Università degli Studi di Padova

Dipartimento di Medicina

SCUOLA DI DOTTORATO DI RICERCA IN SCIENZE MEDICHE, CLINICHE E SPERIMENTALI
INDIRIZZO IN SCIENZE REUMATOLOGICHE
CICLO XXIV

SYNOVIAL INFLAMMATION AND ASSOCIATED INTRA-ARTICULAR PATHOLOGY IN A COHORT OF PATIENTS UNDERGOING ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION FOR TRAUMATIC RUPTURE

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To Mary and Steve

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SUMMARY

Purpose:

Previous epidemiologic studies have established that there is a strong relationship between anterior cruciate ligament (ACL) disruption and the risk for subsequent development of osteoarthritis (OA). Though not normally considered a classical inflammatory arthropathy, OA is often associated with low-grade synovitis. In patients with OA, synovial inflammation is one factor associated with risk of progression of structural joint deterioration and symptoms. The aims of the study were to characterize the histopathological features and chemokine profile of the synovium in patients undergoing ACL reconstruction after traumatic ACL rupture and to determine the relationship between the synovial characteristics and meniscal and/or cartilage abnormalities.

Materials and Methods:

The study was conducted in the context of the ACL registry at the Hospital for Special Surgery (HSS), New York, USA, which maintains extensive records of preclinical, intraoperative and post-operative data for 1200 patients who have undergone ACL reconstruction for traumatic ACL rupture. Synovial biopsies were collected from 40 patients during arthroscopic surgery and processed for histology and RNA extraction. Synovial features of inflammation and degeneration were assessed using a combination histological synovial scoring system developed at HSS in which the following six features were graded: perivascular mononuclear cell infiltration, detritus, mucoid change, fibrosis, increased vascularity and hyperplasia of the synovial lining layer, leading to a total combined maximal score of 15. The following clinical data were also collected: age, sex, body mass index (BMI), date of injury, time to surgery and the intraoperative presence or absence of meniscal and/or cartilage abnormalities. Total RNA extracts were prepared from the suprapatellar synovial samples and the levels of mRNA for four chemokines (IL-8, CCL19, CCL21, and CCL5) and one chemokine receptor (CCR7), shown previously to be associated with synovial inflammation in patients undergoing meniscectomy by microarray pathway analysis (C Scanzello, *Arthritis Rheum 2011*) [1], were measured by real-time qPCR.

Results:

Of the 40 patients undergoing synovial biopsy and analysis, 19 were female and 21 male, with median ages of 20 and 36, respectively. 85% of the patients exhibited histological evidence of synovial inflammation or degeneration in the suprapatellar area: 44.1% of patients scored 1 or 2, 44.1% scored 3 or 4, 5.8% scored 5 or 6, and 5.8% scored 7 or 8. Arthroscopically, meniscal abnormalities (mostly tears) were observed in 19/40 patients (47%), and cartilage defects were observed in 16/40 patients (40%). No association was observed between the body mass index (BMI), date of injury, time to surgery, and frequency of cartilage defects or meniscal pathology. The patients were divided in two groups based on age: 19 of patients older than 30 years and 21 patients younger than 30 years. Meniscal tears were associated with synovial inflammation in patients older than 30 years. We selected 16 synovial suprapatellar biopsies from patients > 30 years for qPCR analysis, 8 from patients without synovial inflammation (grade 0-2) and 8 with synovial inflammation (grade 3-8). The levels of *CCL5* and *CCL19* mRNA were significantly upregulated in biopsy specimens exhibiting inflammation with associated fold-changes of 2.7 (P=0.0426) and 4.7 (P=0.0289), respectively.

Conclusions:

In our study, we observed a high percentage of low-grade synovitis (85%), meniscal tear (47%), and/or cartilage defects (40%) in patients undergoing ACL reconstruction for traumatic ACL rupture. In a cohort of patients > 30-years-old, the presence of synovitis correlated with evidence of a meniscal tear, suggesting a relationship between these two pathologic processes. Preliminary analysis of mRNA expression patterns in this group of patients confirmed the upregulation of *CCL5* and *CCL19*. This is in agreement with previously published data [1]. These two chemokines may play an important contributory role in the pathophysiology of OA particularly in those patients having synovial inflammation.

RIASSUNTO

Scopo dello studio:

I dati riportati in lettura evidenziano una stretta correlazione tra la rottura del legamento crociato anteriore (LCA) e il rischio di sviluppare osteoartrosi (OA). Benché non sia considerata una classica artropatia infiammatoria, l'OA è spesso associata alla presenza di sinovite di basso grado. Nei pazienti affetti da OA, l'infiammazione sinoviale viene considerata un fattore di rischio correlato con l'attività di malattia e con la progressione del danno articolare. Lo scopo dello studio consiste nel caratterizzare la sinovite dei pazienti sottoposti a ricostruzione del LCA post rottura traumatica e nell'identificare la correlazione tra la presenza d'infiammazione sinoviale e la coesistenza di lesioni meniscali e cartilaginee.

Materiali e Metodi:

Lo studio è stato condotto presso l'Hospital for Special Surgery (HSS) di New York, USA, utilizzando il registro per il LCA, che raccoglie i dati clinici, preoperatori ed intraoperatori, di 1200 pazienti sottoposti a ricostruzione del LCA in seguito a rottura traumatica. Su 40 pazienti sono state eseguite biopsie sinoviali in corso di artroscopia ed i campioni raccolti sono stati sottoposti ad istologia e ad estrazione di RNA. La presenza d'infiammazione sinoviale è stata valutata mediante l'utilizzo di uno score istologico composito messo a punto all'HSS. Tale score prende in considerazione la presenza dei seguenti parametri istologici per un punteggio complessivo massimo di 15: infiltrazione perivascolare di cellule mononucleate, detriti, cambiamenti mucoidi, fibrosi, incremento della vascolarizzazione ed iperplasia della membrana sinoviale. Sono stati inoltre raccolti i seguenti dati clinici: età, sesso, indice di massa corporea (BMI), data del trauma, data dell'intervento chirurgico e riscontro intraoperatorio di lesioni meniscali e/o cartilaginee. I livelli di mRNA di 4 chemochine (IL-8, CCL19, CCL21, and CCL5) e di un recettore per le chemochine (CCR7) sono stati misurati sulla sinovia sovrapatellare, tramite real time qPCR. Tali chemochine sono risultate precedentemente associate all'infiammazione sinoviale nei pazienti sottoposti a meniscectomia in uno studio condotto tramite microarray analisi (C Scanzello, *Arthritis Rheum 2011*) [1].

Risultati:

Dei 40 pazienti sottoposti a biopsia sinoviale, 19 sono femmine e 21 maschi, con un'età mediana rispettivamente di 20 e 36 anni. L'85% dei pazienti analizzati presenta segni istologici d'infiammazione sinoviale a livello sovrapatellare: il 44.1% dei pazienti ha uno score tra 1 e 2, il 44.1% uno score tra 3 e 4, il 5.8% score tra 5 e 6, e il 5.8% uno score tra 7 e 8. Durante l'artroscopia si sono riscontrate lesioni meniscali in19/40 pazienti (47%) e cartilaginee in 16/40 pazienti (40%). Non si sono identificate correlazioni tra grado d'infiammazione sinoviale e BMI, data del trauma, data dell'intervento e frequenza delle lesioni meniscali e cartilaginee. A seconda dell'età si è suddiviso i pazienti in due gruppi: 19 pazienti hanno un'età superiore a 30 anni e 21 un'età inferiore ai 30 anni. La presenza di lesioni meniscali è associata all'infiammazione sinoviale nei pazienti con età > 30 anni. Tra le biopsie sinoviali sovrapatellari eseguite su pazienti con età > 30 anni, se ne sono selezionate 16 da sottoporre a qPCR analisi: 8 biopsie provenienti da pazienti senza segni d'infiammazione sinoviale (grado 0-2) e 8 provenienti da pazienti con infiammazione sinoviale (grado 3-8). I livelli di mRNA di *CCL5 e CCL19* risultano significativamente più elevati nei pazienti con infiammazione sinoviale di 2.7 ($p = 0.0426$) e di 4.7 volte ($p = 0.0289$) rispettivamente.

Conclusioni:

Nel nostro studio, i pazienti sottoposti a ricostruzione del LCA per rottura traumatica presentano un'alta percentuale di sinovite di basso grado (85%), lesioni meniscali (47%) e difetti cartilaginei (40%). Nel gruppo di pazienti con età > 30 anni, la presenza di sinovite si correla con il riscontro di lesioni meniscali suggerendo una possibile relazione tra i due processi patologici. L'analisi dell'espressione dell'mRNA in questo gruppo di pazienti, ha confermato un incremento di *CCL5* and *CCL19*, in accordo con quanto riportato in letteratura [1]. Queste due chemochine potrebbero avere un ruolo chiave nella patofisiologia dell'OA, in particolar modo nei pazienti con infiammazione sinoviale.

INTRODUCTION

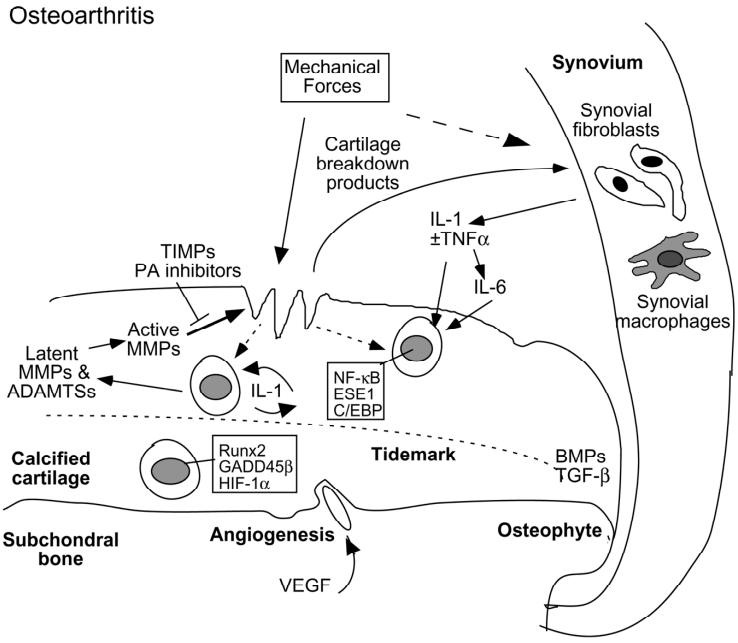
OSTEOARTHRITIS

Osteoarthritis (OA) is the most common joint disorder and the major cause of disability in the adult population. OA is characterized by an inappropriate remodelling of the articular cartilage extracellular matrix (ECM) that results from an imbalance in chondrocyte anabolic and catabolic activities [2]. Articular chondrocytes modify the composition, structure and functional properties of their surrounding ECM in response to mechanical injury, alterations in loading, and biological stimuli such as cytokines and growth and differentiation factors. Current knowledge segregates the risk factors for development of OA into two fundamental mechanisms related either to the adverse effects of ‘abnormal’ loading on normal cartilage or of ‘normal’ loading on ‘abnormal’ cartilage. Aging has been suggested as the primary factor contributing to this ‘abnormal’ state of articular cartilage, although genetic factors that cause disruption of chondrocyte differentiation and function and influence the composition and structure of the cartilage matrix also contribute to abnormal cartilage biomechanical properties, independent of the influence of the aging process [3]. Although cartilage destruction is the hallmark of OA and the degradation of type II collagen is the pivotal event that determines the irreversible progression of OA disease, it is now well established that OA is a whole-joint disorder, involving and affecting all of the joint tissues, including the subchondral bone, menisci and synovial membrane [4] (Fig. 1).

SYNOVITIS

OA is not considered a classical inflammatory arthropathy. This conclusion is, in part, related to the absence of neutrophils in the synovial fluid and the lack of systemic manifestations of inflammation. OA is frequently associated, however, with signs and symptoms of inflammation, including joint pain, swelling and stiffness that may contribute to significant functional impairment and disability [5]. OA is often characterized by low-grade synovitis associated with the presence of mononuclear cell infiltrates and production of inflammatory cytokines [6, 7]. Increasing evidence indicates that synovial inflammation is correlated with joint pain and dysfunction and importantly is a major risk factor for more rapid progression of structural joint deterioration [8]. It has been suggested that synovial inflammation is a factor that

contributes to dysregulation of chondrocyte function, favoring an imbalance between the catabolic and anabolic activities of the chondrocyte in remodeling the cartilage ECM [9].



From MB Goldring, Arthritis Research and Therapy 2009

Figure 1. This schema represents a model that illustrates the role of mechanical loading and biological factors in the pathogenesis of OA joint pathology and depicts the important role of cross-talk between the cartilage and synovium in this process. Products of cartilage matrix breakdown that are released into the synovial fluid act on the synovium to induce synovial inflammation. In turn, activated synovial cells in the inflamed synovium produce catabolic and proinflammatory mediators that lead to excess production of proteolytic enzymes by chondrocytes that are responsible for cartilage breakdown and further release of cartilage degradation products, creating a positive feedback loop.

