

Does equine osteoarthritis have an inflammatory component?

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Osteoarthritis (OA) is a common age-related degenerative joint disease in humans, dogs and horses defined by deterioration of articular cartilage, in addition to changes in bone and soft tissues. Synovitis is a key contributor to joint degradation and plays a fundamental role in the pathophysiology of OA. A novel macroscopic synovitis grossing scoring system was devised for assessment of synovitis severity in the equine metacarpophalangeal joint using parameters such as degree of inflammation and spread of synovitis in different regions of interest (ROI). This was then correlated with associated scores found using the Kawcak Gross Osteoarthritis Scoring System, which quantifies the severity of OA, to determine if there is a correlation between the visible inflammatory component of synovitis and OA severity. Scoring was performed by two independent observers and conducted on images collected from an abattoir as part of the University of Liverpool Equine Musculoskeletal Biobank.

Abstract

OA is one of the most common and disabling skeletal chronic joint disorders affecting humans, horses and dogs causing lameness. Synovitis is observed in osteoarthritic joints and is a key contributor to the pathogenesis of OA through the formation of various catabolic, pro-inflammatory and anti-inflammatory mediators, which alter the balance between extracellular matrix (ECM) degradation and repair. There is no exact answer to the pathophysiology of OA; however, many studies have hinted at the role of synovial inflammation and chronic synovitis as either a primary or secondary change in the disease process of OA. The purpose of this study was to establish if there is a relationship between synovitis and OA. A novel macroscopic synovitis gross scoring system was devised for the purpose of this study for assessment of synovitis severity in the equine metacarpophalangeal joint which was correlation tested with associated scores found using the Kawcak Gross Scoring System. It was hypothesised there was a correlation between synovitis and OA severity. Scoring was performed by two independent observers conducted on images collected from an abattoir as part of the University of Liverpool Equine Musculoskeletal Biobank. Inter-rater reliability was calculated using Cohen's Kappa Score. No significant correlation was found between synovitis score and Kawcak Gross Score from both observers, $r = 0.07$ and $r = 0.09$. Kappa Cohen scoring measured a fair and moderate agreement; Kawcak Gross Scores = 0.38 and Synovitis Scores = 0.68.

Introduction

OA is a chronic age-related degenerative joint disease found in humans, horses and dogs characterized by loss of articular cartilage components, particularly type II collagen, and morphological changes in bone and soft tissues with fibrillation, fissures, ulceration and full thickness loss of joint surface (1). Trauma, sepsis, immobilisation, shoeing, conformation, age-related degeneration and osteochondrosis are some of the numerous aetiologies that may contribute to the development of the disease (2). Often the end result is pain, deformity and decreased function that contribute to the wastage of horses bringing huge economic losses to the equine industry (2).

OA most commonly affects synovial joints and is a key contributor to equine lameness. Synovial joints are composed of articular cartilage-covered articulating bone surfaces, secured by ligaments and a joint capsule filled with synovial fluid. In a healthy animal, remodeling of articular cartilage is balanced by anabolism; however, over time and/or after joint injury, breakdown of extracellular matrix (ECM) components outweighs new matrix synthesis leading to ECM degeneration (2). The disease processes affect the entire joint; synovial membrane, subchondral bone, capsule, ligaments and peri-articular tissues. It is an 'organ' disease of the joint. Rose *et al.* (3) survey found that 33% of all the equine patients had intra-articular lesions related to OA. Tew and Hackett reported that 35% of the

72 equine joints evaluated had evidence of visual cartilage damage (4). This demonstrates the severe impact of OA on synovial joints.

The exact role of the inflammatory component of synovitis in OA is undetermined; however, it has been demonstrated that synovitis is a major contributor to joint degradation and plays a role in the pathophysiology of OA in both humans and horses (5). Inflammation of the synovium is characterised by an infiltration of neutrophils, T lymphocytes, monocytes, in addition to vascularization and hyperplasia of the synovium (6). Blom *et al.* (7) reported that macrophage aggregation in synovium is essential for cartilage damage potentially leading to early OA in mice. Synoviocytes, as well as chondrocytes are the main cell types involved in the pathogenesis of OA implicating the role of synovitis in the disease process (6). Increased levels of pro-inflammatory cytokines produced by synoviocytes, IL-1 β and TNF- α , have been found in synovial fluid of horses with OA when compared to unaffected joints supporting this theory (6).

