

# Synovium scRNA-Seq Reveals Clinically Relevant Inflammatory Responses in Adolescent ACL Reconstruction

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**INTRODUCTION:** Anterior cruciate ligament (ACL) tears are common in the adolescent athletic population, and progression to post-traumatic osteoarthritis (PTOA) occurs in 50-90% of patients. The exact pathogenesis of PTOA after ACL reconstruction remains unknown. Evidence from animal models suggests a critical role of synovial inflammatory infiltration. Single cell RNA sequencing (scRNA-seq) of mouse synovium in ACL rupture models quantified immune cell types and synovial fibroblast/macrophage subtypes present during injury healing (1, 2). Recent transcriptomic exploration of the synovium in OA patients provided valuable information on the immune and resident cells present in a disease state (3-5). The current gap in knowledge is the human synovial immune response in between ACL injury and development of OA. We hypothesized that higher synovium immune cell infiltration at the time of ACL reconstruction would correlate with poorer clinical outcomes. To investigate this, flow cytometry provided a snapshot of the cellular composition of the synovium, scRNA-seq comprehensively examined a subset of those samples, and correlations with clinical outcomes were analyzed.

**METHODS:** Patients aged 12-18 years undergoing primary ACL reconstruction were enrolled in an IRB-approved prospective study and demographic/injury information collected (e.g. concomitant meniscus injury). At the time of surgery, an arthroscopic synovial biopsy from the prefemoral synovium was digested into a single cell suspension for immune profiling by multicolor flow cytometry and scRNA-seq. Flow cytometry data (n = 17) was acquired on a BD IsrFortessa flow cytometer and analyzed in FlowJo v10. Principle Component Analysis (PCA) of flow cytometry immune cell frequencies and hierarchical clustering in R grouped the synovial samples by their immune cell profile. Some cells from 6 of the 17 samples were also used for scRNA-seq. Chromium (10X Genomics) droplet barcoding of cells, RNA library construction, and sequencing of 41,842 cells total was followed by analysis with Cell Ranger and Loupe to generate t-SNE and UMAP visualizations. Cells were identified by known cell type markers and prevalence analyzed via two-way ANOVA. Clinical outcomes were collected from 2-week, 6-week, 3-month, 6-month, and final postoperative clinical visits (range of motion, complications) and from patient surveys (PROMIS, ACL-RSI, and IKDC). Statistical analysis of demographic/injury variables and clinical outcomes included mixed-effects longitudinal analysis, ANOVA, linear regression, and Pearson/Spearman correlations performed in R and GraphPad Prism 10.

**RESULTS:** PCA and hierarchical clustering of flow cytometry immune profiling (n = 17; 9 female/8 male) revealed three clusters/immunotypes explaining 92.62% of the variation (Fig. 1A). The immune cell profile of Type 3 indicated significantly higher immune cell infiltration into the synovium (45% ± 11%, p < 0.005), particularly of adaptive immune cells. Type 1 had lower immune cell infiltration (31.6% ± 3.8%) and adaptive immune contribution while Type 2 was intermediate. Type 1 also had a greater time between injury and surgery (median 99 days, 51-989; p < 0.05) than Type 2 (median 32 days, 21-38) and Type 3 (median 32 days, 13-42), and Type 1 synovium contained more mast cells (2.7 ± 0.78% of total cells, p < 0.05) than Type 2 (1.0 ± 0.39 %) and Type 3 (1.3 ± 0.82%). When a subset of these synovium tissues (n = 6) were analyzed via scRNA-seq, 25 cell types were identified overall (Fig. 1B) with immunotype 3 trending towards more T cells and B cells, plus a higher CD8+ to CD4+ T cell ratio. The overall immune profile pattern shown as a heatmap (Fig. 1C) suggests that Type 3 synovium may experience higher adaptive inflammation compared to Type 1. The clinical outcomes (n = 17; mean follow-up time 8.9 ± 1.4 months) exhibited no differences in any of the PROMs, but knee range of motion was significantly worse for Type 3 in both extension and flexion (Fig. 1D, mixed effects p < 0.05), particularly in the 2-week to 3-month post-op range. Additionally, no complications occurred for patients in Type 1, one case of arthrofibrosis resulting in very mild loss of extension (< 3 degrees) was observed in Type 2, and significant complications occurred in Type 3 including one ACL re-rupture requiring revision and a separate case of arthrofibrosis with a 30 degree flexion contracture requiring lysis of adhesions.

**DISCUSSION:** Our study provides the first analysis of immune cell behavior in the synovium of ACL injured knees at the time of ACL reconstruction. Three immunotypes that were not correlated with patient demographic variables were discovered, with Type 3 presenting higher immune cell infiltration and higher adaptive immune cell contribution. As hypothesized, Type 3 was associated with significantly worse range of motion during the first 3 months post-op as well as more severe complications than either Type 1 or Type 2. These immunotypes could represent intrinsic differences in the response to injury.

Further research with larger cohorts and investigation of long-term outcomes may provide insight into the role of synovial inflammation in post-ACL joint health and degeneration as well as provide strategies for personalized treatments in the prevention of PTOA.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Pediatric and adolescent ACL injuries are common, and the incidence of PTOA is 50-90% after ACL reconstruction. Given the limited PTOA treatment options in the young adult, identifying early indicators and advancing prevention is crucial.

**REFERENCES:** 1. Sebastian (2022). *Front. Immunol.*, 13, 938075. 2. Knights (2023). *Ann Rheum Dis*, 82(2), 272-282. 3. Wood (2019). *JCI insight*, 4(2). 4. Nanus (2021). *EBioMedicine*, 72. 5. Chou (2020). *Sci. Rep.*, 10(1), 10868.

**IMAGES/TABLES:**

Figure 1. (A) 3D graph of the PCA and clustering results from the flow cytometry-based immune profiling shows the three immunotypes grouping the patient samples (n = 17). (B) scRNA-seq t-SNE visualization of cell type clusters for all cells within the synovium at the time of ACL reconstruction (n = 6). (C) Heatmap of cell type distribution based on scRNA-seq (n = 6). (D) Range of motion comparison between immunotypes during healing (n = 17, p < 0.05).

