Histologic Characterization of Synovium in Charcot Neuroarthropathy

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Summary:
The pathology of Charcot neuroarthropathy (CNA) has not been fully understood. This study examined the synovium from Charcot joints, in comparison with synovium from osteoarthritic and normal joints, with histology and immunohistochemistry. The results showed that synovium in CNA had severe synovitis and expanded intimal layer but significantly reduced innervation.

Introduction:
Charcot neuroarthropathy (CNA) is a rapid onset, destructive arthropathy that occurs in patients with underlying neuropathy. It is clinically characterized by an acute inflammatory reaction of the joint that can progress to chronic osteolysis, fracture, dislocation and ultimately joint collapse. Our previous work suggests the synovium is an important mediator of joint damage in CNA. This study aimed to characterize Charcot synovium using histology and immunohistochemistry.

Methods:
Charcot (n=7), osteoarthritis (n=4) and normal synovial tissues (n=3) were collected during foot and ankle surgery and processed for both histologic and immunohistologic staining. Hematoxylin and eosin staining was conducted according to standard procedures. All specimens were scored using the Krenn Synovitis Score, according to intimal layer expansion, resident cell density, and inflammatory infiltrate. PGP9.5 and Cadherin-11 staining were conducted by immunohistochemistry. Rabbit anti-human PGP 9.5 antibody (Abcam) was used at 1:200 dilution in 100 mM TBS with 0.025% Triton-X and rabbit anti-human Cadherin-11 antibody (Invitrogen) was used at 1:100 dilution. Anti-rabbit antibody and ABC reagents were used according to manufacturer’s instructions (Vector Laboratories). Stained nerve fibers were quantified by microscopic observation under random high powered fields.

Results:
Krenn Synovitis Score demonstrated marked synovitis in Charcot synovium compared with both OA and normal specimens. The average Krenn synovitis score was 0.78 ± 0.39, 2.67 ± 0.0, 5.33 ± 0.34 for normal, OA, and Charcot specimens, respectively. Charcot synovium demonstrated 2.37-fold increase of synovitis score compared to OA and a 6.83 fold-increase compared to normal synovium. Synovial innervation was significantly decreased in Charcot synovium compared to both normal and OA synovium. Normal synovium demonstrated 6.57 ± 1.8 PGP9.5-positive structures per HPF, compared to 3.22 ± 0.5 PGP 9.5-positive structures per HPF in OA synovium and 0.44 ± 0.11 PGP 9.5-positive structures in Charcot synovium. Cadherin-11 expression was found almost exclusively in the intimal layer of Charcot synovium where
significant intimal layer proliferation had taken place. Cadherin-11 expression was notably absent from both normal and OA synovium (Figure 1).

**Conclusion:**
The pathogenesis of CNA has not been firmly established since its original description in 1868. This study described both qualitatively and quantitatively the histologic characteristics of the synovium in CAN, in comparison with OA and normal synovium. It was found that there was more severe synovitis in Charcot synovium than in OA synovium. Specifically, there was marked intimal layer expansion with non-significant changes in cellularity and inflammatory infiltration. Cadherin-11, an integrin responsible for synoviocyte invasion, was found almost exclusively in Charcot synovium at areas of high intimal layer proliferation. These findings suggest that Cadherin-11 could be responsible for aggressive changes in Charcot synovium. Furthermore, Charcot synovium had reduced innervation when compared to OA and normal synovium by PGP-9.5 staining. The findings of this study warrant further investigation into synovium for its role in CAN pathology.

![Figure 1. Cadherin-11 expression was demonstrated in the intimal lining of Charcot synovium, but not found in normal or OA synovium. IL, intimal lining; SL, sub-intimal layer](image-url)