at the time of surgery. However, given that each of the studies that have collected synovial fluid COMP levels also documented these levels are highest closer to the time of injury,<sup>19, 58</sup> a time of surgery sample may not truly represent the initial level of damage sustained to the articular cartilage as surgery is not often immediate following AKLI.

Multiple studies have investigated differences in pain, function and symptoms for patients with AKLI with and without BBL.<sup>23, 38, 42, 97</sup> The results of these studies were inconsistent, likely because multiple methods of measuring pain, function and symptoms were utilized or possibly because BBL severity may affect these outcomes. When

controlling for confounding variables (age, BMI and time from injury to data collection) we found patients with BBL to have lower scores on the KOOS-ADL when compared to patients without BBL. These results indicate patients with BBL have a more difficult time performing functional activities related to ADLs due to their knee pathology, when compared to patients without BBL. A recent investigation also found differences in function between patients with and without BBL using the Noyes function scale, with BBL patients having significantly more self-reported functional deficits compared to patients without BBL.<sup>97</sup> Barring any immediate need for surgical reconstruction, most surgeons will wait to perform anterior cruciate ligament reconstruction (ACLR) until the patient has regained full ROM, achieved normal gait and had no effusion.<sup>42</sup> A recent investigation reported patients with BBL had more effusion that lasted longer than patients without BBL, used crutches to ambulate longer than patients without BBL and took longer to regain full ROM compared to patients without BBL.<sup>42</sup> The clinical characteristics that were measured could cause a patient to have difficulty completing their ADLs, especially those activities measured in the KOOS-ADL.<sup>83</sup> It must be noted that we did not measure these characteristics, such as presence of effusion, size of effusion, or ROM deficits in our cohort. Additionally, when controlling for age, BMI and time elapsed from injury to data collection, we reported differences in knee related quality of life, with patients with BBL reporting lower levels on the KOOS-QOL compared to patients without BBL. These results indicate patients with BBL are more aware of their knee problems, have made more modifications to their activities because of their knee, report a lack of confidence in their knee and report more difficulty with their knee compared to the patients without BBL.<sup>83</sup> While all patients with AKLI have some



level impairment, it appears the BBL group may have more impairment, resulting in participation restrictions and activity limitations, as measured via the KOOS-ADL, resulting in a lower health related quality of life.

When controlling for confounding variables, we did not find differences in pain, as measured by the KOOS-P for patients with and without BBL, which supports recent findings where no differences in KOOS-P were found between patients with and without BBL.<sup>23</sup> Additionally, we found no differences in the IKDC-Pain score, which was derived from the IKDC score to represent a 0-10 likert scale used in previous research. These results support a recent investigation that reported only a 0.23 difference between patients with BBL and without BBL.<sup>6</sup> One study measured differences in a likert pain scale, with patients with BBL reporting a pain value of 6.1 compared to a 2.9 reported by patients without BBL.<sup>42</sup> In addition it was reported that the patients with BBL had a larger effusion and decreased ROM.<sup>42</sup> While we did not measure these clinical variables, pain differences detected by Johnson et al.<sup>42</sup> may have been related to increases effusion and the resultant decreased ROM.

We hypothesized the differences in pain, function and symptoms for patients with and without BBL were conflicting because differences may exist based on BBL severity rather than presence. As a result, it is possible that the patients included in studies which reported differences in pain and function<sup>42, 97</sup> contained a cohort of patients with more severe BBL, while the studies that did not detect differences contained cohorts with less severe BBL.<sup>6, 23</sup> Therefore, we also examined whether or not differences in self-reported pain and function exist when patients with BBL were compared according to differing BBL severities. Our results indicated there are differences in function related to sports

and recreation when controlled for confounding variables, as measured by the KOOS-SP, as patients with more severe BBL (CPC-III) reported more difficulty in performing sports related activities compared to less severe BBL (CPC-II). Furthermore, when controlling for confounding variables, our results indicated patients with more severe BBL reported lower scores on the KOOS-QOL compared to patients with less severe lesions and these data in conjunction with a decrease in KOOS-SP also seem appropriate as these patients are unable to participate in meaningful activities and this is reflected in their lower KOOS-QOL scales.

This study was exploratory in nature with the primary aim of determining if a cartilage degradation marker, specifically sCOMP, could detect acute articular cartilage damage associated with BBL and whether or not there would be differences for patients with more severe BBL. We did not detect differences between either of the groups and this could be because sCOMP is not specific enough for the damage that is sustained. Future research studies should explore other markers of articular cartilage degradation that have been utilized in patients with AKLI, such as synovial fluid COMP,<sup>19, 58</sup> CTxII,<sup>13, 53</sup> MMP-3<sup>5, 13</sup> or aggrecan markers.<sup>13, 58</sup> Future research studies should incorporate measures of BBL severity when trying to address the longitudinal outcomes of these lesions. Utilizing a severity classification system or volumetric technique may further elucidate the likelihood of patients developing PTOA following AKLI. Adding a volumetric measurement to the severity classification systems may allow for better quantification of the rate of resolution of BBL for future longitudinal research studies. Finally, in order to better measure the amount of damage occurring to the articular cartilage overlying these lesions, future research studies should incorporate a direct

measurement of articular cartilage damage to identify whether or not there are differences in the amount of damage for each of the BBL severity classifications.

#### Limitations

This study is not without limitations. First, a single radiologist measured the volume of the BBL and assigned the CPC severity for each of the lesions. While we demonstrated sufficient intrarater reliability for each of the measurements, we were unable to compare these results to other raters. Additionally, multiple surgeons viewed and scored the articular cartilage overlying the BBL during arthroscopy. We were unable to determine the intrarater or interrater reliability of these surgeons when assigning these scores to the articular cartilage. While BBL were most commonly identified on MRI diagnosing for ACL injury, we included patients with injuries to PCL and MCL and patients with multiple AKLI. Finally, multiple MRI machines were used for this research study. Patients were not excluded from participating in this research study if they obtained an MRI outside of the University of Kentucky radiology department.

