Injuries to the anterior cruciate ligament (ACL) are common and are most frequently the result of sports participation [1]. In contrast to extra-articular ligaments, complete tears of the ACL fail to heal [2]. Historical reports showed unsatisfactory outcomes following ACL primary repair [3-5] leading to unanimous abandonment of suture repair and widespread adoption of ACL reconstruction. Currently, ACL reconstruction is considered the gold standard of care for ACL injuries, especially for young people who aim to return to high-level sporting activities [6].

In recent years there has been renewed interest in remnant preservation surgery to maintain the biomechanical properties of the native ACL [7-9] followed by advancements in functional tissue engineering and regenerative medicine. However, novel techniques rely on understanding the pathophysiological processes within the injured ligament and its environment. Existing studies that have investigated these processes have mainly been performed on animals [10,11] with few conducted on humans [2,12,13]. Murray and colleagues [12] evaluated 23 ACL remnants after complete rupture in humans and described four different histological phases. Sonnery-Cottet et al. [13] described the histological features of 26 ACL partial tear remnants and showed competent histological structures that support remnant preservation. Nguyen et al. [2] showed that on five patients the human proximal third ACL has an intrinsic healing response with typical histological characteristics similar to the medial collateral ligament. None of these histological studies described the corresponding morphological changes of the ACL remnant.

The purpose of this study was to determine the morphological and corresponding histological appearances of the torn ACL in relation to time after injury. The hypothesis was that after the acute inflammatory phase of injury, the torn ACL undergoes a short reparative phase and a prolonged remodeling phase that ends with adherence to the PCL.

The study was approved by an institutional review board. The study included remnant specimens of torn anterior cruciate ligaments (ACL) from patients who underwent ACL reconstruction surgery in the year 2017. Surgeries were performed by two surgeons (BH, MK) who specialized in arthroscopic procedures. Exclusion criteria were prior knee surgery, tibial eminence fracture, posterior cruciate ligament rupture, and meniscal tears.

**ABSTRACT**  
**Background:** Injuries to the anterior cruciate ligament (ACL) are common and complete tears often fail to heal. ACL reconstruction is considered the surgical gold standard of care for ACL injuries in young active patients.  
**Objectives:** To determine the corresponding morphological and histological features of the torn ACL in different time periods after injury.  
**Methods:** The study included 28 remnant specimens of torn ACLs from patients who had ACL reconstruction surgery of the knee. The remnant pathology was evaluated by its morphology during arthroscopy and by histopathologic measurements.  
**Results:** At surgery there were three progressive and distinct morphological tear patterns. The first pattern was noticed within the first 3 months from injury and showed no scar tissue. The second pattern appeared later and was characterized by the appearance of scar tissue with adherence to the femoral wall. The third pattern was characterized by adhesion of the ACL remnant to the posterior cruciate ligament. The histological changes of the first morphological pattern showed abundance of blood vessels and lymphocytes at the torn femoral end with few irregular collagen fibers. The second and third tear patterns showed decrement in the number of blood vessels and lymphocytes with longitudinally oriented collagen fibers.  
**Conclusions:** The morphological features of the ACL remnant in the first 3 months after injury showed no scar tissue and its histological features had the characteristics of a reparative phase. This phase was followed by a prolonged remodeling phase that ended with attachment of the remnant to the posterior cruciate ligament.

**KEY WORDS:** anterior cruciate ligament (ACL), ACL tear, knee, histology, morphology

**PATIENTS AND METHODS**  
The study was approved by an institutional review board. The study included remnant specimens of torn anterior cruciate ligaments (ACL) from patients who underwent ACL reconstruction surgery in the year 2017. Surgeries were performed by two surgeons (BH, MK) who specialized in arthroscopic procedures. Exclusion criteria were prior knee surgery, tibial eminence fracture, posterior cruciate ligament rupture, and meniscal tears.
inflammatory disease or severely abnormal chondral lesions (International Cartilage Repair Society Grade > 2) of the knee. Twenty-eight ACL remnant specimens met the inclusion criteria for this study.

There were 25 men and three women with an average age of 31.4 ± 12.1 years old. The remnant pathology was evaluated by its morphology during arthroscopy and by histology.

The surgery was done under general anesthesia with the patient in a supine position. A tourniquet and a lateral thigh support were used for the operated leg. Arthroscopy was performed through standard anterolateral and anteromedial portals. The knee compartments were inspected and all pathologies were documented. Specifically, the morphology of the ACL remnant was palpated with a probe and categorized according to Crain's classification [14]. They described and classified the gross appearance of the ACL remnant into four morphological types. In the first type the ACL remnant had attached to the PCL, in the second to the roof of the notch, in the third to the lateral wall of the notch, and in the fourth there was no scarring at all. A modified classification with additional configurations that has almost perfect reliability and may be useful for studies on ACL repair and preservation [15]. Before the reconstruction, the remnant of the ACL was carefully separated from the adjacent tissues and transected from its femoral and tibial attachments with arthroscopic scissors. After harvesting the ligament remnant was marked with a suture at the site of tibial transection and fixed in 4% neutral buffered formalin before it was sent to the pathology department. All specimens were sectioned horizontally into three parts (i.e., femoral end, middle part, and tibial end), which were further longitudinally sectioned and then embedded in paraffin. Slices of five micrometer thick were mounted on glass slides and stained with Hematoxylin & Eosin (H&E).

