European College of Veterinary Surgeons

Short Abstract Form for Publication

1. Title of abstract: **Biochemical markers and radiographic scores as an evaluation for the osteoarticular status of Warmblood stallions**

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Biochemical markers and radiographic scores as an evaluation for the osteoarticular status of Warmblood stallions. Verwilghen D., Busoni V., Salciccia A.., Grulke S.*, Serteyn D. Equine Clinic, Faculty Veterinary Medicine of Liege, Belgium

Establishing the osteoarticular status of the horse is often performed by means of radiological screening of the animals. Widespread blood sampling could potentially be an alternative to this procedure which is time consuming and sometimes technically difficult. The aim of this study was to investigate the relationship between the radiological status of the horses and the levels of biochemical markers (BM) of cartilage degradation and synovial inflammation. **Materials and Methods:** A specific radiological scoring (RS) system was developed and applied on 63 stallions presented for studbook admission. Additionally, groups of horses were established according to the occurrence of osteochondrosis (OC), degenerative joint disease (DJD) and distal interphalangeal joint (DIPJ) effusion. Insulin growth factor-I, myeloperoxidases, Coll2-1 and Coll2-1NO_2 were used as BM. The effects of age and weight on the BM and of the BM on the RS were measured. Mean values of BM between OC positive versus negative, DJD positive versus negative and DIPJ effusion positive versus negative were compared using SAS statistical program. **Results**: No effect of the combined BM was found on the RS. No significant differences between the values of the BM were found within the different radiological classes (RC). However, considering only the Coll2-1NO2 marker, the probability to belong to class A was given by the equation 9.63 -0.31 Coll2-1NO^2 + 0.04 (Coll2-1NO^2)². Equally a tendency (p= 0.06) towards an increase in RC by 0.45 for each increasing unit of Coll2-1NO2 was present. Significant positive correlations were found between Coll2-1 values and Coll2-1 NO^2 values. MPO values were found to be significantly higher in OC negative horses (357.86 ± 64.43 ng/ml) than in OC positive horses (231.17 ± 27.15 ng/ml). IGF-I levels were found significantly lower in the positive DIPJ effusion group (416.05 ± 17.57 ng/ml) compared to the negative DIPJ effusion group (477.1 ± 22.40 ng/ml). Coll2-1 values were significantly higher in the positive DIPJ effusion group (911.04 ± 41.13 nM) compared to the negative DIPJ effusion group (769.04 ± 37.92 nM). Mean values of Coll2-1 were significantly higher in the DJD positive group (901.24 ± 36.75 nM) compared to the DJD negative group (790.590 ± 39.397 nM) **Discussion**: The combination of the blood parameters did not seem to correlate with the used RS system. Coll2-1NO_2 levels however tend to increase with poorer RC and could therefore be used as a useful predictor of the osteoarticular status of the horse. Coll2-1 levels were significantly higher in the degenerative joint disease group. A high percentage of horses with DIPJ effusion was present in this study and was associated with decreased IGF-I and increased Coll2-1 levels.

Long Abstract Form for Blinded Evaluation

(no names or other identification on this form)

Title of abstract: Biochemical markers and radiographic scores as an evaluation for the osteoarticular status of Warmblood stallions

Introduction

The health status of a horse and in particular of its musculoskeletal system is of paramount importance to determine its durability, usability and suitability for future athletic performance. Radiographic evaluation has therefore become a common part of health screenings of young animals, especially those enrolled in future breeding or performance programs. Developmental orthopaedic disorders and early joint disease are one of the frequently diagnosed findings on radiology. Detection of these diseases is a crucial point when applying these radiographic screenings, especially because joint disease often leads to lameness and causes considerable wastage among animals. Establishing the osteoarticular status of horses by widespread blood sampling could potentially be an alternative to imaging techniques. For this study, 4 biochemical markers (BM) (Insulin growth factor-I (IGF-I), Meyloperoxidase (MPO), collagen type II degradation marker Coll2-1 and its nitrated form Coll2-1NO^2) were chosen which have been shown to allow evaluation of modifications of cartilage metabolism as well as having a role in the inflammatory process coinciding with joint diseases. The aim of this study is to investigate the relationship between the radiological status of the horses and the levels of biochemical markers (IGF-I, MPO, Coll2-1 and Coll2-1NO_2) of cartilage degradation and synovial inflammation. Moreover, it was hypothesised that the radiological score could be predicted by the combination of the four biochemical markers used.

