Tenosynovial Histopathology Mirrors Molecular Profiles and Clinical Outcomes in Peroneal Tendon Disease

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INTRODUCTION: Peroneal tendon disease (PerTD) is a major source of lateral ankle pain, encompassing tendinosis, tenosynovitis, tears, and instability, all involving inflammation and/or degeneration of the peroneal tendon and its surrounding tenosynovium (TS). While its role in disorders such as trigger finger and rheumatoid arthritis is well established, its contribution to PerTD remains largely unknown. Currently, no clinical/preclinical PerTD classification system incorporates TS tissue features. Classification systems are important not only for predicting outcomes and guiding treatment options but also as frameworks to study underlying pathology. We therefore aimed to develop a clinically meaningful histopathological classification of the peroneal TS and relate TS phenotypes to molecular signatures and patient-reported outcomes (PROs), providing a framework for biomarker discovery and targeted therapy development.

METHODS: TS specimens were collected intraoperatively (IRB approved, upon informed consent) from patients with PerTD (non-inflamed TS, n=3; inflamed TS with healthy tendon, n=12; inflamed TS with tendinopathy or tear, n=8; 60% female). All following assays used matched samples. Formalin-fixed, paraffin-embedded (FFPE) TS was stained with Hematoxylin & Eosin (H&E) for semiquantitative assessment of lining thickness (uni-/multicellular), cellular infiltration (none/diffuse/nodular), and deep connective tissue reaction (loose/dense; **Fig1A, B**). The classification was developed on a pilot TS data set and then applied, without modification, to the 23 study patients. FFPE Picrosirius Red (PSR)–stained sections were imaged under fluorescence microscopy and tiled into 10,000 µm² sections; per-tile collagen-positive fraction was quantified, with values normalized within each specimen to mitigate overrepresentation from larger tissue sections. Multiplex FFPE immunohistochemistry (IHC) staining (CLIC5, CD68, CD31, DAPI) was acquired on the Akoya PhenoImager. Bulk TS RNA sequencing was performed, and principal component analysis (PCA) on all differentially expressed genes assessed separation among PerTD histological categories. PROMIS Pain Interference and PROMIS Physical Function Scores were obtained preop and at 3, 6, 12, and 24 months postop. *Statistics:* collagen fraction was modeled with mixed-effects beta regression. Estimated marginal means were reported with Tukey adjustment. PROMIS trajectories were compared using Kruskal–Wallis tests with Dunn's correction. All tests were two-sided with α=0.05, performed in R Studio.

RESULTS: Principal Component 2 scores differed significantly by TS histological features: lining thickness (0 vs 1: p=0.008; 0 vs 2: p=0.007), connective tissue density (0 vs 1: p=0.016; **Fig1C**), and nodular cellular infiltration (0vs2: p=0.017; *data not shown*). PSR quantification demonstrated a significant increase in collagen-positive area (p=0.03; **Fig1D**). IHC for CLIC5 (TS lining fibroblasts) and CD68 (TS macrophages) aligned with qualitative findings in H&E imaging, showing a thicker CLIC5-positive lining and a marked increase in CD68 signal within the TS lining (**Fig1E**). Patients with a dense connective-tissue reaction (**Fig1A**) tended to show greater postoperative improvement in PROMIS Physical Function and larger reductions in Pain Interference; trends not observed in those without (**Fig1F**).

DISCUSSION: Our novel histopathological classification differentiates PerTD categories that correspond to distinct molecular profiles, supporting its potential as a meaningful framework for studying TS disease progression. The three histologic categories separated samples along the same principal component, suggesting a shared TS-centric PerTD axis. The strong increase in CD68 signal within the TS lining is consistent with macrophage enrichment. Consistent with observations in articular joint synovium, these patterns may imply a macrophage–fibroblast activation axis linking macrophage enrichment to fibroblast-driven fibrosis. Clinically, the trend toward greater postoperative functional improvement in patients with dense connective-tissue reaction may reflect a benefit from debridement of fibrotic TS tissue. However, this observation is exploratory and requires prospective validation. Future work will incorporate quantitative image analysis to standardize the classification and support preoperative decision-making.

