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Nuclear scintigraphic retrospective study of the C6/7 articular facets of the equine cervical spine

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Summary: The objectives of this study were firstly, to determine an objective grading system of radiopharmaceutical uptake in the C6/7 articular facets of the cervical spine as a predictor of clinical signs in warmblood sporthorses; and secondly to establish an objective absorption coefficient (AC) value that could possibly differentiate between presence or absence of clinical signs in this region. 308 scintigraphic views of the cervical spine in 154 German warmblood horses were evaluated. Scintigrams were divided into clinically normal (60) and clinically abnormal (94) groups of horses. Horses were grouped according to performance type and age. Uptake coefficients using C3/4 as a reference point were established. Sensitivity and specificity for detection of neck pain and radiographic abnormalities were assessed. Receiver operating characteristic (ROC) analysis was used to determine a cutoff point for distinguishing between clinically normal and abnormal horses. An AC value of 1.243 at the C6/7 cervical vertebral facets separated clinically normal from clinically abnormal horses. Values decreased slightly with increasing age of the horses, and were highest in the middle age. There was a significant difference in AC values between clinically normal and abnormal horses in the dressage and show jumping groups. The best predictive values were established for horses in the show jumping category where scintigraphic findings correlated with clinical signs. An AC value of 1.235 at the C6/7 cervical vertebral facets reliably separates pathological from physiological uptake. However, age and performance type of the horses should be taken into account when interpreting these findings.

Keywords: equine, scintigraphy, cervical spine, absorption coefficient, diagnostic imaging

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Introduction

The articular facets of the equine cervical spine are most commonly affected by osteochondrosis lesions or fractures (*Hahn* et al. 2008, *Nout* et al. 2003). Significant cord compression may result from spondylarthrosis of these joints (*Moore* et al. 1994). The extent of cord compression is difficult to accurately assess on radiographs alone; false positive results are encountered rather frequently (*Papageorges* et al. 1987, *Nout* 2003). The proposed sensitivity of spondylarthrosis recognition on radiographs was judged to be only 10–50% in recent literature, although specificity was found to be much higher at 46–90% (*Weller* 2015, *Engel* 2010). To circumvent the reported poor sensitivity, a comparative study of the thoracic spine utilizing radiography and scintigraphy was performed. Increased radiopharmaceutical uptake (IRU) was noted predominantly in joints of horses with a history of back problems (*Gillen* et al. 2009).

The scintigraphic appearance of cervical vertebrae is reportedly highly variable and is dependent on the age and use of the animal (*Weller* 2015). Apart from bone density, biomechanical stresses during movement also play a role in the variability seen (*Dyson* et al. 2003). Some authors are of the opinion that an IRU at C6/7 is abnormal regardless of clinical signs (*Martinelli* et al. 2010). Scintigraphic studies of the equine cervical spine have thus far only been conducted in healthy horses. One of these studies utilised regions of interest (ROI) and absorption coefficients, while the other utilised a customised automated software programme (*Keyl* et al. 2011, *Didierlaurent* et al. 2009).

To the author's knowledge, no study evaluating scintigraphic changes in the cervical spine of abnormal horses has been conducted to date. The objectives of this study are to determine the absorption coefficient of the C6/7 articular facet and to develop more objective evaluation criteria of the cervical spine for future reference. In addition, discrimination between healthy and diseased joints will be investigated, along with associated sensitivity and specificity of the findings.

Materials and Methods

Animals

Warmblood horses were selected retrospectively from the patient list of a private veterinary practice in Germany over a four year period, from 2007 to 2010. The horses were subsequently divided into clinically affected and clinically healthy (unaffected) groups.

The following criteria were used for the selection of the clinically affected group: animals with a history of compromised rideability, with cervical spine pathology suspected on clinical evaluation, or with thoracic limb lameness that could not be

localised to the limbs themselves with the use of regional nerve blocks. Clinical symptoms of this group included unilateral or bilateral neck stiffness with reluctance to work on the bit, muscle atrophy or spasms in the cervical region, hypo- or hyperreflexic cervical muscles, guarding of the neck, irregular or asymmetrical gait and rideability issues such as bucking, rearing or head-shaking.

The control group consisted of horses that showed clinical lameness localisable to the pelvic or thoracic distal limbs using regional nerve blocks, but that were completely clinically normal in the cervical region.

Each group was divided into three age groups: AG 1 included horses 6 to 10 years of age, AG 2 included horses 11 to 15 years of age and AG 3 included horses above 16 years of age. Use categories of the selected horses were divided into Show Jumping (SH 1), Dressage (Dre 2) and Hacking (Ha 3). Animals that had radiographic changes or IRUs on scintigraphic images in articular facets in regions other than C6/7 were excluded from the study.

Radiographic evaluation

Lateral survey radiographs of the cervical spine were taken of all horses using exposure factors of 71 kV and 56 mAs with a stationary digital x-ray unit (Polydorus 100, Siemens AG, Munich, Germany) with a flat panel detector (Canon CXDI 31, Canon Medical Systems, Amstelveen, Netherlands).

Radiographs were evaluated by an experienced vet (SZ) according to prescribed criteria (*Down* and *Henson* 2009). Horses that were graded 1 to 4a were deemed radiographically normal, while those graded 4b and above were deemed radiolographically abnormal.

