Single-Cell RNA Sequencing of Corticosteroid-Induced Osteonecrosis of the Femoral Head

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INTRODUCTION: Osteonecrosis of the femoral head (ONFH) is a prevalent condition with poorly characterized pathophysiology. It affects an estimated 20,000 to 30,000 individuals annually in the United States and accounts for approximately 10% of the 250,000 total hip arthroplasties performed each year. Steroid- and alcohol-induced ONFH represent the two most common nontraumatic causes, accounting for up to 80% of cases. The underlying pathogenesis of ONFH requires further investigation given emerging evidence suggesting distinct pathogenic mechanisms. The purpose of this study is to elucidate the transcriptomic landscape and compare the cellular compositions of femoral head specimens from patients with steroid-induced ONFH, alcohol-induced ONFH, and OA.

METHODS: Femoral heads were collected from patients undergoing total hip arthroplasty for the treatment of collapsed corticosteroid-induced ONFH (n=2, male) and OA (n=3, 1 male 2 female). Alcohol-induced ONFH femoral head sample data were obtained from publicly available data (SRA database -SRP361778) (n=6, unknown sex). Samples were mechanically and enzymatically digested to isolate cells according to previously described protocols. scRNA-seq was performed using 10X genomics 3'UTR gene expression protocol. scRNA-seq data was analyzed using Cell Ranger (v7.0.1), filtered using Seurat (v4.3.0), normalized with SCTransform, and dimensionality reduction was performed using the first 30 principal components (PCs) with UMAP (min_dist = 0.5, n_neighbors = 30). This study is classified as Institutional Review Board exempt as femoral head specimens were de-identified and would otherwise be discarded.

RESULTS SECTION: Comprehensive single-cell transcriptomic analysis revealed several distinctive cellular features that differentiate steroid-induced ONFH from both alcohol-induced ONFH and OA. Notable divergence in metallothionein (MT) was observed between steroid and alcohol-induced ONFH, with alcohol-induced ONFH uniquely demonstrating marked enrichment in MT-expressing CD8 + T cells. Steroid-induced ONFH exhibited significantly fewer mature CD4 + T cells (cluster 0) and CD4 + memory T cells (cluster 10) but contained higher proportions of naïve CD4 + T (cluster 3) cells compared to alcohol-induced ONFH (Figure 1). OA samples contained increased macrophages (cluster 3) compared to steroid-induced ONFH (Figure 2). In contrast, steroid-induced ONFH demonstrated significantly higher neutrophil (cluster 1) numbers compared to both OA and alcohol-induced ONFH (Figure 1). Alcohol-induced ONFH samples contained greater proportions of natural killer (NK) cells (cluster 4) and macrophages (cluster 6) than steroid-induced ONFH. Finally, the vascular compartment displayed increased numbers of endothelial cells (cluster 13) and pericytes (cluster 4) in steroid induced ONFH compared to alcohol-induced (Figure 2). In steroid-induced ONFH, the study specifically detected the expression of inflammatory markers including IL7R, SEMA4D, TNFAIP3, CCR7, and FOXP1, as well as the anti-apoptotic factor BCL2.

DISCUSSION: The notable differences in immune, vascular, and oxidative stress responses between steroid- and alcohol-induced ONFH highlight that these clinically similar conditions arise from distinct pathogenic processes. With MTs serving as important antioxidant and anti-inflammatory mediators protecting against oxidative stress, the finding of reduced expression in steroid-induced ONFH compared to alcohol-induced suggests an increased vulnerability to oxidative damage underlying the pathology. This aligns with prior studies demonstrating that glucocorticoid-induced oxidative stress contributes to osteoblast dysfunction and apoptosis. Alcohol-induced ONFH's robust MT expression suggests a compensatory protective mechanism against alcohol-mediated oxidative injury. Additionally, the difference in T cell maturation between steroid-induced and alcohol-induced ONFH is a previously uncharacterized immune dysregulation. While the difference is likely a product of the established effect of glucocorticoids on thymic function and T cell maturation, reclustering highlighted a reduction in Treg cells and imbalance of Treg/Th17. This dysregulation has been shown to be associated with reduced angiogenesis and increased osteclastogenesis, indicating that this imbalance may play a role in the disease pathogenesis. Furthermore, the neutrophilia found in steroid-induced ONFH supports the theory that neutrophil extracellular traps (NETs), implicated in vascular occlusion and thrombosis, may directly contribute to the ischemic injury in the femoral head. Finally, the expansion of endothelial cells identified in steroid induced compared to alcohol induced ONFH suggest a specific vascular mechanism. This finding challenges the traditional concept that reduced vascularity underlies the glucocorticoid-induced pathology. Instead, it supports recent studies suggesting that endothelial dysfunction and activation are implicated rather than reduced vessel density. The distinct cellular profiles point to fundamentally different disease mechanisms, despite similar clinical features, highlighting the importance of tailoring treatments to the underlying defense mechanism.

SIGNIFICANCE/CLINICAL RELEVANCE: This study reveals distinct cellular and molecular landscapes of steroid-induced osteonecrosis of the femoral head and highlight the mechanisms that differentiate it from alcohol-induced ONFH and osteoarthritis. The findings illuminate specific pathways and potential therapeutic targets for personalized care and treatment of ONFH.

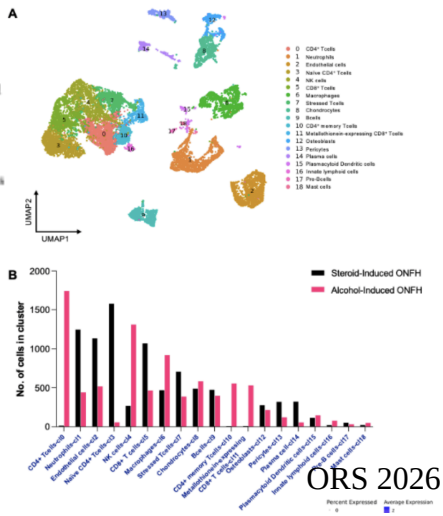

Figure 1: Comparative analysis comparing microenvironment of steroid-induced ONFH to alcohol-induced ONFH
(A) UMAP showing the cell populations identified from the combinatorial analysis of 17,166 cells (8583 cells from each sample cells.
(B) Bar plot demonstrating the distribution of the population.

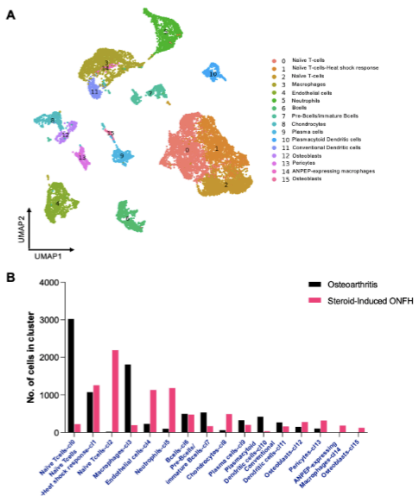

Figure 2: Comparative analysis comparing microenvironment of steroid-induced ONFH to OA
(A) UMAP showing the cell populations identified from the combinatorial analysis of 17166 cells (8583 cells from each sample).
(B) Bar plot demonstrating the distribution of the population.