In most studies, the pathogenesis of the synovitis has been attributed to de-regulated chondrocyte function, which leads to the release of biologically active chondrocyte-derived factors or products from the cartilage extracellular matrix that initiates the synovial inflammation [3, 4, 6-8, 10]. In general, immunohistopathological signs of synovitis in patients with OA or posttraumatic joint injuries are moderate. Compared to controls from healthy individuals, patients with synovitis associated with joint trauma displayed more synovial lining hyperplasia and the presence of infiltrating T-lymphocytes dominated by T-helper cells [11]. In support of this concept, in patients with traumatic meniscal injury, but no radiographic evidence of OA, the synovium

retrieved during meniscectomy is frequently inflamed and inflammation scores are associated with increased pain and dysfunction and a unique chemokine profile [1].

ACL RUPTURE

The articular surface plays an essential role in load transfer across the joint and there is good evidence that conditions that produce increased load transfer and/or altered patterns of load distribution can accelerate the initiation and progression of OA [12]. Rupture of the anterior cruciate ligament (ACL) in the setting of acute injury represents a relatively common occurrence that alters the biomechanical dynamics of the joint, often in a young and otherwise healthy individual. The overall incidence of ACL injury in the general US population is not known, but many estimates suggest there are 80,000 to 100,000 ACL repairs performed in the United States each year [13, 14]. Injuries to the ACL frequently occur in young patients, especially in the athletes, leading to pain and functional impairment in the young or middle-aged adult. Most ACL ruptures occur from noncontact injuries with contact injuries accounting for only about 30 percent of the ACL ruptures. The noncontact injuries occur primarily during deceleration of the lower extremity, with the quadriceps maximally and the hamstrings minimally contracted and the knee at or near full extension. Women have an increased risk of ACL ruptures compared to age-matched males (calculated between 1.4 to 9.5). ACL ruptures occur more frequently during the games compared with practices [15, 16].

ACL RUPTURE AND OSTEOARTHRITIS

Previous epidemiologic studies have established that there is a strong relationship between ACL disruption and the risk for subsequent development of OA [17, 18]. There are an estimated 900,000 cases of knee injuries annually in the United States and posttraumatic OA accounts for 12% of all cases of OA [19]. The reported radiographic rates of OA after an ACL injury vary between 10% and 90% at 10 to 20 years after the ACL injury [20, 21]. As post-traumatic OA primarily affects younger individuals [22], it leads to reduced physical activity and to deconditioning of the musculoskeletal system: the so-called “young patients with old knees”. Joint replacement in this young patient group is complicated by the limited lifespan of the implants. OA risk increases with patient age at the time of the injury and with time from the onset of injury [23]. The presence of additional OA risk factors, such as obesity, joint malalignment or

genetic risk factors, leads to more unfavorable outcomes. Between 60 and 80% of patients with magnetic resonance imaging or arthroscopically documented cartilage injury developed cartilage degeneration within 5 years [24].

OA changes have in part been attributed to increased joint instability and altered joint mechanics that result from ACL disruption. In this environment, the articular cartilage is exposed to abnormal biomechanical forces and it has been suggested that these influences are responsible for deregulated chondrocyte matrix synthesis and repair capacity. Such acute mechanical injury also results in the release of biological mediators such as cytokines, proteolytic enzymes, and reactive oxygen species that may adversely affect chondrocyte survival and synthetic activity [25-26]. Despite restoration of the knee stability through ACL reconstruction, 50-60% of patients eventually experience progressive osteoarthritic changes [27, 28]. It is unclear whether the deterioration in joint integrity reflects limitations in surgical techniques, patient behavioral characteristics, or the influence of biological processes, including intraarticular synovial inflammation. Several studies measured the concentration of cytokines in synovial fluid aspirated from the injured knees of patients with acute, subacute and chronic ACL deficiency. It has been shown that the acute inflammation seen after the ACL rupture is associated with large increases in the concentration of several cytokines. Following the acute trauma, an initial burst of cytokine production has been reported which mirrors that seen in wound healing and includes tumor necrosis factor (TNF)α, IL-6, IL-8, IL-10, interleukin (IL)-1β and IL-1 receptor antagonist (IL-1ra) [29-32]. Subacutely and chronically, the inflammation subsides but does not completely resolve. Instead, a cytokine imbalance persists that has the potential to engender cartilage loss and the eventual development of OA. Interestingly, concentrations of synovial IL-IRa decrease dramatically in patients with chronic ACL deficiency, suggesting the loss of an important protective cytokine and the development of an imbalance between the low levels of IL-1β noted in the chronic group without sufficient neutralizing levels of IL-1Ra [29, 30].

The acute symptoms following joint injury include joint pain and swelling due to intraarticular bleeding, synovial effusion and inflammation [24]. After this initial period, there is a release into the synovial fluid of components of the cartilage matrix, such as cartilage oligomeric matrix protein (COMP), and products of proteoglycan and collagen degradation [33, 34]. In addition, levels of metalloproteinase (MMP)-3 and tissue inhibitor of metalloproteinase (TIMP)-1 that are involved in cartilage remodeling

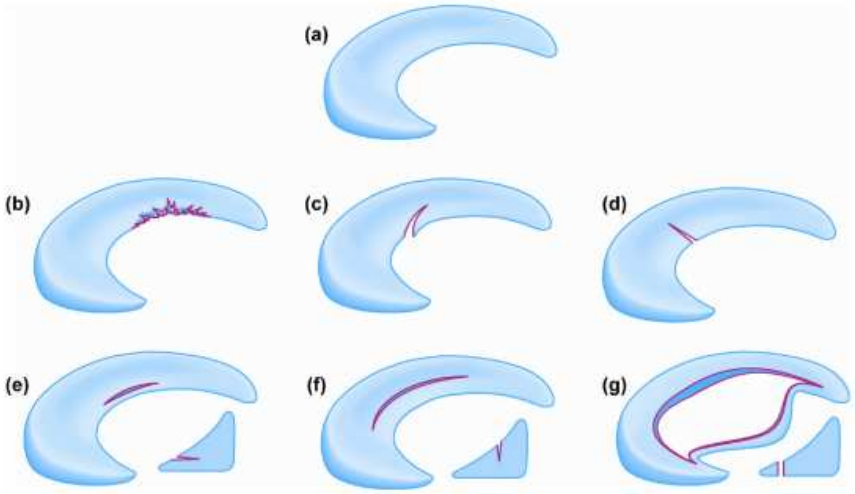
have been reported to be elevated in synovial fluid after ACL injury [35-37]. These observations emphasize the role of events in the time period after the initial trauma in the de-regulation of chondrocyte metabolism and cartilage matrix remodeling. Joint trauma affects all joint tissues to some degree but the damage to articular cartilage appears to be most significant, as it is largely irreversible and may be the major determinant of the subsequent development of OA. There is a certain degree of immediate or irreversible damage, but the days and weeks after injury represent the phase when damage appears to progress most rapidly.

ACL RUPTURE AND ASSOCIATED PHATOLOGY

ACL rupture is a complex event and concomitant meniscal injury, collateral ligament tear, cartilage damage and bone contusion can be detected as a consequence of trauma. Meniscal tears and chondral lesions, occurring as a part of initial injury or subsequent episodes of knee subluxation, lead to an impairment of the load transmission capability of the cartilage and instability of the knee. These are among the factors that contribute to the increased risk for the development of knee osteoarthritis.

Meniscus — Meniscal injuries are commonly seen in association with ACL injury. The incidence of meniscal tears in ACL-deficient knees has been reported in 16-82% of acute ligament injuries and in as many as 96% of knees with chronic ACL deficiency [38]. If meniscal damage occurs at the time of the acute ACL injury, the incidence of lateral meniscus tears is marginally higher than the medial tears. Indeed, the incidence of medial meniscal tears at the time of ACL rupture varies from 25-45% and for the lateral meniscus from 31% to 65% [39]. Numerous mechanisms have been proposed to account for acute rupture of the ACL with the most common mechanism being a noncontact deceleration injury associated with axial loading, in addition to a rotational and a valgus moment culminating in anterolateral subluxation of the tibia. This combination of forces results in impingement of the posterolateral tibial rim and meniscus on the anterolateral femoral condyle and might be expected to load the posterolateral compartment of the knee, including the posterior horn of lateral meniscus. This mechanism has been confirmed by the frequent location of magnetic resonance imaging (MRI)-identified bone bruises in this region following acute ACL injuries [40, 41]. It is likely that this combination of forces results in injury to the lateral meniscus. Instead, medial meniscus tears are more frequent in the chronically ACL-deficient knee

[42, 43]. In the ACL-deficient knee, the medial meniscus assumes an important role in the provision of stability in addition to that of load transmission and becomes an important restraint to A-P translation in the ACL-deficient knee. Loss of the medial meniscus results in increases in A-P translation. The likely sequelae of this are increased shear forces on chondral surfaces and secondary chondral injury. It has been reported that the time course after an ACL injury is associated with an increasing prevalence of meniscal injury [44]. Various types of meniscal tears can occur as the result of degeneration/trauma (Fig. 2). Medial meniscal tears are more frequently peripheral and involve the posterior horn, whereas lateral tears are more commonly radial in pattern involving the posterior horn or mid-lateral third of the meniscus [42, 43].



From Kyriacos A, Engineering the Knee Meniscus, Morgan & Claypool Publishers 2009

Figure. 2. Type of meniscus tears: normal meniscus (a), complex (degenerative) tears (b), oblique tears (c), radial tears (d), horizontal tears (e), vertical-longitudinal tears (f) and bucket-handle tears (g).

Cartilage – The coincidence of articular cartilage and ACL injury has been well described. In acute injuries, cartilage damage likely occurs at the time of the initial trauma. The acute lesions can appear macroscopically as chondral softening, chondral fractures, impaction lesions, creases, crack or flaps and occur principally in the lateral compartment but may involve the medial femoral condyle in 30% of cases. These may be associated with an underlying bone bruise identified on MRI. The incidence of severe full-thickness articular cartilage injury in acute ACL tears has been reported to be between 16% and 46% [45]. The joint kinematics in chronic ACL-deficiency can alter the normal pattern of loading on the articular surface of the knee, leading to

damage. Cartilage defects have been attributed to the altered tibiofemoral biomechanics resulting from recurrent episodes of instability. The literature consistently supports an increasing incidence of cartilage damage with increasing time from injury to surgery [46-48]. Also the severity of cartilage defect graded by Outerbridge score has been correlated with the chronicity of the ACL rupture.

Subchondral bone —With the introduction of MRI, the association of bone and subchondral injury with the acutely ACL injured knee has become well recognized. Traumatic bone bruises, also called bone marrow lesions or marrow edema, represent microfractures of the trabecular bone and are detectable only with magnetic resonance imaging. The reported incidence of associated bone bruises approaches 80% [40, 41, 49, 50]. An “MRI triad” has been described, consisting of ACL disruption, bone bruise to the terminal sulcus of the lateral femoral condyle (LFC), and areas of abnormal signal to the posterolateral corner of the tibial plateau [51]. In histological studies, hemorrhage and edema is found in areas with the bone bruise along with chondrocyte death and structural damage to the overlying hyaline joint cartilage [52]. It has been hypothesized that these lesions maybe the precursor of degenerative changes in the knee.

PURPOSE

Epidemiologic studies have established that there is a strong relationship between ACL disruption and risk for subsequent development of OA. Although ACL reconstruction improves joint stability in the short-term, the long-term benefit of this procedure with respect to prevention or slowing the development of OA remains unclear. The overall goal of this study was to exploit the extensive clinical and laboratory resources at Hospital for Special Surgery (HSS) and to initiate a multidisciplinary study involving clinicians in the Orthopaedics and Rheumatology Departments, clinical investigators and laboratory scientists to define the cellular and molecular processes that contribute to the initiation and progression of OA in patients undergoing ACL reconstructive surgery. This study resulted in the development of a Biological and Clinical Registry (as a subset of the ACL Reconstruction database) for the collection of data from a subset of patients undergoing arthroscopies after post-traumatic ACL injury. Data, including clinical history, pre-operative radiographic and/or MRI evaluations, histological assessments, and intra-operative scoring were collected. The primary aims of the study were to characterize the synovial features in patients undergoing ACL reconstruction after traumatic ACL rupture and to determine the relationship between the presence of synovial inflammation and meniscal and cartilage abnormalities. The extent of synovial inflammation was documented at the time of surgery and synovial tissue biopsies obtained. The histologic and transcriptional profiles of synovial tissue was analyzed and correlated with the patient characteristics, pre- and intra-operative alterations in chondral and meniscal tissues. Particular attention focused on the expression levels of 4 chemokines (IL-8, CCL19, CCL21, and CCL5) and 1 chemokine receptor (CCR7), which were previously shown to be associated with synovial inflammation in patients undergoing meniscal surgery for treatment of acute meniscal injury [1]. We hypothesized that there would be a relationship between synovial inflammation and the presence of meniscal and chondral pathology detected at the time of arthroscopy in patients undergoing ACL repair for ACL disruption. The long-term aim is to establish whether there is a relationship between synovial inflammation and the “state” of the cartilage, meniscus and ligaments on the short- and long-term post-operative clinical course and the onset, severity and rate of progression of OA after ACL reconstruction using the collection of longitudinal post-operative outcomes data in the ACL Registry.

