Equine synovial fluid analysis

Sandra Forsyth, of SVS Laboratories, discusses the ins and outs of testing for and diagnosing joint disease.

INTRODUCTION

Synovial fluid (SF) is a lubricant and conduit for nutrition to chondrocytes of the articular cartilage. Alterations in fluid composition secondary to infection, trauma (acute and chronic) and inflammation interfere with synovial fluid dynamics and may permanently alter joint structure and function. Consequently, it is important that disease is recognised as early as possible, something easier said than done at times.

Diagnosis of joint disease combines findings from physical exam, radiography and fluid analysis. This discussion concentrates on fluid analysis, but results need to be interpreted in conjunction with other findings to gain a clear picture of the disease that is present.

ANALYSIS OF SYNOVIAL FLUID

Gross examination of synovial fluid

SF is clear, pale-yellow and viscous and doesn't clot, but may exhibit thixotropism so that on standing it becomes gelatinous, returning to normal with agitation. Blood contamination during sample collection is common and can alter protein content, nucleated cell count (NCC) and cytology (discussed below). Iatrogenic haemorrhage typically produces a localised area of red in the sample, unlike haemarthrosis in which colour is homogenous and may be red, yellow or brown. In both cases, viscosity is reduced.

In sepsis, SF may be watery, turbid, floccular and variable in colour. The presence of nucleated cells increases turbidity, and once cell count is over 30 x 10^9/l it isn't possible to read printed material (point-size 12, Times New Roman) through the fluid (Fig 1).

Viscosity relates to the quantity of polymerised hyaluronic acid that decreases with inflammation due to dilution, decreased synthesis and degradation. A drop of normal SF stretches 2-5cm between the fingers before breaking; reduced viscosity causes it to break sooner.

Total protein

SF is a dialysate of plasma and is variably reported as having a total protein (TP) that is about 25% of peripheral blood

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concentration, or ≤15-≤25 g/l depending on the reference.

Blood contamination increases SF TP. However, blood must make up more than 50% of a normal SF sample before TP exceeds 25g/l (Roquet et al., 2012).

Nucleated cell count

Nucleated cell count can be determined by automated haematology analyser, but hyaluronidase is often added to prevent the sample from clogging the tubing. While NCC correlates well with a manual cell count, differential counts are inaccurate (Ekmann et al., 2010).

Typically, the upper limit of the NCC reference interval is 1.0 x 10^9/l, although counts from most healthy joints fall between 0.1 and 0.2 x 10^9/l. NCC may increase to 15.0 x 10^9/l following haemorrhage and >30 x 10^9/l in sepsis. However, during the first 24 hours of sepsis, NCC may be as low as 10 x 10^9/l, making it difficult to differentiate from sterile inflammation.

Cytology

Examination of synovial fluid by microscopy shows a magenta-stained background of dense proteoglycan containing occasional mononuclear cells (Fig 2). Typically, cellularity increases and background staining decreases in the presence of pathology.

Red blood cells

When present, RBCs often show a streaming pattern (windrowing) on cytology due to the background proteoglycan. Erythrophagocytosis typically indicates haemorrhage that is at least several hours old, but post-collection erythrophagocytosis may occur in samples with iatrogenic haemorrhage. Macrophages containing haemosiderin indicate haemorrhage that occurred at least 48 hours and up to one month before sampling.

Nucleated cells

Normal SF contains mononuclear cells with neutrophils making up less than 10% of NCC. Inflammation increases the neutrophil percentage, and sepsis often shows concentrations of ≥90%. Blood contamination increases cell count, but even so, NCC over 20 x 10^9/l with TP over 40g/l suggests a septic process (Roquet et al., 2012) (Fig 3).

In septic synovitis, neutrophils may be non-degenerate in morphology, and bacteria are often not seen.

Lavage and synovial fluid analysis

Lavage increases TP, NCC and neutrophil percentage to levels that mimic early infection. Twenty-four hours after through-and-through lavage of healthy joints, TP may rise to 39g/l, NCC to 20 x 10^9/l and neutrophil percentage to 64%. Parameters return towards baseline over the ensuing days but may still be mildly elevated at day five. In these horses, serum and SF serum amyloid A are not expected to increase above baseline (Sanchez et al., 2016).

Instillation of antibiotics, glucocorticoids and hyaluronic acid into a joint may temporarily increase NCC to levels consistent with infection (to 30 x 10^9/l), with the rise usually lasting less than three days. Pentosan and glucosamine increase NCC and TP further, with protein concentrations to 79g/l and cell counts to 50 x 10^9/l reported three days after administration. These parameters then decrease, but remain mildly elevated at seven days (Kwan et al., 2012).