#### Conclusions

The results indicate there are no differences in sCOMP levels when patients are compared according to BBL presence/absence or by differing severities. However, caution must be utilized during interpretation of these results as the group of patients without a BBL and with the least severe BBL (CPC-I) were very small. Additionally, we were able to demonstrate patients with BBL reported a decrease in function related to ADLs compared to patients without BBL and patients with more severe BBL reported a decrease in function related to sports participation compared to patients with less severe BBL. The utilization of a BBL severity classification may further elucidate those patients

that have functional deficits prior to surgical reconstruction. Future research studies with larger cohorts are needed to confirm or dispute the findings within this study.

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Table 4.1: Summary of the Demographic Characteristics (Mean (Standard Deviation (SD) or Count (Percentage)) Collected for the Entire Sample and for the Groups (Bone Bruise Lesion (BBL) present, BBL absent).

	<b>All subjects (N=59)</b>	<b>BBL Present (N=52)</b>	<b>BBL Absent (N=7)</b>
<b>Age</b>			
N	59	52	7
Mean (SD)	21.4 (8.7)	21.0 (8.8)	23.9 (7.8)
<b>Height (cm)</b>			
N	58	51	7
Mean (SD)	174.6 (9.6)	174.0 (9.6)	178.7 (9.1)
<b>Weight (kgs)</b>			
N	58	51	7
Mean (SD)	80.0 (20.3)	77.2 (21.1)	83.6 (12.8)
<b>BMI</b>			
N	58	51	7
Mean (SD)	25.4 (5.2)	25.3 (5.4)	26.3 (3.1)
<b>Injury to MRI (days)</b>			
N	59	52	7
Mean (SD)	4.7 (3.9)	4.7 (3.8)	4.7 (4.6)
<b>MRI to Data (days)</b>			
N	59	52	7
Mean (SD)	6.2 (6.0)	6.0 (5.3)	7.6 (10.4)
<b>Injury to Data (days)</b>			
N	59	52	7
Mean (SD)	10.9 (7.8)	10.7 (7.1)	12.3 (12.6)
<b>Sport</b>			
N	59	52	7
Soccer	14 (23.7)	12 (23.1)	2 (28.6)
Football	11 (18.6)	10 (19.2)	1 (14.3)
Basketball	22 (37.3)	21 (40.4)	1 (14.3)
Other	12 (20.3)	9 (17.3)	3 (42.9)
<b>Race</b>			
N	59	52	7
Caucasian	50 (84.8)	43 (82.7)	7 (100.0)
African American	4 (6.8)	4 (7.7)	0 (0.0)
Other	5 (8.5)	5 (9.6)	0 (0.0)
<b>Injured Side</b>			
N	59	52	7
Left	34 (57.6)	29 (55.8)	5 (71.4)
Right	25 (42.4)	23 (44.2)	2 (28.6)
<b>Gender</b>			
N	59	52	7
Male	32 (54.5)	27 (51.9)	5 (71.4)
Female	27 (45.6)	25 (48.1)	2 (28.6)

Table 4.2: Summary of the Demographic Characteristics (Mean (Standard Deviation (SD), or Count (Percentage)) Collected for the Entire Sample with BBL and for Each of the Severity Groups (Costa-Paz Classification<sup>18</sup> (CPC)-I, CPC-II, CPC-III).

	<b>All subjects (N=52)</b>	<b>CPC-I (N=1)</b>	<b>CPC-II (N= 30)</b>	<b>CPC-III (N=21)</b>
<b>Age</b>				
N	52	1	30	21
Mean (SD)	21.0 (8.8)	15	21.5 (9.0)	20.7 (8.9)
<b>Height (cm)</b>				
N	51	1	30	21
Mean (SD)	174.0 (9.6)	150.0	173.7 (8.9)	175.5 (9.3)
<b>Weight (kgs)</b>				
N	51	1	30	21
Mean (SD)	77.2 (21.1)	63.6	75.6 (19.0)	81.5 (23.9)
<b>BMI</b>				
N	51	1	30	21
Mean (SD)	25.3 (5.4)	28	24.4 (4.4)	26.4 (6.6)
<b>Injury to MRI</b>				
N	52	1	30	21
Mean (SD)	4.7 (3.8)	2	4.1 (3.0)	5.5 (4.8)
<b>MRI to Data</b>				
N	52	1	30	21
Mean (SD)	6.0 (5.3)	11	4.9 (3.7)	7.4 (6.8)
<b>Injury to Data</b>				
N	52	1	30	21
Mean (SD)	10.7 (7.1)	13	9.0 (5.3)	13.1 (8.8)
<b>Sport</b>				
N	52	1	30	21
Soccer	12 (23.1)	1 (100.0)	6 (20.7)	5 (23.8)
Football	10 (19.2)	0 (0.0)	7 (23.3)	3 (14.3)
Basketball	21 (40.4)	0 (0.0)	13 (43.3)	8 (38.1)
Other	9 (17.3)	0 (0.0)	4 (13.3)	5 (23.8)
<b>Race</b>				
N	52	1	30	21
Caucasian	43 (82.7)	1 (100.0)	24 (80.0)	18 (85.7)
African Am.	4 (7.7)	0 (0.0)	3 (10.0)	1 (4.8)
Other	5 (9.6)	0 (0.0)	3 (10.0)	2 (9.5)
<b>Injured Side</b>				
N	52	1	30	21
Left	29 (55.8)	0 (0.0)	20 (66.7)	9 (42.9)
Right	23 (44.2)	1 (100.0)	10 (33.3)	13 (61.9)
<b>Gender</b>				
N	52	1	30	21
Male	27 (52.0)	0 (0.0)	16 (53.3)	11 (52.4)
Female	25 (48.1)	1 (100.0)	14 (46.7)	10 (47.6)

Table 4.3: Summary of the Number of Bone Bruise Lesions (BBL) and Volumetric Measurement for all Lesions, Side (Medial (M) or Lateral (L)), Location (Femoral Condyle (FC) or Tibial Plateau (TP)), Compartment (LFC, MFC, LTP, MTP) and Costa-Paz Classification<sup>18</sup> (CPC) Severities (CPC-I, CPC-II and CPC-III).