Material and Methods:

1. Examined horse population, age and weight groups: Sixty three stallions underwent a standard studbook examination protocol, consisting of a general clinical examination, an orthopaedic assessment, an upper airway endoscopy and a radiographic protocol. For the current study only the radiographic protocol was considered. The group had a mean age of 2.55 years.. Only two animals were 6 years old and one 5 years old, all others ranged between 2 and 4 years of age. Twenty two were of sBs (Belgian sport horses) origin, eleven were BWP's (Belgian Warmblood horses), five KWPN (Dutch Warmblood horses), three Hanoverian, seven Holstein, seven Selle Francais and nine were from other Studbooks. Their mean weight was 570 ± 60 kg. Informed owner's consent was obtained before enrolling the horses in the study. Three age (all 2 year olds (n = 43), all 3 year olds (n = 10), 4 year olds and older (n = 10)) and weight (below 550 kg (n= 14), between 550 and 600 kg (n = 24), above 600 kg (n = 25)) groups were established to process the data.

2. Radiographic examination (RE) and radiological classification: All RE were performed following a standardised procedure. All horses were sedated for the RE using detomidine (10ug/kg IV) alone or combined with butorphanol (0.02 mg/kg IV). The following views were taken: dorso 60° proximo-palmarodistal oblique and weight bearing LM views of the front feet, LM views of the 4 fetlocks, LM and PLDMO oblique views of the hocks and a LM view of the stifles. Supplementary views were however taken when doubts arose on the obtained findings. Radiographs were read and scored by consensus opinion of two experienced readers (a senior ECVDI certified radiologist and a final-year radiology resident). Scores (RS) were allocated to all radiographic findings recorded using a severity grading system adapted from the one described by Robert et al. (2006).Higher scores indicate greater severity of radiographic changes and higher presumed clinical significance. Horses having a total RS between 0 and 3 were assigned to radiological class (RC) A = excellent/good, those having a total score between 4 and 6 were assigned to RC B = average/fair and those having a total score equal or superior than 7 were assigned to RC C = poor.

Additionally to the scoring system, three separate groups were created. One group consisting of horses with OC lesions, including horses with radiological signs of OC, OCD, cyst-like lesions, osteochondral fragments at the distal border of the navicular bone, the processus extensorius and in the fetlock, together with well defined defects of the sagittal ridge of the third metacarpus/tarsus. Another group of horses suffering from degenerative joint disease (DJD) was established. This group included all horses suffering from DJD in any of the examined joints, not taking severity of the lesions into account. A third group was made including all horses showing radiographic evidence of distal interphalangeal joint (DIPJ) effusion on the LM view of one or both front feet, not taking the severity of the effusion into account.

4. Blood samples Venous blood samples were collected from the jugular veins into tubes (Monovette 9ml, Sarstedt) containing EDTA (1.6 mg/ml blood) for the Coll 2-1, Coll 2-1NO_2 and MPO assays or heparin (15 I.U./ml blood) for the IGF-I assay in all but seven horses. Sample collection took place in the morning in order to exclude any effects of possible circadian variation (Jackson et al., 2003a). Blood was centrifuged at 7000 g for 10 minutes at room temperature. The plasma was aliquoted and frozen at -20° C within 60 minutes from sampling. Plasma samples were only thawed immediately prior to assays.

5. IGF-I assay: As described in the instructions of the Equine IGF-I ELISA kit (BiopTis, Liege, Belgium

6. MPO assay : MPO was assayed with an original specific enzyme-linked immunosorbent assay (Equine MPO-ELISA kit, BiopTis, Liege, Belgium) developed by Franck *et al*. (2005).

7. Coll2-1 and Coll2-1NO_2 assays: The Coll2-1 and Coll2-1NO_2 assays are two competitive immunoassays previously described in detail by Deberg *et al.* (2005).

8. Statistical analysis

All data was analysed using SAS commercial software (Statistical Analysis System, SAS Institute GmbH Heidelberg, Germany). The radiographic data used for statistical analyses included the OC, DJD and DIPJ effusion group and the overall RS of the horse (sum of all scores of each individual finding of a horse) and his attributed A, B or C class. The effects of age and weight on the BM and of the BM on the RS and RC were measured using GLM procedure. Correlations between biochemical markers and radiological grade and class were measured using Pearson's correlations. Mean values of BM between groups of OC positive versus negative, DJD positive versus negative and joint effusion of the DIPJ positive versus negative were compared using t-test procedure. Significance was set at $P < 0.05$.