The exact cause of OA is unknown; however, numerous studies have hinted at the possibility of synovitis being a primary or secondary process; linked to initiation and propagation of OA as it is induced by the innate immune system following the cartilage damage and may be essential for further OA development (6, 8). Sellam *et al.* (9)

	0 (healthy)	1 (mild)	2 (moderate)	3 (severe)
Degree of inflammation	Thin and transparent synovial membrane (SM), no inflammation seen	Light inflamed tissue with slight red hue	Darker inflamed tissue with a darker red hue	Bright red inflamed tissue
Degree of spread of synovitis	No synovitis seen in SM	Less than 50% seen with synovitis	Greater than 50% seen with synovitis	Synovial membrane completely covered and seen with synovitis

Table 1. Macroscopic Synovitis Scoring System. Scores were determined from the degree of inflammation and the degree of spread of synovitis with scores from 0; healthy to 3; severe synovitis.

study on human osteoarthritic joints states that synovitis is a pivotal factor in the progression of the disease, despite not being a prerequisite for development of OA.

Several methods have been devised to quantify the severity of OA, both grossly and histologically (10). This study utilised the Kawcak Macroscopic Gross Scoring System using parameters such as wear lines, erosion and palmar arthrosis (10). Currently, there is no system to grade synovitis grossly from an image of a joint that is not taken following arthroscopy; therefore, a Synovitis Macroscopic Scoring System (Table 1) was developed for the purpose of this study.

This study's aim was to determine if there was a correlation between synovitis and gross OA score as measured by a Synovitis Scoring System created for this study and the Kawcak Macroscopic Scoring System applied to the equine MCP (fetlock) joint which is the articulation between the third metacarpal, proximal phalanx and proximal sesamoid bones enabling flexion and extension.

Methods

Sampling

Equine MCP joints were collected from an abattoir, as a by-product of the agricultural industry. The Animal (Scientific Procedures) Act 1986 Schedule 2 does not state collection from these sources as scientific procedures, thus ethical approval was not required for this research. These samples were whole MCP joints of 72 horses of varying breeds, gender and age. Ages of the horses were determined from their passport. The equine age average \pm standard deviation was 12.94 ± 7.64 and the range was 3-35 years old.

Synovitis Scoring System

A Synovitis Scoring System was developed for the purpose of this research paper, due to the lack of current gross macroscopic synovitis scoring systems. McIlwraith *et al.* (1) microscopic synoviopathy grading system formed the basis of the grading criteria. This was then furthered through direct observation of differences in between the equine MCP joint photographs. Synovitis scoring was then

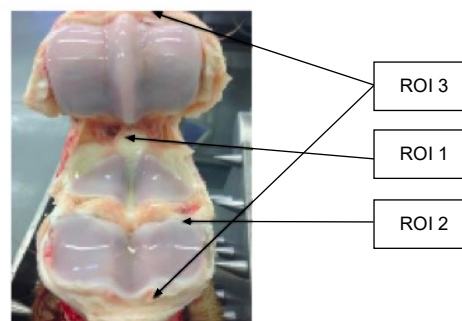


Figure 1. Equine metacarpophalangeal (MCP) joint with three regions of interest (ROI) labelled ROI 1, ROI 2 and ROI 3 on which synovitis scoring was conducted. This MCP joint enables flexion and extension articulating the third metacarpal, phalanx and sesamoid bones.

chosen to be undertaken on three regions of interest (ROI) of the MCP joint (Fig. 1) (4). Grades ranging from healthy to severe were created for the following changes: degree of inflammation and degree of spread of synovitis. A cumulative score was then determined. For this scoring system, a higher score indicates a more severe synovitis.

Gross Scoring

Each horse's MCP joint was scored twice (on consecutive days) macroscopically using the Kawcak Gross Scoring System (10) and Synovitis Scoring System (Table 1). The average of the two scores of each of the system was used for data analysis. Each joint was scored twice by each rater to identify the reliability of the results.

Statistical Analysis

All statistical analysis was undertaken using GraphPad Prism Version 8 or Microsoft Excel. GraphPad Prism was used to create the scatter plots in Fig. 2 and 3. Microsoft Excel was used for data collection. Correlation analysis was performed using Pearson's Correlation Analysis. Cohen's Kappa score was calculated online which allows calculations of inter-rater reliability to be made (11). Cohen's Kappa scores are divided into degrees of agreement; 0.01-0.20 as none to slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1.00 as almost perfect agreement.

