# Comprehensive Study of Synovial Fluid and Membrane in Horses Affected by Inflammatory and Septic Synovitis


## ABSTRACT

**Background:** Septic synovitis in horses is a frequent emergency with major prognostic consequences. Rapid distinction from non-septic synovitis remains challenging. Arthroscopy provides a descriptive evaluation, while histology offers complementary information on inflammation. The role of cytokines in non-septic arthritis is documented, but few studies have assessed their involvement in septic synovitis.

**Objectives:** To identify inflammatory factors associated with septic and non-septic synovitis, evaluate correlations between arthroscopic and histological scoring, and determine relevant synovial fluid biomarkers.

**Methods:** Seventy horses were divided into three groups: Group 1, elective or therapeutic arthroscopy (n=26); Group 2, septic synovitis requiring arthroscopic lavage (n=25); and Group 3, periarticular injuries without synovial involvement (n=19). Synovial fluid was analyzed for cytology and 23 cytokines using a MILLIPLEX® panel. Groups 1 and 2 also underwent synovial biopsies. Arthroscopic scores (0–12) were designed for this study; histological scores (0–15) followed a standardized system. Data were analyzed with parametric and non-parametric tests, Spearman correlation, and principal component analysis.

**Results:** Septic synovitis showed higher synovial cellularity, protein, and neutrophils than other groups ($p<0.01$). Arthroscopic (8.0 vs 6.0; $p=0.004$) and histological scores (9.0 vs 5.6; $p=0.006$) were higher in septic cases, with moderate correlation ($r=0.39$; $p=0.02$). IL-1β, IL-6, TNF-α, and IL-10 were consistently elevated in septic synovitis, correlated with cytology and scoring, and were the main discriminators in PCA.

**Limitations:** Unequal group sizes, lack of a healthy control group, and heterogeneity in horses.

**Conclusions:** Cytology provides rapid diagnostic insight, but histology adds accuracy. The cytokine quartet IL-1β, IL-6, TNF-α, and IL-10 represents a promising biomarker panel for septic synovitis and supports development of rapid diagnostic assays for clinical use.



**Keywords:** Horse, Cytokine, Biomarkers, Synovial fluid, Synovitis, Joint inflammation


## INTRODUCTION

Synovial disorders are a major cause of lameness in horses, limiting performance and career longevity (Chevalier & Richette, 2005). Loss of synovial homeostasis, whether traumatic, degenerative, or septic, leads to cartilage degradation, chronic inflammation, and joint dysfunction (Rahmati et al., 2016). Among these, septic synovitis is the most severe, as it can rapidly become life-threatening if not diagnosed and treated early (Morton, 2005).

Current diagnostic tools include synovial fluid cytology and bacteriology. Cytology provides rapid information but shows overlap between septic and non-septic cases, while bacterial culture remains the gold standard but requires several days. Arthroscopy and histopathology allow direct grading of synovial inflammation (Agreste et al., 2021; McIlwraith et al., 2010), but their invasiveness limits routine use.

Cytokines are central mediators of joint inflammation. Their involvement has been well described in non-septic conditions such as osteoarthritis (Bertone et al., 2001), but their presence and profile in equine septic synovitis

have never been characterized in vivo. Identifying specific cytokine signatures in this context could provide new biomarkers for early differentiation between septic and non-septic synovitis, and ultimately guide faster therapeutic decisions through point-of-care diagnostic tools.

The aim of this exploratory study was to evaluate a multimodal diagnostic approach combining cytology, arthroscopy, histology, and cytokine profiling in horses with synovitis. Specifically, we sought to (1) describe correlations between the novel arthroscopic score developed for this study and established histological grading, and (2) identify cytokines associated with septic synovitis that could serve as future bedside diagnostic tools.