Results:

Prevalence of lesions, distribution of classes and grades, lameness grades, mean blood values for IGF-I, MPO, Coll2-1 and Coll2-1NO2. Forty stallions (63.49%) were attributed a class A, 15 (23.81%) a class B and 8 (12.70%) a class C after adding their individual RS. The distribution of the RS and RC, the lameness grades and mean blood parameters and their standard error of means (SEM) are given in Table 1. Twenty three (36.51%) of the horses showed signs of OC and 20 (31.75%) showed signs of DJD. In 28 (44.44%) horses radiological signs of joint effusion of the DIPJ were found, of these 15 belonged to the A class, 9 to the B and 4 to the C class. Mean values for IGF-I, MPO, Coll2-1 and Coll2-1 NO2 for the OC, DJD and DIPJ effusion groups are listed in Table 2.

Effects of blood markers on radiological class, scores.

No effect of the combined BM was found on the RS or RC. No significant differences between the values of the BM were found within the different RS or RC. However, considering only the Coll2-1NO2 marker, the probability to belong to class A was given by the equation $9.63 - 0.31\ \text{Coll2-1NO}^2 + 0.04\ (\text{Coll2-1NO}^2)^2$. Equally a tendency ($p = 0.06$) towards an increase in RC by 0.45 for each increasing unit of Coll2-1NO^2 was present. Significant positive correlations were found between Coll2-1 values and Coll2-1 NO^2 values ($r = 0.61$). MPO values were found to be significantly higher in OC negative horses (357.86 ± 64.43 ng/ml) than in OC positive horses (231.17 ± 27.15 ng/ml). IGF-I levels were found significantly lower in the positive DIPJ effusion group (416.05 ± 17.57 ng/ml) compared to the negative DIPJ effusion group (477.1 ± 22.40 ng/ml). Coll2-1 values were significantly higher in the positive DIPJ effusion group (911.04 ± 41.13 nM) compared to the negative DIPJ effusion group (769.04 ± 37.92 nM). Mean values of Coll2-1 were significantly higher in the DJD positive group (901.246 ± 36.75 nM) compared to the DJD negative group (790.590 ± 39.397 nM).

Discussion:

Robert et al. (2006) stated that the RS, similar to the one used in the present study, is a synthesis parameter and provides an overall assessment of the horse. This study however, did not aim at evaluating performance, but at using the RS system in an attempt to correlate it to BM involved in the osteoarticular status of the animal. Results failed to demonstrate the hypothesis that the RS could be predicted by the combination of the selected BM.

A relatively high prevalence of synovial effusion of the DIPJ was detected on radiographs (44.44% of horses). This may be a consequence of overestimation of this abnormality on x-rays, or may indicate that this joint is subjected to high workload in young horses at the beginning of their training. This hypothesis is supported by the lower mean IGF-I levels found in the DIPJ effusion group in the current study and by work realised by Jackson et al. (2003) who found similar low serum levels in horses submitted to 20 weeks of treadmill exercise. No significant correlation was found between Coll2-1 and the RS. Horses from the current study ranged from 2 to 6 year old (mean age 2.56 year) and primary joint disease with remodelling of the articular surface and loss of cartilage mostly occurs in older horses or is in relation with OC lesions found in young animals. OC, a disease specific for developing animals, has shown to develop at very young age also beneath the age range of this population. Cartilage breakdown and collagen degradation in relation with OC pathologies might already be completed in the age group studied here. Billinghurst et al. (2004) supported this theory by finding loss of correlation between type-II collagen markers of degradation and OC lesions at 11 months in contrast to lesions at 5 months.

The tendency for Coll2-1 NO_2 to be more elevated in a less favourable RC coincides with all the findings previously described on this marker. In human medicine, this marker was shown to be increased in patients with OA and rheumatoid arthritis and to be a good marker for OA progression over a 1 year evaluation (Deberg et al., 2005, 2007). In the equine population, the marker was shown to be significantly higher in populations suffering from OC (Gangl et al., 2007) and juvenile DJD (Lejeune et al., 2007) indicating it is a useful marker for both diseases diagnosed by radiology.

In conclusion, only Coll2-1NO_2, a marker reflecting degradation of cartilage collagen and inflammation was shown a potentially useful predictor of the osteoarticular status of the horse. Larger population of horses, spread over a bigger age range should be used to confirm this hypothesis. To address some of the other remaining questions of this study, a longitudinal set up, involving repeated sampling and RS of a group of animals over time should be considered. Moreover, as chondrocytes in articular cartilage that are subjected to exercise as well as to osteoarthritic inflammation, not only regulate degradation but also synthesis of extracellular matrix components (Skioldebrand et al., 2006), biomarkers reflecting type II collagen and aggrecan synthesis would be interesting to be studied further in this population.

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I. Check session: (only one)

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