PATIENTS AND METHODS

Patients—The study was conducted in the context of the ACL Registry at the Hospital for Special Surgery, New York; which is an extensive collection of preclinical, intraoperative and post-operative data for patients who have undergone ACL reconstruction for traumatic ACL rupture. Institutional Review Board approval was obtained for this study protocol prior to initiating the investigation and all subjects provided written informed consent, including permission for retrieving joint samples. Inclusion criteria included patients of age greater than 12 years and with arthroscopic/surgical evidence of ACL injury without or with synovial fluid effusion, inflamed synovium and cartilage damage. The exclusion criteria included history of tumor and infection in the index joint.

Questionnaire packet and clinical data— As a part of the ACL Registry, a questionnaire packet containing the following forms was given to the patients: Short Form (SF)-36 Health Survey, Lysholm Knee Scale and Marx Activity Scale. The SF-36 Health Survey (Fig. 3) is a generic outcome measure designed to examine a person's perceived health status. Eight domains of health are assessed by SF-36: physical functioning, role limitations due to physical health, bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional problems, and mental health. In contrast, Lysholm Knee Scale (Fig. 3) is a validated knee-specific instruments for measuring symptoms and functional disability, while Marx Activity Scale indicates patient's activity levels. (Fig. 4). In addition, clinical information, including age, sex, BMI, date of injury and time to surgery, previous surgery in the operating or in the contralateral knee, mechanism of injury, sport/activity of injury were collected by the surgeon. Three experienced orthopedic surgeons were involved in the study. Magnetic resonance imaging (MRI) of the affected joint was obtained by request of the treating surgeons and parameters such as the presence and location of bone marrow lesions were recorded pre-operatively. Clinical examination to grade knee stability was performed under anesthesia just before the surgery. Knee alignment, range of motion, Lachman Test, Pivot Shift Test, and presence of knee effusion were evaluated by the surgeons. The ACL Registry follow-up requires questionnaire packet filling at 1, 2, 5, and 10 years post-operation and a plain radiograph at 6 weeks post-operatively per usual standard of care.

Figure 3. SF-36

1. In general, would you say your health is: ❑Excellent ❑Very Good ❑Good ❑Fair ❑Poor

2. Compared to one year ago, how would you rate your health in general now?

   ❑Much better now than 1 year ago ❑Somewhat better now than 1 year ago ❑About the same as 1 year ago

   ❑Somewhat worse now than 1 year ago ❑Much worse now than 1 year ago

3. The following items 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 All
a.	Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	❑	❑	❑
b.	Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	❑	❑	❑
c.	Lifting or carrying groceries	❑	❑	❑
d.	Climbing several flights of stairs	❑	❑	❑
e.	Climbing one flight of stairs	❑	❑	❑
f.	Bending, kneeling or stooping	❑	❑	❑
g.	Walking more than a mile	❑	❑	❑
h.	Walking several blocks	❑	❑	❑
i.	Walking one block	❑	❑	❑
j.	Bathing or dressing yourself	❑	❑	❑

4. During the <u>past 4 weeks</u>, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

		YES	NO
a.	Cut down on the amount of time you spent on work or other activities	❑	❑
b.	Accomplished less than you would like	❑	❑
c.	Were limited in the kind of work or other activities	❑	❑
d.	Had difficulty performing the work or other activities (for example, it took extra effort)	❑	❑

5. During the <u>past 4 weeks</u>, 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)?

		YES	NO
a.	Cut down on the amount of time you spent on work or other activities	❑	❑
b.	Accomplished less than you would like	❑	❑
c.	Didn't do work or other activities as carefully as usual	❑	❑

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

❑Not At All ❑Slightly ❑Moderately ❑Quite a Bit ❑Extremely

7. How much bodily pain have you had during the past 4 weeks?

❑None ❑Very Mild ❑Mild ❑Moderate ❑Severe ❑Very Severe

8. 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

9. 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	A good bit of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of pep?	❑	❑	❑	❑	❑	❑
b. Have you been very nervous?	❑	❑	❑	❑	❑	❑
c. Have you felt calm and peaceful?	❑	❑	❑	❑	❑	❑
d. Did you have a lot of energy?	❑	❑	❑	❑	❑	❑
e. Have you felt down-hearted and blue?	❑	❑	❑	❑	❑	❑
f. Did you feel worn out?	❑	❑	❑	❑	❑	❑
g. Have you been a happy person	❑	❑	❑	❑	❑	❑
h. Did you feel tired?	❑	❑	❑	❑	❑	❑

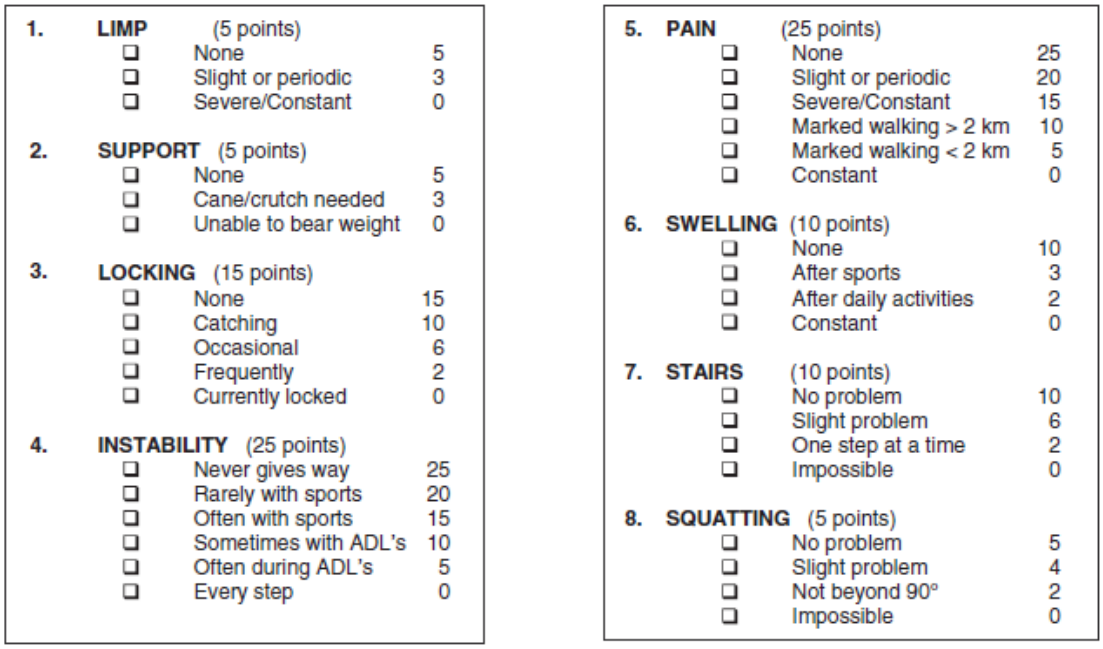
10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

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

11. How TRUE or FALSE is each of the following statements for you?

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
a. I seem to get sick a little easier than other people	❑	❑	❑	❑	❑
b. I am as healthy as anybody I know	❑	❑	❑	❑	❑
c. I expect my health to get worse	❑	❑	❑	❑	❑
d. My health is excellent	❑	❑	❑	❑	❑

Figure 4– LYSHOLM KNEE SCALE



1. **LIMP** (5 points)
   - ❑ None 5
   - ❑ Slight or periodic 3
   - ❑ Severe/Constant 0

2. **SUPPORT** (5 points)
   - ❑ None 5
   - ❑ Cane/crutch needed 3
   - ❑ Unable to bear weight 0

3. **LOCKING** (15 points)
   - ❑ None 15
   - ❑ Catching 10
   - ❑ Occasional 6
   - ❑ Frequently 2
   - ❑ Currently locked 0

4. **INSTABILITY** (25 points)
   - ❑ Never gives way 25
   - ❑ Rarely with sports 20
   - ❑ Often with sports 15
   - ❑ Sometimes with ADL's 10
   - ❑ Often during ADL's 5
   - ❑ Every step 0

5. **PAIN** (25 points)
   - ❑ None 25
   - ❑ Slight or periodic 20
   - ❑ Severe/Constant 15
   - ❑ Marked walking > 2 km 10
   - ❑ Marked walking < 2 km 5
   - ❑ Constant 0

6. **SWELLING** (10 points)
   - ❑ None 10
   - ❑ After sports 3
   - ❑ After daily activities 2
   - ❑ Constant 0

7. **STAIRS** (10 points)
   - ❑ No problem 10
   - ❑ Slight problem 6
   - ❑ One step at a time 2
   - ❑ Impossible 0

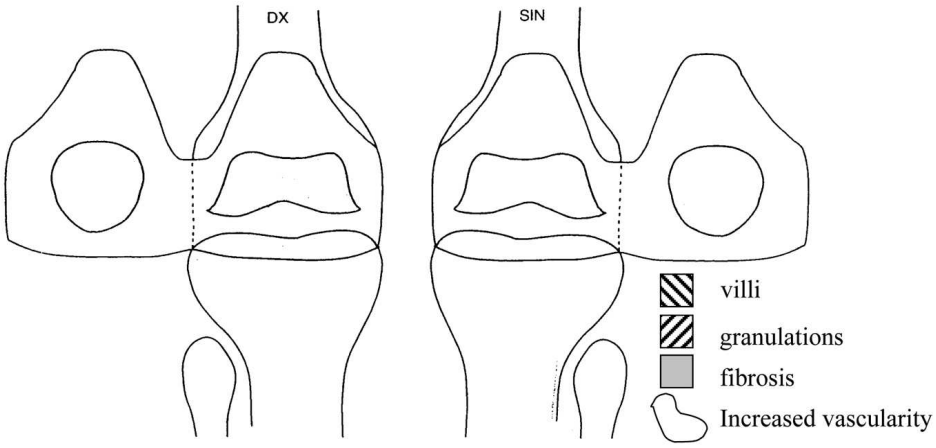
8. **SQUATTING** (5 points)
   - ❑ No problem 5
   - ❑ Slight problem 4
   - ❑ Not beyond 90° 2
   - ❑ Impossible 0

Figure 5– MARX ACTIVITY SCORE

	Less than one time in a month	One time in a month	One time in a week	2 or 3 times in a week	4 or more times in a week
Running: running while playing a sport or jogging	0	1	2	3	4
Cutting: changing directions while running	0	1	2	3	4
Deceleration: coming to a quick stop while running	0	1	2	3	4
Pivoting: turning your body with your foot planted while playing sport; For example: skiing, skating, kicking, throwing, hitting a ball (golf, tennis, squash), etc.	0	1	2	3	4

Synovial intraoperative macroscopic scoring— The presence of synovitis was documented by the surgeon in four different areas (suprapatellar, peri-ACL, lateral and medial compartment) during arthroscopic surgery for ACL reconstruction (Fig. 6). Synovitis was scored in each area using a scale of 0 to 4 based on the following visual parameters: hypertrophy, vascularity and hyperemia, and representative photographs were obtained [53]. *Hypertrophy* was a parameter that graded visible synovial mass. The lowest score (0) represented a thin and transparent synovial membrane, while the presence of villi gave the maximum score (4). This parameter did not take into account the present or past inflammation activity; white fibrotic villi scored the same as active hypervascularised villi. *Vascularity* was a parameter that graded density of visible vessels. Fibrotic tissue scored 0, while densely packed vessels scored 4. Hyperemia was not included in this parameter. *Hyperemia* was a parameter that graded every visible aspect of synovitis as assessed globally by the surgeons. A higher score represented a higher degree of inflammation. White fibrotic tissue scored 0, as normal synovial membrane. The macroscopic scoring instructions were given to three surgeons before initiating the study and arthroscopists were trained by using reference image scores. The printed photographs of synovium obtained during arthroscopy were successively reviewed by all the surgeons separately in such a way as to reduce the intra- and inter-reviewer variation. The presence of synovitis was considered mild if the sum of the three parameters for all four compartments was < 3, mild to moderate 4-6, moderate 7-9, and severe 10-12. The presence, size, depth, and location of chondral and meniscus injury based on direct arthroscopic visualization during ACL reconstruction was recorded by the surgeon in the Associated Pathology Forms (Fig. 7 e Fig. 8). Cartilage damage was assessed intra-operatively using the Outerbridge scoring system [54]. A grade I showed softening and swelling of the articular cartilage. A grade II lesion showed fragmentation and fissuring in an area up to half an inch in diameter. Grade III lesions showed the same features as grade II lesions, but an area more than half an inch in diameter was involved. A grade IV lesion showed a full-thickness erosion of the articular cartilage to subchondral bone. The worst score from all three compartments (medial, lateral, patello-femoral) was utilized in this analysis.