## MATERIALS AND METHODS

This prospective clinical study included 70 horses. Horses were divided into three groups: Group 1, non-septic synovial affections undergoing elective or therapeutic arthroscopy (n=26); Group 2, confirmed septic synovitis requiring arthroscopic lavage (n=25); and Group 3, periarticular injuries without synovial involvement (n=19). No exclusion criteria were applied for age or breed. Septic synovitis was diagnosed based on synovial cytology (white blood cell count >30,000/µL, protein >40 g/L, neutrophils >80%), and/or clinical signs such as synovial leakage and positive distension test. When sufficient synovial fluid was available, a bacteriological analysis was sent to confirm the diagnosis of sepsis

**Sample collection, arthroscopy and histology:** Synovial fluid was collected aseptically before arthroscopy or on admission. Samples were analyzed for cytology (nucleated cell count, protein concentration, neutrophil percentage) and stored at −80 °C for cytokine quantification. Groups 1 and 2 underwent arthroscopy. Macroscopic assessment of synovitis was scored using a novel semi-quantitative system

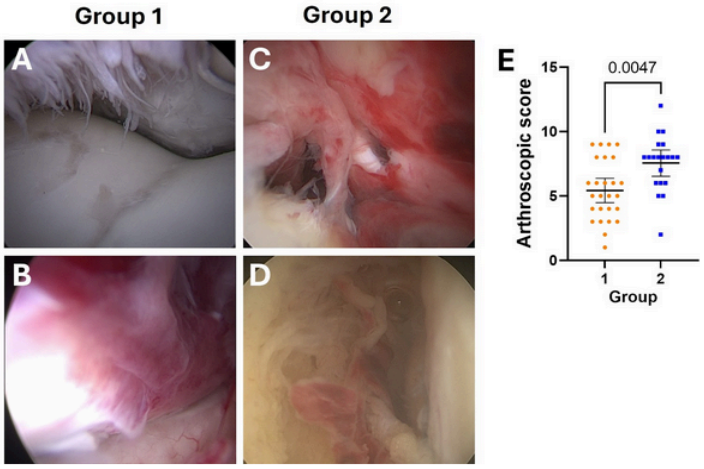


**Figure 1**: Arthroscopic images from Group 1 (**A,B**) and Group 2 (**C,D**), associated with different macroscopic score (MS). **A**. Moderate to severe lesion, MS = 8/12; **B**. moderate to severe inflammation, MS = 9/12; **C**. Severe inflammation, MS = 12/12; **D**. Moderate to severe inflammation, MS = 8/12. **E**: Arthroscopic scores from Group 1 and Group 2; p-values were calculated using the Mann Whitney test, mean difference with 95% confidence intervals are represented. (Arrowhead: synovial membrane thickening; *: cartilage erosion; black arrow: fibrin; star: haemorrhage).

developed for this study (range 0–12), based on synovial membrane villous hypertrophy, cartilage damage, synovial liquid appearance and quantity, articular capsule thickness and the presence of intra-synovial haemorrhage, fibrin and periarticular lesions. Synovial biopsies were collected during arthroscopy, fixed, and stained for histology. Samples were graded using a modified OARSI/Krenn system (range 0–15), evaluating intimal hyperplasia, inflammatory infiltration, stromal density, vascularity, and neutrophil presence. Scores were assigned through double or triple-blind evaluation.

**Cytokine quantification:** Expression of 23 cytokines and chemokines was measured with a MILLIPLEX® equine panel (Luminex® technology) on synovial fluid sample.

**Statistics:** Data were analyzed using Student, Mann-Whitney, and Kruskal-Wallis tests as appropriate. Correlations were assessed by Spearman analysis. Principal component analysis

was used to assess clustering of groups according to cytokine profiles. Statistical significance was set at $p<0.05$.