	<b>Number of BBL</b>	<b>Mean (SD) cm<sup>3</sup></b>
<b>Total</b>	143	4.4 (8.1)
<b>Side</b>		
L	91(63.2%)	6.0 (9.7)
M	52 (36.8%)	1.8 (1.9)
<b>Location</b>		
FC	62 (43.1%)	5.6 (10.7)
TP	81 (56.9%)	3.5 (5.2)
<b>Compartment</b>		
LFC	43 (29.9%)	7.5 (12.3)
MFC	19 (13.2%)	1.3 (1.6)
LTP	48 (33.3%)	4.6 (6.4)
MTP	33 (22.9%)	2.0 (2.1)
<b>Severity</b>		
CPC-I	8 (5.6%)	4.4 (5.5)
CPC-II	112 (77.8%)	3.0 (5.5)
CPC-III	23 (16.0%)	11.3 (14.1)

Table 4.4: Summary of the Dependent Variables (Mean (Standard Deviation)\* Collected for the Entire Sample and for Each Group (Bone Bruise Lesion (BBL) Present, BBL Absent).

	<b>All Subjects (N=59)</b>	<b>BBL Present (N=52)</b>	<b>BBL Absent (N=7)</b>
<b>sCOMP (ng/mL)</b>			
N	51	45	6
Mean (SD)	1379.7 (318.8)	1362.5 (310.0)	1509.1 (384.6)
<b>IKDC</b>			
N	58	51	7
Mean (SD)	35.5 (13.6)	35.0 (14.4)	38.9 (10.4)
<b>IKDC-Pain</b>			
N	58	51	7
Mean (SD)	5.8 (2.6)	5.8 (2.7)	6.1 (2.1)
<b>IKDC-Function</b>			
N	58	51	7
Mean (SD)	4.3 (1.9)	4.3 (2.0)	4.4 (1.1)
<b>KOOS-P</b>			
N	58	51	7
Mean (SD)	57.9 (18.3)	57.0 (18.9)	64.7 (11.8)
<b>KOOS-S</b>			
N	58	51	7
Mean (SD)	57.7 (10.5)	56.6 (10.0)	66.9 (10.1)
<b>KOOS-ADL</b>			
N	58	51	7
Mean (SD)	62.6 (20.7)	60.9 (20.9)	75 (14.2)
<b>KOOS-SP</b>			
N	58	51	7
Mean (SD)	17.5 (22.3)	16.0 (20.3)	28.6 (33.9)
<b>KOOS-QOL</b>			
N	58	51	7
Mean (SD)	32.1 (18.1)	30.3 (17.7)	45.3 (16.4)
<b>SF-Mental</b>			
N	58	51	7
Mean (SD)	50.3 (9.8)	49.6 (10.2)	53.2 (5.6)
<b>SF-Physical</b>			
N	58	51	7
Mean (SD)	36.5 (8.7)	36.3 (8.5)	38.6 (10.5)
<b>BB Total Volume (cm<sup>3</sup>)<sup>†</sup></b>			
N	59	52	7
Mean (SD)	10.8 (16.2)	12.2 (3.8)	0 (0.0)



Table 4.4 (continued)

**Articular Cartilage  
Damage**

N	47	47	4
Normal	33 (70.2)	33 (70.2)	0 (0.0)
Softening	8 (17.0)	8 (17.0)	0 (0.0)
Fissuring	5 (10.6)	5 (10.6)	0 (0.0)
Fibrillation	0 (0.0)	0 (0.0)	0 (0.0)
Defect	1 (2.1)	1 (2.1)	0 (0.0)

\* sCOMP: serum cartilage oligomeric matrix protein, IKDC: International Knee Documentation Committee Subjective Knee Form, IKDC- Pain: International Knee Documentation Committee Subjective Knee Form Pain Scale, IKDC-Function: International Knee Documentation Committee Subjective Knee Form Function Scale, KOOS-P: Knee Injury and Osteoarthritis Outcome Score-Pain, KOOS-S: Knee Injury and Osteoarthritis Outcome Score- Symptoms, KOOS-ADL: Knee Injury and Osteoarthritis Outcome Score- Activities of Daily Living, KOOS-SP: Knee Injury and Osteoarthritis Outcome Score- Sports and Recreation, KOOS-QOL: Knee Injury and Osteoarthritis Outcome Score- Quality of Life, SF-Mental: 12-Item Short Form Health Survey Mental Component Score, SF-Physical: 12-Item Short Form Health Survey Physical Component Score.

† Total volume is the sum of the volume for each of the lesions present on MRI for the patient.

Table 4.5: Summary of the Dependent Variables\* (Mean (Standard Deviation (SD)), or Count (Percentage)) Collected for the Entire Sample with Bone Bruise Lesions (BBL) and for each Severity (Costa-Paz Classification<sup>18</sup> (CPC)-I, CPC-II, CPC-III).