Figure 6 - SYNOVITIS MASCROSCOPIC SCORE FORM

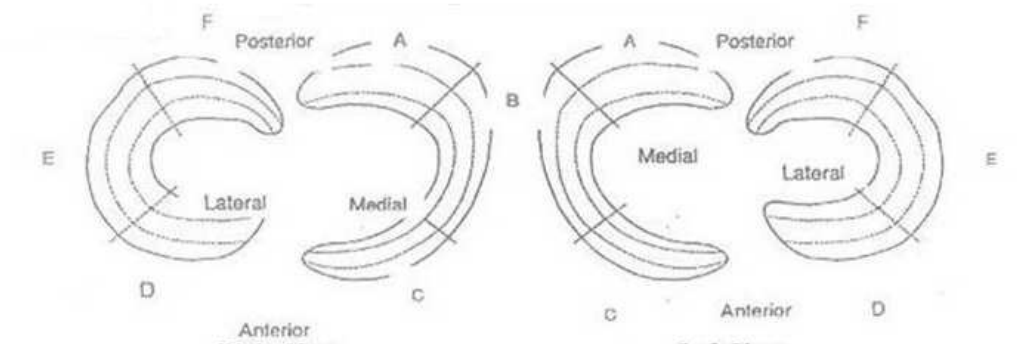


Suprapatellar	Hypertropy	□0	□1	□2	□3	□4
	Vascularity	□0	□1	□2	□3	□4
	Synovitis	□0	□1	□2	□3	□4
	Pictures	□yes	□no	Biopsies n°_____		
Lateral compartment	Hypertropy	□0	□1	□2	□3	□4
	Vascularity	□0	□1	□2	□3	□4
	Synovitis	□0	□1	□2	□3	□4
	Pictures	□yes	□no	Biopsies n°_____		
Medial compartment	Hypertropy	□0	□1	□2	□3	□4
	Vascularity	□0	□1	□2	□3	□4
	Synovitis	□0	□1	□2	□3	□4
	Pictures	□yes	□no	Biopsies n°_____		
Peri-ACL	Hypertropy	□0	□1	□2	□3	□4
	Vascularity	□0	□1	□2	□3	□4
	Synovitis	□0	□1	□2	□3	□4
	Pictures	□yes	□no	Biopsies n°_____		

Adapted from af Klint, Arthritis Research & Therapy 2009

ASSOCIATED PATHOLOGY FORMS:

Figure 7 - MENISCAL TEAR FORM



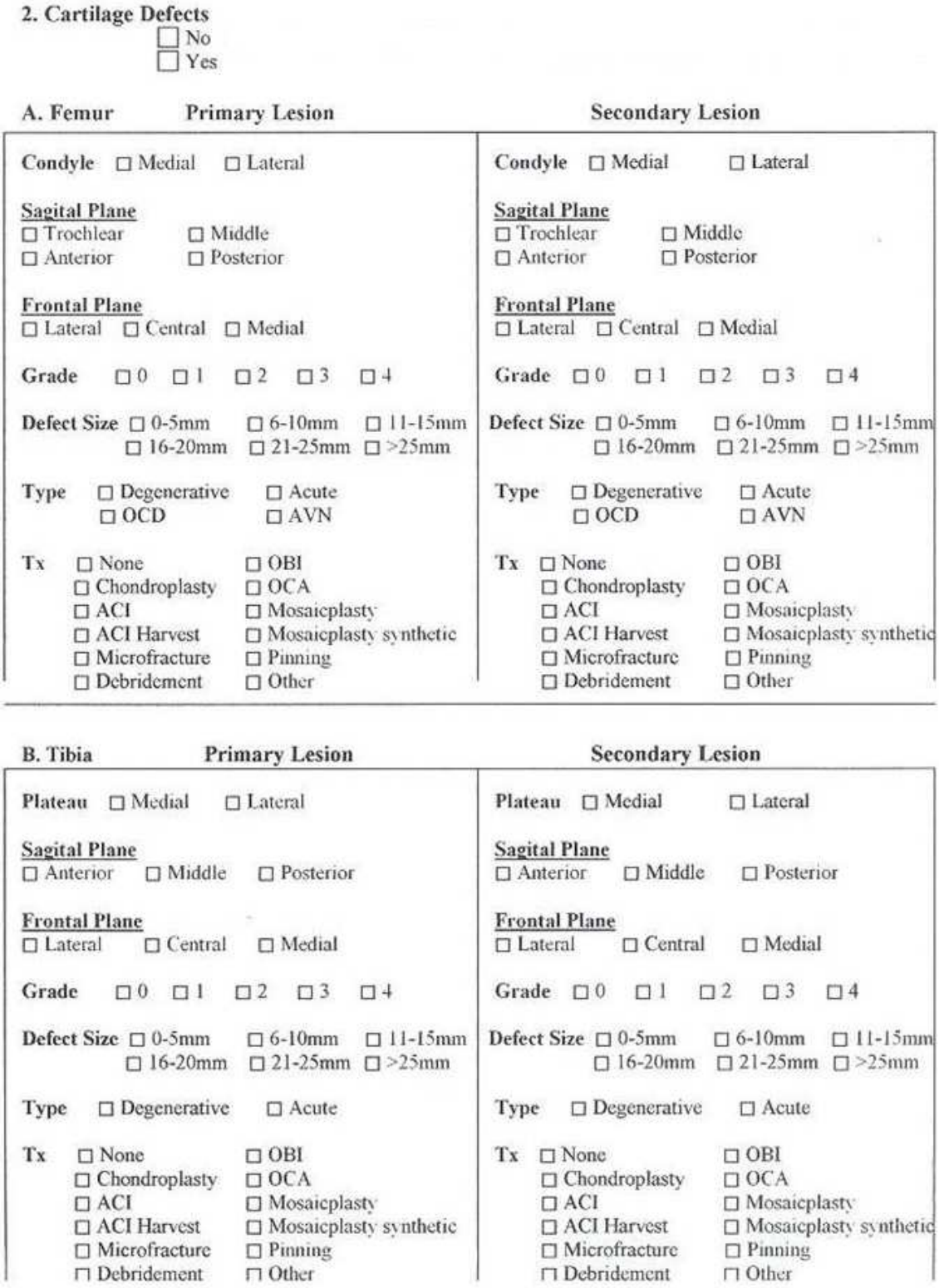
1. Meniscal Tear
☐ Yes ☐ No (If No, proceed to Cartilage (#2))

# of MEDIAL Tears ☐ 0 ☐ 1 ☐ 2 ☐ 3

# of LATERAL Tears ☐ 0 ☐ 1 ☐ 2 ☐ 3

	MEDIAL	LATERAL
Location	☐ A ☐ B ☐ C ☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ D ☐ E ☐ F ☐ 0 ☐ 1 ☐ 2 ☐ 3
Thickness	☐ Partial ☐ Femoral ☐ Tibial ☐ Full Thickness	☐ Partial ☐ Femoral ☐ Tibial ☐ Full Thickness
Type	☐ Radial ☐ Oblique ☐ Longitudinal-vertical ☐ Bucket handle-displaced ☐ Horizontal ☐ Flap ☐ Complex	☐ Radial ☐ Oblique ☐ Longitudinal-vertical ☐ Bucket handle-displaced ☐ Horizontal ☐ Flap ☐ Complex
Length	☐ 0-9mm ☐ 10-19mm ☐ +20mm	☐ 0-9mm ☐ 10-19mm ☐ +20mm
Treatment	☐ None ☐ Excision ☐ Repair ☐ Healing Response	☐ None ☐ Excision ☐ Repair ☐ Healing Response
Meniscus Repair Technique	☐ All-in ☐ Fast Fix ☐ Rapid Lock ☐ Inside-out ☐ Both inside-out & all-in ☐ Outside-in ☐ Other	☐ All-in ☐ Fast Fix ☐ Rapid Lock ☐ Inside-out ☐ Both inside-out & all-in ☐ Outside-in ☐ Other
Location of Excision	☐ A ☐ B ☐ C ☐ 0 ☐ 1 ☐ 2 ☐ 3	☐ D ☐ E ☐ F ☐ 0 ☐ 1 ☐ 2 ☐ 3

Figure 8 – CARTILAGE DEFECT FORM



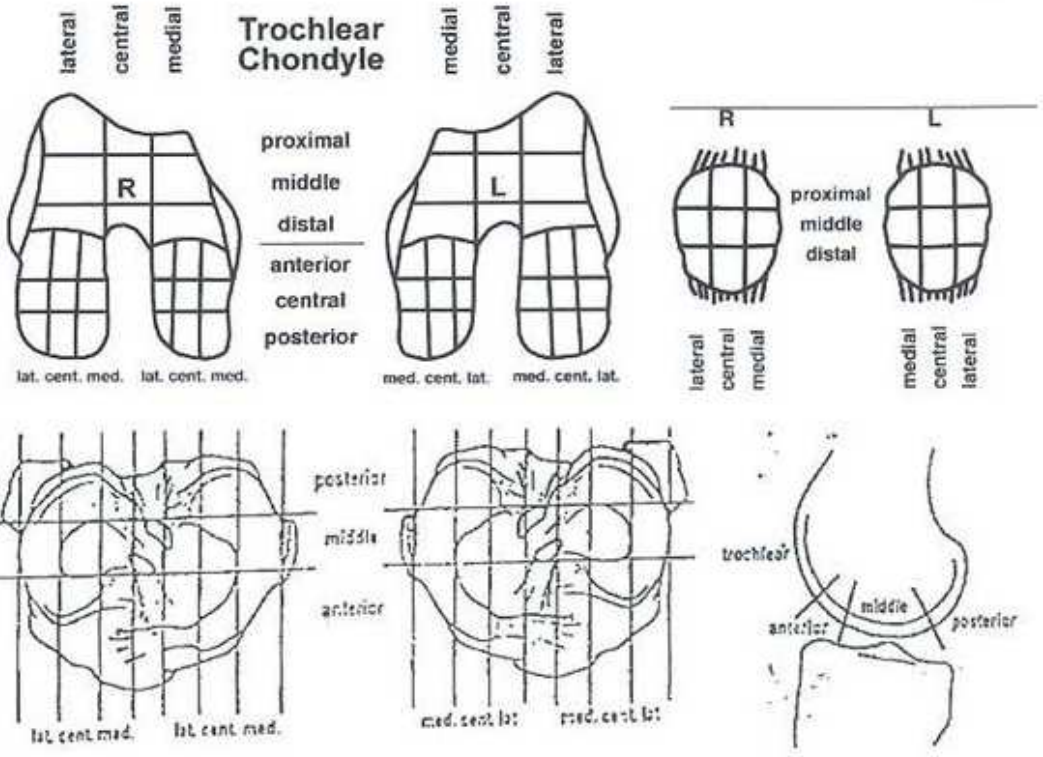
2. Cartilage Defects
☐ No
☐ Yes

A. Femur	Primary Lesion	Secondary Lesion
Condyle	☐ Medial ☐ Lateral	☐ Medial ☐ Lateral
Sagital Plane	☐ Trochlear ☐ Middle ☐ Anterior ☐ Posterior	☐ Trochlear ☐ Middle ☐ Anterior ☐ Posterior
Frontal Plane	☐ Lateral ☐ Central ☐ Medial	☐ Lateral ☐ Central ☐ Medial
Grade	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4
Defect Size	☐ 0-5mm ☐ 6-10mm ☐ 11-15mm ☐ 16-20mm ☐ 21-25mm ☐ >25mm	☐ 0-5mm ☐ 6-10mm ☐ 11-15mm ☐ 16-20mm ☐ 21-25mm ☐ >25mm
Type	☐ Degenerative ☐ Acute ☐ OCD ☐ AVN	☐ Degenerative ☐ Acute ☐ OCD ☐ AVN
Tx	☐ None ☐ OBI ☐ Chondroplasty ☐ OCA ☐ ACI ☐ Mosaicplasty ☐ ACI Harvest ☐ Mosaicplasty synthetic ☐ Microfracture ☐ Pinning ☐ Debridement ☐ Other	☐ None ☐ OBI ☐ Chondroplasty ☐ OCA ☐ ACI ☐ Mosaicplasty ☐ ACI Harvest ☐ Mosaicplasty synthetic ☐ Microfracture ☐ Pinning ☐ Debridement ☐ Other

B. Tibia	Primary Lesion	Secondary Lesion
Plateau	☐ Medial ☐ Lateral	☐ Medial ☐ Lateral
Sagital Plane	☐ Anterior ☐ Middle ☐ Posterior	☐ Anterior ☐ Middle ☐ Posterior
Frontal Plane	☐ Lateral ☐ Central ☐ Medial	☐ Lateral ☐ Central ☐ Medial
Grade	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4
Defect Size	☐ 0-5mm ☐ 6-10mm ☐ 11-15mm ☐ 16-20mm ☐ 21-25mm ☐ >25mm	☐ 0-5mm ☐ 6-10mm ☐ 11-15mm ☐ 16-20mm ☐ 21-25mm ☐ >25mm
Type	☐ Degenerative ☐ Acute	☐ Degenerative ☐ Acute
Tx	☐ None ☐ OBI ☐ Chondroplasty ☐ OCA ☐ ACI ☐ Mosaicplasty ☐ ACI Harvest ☐ Mosaicplasty synthetic ☐ Microfracture ☐ Pinning ☐ Debridement ☐ Other	☐ None ☐ OBI ☐ Chondroplasty ☐ OCA ☐ ACI ☐ Mosaicplasty ☐ ACI Harvest ☐ Mosaicplasty synthetic ☐ Microfracture ☐ Pinning ☐ Debridement ☐ Other