Results

Synovitis Scoring System

Correlation analysis

There was no significant correlation found between the Kawcak Gross Score and Synovitis Score by both raters; $r = 0.09$, $P = 0.57$ (Fig. 2A) and $r = 0.07$, $P = 0.56$ (Fig. 3A). A weak significant positive correlation between the Kawcak Gross Score and age was found; $r = 0.32$, $P = 0.01$ (Fig. 2B) and $r = 0.35$, $P = 0.0004$ (Fig. 3B). No significant correlation was found between Synovitis Score and age; $r = -0.13$, $P = 0.35$ (Fig. 2C) and $r = -0.20$, $P = 0.14$ (Fig. 3C).

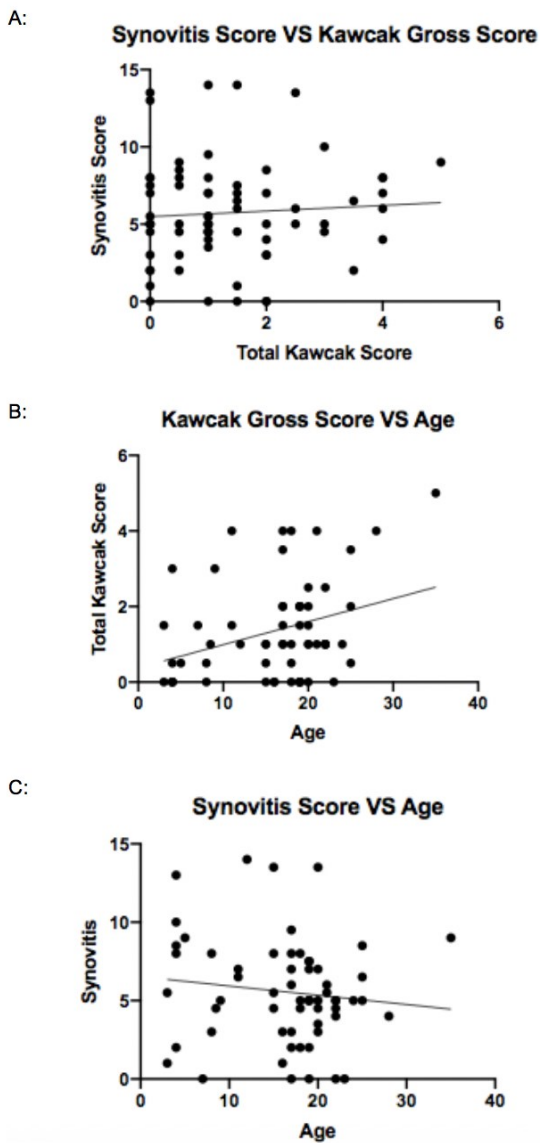


Figure 2. Scatter plots of scoring systems and age for rater 1. (A) Scatter plot of Synovitis and Kawcak Gross Scores; $r = 0.094$ and $P = 0.57$. (B) Scatter plot of Kawcak Gross Score and age (in years); $r = 0.32$ and $P = 0.012$. (C) Scatter plot of Synovitis Score and age; $r = -0.13$ and $P = 0.35$.

Cohen's Kappa Score

Calculation of the Cohen's Kappa Score was used to determine inter-rater scoring reliability. The Kappa values for inter-rater reliability of the scores provided fair (Kawcak Gross Scores = 0.38) and substantial agreement (Synovitis Scores = 0.68).

Discussion

This study was carried out to establish the relationship between gross synovitis and gross OA severity in the MCP joints of the equine biobanked samples. The severity of OA in these samples was principally low grade, early, age-related OA. Gross scoring was conducted on photographs of MCP joints from an abattoir, archived in the University of Liverpool Equine Musculoskeletal Biobank. Limited clinical and histological data was available on these horses, however for some horses details of age, gender and breed