	<b>All subjects (N=52)</b>	<b>CPC-I (N=1)</b>	<b>CPC-II (N= 30)</b>	<b>CPC-III (N=21)</b>
<b>sCOMP (ng/mL)</b>				
N	45	1	25	19
Mean (SD)	1362.5 (310.0)	1125	1407.9 (322.5)	1315.2 (296.5)
<b>IKDC</b>				
N	51	1	29	21
Mean (SD)	35.0 (14.4)	29.9	34.9 (11.3)	35.5 (18.3)
<b>IKDC- Pain</b>				
N	51	1	29	21
Mean (SD)	5.8 (2.7)	10	5.2 (2.7)	6.4 (2.4)
<b>IKDC- Function</b>				
N	51	1	29	21
Mean (SD)	4.3 (2.0)	10	3.9 (1.7)	4.6 (1.9)
<b>KOOS-P</b>				
N	51	1	29	21
Mean (SD)	57.0 (18.9)	33	58.7 (17.3)	55.8 (21.1)
<b>KOOS-S</b>				
N	51	1	29	21
Mean (SD)	56.5 (10.0)	61	55.5 (8.1)	57.5 (12.3)
<b>KOOS-ADL</b>				
N	51	1	29	21
Mean (SD)	60.9 (20.9)	54	61.7 (17.3)	60.2 (25.9)
<b>KOOS-SP</b>				
N	51	1	29	21
Mean (SD)	16.0 (20.3)	0	17.9 (18.5)	14.0 (23.0)
<b>KOOS-QOL</b>				
N	51	1	29	21
Mean (SD)	30.3 (17.7)	13	32.2 (17.9)	28.3 (17.6)
<b>SF-Mental</b>				
N	51	1	29	21
Mean (SD)	49.8 (10.2)	45.67	48.9 (10.4)	51.4 (10.2)
<b>SF-Physical</b>				
N	51	1	29	21
Mean (SD)	36.3 (8.5)	38.8	36.9 (8.5)	35.3 (8.8)
<b>BB Total Volume (cm<sup>3</sup>)<sup>†</sup></b>				
N	52	1	30	22
Mean (SD)	12.2 (16.8)	1.7	8.6 (11.1)	18.0 (21.8)

Table 4.5 (continued)

**Articular  
Cartilage Damage**

N	47	1	28	18
Normal	33 (70.2)	1 (100.0)	22 (85.2)	10 (55.6)
Softening	8 (17.0)	0 (0.0)	3 (11.1)	5 (27.8)
Fissuring	5 (10.6)	0 (0.0)	2 (7.4)	3 (16.7)
Fibrillation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Defect	1 (2.1)	0 (0.0)	1 (3.7)	0 (0.0)

\* sCOMP: serum cartilage oligomeric matrix protein, IKDC: International Knee Documentation Committee Subjective Knee Form, IKDC-Pain: International Knee Documentation Committee Subjective Knee Form Pain Scale, IKDC-Function: International Knee Documentation Committee Subjective Knee Form Function Scale, KOOS-P: Knee Injury and Osteoarthritis Outcome Score-Pain, KOOS-S: Knee Injury and Osteoarthritis and Outcome Score- Symptoms, KOOS-ADL: Knee Injury and Osteoarthritis Outcome Score- Activities of Daily Living, KOOS-SP: Knee Injury and Osteoarthritis Outcome Score- Sports and Recreation, KOOS-QOL: Knee Injury and Osteoarthritis Outcome Score- Quality of Life, SF-Mental: 12-Item Short Form Health Survey Mental Component Score, SF-Physical: Study 12-Item Short Form Health Survey Physical Component Score.

† Total volume is the sum of the volume for each of the lesions present on MRI for the patient.

## Chapter Five: Summary

### Purposes, Aims and Hypotheses

The purposes of this dissertation were to determine the inter and intraday reliability of serum cartilage oligomeric matrix protein (sCOMP) in a physically active cohort; to document the stability of sCOMP in collegiate athletes during athletic participation and following acute knee ligament injury (AKLI); to determine if differences in sCOMP levels and self-reported pain and function exist for patients following AKLI with bone bruise lesions (BBL) and without BBL, and when compared according to differing BBL severities. The following specific aims and hypothesis were examined throughout the different chapters of this dissertation:

1. To determine the inter and intraday reliability of sCOMP in a physically active cohort.

*Hypothesis:* Serum COMP will have acceptable inter and intraday reliability in a physically active cohort.

2. To document the stability of sCOMP over the duration of an athletic season and to determine if differences are present following AKLI.

*Hypothesis<sub>1</sub>:* Serum COMP will remain stable over the duration of an athletic season.

*Hypothesis<sub>2</sub>:* Serum COMP levels will increase following AKLI.

3. To determine if differences in sCOMP levels exist between patients with BBL and without BBL following AKLI.

*Hypothesis:* Patients with BBL will have higher sCOMP levels compared to patients without BBL.

4. To determine if differences in sCOMP levels exist when patients are compared according to differing BBL severities (Costa-Paz Classification (CPC)-I, CPC-II, CPC-III) following AKLI.

*Hypothesis:* Patients with more severe BBL (CPC-III) will have higher sCOMP levels compared to patients with less severe BBL (CPC-I or CPC-II).

5. To determine if differences in self-reported pain and function exist between patients with BBL and without BBL.

*Hypothesis:* Patients with BBL will have higher levels of self-reported pain and more functional limitations compared to patients without BBL.

6. To determine if differences in self-reported pain and function exist when patients are compared according to differing BBL severities (CPC-I, CPC-II, CPC-III) following ALKI.

*Hypothesis:* Patients with more severe BBL (CPC-III) will report higher levels of self-reported pain and functional limitations compared to patients with less severe BBL (CPC-I or CPC-II).

#### Summary of Findings

The following are summaries of the findings for each of the specific aims:

1. To determine the inter and intraday reliability of sCOMP in a non-elite, physically active cohort.

*Finding:* The hypothesis was confirmed, the results of this study demonstrated strong inter and intraday reliability for sCOMP values in a

healthy, physically active cohort with no history of lower extremity surgery.

2. To document the stability of sCOMP over the duration of an athletic season and to determine if differences are present following AKLI.

*Finding<sub>1</sub>*: The hypothesis was rejected; the results demonstrated sCOMP levels do not remain stable in healthy collegiate level soccer players over the duration of a soccer season. However, the difference between each of the time points was less than the calculated intersession MDC value (464.6 ng/mL), indicating these elevations were not clinically meaningful.

*Finding<sub>2</sub>*: The second hypothesis was confirmed as the results demonstrated significant elevations in sCOMP immediately following AKLI. However, these results must be interpreted with caution as only one subject sustained an ACL injury. Interestingly the results demonstrated a significant elevation in sCOMP levels prior to AKLI.

3. To determine if differences in sCOMP levels exist between patients with BBL and without BBL following AKLI.

*Finding*: The hypothesis was rejected; there were no differences in sCOMP levels between patients with and without BBL.

4. To determine if differences in sCOMP levels exist when patients are compared according to differing BBL severities (CPC-I, CPC-II, CPC-III) following AKLI.