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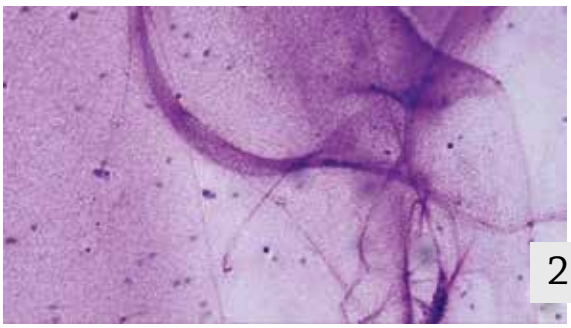

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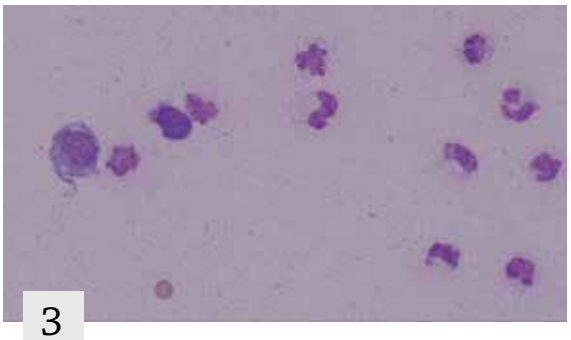

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FIGURE 1: Synovial fluid with an elevated NCC.

FIGURE 2: Normal synovial fluid with dense proteoglycan background.

FIGURE 3: Septic arthritis.

WHEN NCC AND PROTEIN ARE HIGH, SEPTIC ARTHRITIS IS RELATIVELY EASY TO DIAGNOSE, **WHEREAS EARLY INFECTION IS DIFFICULT.**

DIFFERENTIATING ACUTE NON-SEPTIC FROM SEPSIS SYNOVITIS BASED ON LABORATORY PARAMETERS CAN BE CHALLENGING, **AND RESEARCHERS ARE CONSTANTLY INVESTIGATING TESTS THAT IMPROVE DIAGNOSIS.**

Septic arthropathies

When NCC and protein are high, septic arthritis is relatively easy to diagnose, whereas early infection is difficult. Ludwig et al. (2016) determined that after introduction of *Staphylococcus aureus* into a joint, NCC is above the upper limit reference by six hours, but some animals may have relatively modest counts (~6.5 x 10^9/l) at 12 hours post-infection, rising to over 100 x 10^9/l by 24 hours.

Culture

Culture is considered the gold standard for detecting sepsis. However, it is far from perfect for SF. Culturing from a swab or fluid produces a positive result in 23.3% of infected synovitis cases, increasing to 78.9% when samples are placed in a paediatric blood culture bottle on collection (Dumoulin et al., 2010). It is likely that E-swabs® are less useful than blood culture bottles, but more effective than traditional swabs. However, there are no comparative studies in horses.

TESTS THAT ARE NEW AND ON THE HORIZON

Differentiating acute non-septic from septic synovitis based on laboratory parameters can be challenging, and researchers are constantly investigating tests that improve diagnosis.

Myeloperoxidase

Myeloperoxidase (MPO) is an enzyme involved in oxygen radical formation that is released by neutrophils during bacterial deactivation, and has been evaluated as a biomarker of synovial sepsis in horses. MPO is substantially higher in infected compared to healthy osteochondritis dissecans and traumatic joints (Wauters et al., 2013). To date, this test is not commercially available.

PCR for 16S rRNA gene

The 16s rRNA gene is found in all bacteria and is a useful marker for the presence of sepsis. According to Pille et al. (2007) the gene is found in 79-83% of SF with penetrating wounds and iatrogenic infections, and 100% of samples from foals with polyarthritis and adults with idiopathic septic arthritis. Specificity is not 100% because sample contamination producing false positive results occasionally occurs. It is possible to obtain 16S rRNA PCR in New Zealand diagnostic laboratories, but culture is concomitantly required to identify the organism and determine antibiotic sensitivities.

Synovial fluid serum amyloid A

A study investigating the use of synovial fluid serum amyloid A found that it is slower to rise than that in serum and cannot always differentiate sterile from septic synovitis (Ludwig et al., 2016).

D-dimers

In diseased joints there is extravasation of protein, including fibrinogen and fibrin into the synovial cavity through inflamed vasculature. Fibrinolysis is initiated by intra-articular macrophages causing breakdown of fibrin to degradation products, including D-dimers. A study in foals found that SF D-dimer concentration was significantly higher in septic synovitis compared to joints from healthy foals and ill foals without arthritis (Ribera et al., 2011). This test is not currently available in New Zealand diagnostic laboratories.

SUMMARY

Synovial fluid analysis remains an important component of joint disease evaluation, but standard parameters may be insufficient to provide a definitive diagnosis. PCR for 16s rRNA is a viable option for detecting sepsis, while other tests on the horizon may prove to be as useful in the future. VS

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