C. Patella	Primary Lesion	Secondary Lesion
	Sagital Plane ☐ Distal ☐ Middle ☐ Proximal	**Sagital Plane** ☐ Distal ☐ Middle ☐ Proximal
	Frontal Plane ☐ Lateral ☐ Central ☐ Medial	**Frontal Plane** ☐ Lateral ☐ Central ☐ Medial
	Grade ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4	**Grade** ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4
	Defect Size ☐ 0-5mm ☐ 6-10mm ☐ 11-15mm ☐ 16-20mm ☐ 21-25mm ☐ >25mm	**Defect Size** ☐ 0-5mm ☐ 6-10mm ☐ 11-15mm ☐ 16-20mm ☐ 21-25mm ☐ >25mm
	Type ☐ Degenerative ☐ Acute	**Type** ☐ Degenerative ☐ Acute
	Tx ☐ None ☐ OBI ☐ Chondroplasty ☐ OCA ☐ ACI ☐ Mosaicplasty ☐ ACI Harvest ☐ Mosaicplasty synthetic ☐ Microfracture ☐ Pinning ☐ Debridement ☐ Other	**Tx** ☐ None ☐ OBI ☐ Chondroplasty ☐ OCA ☐ ACI ☐ Mosaicplasty ☐ ACI Harvest ☐ Mosaicplasty synthetic ☐ Microfracture ☐ Pinning ☐ Debridement ☐ Other

Grade lesion of each section by WORST GRADE involved via I-IV Outerbridge scale using "1,2,3,4"



Synovial tissue collection and processing— Synovial biopsies were collected during arthroscopic surgery in the same four locations (suprapatellar, peri-ACL, lateral and medial compartment) where surgeons scored the presence of synovitis and recorded the pictures. Biopsy specimens were obtained from areas of synovium that appeared to be inflamed or thickened. When no inflammation was apparent, biopsy specimens were obtained from the standard location: the femoral aspects of the medial and lateral gutters and the central supratrochlear region in the suprapatellar pouch. The synovial samples were processed for histology and RNA extraction. Synovial biopsies were stored in 4% paraformaldehyde, embedded in paraffin, and sliced in 6 μm sections. Tissue sections were deparaffinized in xylene, rehydrated through an ethanol series, and stained with hematoxylin and eosin for microscopic analysis. Synovial samples for RNA extraction were stored in RNAlater at -80° C until use.

Synovial histological microscopic scoring— Stained sections were coded by an experienced histopathologist (ED) who was blinded to all data. To reduce the inter-reader variation, synovial specimens were also scored by another reader. To standardize evaluations, only sections containing a clearly recognizable lining layer with underlying vascularised subintima were analyzed. The synovial features of inflammation and degeneration were assessed using a combination histological synovial scoring system developed at our institution [1, 55] in which the following six features were graded: perivascular mononuclear cell infiltration, increased vascularity, detritus, mucoid change, fibrosis, and hyperplasia of the synovial lining layer leading to a total combined score of 15 (Table I). *Lymphocytic inflammation* was graded based on perivascular mononuclear cell infiltration in synovium as follows: grade 0=none, grade 1=mild (0-1 perivascular aggregates per low-power field); grade 2=moderate (> 1 perivascular aggregate per low power field with or without focal interstitial infiltration); grade 3=marked aggregates (both perivascular and interstitial). *Vascularity* was scored as follows: grade 0=normal, grade 1=mild increased, grade 2= markedly increased. The presence of *detritus* was assessed using the following score: grade=0 absent, grade 1=small particulate, grade 2= large particulate. The *fibrosis* was evaluated as follows: grade=0 absent, grade 1=focal/perivascular, grade 2= widespread. The presence of *mucoid change* was assessed using the following score: grade=0 none, grade 1=slight (perivascular or focal interstitial), grade 2=moderate (perivascular and focal interstitial), grade 3=marked (perivascular and widespread interstitial), grade 4= myxomatous.

Synovial hyperplasia was scored considering the thickness of the synovial lining layer: grade 0=normal (up to 2 cell layers thick), grade 1=hyperplasia I (3-4 cells thick), grade 2= hyperplasia II (> 4 cell thick). Synovial hyperplasia was evaluated at high power (25 x objective), while the rest of the parameters were assessed at lower magnification (5 x objective).

Real-time Reverse Transcription-PCR Analysis—Total RNA was extracted from homogenized synovial membrane samples using PerfectPure RNA Fibrous Tissue kits (5Prime Inc.). All the RNA was DNase treated, oligo(dT) primed, and complementary DNA (cDNA) was synthesized with Superscript III reverse transcriptase (Invitrogen Life Technologies). Amplifications were carried out using SYBR Green I-based real-time PCR on the Opticon 2 Real Time PCR Detector System (BioRad).

Suprapatellar synovial levels of mRNA for 4 chemokines (interleukin-8 [IL-8], CCL19, CCL21, and CCL5) and 1 chemokine receptor (CCR7), shown previously to be associated with synovial inflammation in patients undergoing meniscectomy [1], were measured by real time quantitative (q)PCR. Gene-specific primer sequences were obtained from our collaborator C. Scanzello (Table II). In addition, Real time qPCR was used to assess gene expression levels of matrix metalloproteinase 13 (MMP-13) and inflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor α (TNFα) and interleukin-15 (IL-15). After normalizing to GAPDH, expression levels were calculated relative to the mean value for each gene product.

Statistical analysis— Given the small sample size and some irregularly distributed variables, nonparametric tests were used. Data were statistically analyzed using SPSS 15.0I statistical package (Windows version). Differences between groups were evaluated with Mann-Whitney *t*-tests and Spearman's correlation coefficients were calculated. P-values <0.05 were considered to be statistically significant.

Table I. Histological synovitis score

At 5X (must be present in at least 2 out of 5 fields)	
1) Lymphocytic Inflammation: □ (0) none □ (1) mild (0-1 perivascular aggregates per low power field) □ (2) moderate (>1 perivascular aggregate per low power field and focal interstitial infiltration) □ (3) marked (both perivascular and widespread interstitial aggregates)	**2) Vascularity:** □ (0) normal □ (1) mildly increased □ (2) markedly increased
3) Detritus: □ (0) absent □ (1) small particulate □ (2) large particulate	**4) Fibrosis:** □ (0) absent □ (1) focal / perivascular Yes / No: Band-like & superficial □ (2) widespread
5) Mucoid change: □ (0) none □ (1) slight (perivascular or focal interstitial) □ (2) moderate (perivascular and focal interstitial) □ (3) marked (perivascular and widespread interstitial) □ (4) myxomatous	**PICTURES/NOTES: Y/N** _____ _____ _____
At 25X	**PRESENT OR ABSENT**
6) Synovial Hyperplasia: □ (0) Normal (up to 2 cell layers thick) □ (1) Hyperplasia I (3-4 cells thick) □ (2) Hyperplasia II (> 4 cell thick)	1) Fibrin 2) Foreign body giant cells 3) Granulation tissue 4) Synovial Giant Cells 5) > 10% plasma cells within lymphocytic aggregates 6) > 50% plasma cells 7) Germinal centers 8) Russell bodies 9) Synovial lining cell activation 10) Synovial chondrometaplasia 11) Mast Cells

Gene	Primer sequence
IL-8	Forward: 5'-ATGACTTCCAAGCTGGCCGTGGCT-3' Reverse: 5'-CTCAGCCCTCTTCAAAAACTTCTC-3'
CCL19	Forward: 5'-CCCTGCTACTGGCCCTCAG-3' Reverse: 5'-AGGCAGCAGTCTTCAGCATCA-3
CCL21	Forward: 5'-CAAGACACCATCCCCACA-3' Reverse: 5'-TGTGACCGCTCAGTCCTC-3'
CCL5	Forward: 5'-GCGCTCCTGCATCTGCCTCC-3' Reverse: 5'-CGGGTGACAAAGACGACTGCTGG-3
CCR7	Forward: 5'-GAAAGTCCAGAAACTGTTCCCACCTGC-3' Reverse: 5'-TTCCTCACCAAGCCAAGAAGTCTCCCC-3'
IL-1β	Forward: 5'- CCGGGACTCACAGCAAAA-3' Reverse: 5'- GGACATGGAGAACACCACTTG-3'
IL-15	Forward: 5'-GACAAGCCTGTAGCCCATGT-3' Reverse: 5'-TCTCAGCTCCACGCCATT-3'
TNFα	Forward: 5'-CACAGAAATCAATGTTAGCAGATAGC-3' Reverse: 5'-TTGAAGAAGAGCTGGCTATGG-3'
MMP13	Forward: 5'-TCAGGAAACCAGGTCTGGAG-3' Reverse: 5'-TGACGCGAACAATACGGTTA-3'
GAPDH	Forward:5'- CAACGGATTTGGTCGTATT-3' Reverse: 5'-GATGGCAACAATATCCACTT-3'

Table II. Primer sequences used for quantitative PCR

RESULTS

Of 40 patients undergoing ACL reconstruction and synovial biopsy, 19 were female and 21 male, with median ages of 20 and 36, respectively. In the female population there were two age peaks: one younger, ranging from 10 to 19 years, and the other older, ranging from 40 to 59 years. The male population had a broader age distribution ranging between 20 to 60 years (Fig. 9). Demographic characteristics of these patients are presented in Table III. Median age in the total group was 31.5 years (interquartile range = 19.5 to 45.8 years). Median BMI was 24.3 h/cm² (interquartile range = 22.3 to 26.5 h/cm²). Male BMI was significantly higher compared to female BMI (median = 26.4 versus 22.7; $p<0.001$). The patients were divided in two groups based on age: 19 patients older than 30 years and 21 patients younger than 30 years. BMI was significantly different between patients younger and older than 30 years (median = 23.5 versus 24.7; $p <0.05$). The median time from injury to surgery in the total group was 65 days with a range between 13 days and 19.6 years. 55% of patients were injured on the right knee and 42.5% on the left knee, with an equal distribution between male and female. 33 patients had a complete ACL rupture, while 4 had a partial rupture. The mechanism of the injury was non-contact in 33 patients (82.5%) and contact in 6 (15%). Patients had participated in the following sports: skiing (32.5%), soccer (17.5%), basketball (17.5%), football (2.5%), lacrosse (2.5%), and other (27.5%). Associated pathology data are shown in Table IV.

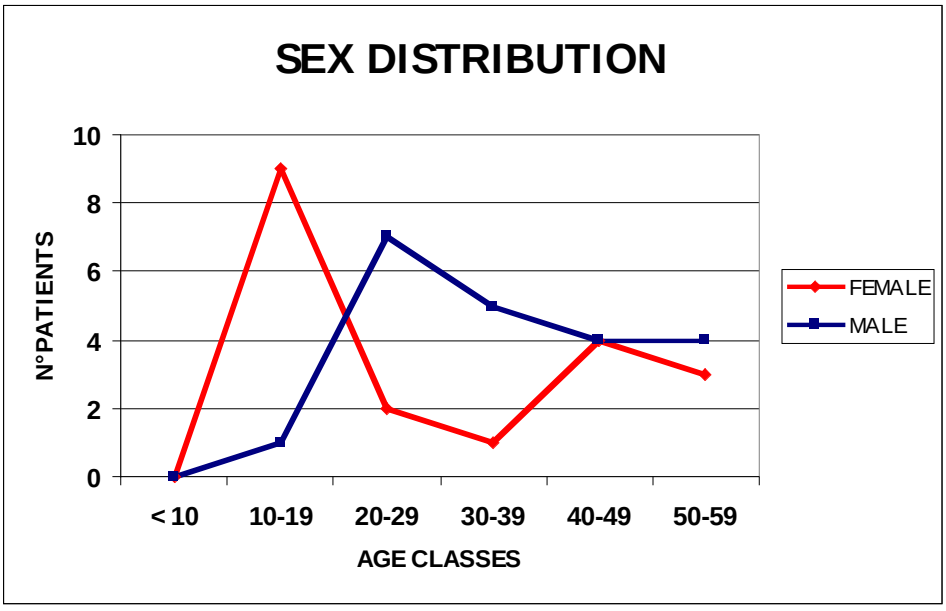


Figure 9. Sex distribution of patients and age classes.

Table III. Characteristics of patients undergoing synovial biopsy.