*Finding*: The hypothesis was rejected; there were no differences in sCOMP levels when patients were compared according to differing BBL severities.

5. To determine if differences in self-reported pain and function exist between patients with BBL and without BBL.

*Finding:* The hypothesis was confirmed for the KOOS-ADL, as patients with BBL reported more functional limitations related to knee symptoms when performing ADLs when compared to patients without BBL. For all other variables of function (KOOS-SP, IKDC-Function, SF-Physical), the hypothesis was rejected. Additionally, for the variables that were used to measure pain (KOOS-P and IKDC- Pain), the hypothesis was rejected.

6. To determine if differences in self-reported pain and function exist when patients are compared according to differing BBL severities (BBL absent, CPC-I, CPC-II, CPC-III) following ALKI.

*Finding:* The hypothesis was confirmed for the KOOS-SP, as patients with BBL reported more functional limitations when performing sports related activities when compared to patients without BBL. For all other variables of function (KOOS-ADL, IKDC-Function, SF-Physical), the hypothesis was rejected. Additionally, for the variables that were used to measure pain (KOOS-P and IKDC-Pain), the hypothesis was rejected.

#### Synthesis of Results and Future Research Implications

From these studies several conclusions and suggestions for future research were able to be made.

1. Serum COMP is a reliable marker in subjects who participate in physical activity. Therefore, increases in sCOMP levels are likely due to articular

cartilage damage or increased turnover and are not due to day to day fluctuations.<sup>36</sup> In addition, these results supported previous recommendations that sCOMP levels can be collected at different time points throughout the day for clinical research studies.<sup>1</sup> In addition, our results support the collection of non-fasting samples for future research studies as consuming meals throughout the day was not controlled in our sample. The ability to collect samples during the waking hours and regardless of whether or not patients have consumed food is important for the utility of this marker in clinical populations and the clinical setting.

2. Serum COMP levels do not remain stable over the duration of an athletic season. While these elevations were not considered clinically meaningful when employing the calculated MDC value, knowledge of this phenomenon is important for future research studies. While the amount of participation the athletes were engaged in was not quantified, understanding that biomarkers are increased at different points in the season may be important in understanding the results of future clinical research studies investigating changes in biomarkers following AKLI. Additionally, future research studies should aim to determine whether or not fluctuations in sCOMP levels are due to increase in turnover or irreversible joint damage as this would shed light on the findings of this study.
3. Our results confirm previous results that documented increases in sCOMP levels following AKLI.<sup>47</sup> While there was only one subject included in this analysis, the results indicate that there are measurable differences in sCOMP



following AKLI compared to a cohort who, a) had no history of lower extremity surgery and b) participated in the same levels of athletic activity. However, we must point out that the injured serum was collected roughly one hour following her injury and there was a decrease in her sCOMP levels at her time of surgery. These results further demonstrate sCOMP levels are most elevated immediately after injury and decrease as time elapses from the injury.<sup>13, 58</sup> These results, in combination with previous research studies, support the use of sCOMP as a biomarker for understanding the degree of acute articular damage following AKLI.<sup>24</sup> Furthermore, these results indicated that sCOMP levels are elevated immediately prior to AKLI. Again, while this phenomenon was observed in only one subject, future research studies investigating the utility of this marker or other markers as predictors of AKLI may be necessary.

4. There were no measurable differences in sCOMP levels between patients with and without BBL or between differing BBL severities (CPC-II and CPC-III) following AKLI. Differences may have been elusive because sCOMP was not specific enough to detect such small changes or too much time elapsed from time of injury to serum collection. Should this be true; one must question the use of this marker for future clinical research studies as the need to collect these samples relatively acutely may not be feasible for some researchers or clinicians.
5. There were differences in the KOOS-ADL for patients with BBL when compared to patients without BBL. However, we detected no differences in

pain between the two groups. Additionally, differences in KOOS-SP were detected for patients with more severe BBL when compared to patients without BBL. Furthermore, we detected no differences in pain between the two severity groups. Keeping in mind that our BBL absent group was relatively small (n=7) and we had only one patient in the CPC-I group, these findings should be reviewed with caution. However, based on these results, integrating a BBL severity classification into future research studies may be able to better explain clinical phenomena, such as differences in self-reported pain and function.

### Conclusions

This dissertation investigated the use of sCOMP as a biomarker for acute articular cartilage damage. Serum COMP is a biomarker that has been studied extensively in the osteoarthritis literature,<sup>15, 43</sup> a chronic articular cartilage disease. Recent literature has suggested that people who participate in intense exercise<sup>12, 48</sup> or for those who succumb to AKLI, regardless of whether they have ligament reconstruction or not,<sup>54, 57, 91</sup> may develop degenerative articular cartilage disease. We hypothesized sCOMP would be a viable marker to better understand acute articular cartilage damage associated with exercise and ALKI.

Serum COMP levels demonstrate strong inter and intraday reliability in subjects who engage in physical activity. We noted that sCOMP levels did not remain stable over the duration of an athletic season. While these changes did not exceed the calculated MDC value, sCOMP may be useful to document the long term effects of exercise on articular cartilage. Additionally, differences were noted following AKLI in one subject

when compared to an uninjured cohort. However, we did not detect differences in sCOMP levels between patients with and without BBL, or differing BBL severities. While these results were insignificant, there may exist additional biomarkers that may be appropriate such as synovial fluid COMP. Synovial fluid COMP may be a more specific marker of acute cartilage damage that requires further analysis. Additionally, while a majority of these patients did not have visible articular cartilage damage when viewed during arthroscopy, a more specific documentation of articular cartilage injury overlying these lesions, such as biopsies, may be necessary to further understand the damage that is sustained to the overlying articular cartilage. In summary, while it appears sCOMP maybe not be a viable marker for understanding the acute articular cartilage damage associated with AKLI, it does appear to be useful for studies investigating the effects of intense exercise on articular cartilage.