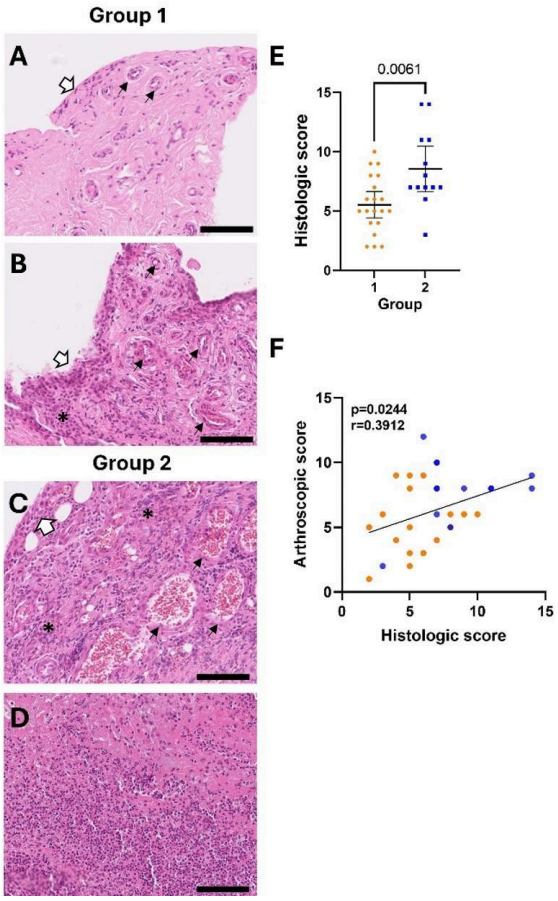


**Figure 2**: Histological section of Group 1 (**A,B**) and Group 2 (**C,D**) associated with different histological score (HS), **A**. Absence of signs of inflammation, HS = 0/15; **B**. Moderate to severe inflammation, HS = 10/15, **C,D**. Severe inflammation, HS = 14/15. (Scale bar : 100µm). **E**. Histological score from Group 1 and 2, p-value was calculated using the Mann Whitney test, mean difference with 95% confidence intervals is represented. **F**. Correlation between arthroscopic and histological score, p-value was calculated using the Spearman test. Orange dots: Group 1; blue dots: Group 2**.** (Hollow arrow: lining; *: inflammatory aggregates; black arrow: blood vessels).

## RESULTS

Synovial fluid cytology showed significantly higher cell counts, protein concentrations, and neutrophil proportions in septic synovitis (Group 2) compared with non-septic groups ($p<0.01$).

Arthroscopic evaluation revealed more severe synovitis in septic cases (mean score 8.0 vs 6.0; p=0.004) (Fig. 1). Histological grading confirmed this difference (mean 9.0 vs 5.6; p=0.006), with marked neutrophil infiltration in septic samples (Fig. 2). Arthroscopic and histological scores were moderately correlated (r=0.39; p=0.024).

Cytokine analysis identified IL-1β, IL-6, TNF-α, and IL-10 as consistently elevated in septic synovitis ($p<0.01$). Principal component analysis demonstrated clear separation between septic and non-septic cases, with the four cytokines contributing most to group discrimination (Fig. 3). Significant positive correlations were observed between cytological parameters, arthroscopic scores, histological grading, and cytokine concentrations across all groups (Fig. 4).

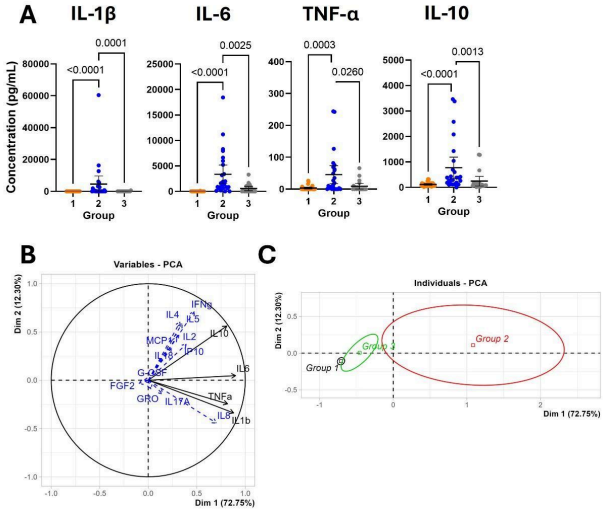


**Figure 3: A.** Concentration (pg/mL) of IL-1β, IL-6, TNF-α and IL-10. p-values were calculated using the Kruskall-Wallis test and Dunn's post-test, mean difference with 95% confidence intervals are represented. **B**. Graph of variable of the principal component analysis (PCA), loading plots with the contribution of each analyte analysed in the PC are represented. **C.** Graph of individuals of the PCA, confidence ellipses are represented for each group.