PATIENTS UNDERGOING SYNOVIAL BIOPSY (N°=40)	
Age, median (interquartile range) years	31.5 (19.5-45.8)
BMI, median (interquartile range) kg/m²	24.3 (22.3-26.5)
N° women/n° men	19/21
Age women median (interquartile range) years	20 (16.5-46)
Age men median (interquartile range) years	36 (27-45)
N° complete (%)/partial rupture/NA	33/4/3
Time from injury to surgery, median (interquartile range) days	65 (39.5-178.5)
Previous meniscectomy	3
N° right/Left knees/NA	22(55%)/17(42.5%)/1
N° right knees women/left	9/10
N° non-contact injuries (%)/Contact/NA	33 (82.5%)/6 (15%)/1
SPORTS	
N° basketball (%)	7 (17.5%)
N° football (%)	1 (2.5%)
N° ski (%)	13 (32.5%)
N° soccer (%)	7 (17.5%)
N° lacrosse (%)	1 (2.5%)
N° other (%)	11 (27.5%)

BMI = body max index, NA = not applicable

Exam under anesthesia— The knee alignments were normal in 37 patients, varus in 2 patients, and valgus in 1 patient. The ranges of motion for knee flexion were described by the surgeons as follows: 150° in 7.5%, 145° in 20%, 140° in 67.5% and 135°in 5% of patients. The ranges of motion for knee extension were normal in 82.5% of patients and recurvatum in 17.5% of patients. Patients had effusion at physical exam in 20% of the cases. Scores graded by the Lachman Test were as follows: 1 in 6 cases, 2 in 33 cases, and 3 in one case. 7.5% of the patients had a Pivot shift test of 0, 22.5% of 1, 67.5% of 2 and 2.5% of 3.

Tab IV. Associated pathology data

BONE BRUISE (N°28 patients/NA 4)	
N° LFC/LTP/MFC/MTP	24/27/1/3
MACROSCOPIC SYNOVITIS (N° 39 patients)	
N° Mild (< 3)	20 (51.2%)
N° Mild to moderate (4-6)	17 (43.5%)
N° Moderate (7-9)	2 (5%)
N° Severe (10-12)	0
SITE OF MACROSCOPIC SYNOVITIS > 3 (N°19 patients)	
N° Suprapatellar	16 (84.2%)
N° Only suprapatellar	9 (47.3%)
N° Peri-ACL	8 (42.1%)
N° Medial compartment	3 (15.7%)
N° Lateral compartment	4 (21%)
SUPRAPATELLAR HISTOLOGICAL SYNOVITIS (n° 40 patients)	
N° score 0	6 (15%)
N° score 1-2	15 (37.5%)
N° score 3-4	15 (37.5%)
N° score 5-6	2 (5%)
N° score 7-8	2 (5%)
MICROSCOPIC SYNOVITIS(n° 34 patients)	
N° grade 1-2/3-4/5-6/7-8	15/15/2/2
MENISCAL TEARS (N°19 patients)	
N° Medial/Lateral/Bilateral	11/6/2
CARTILAGE DEFECT (N°16 patients)	
N° Single/Multiple	10/6
Outerbridge scores I/II/III/IV/NA	1/9/3/2/1

LFC = lateral femoral condyle, LTP = lateral tibial plateau, MFC = medial femoral condyle, MTP=medial tibial plateau, NA = not applicable

Synovial intraoperative macroscopic scoring— Visual evidence of synovitis was evident in 97.5% of the patients, as, graded by the surgeons during the arthroscopy as follows: 51.2% mild, 43.5% mild to moderate, and 5.8% moderate (Fig. 10). No patient had severe macroscopic synovitis. Of the 19 patients with scores of >3, synovitis was most frequently observed in the suprapatellar area (84.2%), followed by peri-ACL (42.1%), medial (15.7%) and lateral compartments (21%). In 47.3% of patients (9/19), the suprapatellar region was the only site of synovitis.

Figure A

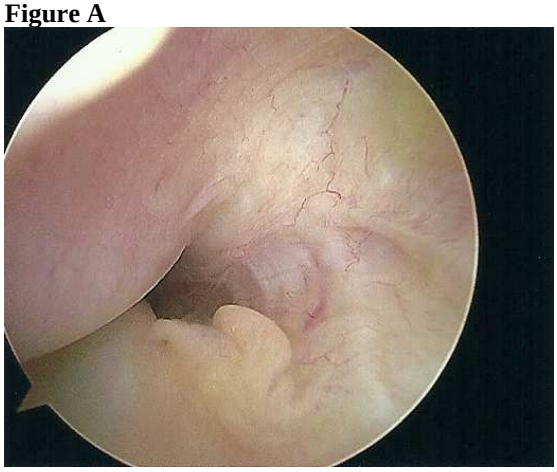
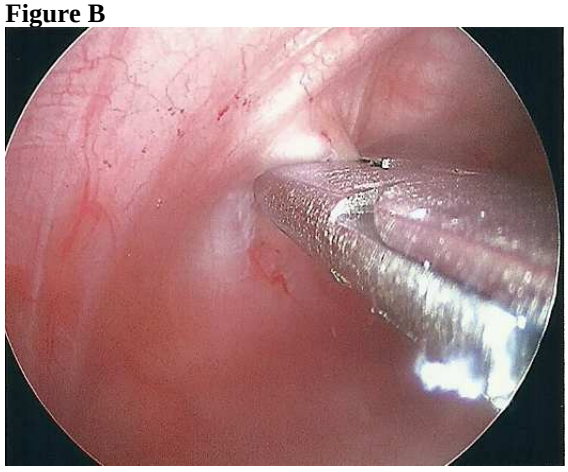


Figure B



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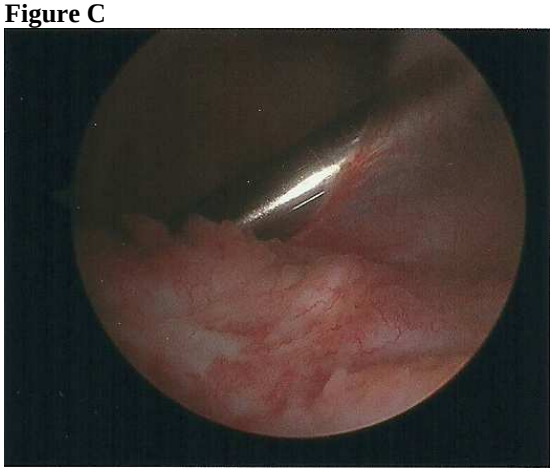


Figure D

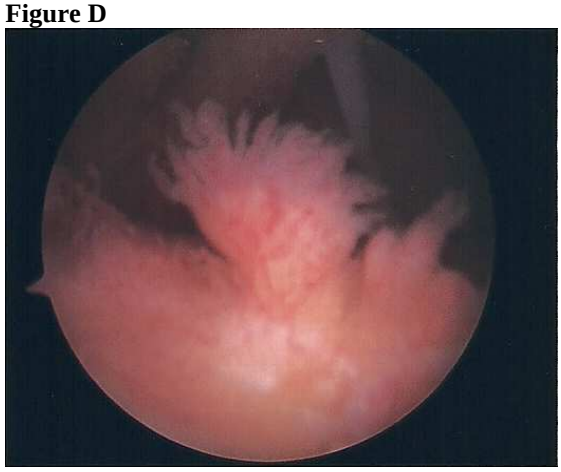


Figure 10. Intraoperative representative photographs from patients with mild (**B**), mild to moderate (**C**), and moderate (**D**) synovial inflammation are shown. Normal synovial membrane is depicted in picture **A**.

Synovial histological microscopic scoring— Since the macroscopic intraoperative scoring showed a prevalent involvement of suprapatellar synovium, histological scoring was assessed only in this area. 15% (n=6) of patients did not have any sign of synovial suprapatellar inflammation. Based on the composite histological synovial scoring system, the remainder had grade 1-2 (n= 15, 37.5%), grade 3-4 (n=15, 35.5%), grade 5-6 (n=2, 5%) and grade 7-8 (n=2, 5%) (Fig. 12). Microscopic synovitis scoring was negatively associated by Spearman's correlation with time from injury to surgery (r = -0.358, p = 0.027) (Fig. 11). No significant association was found between macroscopic and microscopic combined scoring, but macroscopic hyperemia statistically correlated with microscopic synovial hyperplasia (r = 0.316, p = 0.0465).

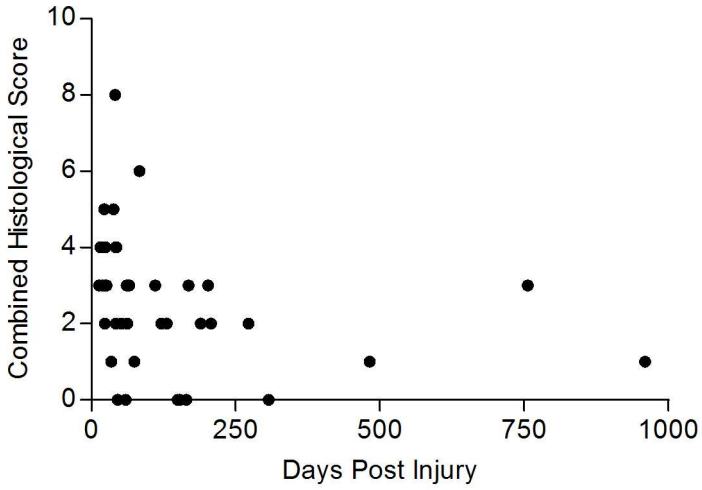


Figure 11. Correlation between time from injury and histological scoring.

Figure A

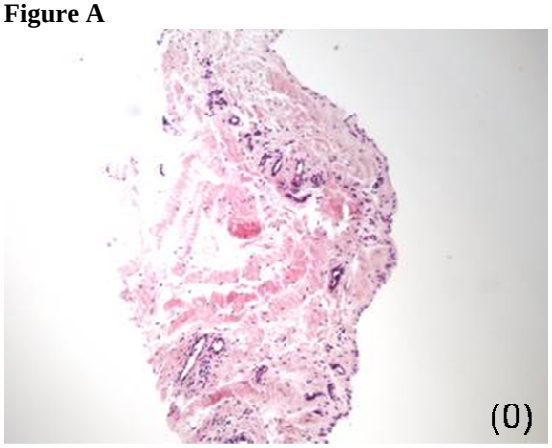
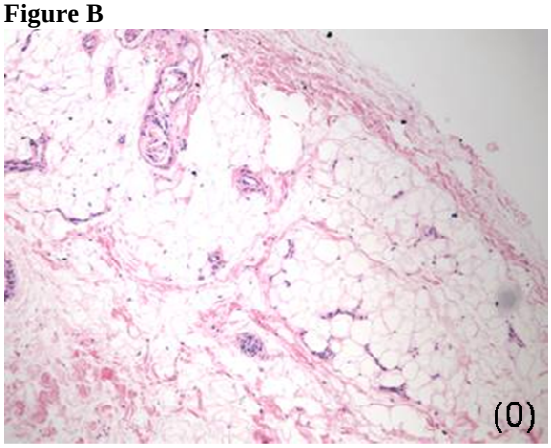


Figure B



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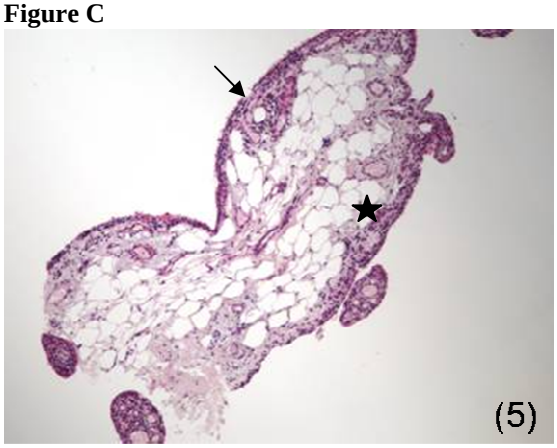