Appendix A: International Knee Documentation Committee (IKDC)

Patient Name \_\_\_\_\_ ID \_\_\_\_\_ Side Right Left

Date of review: \_\_/\_\_/\_\_ OR Follow up period: PreOp OR \_\_ weeks/months/years (circle one)



**SYMPTOMS\*:**

\*Grade symptoms at the highest activity level at which you think you could function without significant symptoms, even if you are not actually performing activities at this level.


1. What is the highest level of activity that you can perform without significant knee pain?

- Very strenuous activities like jumping or pivoting as in basketball or soccer
- Strenuous activities like heavy physical work, skiing or tennis
- Moderate activities like moderate physical work, running or jogging
- Light activities like walking, housework or yard work
- Unable to perform any of the above activities due to knee pain

2. During the past 4 weeks, or since your injury, how often have you had pain?

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

3. If you have pain, how severe is it?

- No pain 0 1 2 3 4 5 6 7 8 9 10 Worst Pain Imaginable
-             

4. During the past 4 weeks, or since your injury, how stiff or swollen was your knee?

- Not at all  Mildly  Moderately  Very  Extremely

5. What is the highest level of activity you can perform without significant swelling in your knee?

- Very strenuous activities like jumping or pivoting as in basketball or soccer
- Strenuous activities like heavy physical work, skiing or tennis
- Moderate activities like moderate physical work, running or jogging
- Light activities like walking, housework, or yard work
- Unable to perform any of the above activities due to knee swelling

6. During the past 4 weeks, or since your injury, did your knee lock or catch?

- Yes  No

7. What is the highest level of activity you can perform without significant giving way in your knee?

- Very strenuous activities like jumping or pivoting as in basketball or soccer
- Strenuous activities like heavy physical work, skiing or tennis
- Moderate activities like moderate physical work, running or jogging
- Light activities like walking, housework or yard work
- Unable to perform any of the above activities due to giving way of the knee

**SPORTS ACTIVITIES:**

8. What is the highest level of activity you can participate in on a regular basis?

- Very strenuous activities like jumping or pivoting as in basketball or soccer
- Strenuous activities like heavy physical work, skiing or tennis
- Moderate activities like moderate physical work, running or jogging
- Light activities like walking, housework or yard work
- Unable to perform any of the above activities due to knee



9. How does your knee affect your ability to:

	Not difficult at all	Minimally difficult	Moderately Difficult	Extremely difficult	Unable to do
a. Go up stairs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b. Go down stairs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c. Kneel on the front of your knee	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d. Squat	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e. Sit with your knee bent	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
f. Rise from a chair	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
g. Run straight ahead	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
h. Jump and land on your involved leg	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
i. Stop and start quickly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>



**FUNCTION:**

10. How would you rate the function of your knee on a scale of 0 to 10 with 10 being normal, excellent function and 0 being the inability to perform any of your usual daily activities which may include sports?

**FUNCTION PRIOR TO YOUR KNEE INJURY:**

Cannot perform daily activities	0	1	2	3	4	5	6	7	8	9	10	No limitation
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

**CURRENT FUNCTION OF YOUR KNEE:**

Cannot perform daily activities	0	1	2	3	4	5	6	7	8	9	10	No limitation
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

## Appendix B: Knee Injury and Osteoarthritis Outcome Survey

**Patient Name** \_\_\_\_\_ **Patient ID** \_\_\_\_\_  
**Side**  Right  Left **Study Name** \_\_\_\_\_ **Study Number** \_\_\_\_\_  
**Date** \_\_\_\_/\_\_\_\_/\_\_\_\_ or **PreOp** weeks/months/years (circle one)  
**Filled in by:**  Operating Dr.  Other MD  Research Assistant  Questionnaire  Other  
**Reviewer Name:** \_\_\_\_\_ **Next Visit Due** \_\_\_\_/\_\_\_\_/\_\_\_\_

**INSTRUCTIONS:** This survey asks for your view about your knee. This information will help us keep track of how you feel about your knee and how well you are able to do your usual activities. Answer every question by ticking the appropriate box, only one box for each question. If you are unsure about how to answer a question, please give the best answer you can.

### Symptoms

These questions should be answered thinking of your knee symptoms during the **last week**.

S1. Do you have swelling in your knee?

Never  Rarely  Sometimes  Often

S2. Do you feel grinding, hear clicking or any other type of noise when your knee moves?

Never  Rarely  Sometimes  Often

S3. Does your knee catch or hang up when moving?

Never  Rarely  Sometimes  Often

S4. Can you straighten your knee fully?

Always  Often  Sometimes  Rarely

S5. Can you bend your knee fully?

Always  Often  Sometimes  Rarely

### Stiffness

The following questions concern the amount of joint stiffness you have experienced during the **last week** in your knee. Stiffness is a sensation of restriction or slowness in the ease with which you move your knee joint.

S6. How severe is your knee joint stiffness after first wakening in the morning?

None  Mild  Moderate  Severe

S7. How severe is your knee stiffness after sitting, lying or resting later in the day?

None  Mild  Moderate  Severe

### Pain

P1. How often do you experience knee pain?

Never  Monthly  Weekly  Daily

What amount of knee pain have you experienced the **last week** during the following activities?

P2. Twisting/pivoting on your knee

None  Mild  Moderate  Severe

P3. Straightening knee fully

None  Mild  Moderate  Severe

P4. Bending knee fully

None  Mild  Moderate  Severe

**Pain, continued**

P5. Walking on flat surface	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
P6. Going up or down stairs	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
P7. At night while in bed	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
P8. Sitting or lying	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
P9. Standing upright	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe

**Function, daily living**

The following questions concern your physical function. By this we mean your ability to move around and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your knee.

A1. Descending stairs	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A2. Ascending stairs	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A3. Rising from sitting	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A4. Standing	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A5. Bending to floor/pick up an object	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A6. Walking on flat surface	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A7. Getting in/out of car	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A8. Going shopping	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A9. Putting on socks/stockings	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A10. Rising from bed	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A11. Taking off socks/stockings	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A12. Lying in bed (turning over, maintaining knee position)	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe
A13. Getting in/out of bath	<input type="checkbox"/> None	<input type="checkbox"/> Mild	<input type="checkbox"/> Moderate	<input type="checkbox"/> Severe

For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your knee.