Histological slides were examined with use of a microscope (Nikon eclipse 50×, Nikon, Tokyo, Japan). For the histomorphometric measurements, each section from the three sites was evaluated. Inflammatory cells and blood vessels were counted in five consecutive × 20 fields. The average count was recorded and a calculation was made accordingly. The evaluation also included the presence of collagen fibers (and their orientation), synovial proliferation, hyaline degeneration, calcifications, and fatty infiltration. Descriptive statistics were used to present mean and standard deviation values (Excel spreadsheet, Microsoft Excel, Version 2013, Microsoft Corp, Richmond, CA, USA).

RESULTS

The average time length from injury to surgery was 11.4 months (range 1 to 36 months). At surgery all patients had complete tears of the ACL that were detached from the femoral notch wall. Three progressive and distinct morphological tear patterns
were observed during arthroscopy. The first pattern was noticed within an average time of 2.6 months from injury and showed no scar tissue [Figure 1A]. The second pattern appeared later within an average time of 5.1 months from injury and was characterized by the appearance of scar tissue with adhesion to the femoral wall [Figure 1B]. The third and last pattern appeared within an average time of 14.2 months from injury and was characterized by adhesion of the ACL remnant to the posterior cruciate ligament [Figure 1C].

The microscopic evaluation of the first tear morphological pattern (no scar tissue) showed abundance of blood vessels and lymphocytes at the torn femoral end with few irregular collagen fibers [Table 1]. The second and third tear patterns (attachment to the femoral wall and to the posterior cruciate ligament, respectively) showed decrement in the number of blood vessels and lymphocytes with longitudinally oriented collagen fibers [Figure 4].

Calcifications and synovial proliferation were seen in the first and second tear patterns while fat infiltration and hyaline degeneration were seen in the third tear pattern. These histopathological features of the femoral end were also observed in the middle part of the ACL remnant but to a lesser extent. The tibial end was seen intact during arthroscopy and there were no significant differences in histology between patients. Specifically all remnants had longitudinally oriented collagen fibers and only few blood vessels and lymphocytes at their tibial end.

Overall, there were no typical acute inflammatory phase cells because surgery was performed at a later stage with a minimum of 4 weeks from injury. ACL reconstruction surgery is usually performed following four weeks from injury to avoid postoperative adhesions (i.e., arthrofibrosis).

**DISCUSSION**

The corresponding morphological and histological changes of a torn ACL in the current study show a short reparative phase within 3 months from injury followed by a prolonged remodeling phase of the ruptured anterior cruciate ligament that ends with attachment of the remnant to the posterior cruciate ligament. Former studies on human ACL histology are scarce and include small cohorts of patients. Murray et al. [12] studied the histological features of 23 ruptured ligaments retrieved at the time of open reconstruction for the treatment of ligament injury. They concluded that after rupture, the human anterior cruciate ligament undergoes four histological phases: inflammation, reparative regeneration, proliferation, and remodeling. They emphasized several differences in response to injury from other dense connective tissues: absence of fibrin clot, formation of a synovial cell layer with retraction capability on the surface of the ruptured ends, the lack of any tissue bridging the rupture site and the presence of an epi-ligamentous reparative phase. Some of the ligaments were retrieved at 10 days to 3 weeks from injury. Compared to Murray’s study [12], there was no evidence of acute inflammation in any of the remnants of the present study because all patients were operated on at a minimum of 4 weeks from injury to avoid knee arthrofibrosis. The histological features of the remnants that were excised at the first 3 months after injury were characterized by many lymphocytes, blood vessels, and non-organized collagen fibers. Remnants from later periods after injury showed decreased numbers of lymphocytes and blood vessels and appearance of organized collagen fibers. The latest morphological tear pattern (attachment of the remnant to the PCL) was characterized by fat infiltration and hyaline degeneration. These changes correspond to Murray’s description.