Figure D

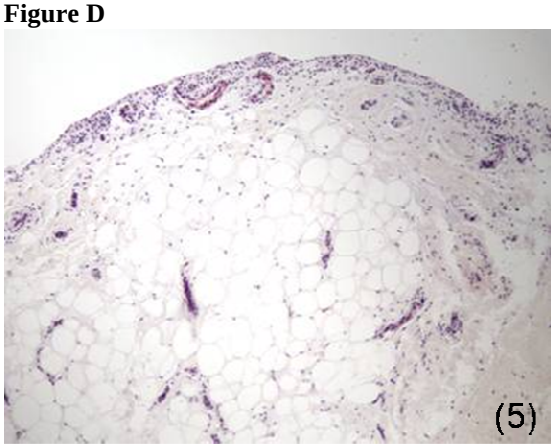


Figure E

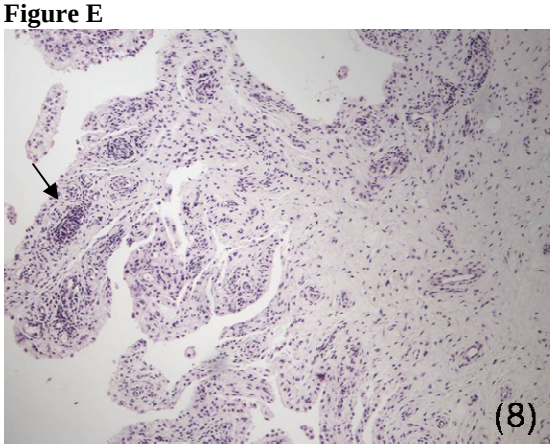


Figure F

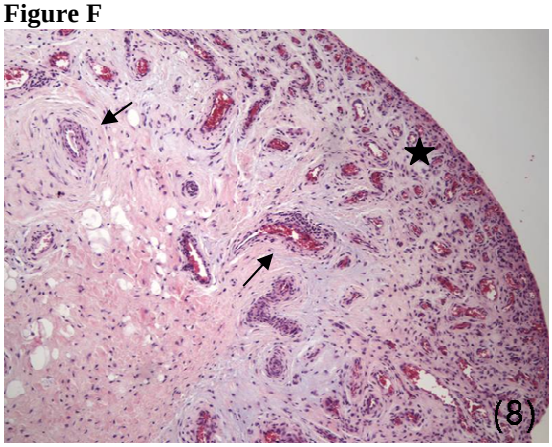


Figure 12. Histological analysis of synovial membrane inflammation in patients undergoing ACL reconstruction for ACL rupture. Synovial biopsy specimens obtained at the time of the surgery were fixed, embedded, and thin-sectioned before being stained with hematoxylin and eosin. Inflammation was graded using a combined histological scoring system described in Patients and Methods. Photomicrographs of representative section from patients with grade 0 (**A** and **B**), grade 5 (**C** and **D**), and grade 8 (**E** and **F**) are shown. Higher synovitis score pictures show increasing perivascular mononuclear cell accumulation (↟) and thickening in the lining cell hyperplasia (★). Original magnification x 10

Cartilage defect— Arthroscopically, cartilage defects were observed in 16/40 patients (40%), 7 female and 9 male. 10 patients exhibited a single cartilage lesion, 4 patients had two and 2 had three separate chondral defects in the knee. Only one patient exhibited Outerbridge grade I. The remainder had grade II (n=9), grade III (n=3) and grade IV (n=2) (Fig 13). The cartilage defect grade of one patient was not reported. Of 24 cartilage lesions, 13 (54.1%) were located in the medial femoral condyle (MFC), 6 (25%) in the patella, 3 (12.5%) in the lateral tibial plateau (LTP), 1 (4.1%) in the lateral femoral condyle (LFC) and 1 (4.1%) in the medial tibial plateau (MTP). 2 (8.6%) cartilage defects measured 0-5 mm, 12 (52.1%) 5-10 mm, 6 (26%) 10-15 mm, 1 (4.3%) 15-20 mm, and 2 (8.6%) 20-25 mm. The presence of a cartilage defect was associated with both age ($p < 0.001$) and BMI ($p < 0.05$). Median BMI was 25.7 (interquartile range 24-28.4) in the cartilage defect group and 23.1 (21.4-24.9) in the group without cartilage lesions. Patients with cartilage defects were older than those with normal articular surfaces (median age 46 versus 23.5 respectively). In addition, in patients with cartilage lesions, the median of the time from injury to surgery was significantly greater when compared with patients with normal articular surfaces, 183 (interquartile range 70-550) days and 43 (interquartile range 30-96) days, respectively ($p < 0.05$). Of patients with cartilage defects 37.5% (n=6) had a meniscal tear (6 with medial tears, and 1 with both medial and lateral tears). Outerbridge score showed a positive association with age, BMI and time from injury to surgery ($r = 0.542$, $p < 0.001$; $r = 0.423$, $p = 0.009$; $r = 0.536$, $p = 0.001$). No association was observed between cartilage defect and synovitis scoring, either macroscopically or microscopically, Lysholm Knee Scale or Marx Activity Scale.

Figure A

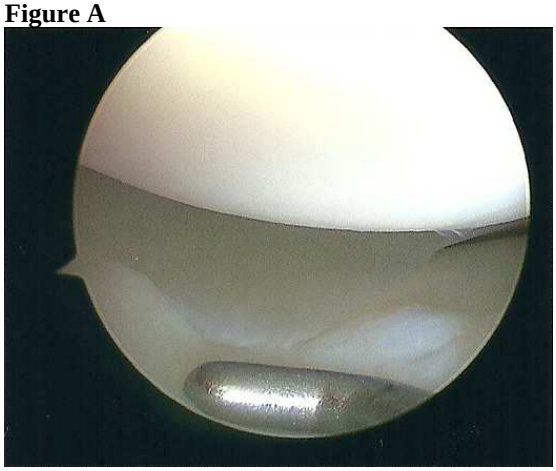


Figure B

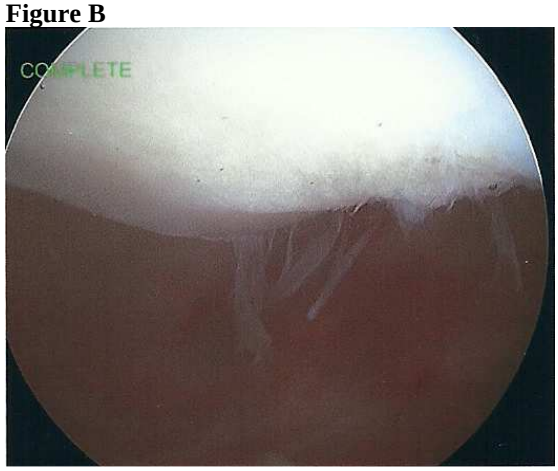


Figure C

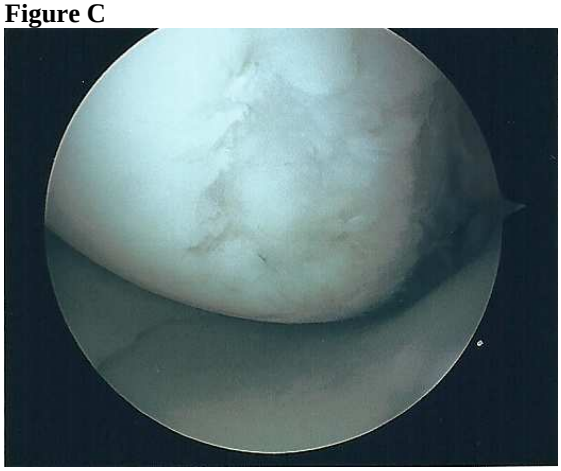


Figure D

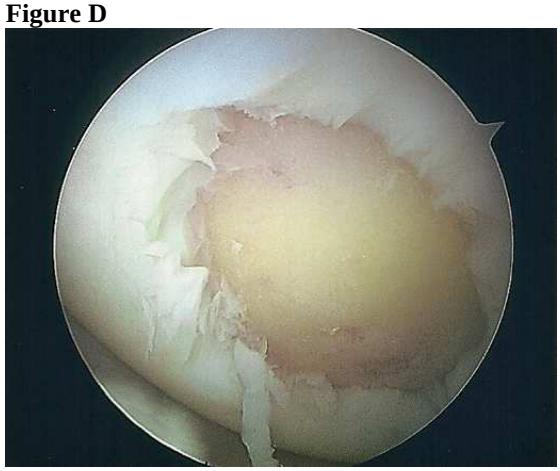


Figure 13. Intraoperative representative photographs from patients with cartilage damage assessed by Outerbridge scoring system are shown: grade I = softening and swelling (**A**), grade II = fragmentation and fissuring in an area up to half an inch in diameter (**B**), grade III = = fragmentation and fissuring in an area more than half an inch in diameter (**C**), grade IV = full-thickness erosion of the articular cartilage to subchondral bone (**D**).

Meniscal tear— Arthroscopically, meniscal tears were observed in 19/40 patients (47.5%), 8 female and 11 male. Most (57.9%, n=11) had medial meniscal tears; 6 had lateral tears, and 2 had both medial and lateral tears. Of 21 meniscal lesions 9 (42%) were classified as complex, 8 (38%) as full thickness, and 3 (14.3%) as partial, located in the tibial plateau. One surgical report was not available. 31.8% of the meniscal tears were longitudinal-vertical, 18.2% bucket handle-displaced, 18.2% complex, 13.6% horizontal, 9% oblique, and 9% radial. The meniscal tear lengths were 0-9 mm in 63.6% of patients, 10-19 mm in 27.3%, and > 20 mm in 9%. The presence of meniscal tear was associated with synovial inflammation in patients older than 30 years ($p < 0.05$) (Fig. 14). Medial meniscal tears were predominant in patients with cartilage defects (6/7), while 5 patients without cartilage defects had medial tears, 6 had lateral tears, and 1 had

both lateral and medial tear. No association was observed between meniscal tear and age, BMI, days from injury, macroscopic and microscopic synovitis scoring, Outerbridge score, SF-36, Lysholm Knee Scale, or Marx Activity Scale.

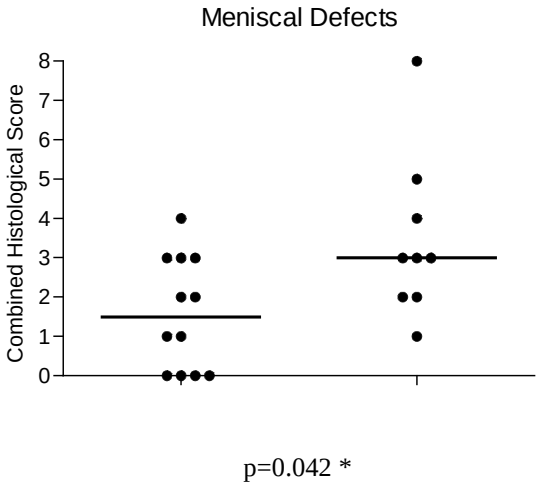


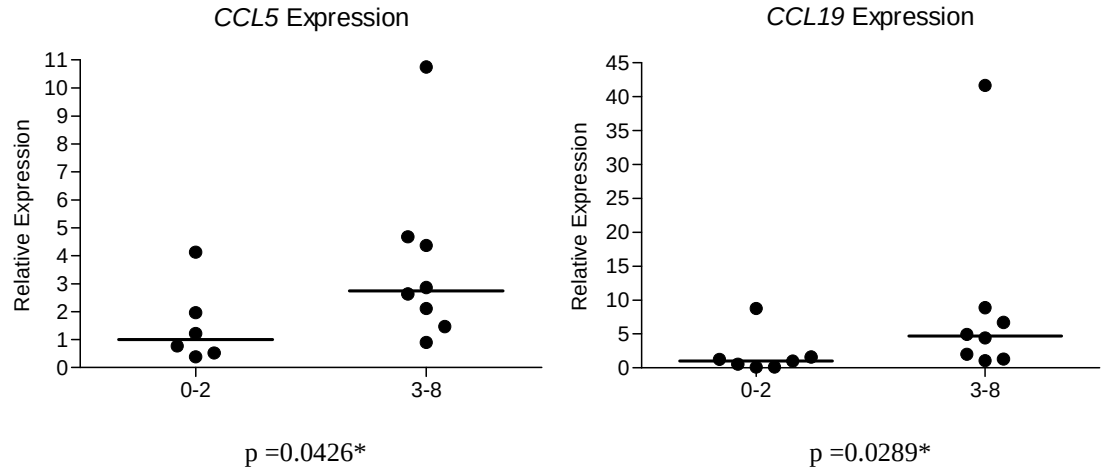
Fig. 14. Association between histological synovitis and meniscal defect in patients > 30 yrs.

Bone bruise— 28(77.7%) patients had a bone "bruise" assessed by the presence of a bone marrow lesion on MRI. The presence of a bone bruise was not reported in the chart of 4 patients. The bone bruises was predominantly located in the lateral compartment. 27 patients had bone marrow lesions in the LTP, 24 in the LFC, 3 in the MTP and 1 in the MFC.

Real Time PCR data— 16 synovial suprapatellar biopsies from patients > 30 yr were chosen for qPCR analysis, 8 from patients without synovial inflammation (grade 0-2) and 8 with synovial inflammation (grade 3-8). The levels of *CCL5*, *CCL19* and *MMP13* mRNA were significantly upregulated in biopsy specimens exhibiting inflammation with associated fold-changes of 2.7 (p = 0.0426), 4.7 (p = 0.0289), and 3.9 (p = 0.0033) respectively (Fig.15). CCR7 mRNA levels correlated with microscopic synovitis by Spearmen's correlation. (r=0.682, p=0.05). No difference in expression level of *IL-8, CCL21, IL-1β or TNFα* was observed considering the presence of absence of synovial inflammation.

MMP13 expression levels were associated with *CCL5* mRNA levels (r = 0.717, P = 0.030). In addition, *MMP13* expression correlated negatively with the time from injury

to surgery and positively with histological inflammation (r = -0.524, P= 0.037, r = 0.791, P < 0.0001 respectively). *CCL5* mRNA levels correlated with *CCL19* mRNA levels and with the grade of synovial inflammation measured by the histological combined scoring (r = 0.569, P= 0.034, r = 0.604, P = 0.022, respectively).