A14. Sitting

None Mild Moderate Severe

A15. Getting on/off toilet

None Mild Moderate Severe

A16. Heavy domestic duties (moving heavy boxes, scrubbing floors, etc)

None Mild Moderate Severe

A17. Light domestic duties (cooking, dusting, etc)

None Mild Moderate Severe

### Function, sports and recreational activities

The following questions concern your physical function when being active on a higher level. The questions should be answered thinking of what degree of difficulty you have experienced during the **last week** due to your knee.

SP1. Squatting

None Mild Moderate Severe

SP2. Running

None Mild Moderate Severe

SP3. Jumping

None Mild Moderate Severe

SP4. Twisting/pivoting on your injured knee

None Mild Moderate Severe

SP5. Kneeling

None Mild Moderate Severe

### Quality of Life

Q1. How often are you aware of your knee problem?

Never Monthly Weekly Daily

Q2. Have you modified your life style to avoid potentially damaging activities to your knee?

Not at all Mildly Moderately Severely

Q3. How much are you troubled with lack of confidence in your knee?

Not at all Mildly Moderately Severely

Q4. In general, how much difficulty do you have with your knee?

None Mild Moderate Severe

Thank you very much for completing all the questions in this questionnaire.



Appendix C: 12 Item Short Form Health Survey

Patient Name \_\_\_\_\_ Patient ID \_\_\_\_\_  
 Side  Right  Left Study Name \_\_\_\_\_ Study Number \_\_\_\_\_ Date \_\_\_\_/\_\_\_\_/\_\_\_\_ or PreOp  
 weeks/months/years (circle one)  
 Filled in by:  Operating Dr.  Other MD  Research Assistant  Questionnaire  Other  
 Reviewer Name: \_\_\_\_\_ Next Visit Due \_\_\_\_/\_\_\_\_/\_\_\_\_

1) In general, would you say your health is:

- Excellent
- Very Good
- Good
- Fair
- Poor

2) The following questions are about activities you might do during a typical day.

Does your health now limit you in these activities?

If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at al
Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Climbing <u>several</u> flights of stairs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

3) During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the tim	None of the time
<u>Accomplished less</u> than you would like	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Were limited in the <u>kind</u> of work or other activities	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

4) During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
<u>Accomplished less</u> than you would like	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Did work or other activities <u>less carefully than usual</u>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

5) During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

- Not at All
- A Little Bit
- Moderately
- Quite a Bit
- Extremely

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the past 4 weeks...

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
Have you felt calm and peaceful?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Did you have a lot of energy?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Have you felt downhearted and depressed?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?**

- All of the time
- Most of the time
- Some of the time
- A little of the time
- None of the time

This form includes questions from the SF-36™ Health Survey. Reproduced with the permission of the Medical Outcomes Trust, Copyright © 1992.

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Vita  
Johanna M. Hoch, ATC

**General information**

Birth place and date: Brookville, OH 07/22/1983

Certificate or Specialty Board Licensure:

National Athletic Trainer's Association Board of Certification #030602048

**I. Education**

- |                |                                                                                                                                                                                                                                                               |
|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2009 – Present | <u>The University of Kentucky</u> , College of Health Sciences<br>Doctor of Philosophy, Rehabilitation Sciences<br>Expected Completion: May 2012<br>Dissertation: Serum cartilage oligomeric matrix protein: a biomarker for acute articular cartilage damage |
| 2006– 2008     | <u>Ohio University</u> , College of Health and Human Sciences<br>Master of Science, Recreation and Sports with a concentration in Athletic Training                                                                                                           |
| 2002-2006      | <u>Ohio Northern University</u> , College of Arts & Sciences<br>Bachelor of Science, Athletic Training                                                                                                                                                        |

**II. Professional Experiences**

- |                      |                                                                                                                                                                                             |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| August 2009- Present | Graduate Assistant, DonJoy Orthopaedics and Orthopaedics and Sports Medicine<br>Advisor: Carl Mattacola, PhD, ATC, FNATA<br>Department of Rehabilitation Sciences<br>University of Kentucky |
| 2008-2009            | Visiting Assistant Professor<br>Undergraduate Athletic Training Program<br>Department of Exercise and Sports Sciences<br>Eastern Kentucky University                                        |
| 2006-2008            | Graduate Assistant Athletic Trainer<br>Ohio University                                                                                                                                      |

### III. Teaching Activity

#### University of Kentucky

KHP 720: Sports Medicine- Teaching Assistant

#### Eastern Kentucky University

ATR 100- Introduction to Athletic Training- Instructor of Record

ATR 201- Practicum I: Taping and Bracing- Instructor of Record

ATR 202- Practicum II: Lower Extremity Evaluation- Instructor of Record

ATR 211- Lower Extremity Evaluation- Instructor of Record

ATR 302: Practicum IV: Modalities- Instructor of Record

PHE 212: Care and Prevention of Athletic Injuries- Instructor of Record

#### Ohio University

RSAT 155- Introduction to Athletic Training- Teaching Assistant

RSAT 160- Practical Aspects in Athletic Training- Teaching Assistant

RSAT 180B- Practical Applications in Athletic Training II- Instructor of Record

RSAT 180C- Practical Applications in Athletic Training III- Instructor of Record

RSAT 220- Physical Examination of the Lower Extremity- Teaching Assistant

RSAT 230- Physical Examination of the Head and Spine- Teaching Assistant

RSAT 280A- Practical Applications in Athletic Training IV- Instructor of Record

RSAT 370- Applied Human Anatomy Prosection- Teaching Assistant

### IV. Honors

2012 Robinson Graduate Award for Research Creativity

2012 University of Florida Neuromuscular Plasticity Scholar Award

2011 ACSM Biomechanics Interest Group (BIG) Student Travel Award

2011 Academic Excellence Scholarship, University of Kentucky

2011 Wright Scholarship, University of Scholarship

2010 Wright Scholarship, University of Kentucky

### V. Speaking Engagements/ Presentations

#### Peer Reviewed

June 2012 National Athletic Trainer's Associate Annual Meeting and Symposium, St. Louis, MO

Oral: *A descriptive analysis of bone bruise presence and severity based on location for patients with acute knee injury.*

Hoch JM, Montgomery J, Johnson DL, Mattacola CG, Lattermann C.