<table>
<thead>
<tr>
<th>Morphological patterns of ACL remnant</th>
<th>No scar tissue</th>
<th>Attachment to femoral wall</th>
<th>Attachment to PCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>8</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Months from injury</td>
<td>2.6 ± 1.3</td>
<td>5.1 ± 4.2</td>
<td>14.2 ± 11.8</td>
</tr>
<tr>
<td>Average number of blood vessels</td>
<td>32.4 ± 12.7</td>
<td>26.1 ± 9.5</td>
<td>20.6 ± 8.1</td>
</tr>
<tr>
<td>Average number of lymphocyte cells</td>
<td>26.1 ± 13</td>
<td>9.3 ± 10.1</td>
<td>1.2 ± 2.9</td>
</tr>
<tr>
<td>Collagen fibers orientation</td>
<td>Non-organized</td>
<td>Longitudinal orientation</td>
<td>Longitudinal orientation</td>
</tr>
<tr>
<td>Synovial proliferation</td>
<td>2 of 8</td>
<td>4 of 8</td>
<td>none</td>
</tr>
<tr>
<td>Specimens with calcifications</td>
<td>6 of 8</td>
<td>5 of 8</td>
<td>none</td>
</tr>
<tr>
<td>Specimens with fat infiltration</td>
<td>none</td>
<td>none</td>
<td>8 of 12</td>
</tr>
<tr>
<td>Specimens with hyaline degeneration</td>
<td>5 of 8</td>
<td>5 of 8</td>
<td>10 of 12</td>
</tr>
</tbody>
</table>

ACL = anterior cruciate ligament, PCL = posterior cruciate ligament

**Table 1. Morphological patterns and histopathology of the ACL remnant**
of the reparative, proliferative and remodeling phases, respectively.

In the present study the gross morphology of the ACL remnant showed three patterns. The first pattern showed no scar tissue within 3 months from the injury followed by a second pattern that was characterized by attachment to the femoral notch wall. The last pattern was characterized by attachment of the remnant to the posterior cruciate ligament (PCL) within 14 months from the injury. These observations are similar to Crain et al. [13] who have investigated 48 patients undergoing ACL reconstructions. They described and classified the ACL remnant gross appearance into four morphological types. In the first type the ACL remnant had attached to the PCL, in the second to the roof of the notch, in the third to the lateral wall of the notch, and in the fourth there was no scarring at all. Since its publication, Crain’s classification of the ACL remnant has been used as a standard.

In a recent study by Kirizuki et al. [16] ACL remnant tissues were harvested from patients who received primary ACL reconstruction within 3 months after injury. The tissues were evaluated according to the Crain classification and the potential for proliferation and differentiation was assessed. The authors concluded that in the subacute phase, ACL remnant tissue of the non-reattachment group possibly has a higher healing potential than that of the reattachment group. This conclusion complements our observation of a non-scar tissue pattern shortly after the injury.

Many authors have utilized morphologic classifications to investigate biomechanical qualities of ACL remnants and the outcomes of remnant preservation during ACL reconstruction surgery [17,18]. In recent years surgeons have published techniques that try to preserve the ACL remnant [19]. The purpose of preservation is to maintain the original anatomical, biological, and mechanical properties, such as proprioception [8], and to avoid postoperative osteoarthritis. However, studies so far have failed to prove any clinical benefit of remnant preservation during ACL reconstruction [20]. It is probable that the success of any preservation technique depends on timing. A group of investigators showed that the highest potential for remnant healing is at the first 3 months after injury [21]. The present study showed a short reparative phase and a prolonged remodeling phase. Although the transition between phases differed between patients, the reparative phase (by histology) in this study was limited to the first 3 months and appeared (by arthroscopy) as stump without scar tissue.

Recent advancements in functional tissue engineering and regenerative medicine have resulted in a renewed interest in ACL repair [22]. The inability of an injured ACL to heal compared to some other extra-articular ligaments is related to several factors such as the "hostile" environment of synovial fluid, the post-injury response, and mechanical characteristics [23]. Bio-enhanced ACL repair is a new paradigm in ACL surgery that aspires to overcome the above obstacles by the use of bioactive scaffold as a carrier for cells, growth factors and enzymes to optimize tissue healing. In a randomized trial on an animal model, the biomechanical outcome of bio-enhanced ACL repair was found to be equal to that of ACL reconstruction [24]. It may be that in the near future these novel techniques performed in proper timing from injury may restore the torn ACL close to its original state.

To the best of our knowledge, our study is one of few that evaluated both morphology and histology of the torn ACL in different time periods from injury.

LIMITATIONS
The study lacked a control group, included few female patients, and was limited in overall sample size. Table 1 practically shows substantial overlap between the groups in some variables. To avoid the risk of arthrofibrosis, almost all patients underwent the ACL reconstruction surgery at least 3–4 weeks after injury which it can be a crucial in the primary healing process.

It is possible to use sacrificed ACLs from osteoarthritic knees before knee arthroplasties as controls; however, these samples are usually degenerative and not always sufficient. Most of the patients in the study were men, which reflects a predisposition in one medical center and therefore it remains to investigate the ACL remnant morphology in a larger female population.

CONCLUSIONS
In the first 3 months after injury the morphological features of the ACL remnant shows no scar tissue and its histological features have the characteristics of a reparative phase. This phase
is followed by a prolonged remodeling phase that ends with attachment of the remnant to the posterior cruciate ligament.

References