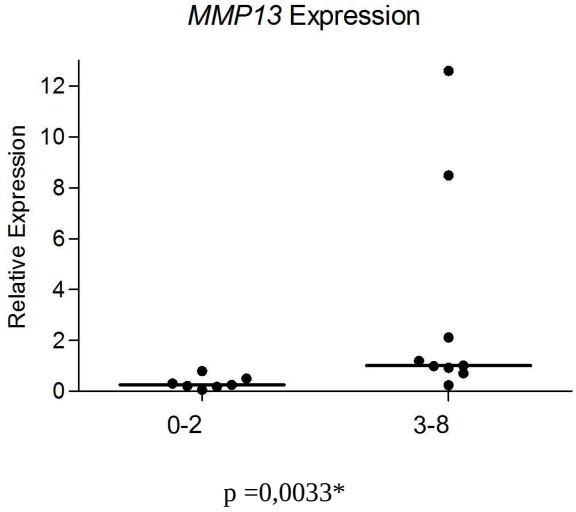


Fig. 15. The levels of *CCL5*, *CCL19* and *MMP13* mRNA are upregulated in patients with synovial inflammation.

SF-36, **Lysholm Knee Scale and Marx Activity Scale.**

Lower Marx activity scores were observed in male than in female patients (median 12 versus 16, p = 0.013). Moreover, Marx activity scores were higher in patients younger than 30 years than in older patients (median 16 versus 11.5, p < 0.001). Marx activity score negatively correlated with IL-15 mRNA levels (r=-o.669 and p=0.009).

Physical component summary of SF-36 was significatively higher in patients older than 30 years compared with those younger than 30 years (median = 50.7 vs 40.4, p = 0.023). The seventh domain of SF-36, corresponding to role limitations due to physical health, was associated with the presence of cartilage defect (P = 0.013) and correlated with the Outerbridge score (r = 0.360, P = 0.043). The eighth domain of SF-36 corresponding to mental health was correlated with *IL-8* mRNA levels (r = 0.707, P = 0.010). No significant difference was observed in Lysholm scores.

DISCUSSION

Most investigations into the pathogenesis of OA have focused on the degeneration of articular cartilage and associated changes in the subchondral bone. However, changes in the synovial membrane also occur. Multiple studies indicate that synovitis is a common finding in patients with early and late OA [6, 7, 55], and there is emerging evidence that synovial inflammation associated with joint injury may precede the development of clinically overt joint disease [1]. The presence of synovitis has been shown to correlate with OA symptoms and importantly is a risk factor for OA disease progression [8, 56-59].

Previous epidemiologic studies have established that there is a strong relationship between ACL disruption and the risk for subsequent development of OA [20, 21, 60, 61]. ACL rupture affects joint kinematics, increasing the anterior tibial displacement and consequently the forces applied mainly on the medial side of the knee [39]. But in addition, increased levels of inflammatory cytokines have been detected in the synovial fluid of patients with acute ACL deficiency, indicating the potential role of biological factors in joint deterioration [29-32]. Of particular note is the observation of a persistent and evolving disturbance in the cytokine profile consisting of an imbalance between pro-inflammatory cytokines and cytokine antagonists in patients with chronic ACL deficiency [29, 30]. This suggests that in addition to the effects of the adverse biomechanical environment biological processes also contribute to the development of osteoarthritic changes after ACL rupture and indicate a potential role of synovitis in the pathogenesis of this form of post-traumatic OA.

The characteristics of synovitis associated with ACL rupture have not been extensively investigated. Lindblad et al studied synovitis in 4 patients undergoing arthroscopy for acute joint trauma (2 ACL ruptures and 2 meniscal tears). Biopsies were obtained from sites demonstrating the greatest inflammatory intensity and analyzed by immunohistopathology. Extensive inflammation was not observed and immunohistophatological signs of synovitis were only moderate [11].

Our study was undertaken to more definitively determine the prevalence and molecular features of synovial inflammation in patients undergoing ACL reconstruction for clinically documented traumatic knee injury associated with MRI evidence of ACL rupture. In our study, we observed a high percentage of low-grade synovitis both evaluated by macroscopic visual (97.5%) and histological scoring (85%). Of note, the

synovial pathology was most commonly observed in the suprapatellar region remote from associated meniscal, cartilage or ACL injury sites suggesting that the synovial changes could represent a more generalized response to the injury or that certain sites in the joint may be uniquely sensitive to the effects of pro-inflammatory factors produced in response to the injury and associated alterations in the meniscal and cartilage tissues.

Our findings are consistent with the results of other studies showing that inflammation is localized in the suprapatellar location [1, 62]. However, in our population, synovitis macroscopic scoring did not correlate with the microscopic scoring. In part, this may be related to the limited extent of the macroscopic synovial inflammation and the fact that the microscopic score was obtained only from selected sites. Lindbland et al [63] found a correlation between visual and histological scoring of synovitis, but the study was conducted in patients with inflammatory arthritis who exhibited more extensive and a higher grade of synovial inflammation compared to our patients with traumatic joint injuries. Although, experienced arthroscopists were involved in our study, we highlight the difficulty in distinguishing more from less inflamed areas in the setting of a low-grade synovitis. In contrast to our findings, Loeuille et all found a slight but significant correlation between the results of synovial macroscopic evaluation and the mean total composite microscopy score in patients who fulfilled the American College of Rheumatololgy criteria for knee OA [64].

In our study macroscopic hyperemia correlated with microscopic synovial hyperplasia. In the Lindblad study hyperemia was the macroscopic finding that contributed most significantly to the correlation between macroscopic and microscopic scoring. They also noted that lymphocytic infiltration rather than lining cell hyperplasia was the optimal parameter for quantifying microscopic inflammatory activity.

Surprisingly, we observed an unexpectedly high percentage of the patients exhibiting evidence of meniscal (48%) and/or cartilage pathology (40%). 54% of cartilage lesions were located in the medial femoral condyle (MFC), suggesting that cartilage defects may predate the ACL ruptures or be the consequence of the chronic deficiency. These data are consistent with other studies in OA patients showing that cartilage defects are more commonly localized to the MFC [1], even if the distribution of the acute osteochondral injuries affected predominantly the lateral compartment [65]. Studies by Tandogan et al. also found a high prevalence of osteochondral injuries in the medial tibial femoral compartment [66]. In their study, the average time from acute injury to

surgery was 19 months, indicating that many of the patients had chronic rather than acute ACL deficiency.

The development of cartilage lesions in the medial compartment in patients with chronic ACL deficiency contrasts with the location of cartilage injuries in patients with acute ACL rupture. In patients with acute ACL ruptures bone bruise lesions detected by MRI are most commonly localized to the lateral compartment. Indeed, in our study bone marrow edema was most frequently located in the LTP and in the LFC. The development of acute lateral compartment injury is thought to be due to the lateral subluxation of the tibial articular surface hitting the LFC at the moment of the trauma. These bony lesions may heal, but the initial injury may produce irreversible changes in the knee with alteration in load-bearing properties. The medial compartment, including the cartilage and meniscus, absorbs the additional loads and therefore there is an increased risk of sustaining damage and developing a secondary lesion. Indeed, in our population medial meniscal tears were more commonly observed (58%), compared to lateral tears (28%).

In accordance with other investigations, our study showed that articular cartilage degeneration was most commonly associated with chronic ACL deficiency [67, 68]. Moreover, the severity of cartilage lesions measured by Outerbridge score correlated with age, BMI and time from injury to surgery. The correlation between the presence/grade of the cartilage defects and the time from injury and the high incidence of medial meniscal and cartilage lesions indicate the important role of altered loading in the setting of chronic ACL deficiency. Although we found no overall association between the presence of synovitis and cartilage or meniscal defects, in the cohort of patients > 30 years old, the presence of synovial inflammation correlated with evidence of a meniscal tear, suggesting a relationship between these two pathologic processes. Preliminary analysis of mRNA expression patterns in this group of patients confirmed the upregulation of *CCL5* and *CCL19*. This is in agreement with previously published data [1]. Moreover, *CCL5* levels were correlated with *CCL19* levels and with the grade of microscopic synovial inflammation.

Chemokines were initially identified as chemoattractants but are now implicated in many biological processes. They play a crucial role in tissue homeostasis, especially in the immune system. Inappropriate activation of the chemokine network is associated with a variety of pathological conditions, including inflammatory arthritis and other autoimmune and inflammatory conditions. CCL5 acts predominantly on mononuclear

cells, such as T cells, monocytes, and macrophages. Recent studies demonstrate that many types of cells produce CCL5, especially in the presence of inflammatory cytokines, such as IL-1β and TNF-α [69]. CCL19 is expressed in lymph nodes and fibroblastic reticular cells and is particularly highly expressed in the T cell–rich areas. More recently it has been shown to act as chemotactic for rheumatoid arthritic synovial tissue fibroblasts [70] and elevated levels of CCL5 and CCL19 have been detected in synovial fluids from patients with rheumatoid and osteoarthritis [71, 72].

The potential important contributory role of these two chemokines in the pathophysiology of OA, particularly in those patients having synovial inflammation, was confirmed by the finding that mRNA levels of their receptor, CCR7, correlated with the microscopic synovitis scoring.

Surprisingly, in our study *IL-1β* and *TNFα* expression was not unregulated in association with synovial inflammation and mRNA levels did not correlate with the time from injury to surgery. This is in agreement with the data reported by Scanzello et al [1], which also did not show a correlation between these two inflammatory cytokines and the grade of synovial inflammation in patients undergoing meniscectomy for meniscal tears.

Interestingly, *MMP13* mRNA levels were unregulated in synovial samples exhibiting inflammation and the levels correlated with *CCL5* expression. MMP-13 is the major type II collagen-degrading collagenase [73] and clinical studies have shown a correlation between expression levels of MMP-13 and articular cartilage destruction, suggesting that increased MMP-13 may play a dominant role in the progression of cartilage degradation in OA [74].

The pathogenic processes following ACL rupture can be separated temporally into the immediate events that are related to mechanical impact, an acute post-traumatic phase with prominent inflammation and a chronic phase [24]. Both the histological synovial inflammation and the MMP-13 mRNA levels were negatively correlated with the time from the injury to the surgery. These findings reflect the natural history of ACL deficiency with an initial acute inflammatory response followed by a chronic phase where metabolic changes in cartilage and other joint structures slowly progress through a long clinically asymptomatic latency period to a symptomatic phase with joint pain and dysfunction.

We investigated whether inflammation was associated with preoperative joint symptoms and dysfunction, but no difference in SF-36, Marx activity scale and

Lysholm scores were observed. The Marx activity score measures the peak of the activity levels in the past year to obtain a more accurate estimate of a patient's baseline activity when participating in a sport. A lower Marx activity score indicates lower patient activity levels. The highest level of activity required by the patient to participate in a sport is important for quantifying activity without confusing the frequency of activity with ability. Marx activity scale was associated with age as previously described [75].

Patients enrolled in this study are being followed up for 10 years, and their clinical course and joint pathology assessed to determine whether the synovial pathology and/or chemokine expression patterns that were detected in this study are associated with short- and long-term outcomes after arthroscopy. ACL rupture is a complex event in which different variables can play an important role in the natural history. Acute and chronic phases have been well recognized. In addition, meniscal tears and cartilage defects can predate the ACL injury, or be the consequence of the altered mechanical loading and disturbance in joint biology in the setting of chronic ACL deficiency.

Limitations of our present study include the small sample size and the heterogonous population of patients that required careful stratification into subgroups according to clinical and intra-operative features. In addition, attention was focused on only a limited number of inflammatory mediators and molecules involved in cartilage, ligament and bone remodelling. Further analyses employing, for example, transcriptional profiling of RNA from archived synovial samples will be needed to identify additional regulatory molecules and pathways. Additional information also is needed regarding the cellular source of the chemokines and the factors responsible for their induction. In part, this can be accomplished with the use of immunohistochemistry and in situ hybridization on our stored samples and the use of in vitro cell culture models to identify the mediators of the inflammatory events. Also, although physical exams were performed on patients, we do not have formal kinetic analyses to assess the role of biomechanical factors in the short- and long-term outcomes.

However, given the role of CCL5 and CCL19 in the recruitment of inflammatory cells, we speculate that these chemokines may contribute to the development of synovial inflammation in patients with ACL rupture. Moreover, MMP-13, the major type II collagen-degrading collagenase, may play an important role in cartilage degradation following ACL rupture. In addition to the complex interactions between biomechanics and altered cartilage biology that initiate OA, synovial inflammation may be an

additional mechanism contributing to disease progression in a subset of patients. Elucidation of the cellular mechanisms that account for CCL5, CCL19 and MMP13 production and their role in inflammation and progression of joint deterioration after ACL rupture have the potential to add to our understanding of pathogenesis of post-traumatic OA and allow the identification of patients who may benefit from more aggressive anti-inflammatory or related forms of pharmacologic therapy. Follow-up studies utilizing the ACL registry will permit establishment of the relationship of the intra-articular pathology to the long-term clinical outcomes and, more specifically, to define the role of synovial inflammation in these events.

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