- June 2012 National Athletic Trainer's Associate Annual Meeting and Symposium, St. Louis, MO  
Poster: *The influence of exercise intensity on serum cartilage oligomeric matrix protein (sCOMP)*. Mateer JL, Hoch JM, Mattacola CG, Li HF, Bush HM, Lattermann C.
- May 2012 American College of Sports Medicine, San Francisco, CA  
Poster: *Normative serum cartilage oligomeric matrix protein (sCOMP) levels in an uninjured, physically active population*. Hoch JM, Mateer JL, Mattacola CG, Lattermann C.
- May 2012 International Cartilage Repair Society World Congress, Montreal, Canada  
Oral: *Weekly changes in serum cartilage oligomeric matrix protein (sCOMP) levels for soccer athletes over a 10 week spring soccer season*. Hoch JM, Mateer JL, Mattacola CG, Lattermann C.
- May 2012 International Cartilage Repair Society World Congress, Montreal, Canada  
Oral: *Systematic review and meta-analysis of the responsiveness of patient report outcome instruments following autologous chondrocyte implantation*. Hoch JM, Mateer JL, Mattacola CG, Lattermann C.
- June 2011 National Athletic Trainer's Associate Annual Meeting and Symposium, New Orleans, LA  
Researchers Forum: *Demystifying the clinical and translational research process: integrating clinicians and capturing the appropriate outcomes*. Hoch JM, Howard JS, Mattacola CG.
- June 2011 National Athletic Trainer's Associate Annual Meeting and Symposium, New Orleans, LA  
Poster: *Serum cartilage oligomeric matrix protein and patient reported outcomes in division I soccer athletes over the course of an athletic soccer season*. Hoch JM, Mattacola CG, Silkman CL, Stephenson DR, Bush HM, Lattermann C.
- May 2011 American College of Sports Medicine, Denver, CO  
Poster: *Reliability and minimal detectable change of serum cartilage oligomeric matrix protein in an athletic population* Hoch JM, Mateer JL, Mattacola CG, Lattermann C.
- May 2011 American College of Sports Medicine, Denver, CO  
Poster: *Dorsiflexion range of motion and dynamic balance asymmetry in adolescents*. McKeon PO, Hoch MC, Silkman CL, Hoch JM, Medina-McKeon JM.
- Sept 2010 International Cartilage Repair Society World Congress, Barcelona, Spain  
Oral: *Serum cartilage oligomeric matrix protein is elevated in patients with knee osteoarthritis: a systematic review*. Hoch JM, Mattacola CG, Howard JS, Lattermann C.

- Sept 2010 International Cartilage Repair Society World Congress, Barcelona, Spain  
 Oral: *Changes in functional performance during walking, squatting, rising, and stepping following autologous chondrocyte implantation.* Howard JS, Mattacola CG, Hoch JM, Lattermann C.
- Sept 2010 International Cartilage Repair Society World Congress, Barcelona, Spain  
 Oral: *Time-line for self-reported changes in function following autologous chondrocyte implantation (ACI) over one year.* Lattermann C, Howard JS, Hoch JM, Mattacola CG,
- Sept 2010 International Cartilage Repair Society World Congress, Barcelona, Spain  
 Oral: *Comparison of knee strength pre-operatively, and 6 and 12 months post-operatively following autologous chondrocyte implantation (ACI).* Howard JS, Mattacola CG, Hoch JM, Lattermann C.

## VI. Research Creative Productivity

### Publications: Peer Reviewed Journals

Hoch JM, Mattacola CG. The effect of bone bruises on pain in patients with traumatic knee injury. *Journal of Sport Rehabilitation*.2012, 21:79-82.

Hoch JM, Mattacola CG, McKeon JM, Howard JS, Lattermann C. Serum cartilage oligomeric matrix protein (sCOMP) is elevated in patients with knee osteoarthritis: a systematic review and meta-analysis. *Osteoarthritis and Cartilage* 2011;19:1396-1404. Doi:/10.1016/j.joca.2011.09.005.

Hoch JM, Mattacola CG, McKeon JM, Shay JN, Lattermann C. Determination of the interday and intraday reliability of serum cartilage oligomeric matrix protein in a physically active population. *Cartilage*.2011,2(4):394-398.

Hoch JM, White K, Starkey C, Krause BA. Delegation and empowerment in CAATE accredited athletic training education programs. *Athletic Training Education Journal*. 2009,4:139-143.

### Grant Activity

- 2011 American College of Sports Medicine Doctoral Student Research Grant  
 Project: *Serum COMP: a biomarker for acute articular cartilage damage.*  
 Investigators: Hoch JM, Mattacola CG, McKeon JM, Bush HM, Hewett TE, Gonzalez G, Lattermann C. Submitted to: American College of Sports  
*Funded: \$5,000.00.*

- 2012 University of Kentucky Department of Orthopaedics and Sports  
Medicine  
Project: *Serum COMP analysis in athletes and bone bruise analysis in ACL patients.*  
Investigators: Shah JN, Hoch JM, Lattermann C.  
*Funded: \$8,500.00,*
- 2010 University of College of Health Sciences Pilot Funding Program  
Project: *Determination of the inter- and intra-day reliability of serum cartilage oligomeric matrix protein in a physically active cohort.*  
Investigators: Mattacola CG, Hoch JM, McKeon JM, Lattermann C.  
*Funded: \$1,970.00.*
- 2009 University of Kentucky Clinical and Translational Sciences Seed  
Grant  
Project: *Determination of the stability of serum cartilage oligomeric matrix protein (COMP) over a period of physical activity, and in the occurrence of injury.*  
Investigators: Hoch JM, Mattacola CG, Bush HM, Lattermann C.  
*Funded: \$5,000.00.*