SIGNIFICANCE/CLINICAL RELEVANCE: Our novel histopathological classification captures distinct TS phenotypes with corresponding molecular signatures, supporting biomarker discovery and hypothesis generation for targeted therapy development. By correlating with postoperative outcomes, the classification could, in the future, serve as a clinical tool to guide therapeutic decision-making.

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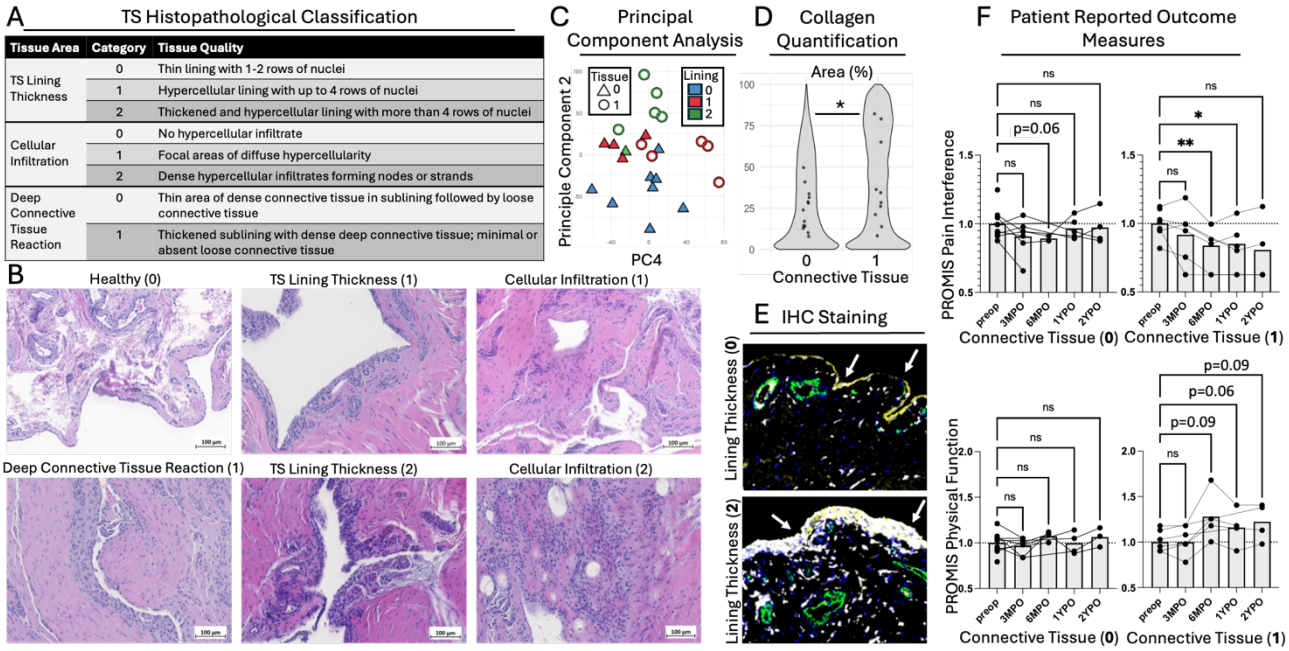

Figure 1: (**A**) TS histopathological classification derived from H&E-stained sections, (**B**) with representative images. (**C**) PCA of bulk TS RNA-seq; Principal Component 2 separates specimens by histopathologic category. (**D**) FFPE PSR-stained TS stratified by deep connective-tissue reaction (0 vs 1); collagen-positive area quantified per 100 µm × 100 µm tile. (**E**) FFPE multiplex IHC of TS stratified by lining thickness (0 vs 2). Channels: DAPI (blue, nuclei), CD31 (green, vessel endothelium), CLIC5 (yellow, TS lining fibroblast), CD68 (white, TS macrophages); white arrows indicate the TS lining. (**F**) PROMIS Pain Interference and Physical Function measured preop and at 3, 6, 12, and 24 months postop, stratified by deep connective-tissue reaction. * p<0.05; ** p<0.01