## DISCUSSION AND CONCLUSION

This study shows that combining cytology, arthroscopy, histology, and cytokine profiling improves discrimination between septic and non-septic synovitis in horses. Cytology remains a rapid first-line tool but lacks specificity (Morton et al., 2005), while arthroscopy and histology provide complementary insights though limited by their invasiveness (Agreste et al., 2021).

A concise cytokine panel, IL-1β, IL-6, TNF-α, and IL-10, was identified as strongly associated with septic synovitis. While cytokines have been studied in non-septic arthritis (Bertone et al., 2001; Rahmati et al., 2016), this is the first in vivo characterization of their presence in equine septic synovitis. Multivariate analysis confirmed their discriminative value, highlighting their potential as practical biomarkers.

Clinically, these results support the development of rapid immunoassays targeting this cytokine quartet, enabling earlier diagnosis, guiding treatment choices, and contributing to antimicrobial stewardship.

Limitations include moderate sample size and reliance on laboratory-based assays. Further studies should establish diagnostic thresholds and assess the feasibility of point-of-care tests in practice.

In conclusion, this exploratory study demonstrates that a multimodal diagnostic workflow strengthens diagnostic confidence. The cytokine quartet level of IL-1β, IL-6, TNF-α, and IL-10 emerges as a promising biomarker panel for early recognition of septic synovitis and improved clinical management.

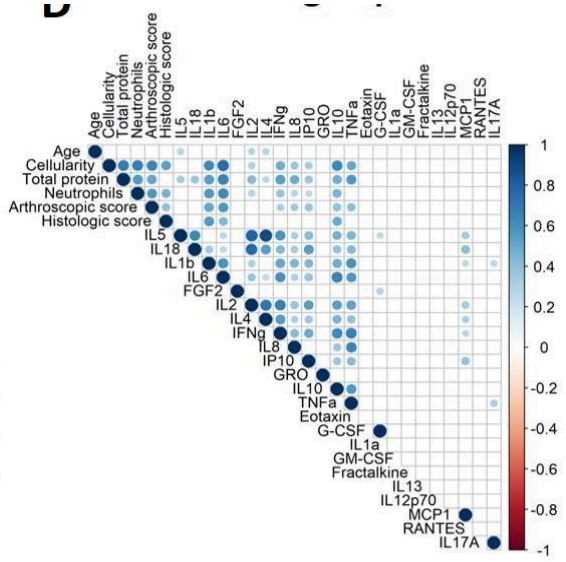


**Figure 4 :** Correlation matrix of all individuals, Scale bar represents the correlation coefficient and p-value > 0.05 are not shown.

## REFERENCES


Agreste, F. R., et al. (2021). Arthroscopic evaluation of the synovial membrane and its relationship with histological changes and biomarkers in equine joint disease. Research in Veterinary Science, 140, 212–220. https://doi.org/10.1016/j.rvsc.2021.09.003

Bertone, A. L., et al. (2001). Synovial fluid cytokines and eicosanoids as markers of joint disease in horses. Veterinary Surgery, 30(6), 528–538. https://doi.org/10.1053/jvet.2001.28430McIlwraith,

C. W., Frisbie, D. D., Kawcak, C. E., Fuller, C. J., Hurtig, M., & Cruz, A. (2010). The OARSI histopathology initiative—Recommendations for histological assessments of osteoarthritis in the horse. Osteoarthritis and Cartilage, 18(S1), S93–S105. https://doi.org/10.1016/j.joca.2010.05.031

Morton, A. J. (2005). Diagnosis and treatment of septic arthritis. Veterinary Clinics of North America: Equine Practice, 21(4), 627–649. https://doi.org/10.1016/j.cveq.2005.08.001

Chevalier, X., & Richette, P. (2005). Normal joint cartilage: Anatomy, physiology, metabolism, ageing. EMC-Rhumatologie Orthopédie, 2(1), 41–58. https://doi.org/10.1016/j.emcrho.2004.10.005

Rahmati, M., et al. (2016). Inflammatory mediators in osteoarthritis: A critical review of the state-of-the-art, current prospects, and future challenges. Bone, 85, 81–90. https://doi.org/10.1016/j.bone.2016.01.019