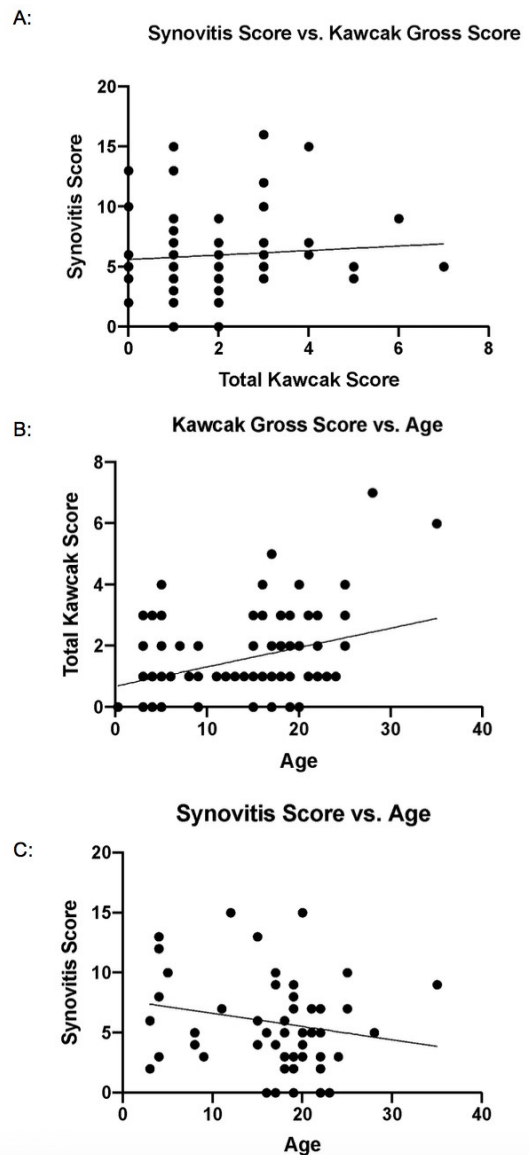


Figure 3. Scatter plots of scoring systems and age for rater 2. (A) Scatter plot of Synovitis and Kawcak Gross Scores; $r = 0.07$ and $P = 0.56$. (B) Scatter plot of Kawcak Gross Score and age (years); $r = 0.35$ and $P = 0.00038$. (C) Scatter plot of Synovitis Score and age; $r = -0.20$ and $P = 0.14$.

were known. It was hypothesised that there was an association between OA severity (as assessed by gross scoring) and synovitis.

There is a lack of studies within the literature investigating if there is an inflammatory component to equine OA. However, in human medicine, there have been several studies concluding that synovitis is an independent cause of OA; a higher synovitis score increases the risk of incidence of OA (13). In horses there are other potential reasons for synovial membrane inflammation such as navel ill in foals, sepsis and other infections. There have been many other studies researching the relationship between OA and increasing age that have found similar results (14).

The results demonstrated that there was no correlation (r -value less than 0.30) between both the Kawcak Gross Score and Synovitis Score and between Synovitis Score and age. However, there was a significant correlation (r -value more than 0.30) between Kawcak Gross Score and

age (15). This is often described as age-related OA and is a phenomenon that has been researched and observed previously in both human, canine and equine literature (14, 16).

There are a number of limitations to this study. There may have been potential errors introduced during data collection, as it was undertaken using digitalised photos. There are many variables that could have impacted on the quality of the photos, some of which include lighting and the angle at which the photos were taken. This may have inaccurately depicted the extent of macroscopic and synovitis changes in the MCP joint of horses. There was variability in the quality of the images, making it difficult to score some of them. This may have affected the credibility of the data, as unclear photo samples had to be discounted from the overall data analysis. Additionally, the quality of the photos displayed for each of the two raters may have varied depending on the device they have used to view the images. This may have contributed to reducing the agreement between the raters, especially that the scoring system was subjective.

A major limitation to this study is that all the samples were collected from an abattoir. This meant that there was a lack of clinical records for the populations of horses studied. It was unknown whether the donors were lame or had previous clinical complications affecting the state of their MCP joints, which may have created a bias towards the results collected. Since these were abattoir samples the OA changes were most likely to be low grade and predominantly age related potentially skewing the data. Carrying out another study with racehorses using the same methodology could potentially obtain data that is a better representation of a wider range of OA, as then the levels of OA should be more varied across the whole population.

For the future, it would be much more helpful if the photos were all taken using a set format and approach; making sure that the photos are in good quality and all ROIs are clearly visible. Additional studies such as histology and further parameters such as hypertrophy and hyperplasia could be included in the scoring systems making the results stronger in credibility.

Conclusion

In summary, this study demonstrated that there was no correlation between both the Kawcak Gross Score and Synovitis Score and between Synovitis Score and age. However, it was clear that the macroscopic changes of OA in the MCP joint increases with age. This study would benefit from clinical history, signs and larger variation in severity of OA.

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