Scintigraphic evaluation

Horses were routinely lunged 15 minutes before intravenous administration of the radiopharmaceutical ^{99m}Tc-MDP at the recommended dose of 1 GBq/100kg of body mass. The bone phase of the scintigraphic study commenced at 2 hours post injection. Horses were sedated with a combined intravenous injection of medetomidin (Domosedan, Animal Health, New York, NY) at a dose of 0.01 mg/kg of body mass and butorphanol (Torbugesic, Fort Dodge Veterinär GmbH, Würselen, Germany) at a dose of 0.01–0.02 mg/kg of body mass. The scintigraphic images were obtained with an Equine Scanner (MIE, Seth, Germany) scintigraphy unit. Motion correction software Scintron®4 (MIE, Seth, Germany) was used.

The head of the horse was stabilised on a head stand and held in position by veterinary personnel. Four scintigraphic views were performed: two cranially and two caudally on each side of the cervical spine. The cranial views were performed in order to exclude any concurrent pathology at other sites. The thoracic limb closest to the camera was retracted slightly to enable optimal camera placement. Parallel alignment as possible with the cervical spine was ensured. A 256×256 matrix and a dynamic image acquistion of 60 seconds (1 frame per second) were selected.

Scintigraphic images were evaluated in grayscale with Regions of Interest (ROIs=counts/pixel/cm^2) placed strategically within the area of C6/7 (see below). The absorption coefficient was calculated as follows:

$$\text{Absorption coefficient (AC)} = \frac{\text{ROI at C6/7 (cpm/cm}^2\text{)}}{\text{ROI at reference area}}$$

A reference area of C3/4 was used because it is the most suitable comparison area for C6/7 (*Keyl* et al. 2011). Scintigraphic images in DICOM format were uploaded to Photoshop (Version CS5, Adobe Systems, San Jose, California, USA IBM) and ROIs were determined at each of the following anatomical regions: the articular facets of C3/4, C5/6, C6/7 and C7/T1; and the vertebral bodies of C3, C4 and C6. Reference ROIs were determined at the anatomical locations previously described (*Keyl* et al. 2011). All absorption coefficients of all facet joints were correlated to each other, and then C 6/7 was choosen. These absorption coeffiecients represented the highest median difference between the affected and non-affected groups. Regions of Interest were extracted and exported as binary masks.

The values of all ratios were then submitted to receiver operating characteristic (ROC) analysis. This procedure calculated a cut-point for distinguishing between healthy (<=cut-point) and diseased (>cut-point) animals and all animals were assigned to a group based on this cut-point value.

Statistics

Statistical analysis was performed using IBM SPSS Statistics (Version 22, IBM Corp., Armonk, New York, USA) and results were visualized using Mathematica (Version 10.1, Wolfram Research, Inc., Champaign, Illinois; USA). Descriptive statistics for ratio values were calculated and box plots were created. Data were assessed for normality using the Shapiro-Wilk test. Group comparisons were performed using the Kruskal-Wallis and Mann-Whitney U tests. Cross tables were created to identify dependence between dichotomous variables using Fisher's exact test. Spearman rank correlation analysis was performed to determine the correlation coefficient (r). Sensitivity, specificity, positive predictive value and negative predictive value, as well as the respective 95% confidence intervals, were calculated. Sensitivity and specifity for scintigraphy, for clinical detection of pain and radiographic lesions were assessed, and for radiography in detection of scintigraphic lesions.

ROC analysis was performed to obtain the value for area under the curve (AUC) which was used to determine a cutoff point for distinguishing between clinically normal and abnormal horses. The level of significance was set at $p < 0.05$ and post-hoc Bonferroni correction was applied for multiple pair comparisons.

Results

A total of 154 horses fulfilled the selection criteria. Thus, 308 images of the caudal cervical regions and both articular facets of C6/7 (with C3/4 as reference values) were evaluated.

The clinically affected group consisted of 94 horses (64 geldings, 24 mares and 6 stallions). Forty-five of these horses were used for dressage, 25 were used for jumping and 24 were used as hacks. Twenty-one horses were 6–10 years of age, 58 horses were 11–15 years of age and 15 horses were 16 years of age or older.

The unaffected healthy group consisted of 60 horses. In this group, 31 were used for dressage, 19 were used for jumping and 10 were used for hacking. Eleven horses were between 6 and 10 years of age, 30 horses were between 11 and 15 years of age, and 19 horses were 16 years of age or older.

ROC analysis showed that the AC value separating clinically normall from clinically abnormal horses at the C6/7 cervical vertebral facets was 1.243 (AUC = 0.794).

In the unaffected group, an inverse relationship between the AC and age was observed. Conversely, all ages in the affected group showed an increase in the median value of AC. The spread of the values was greater in the affected group than in the unaffected group. The highest AC values were found within the middle-aged affected group (Figure 1). A small number of outliers with AC values of up to 1.7 were noted in both groups. There was a significant difference between affected and unaffected animals only in AG 2 ($P < 0.001$).

Performance categories impacted the difference in AC between clinically affected and clinically normal horses in the varying age groups. There was a significant difference between affected and unaffected animals in the show-jumping ($P < 0.001$) and dressage ($P < 0.001$) categories, although a certain degree of overlap was observed in the dressage and hacking categories (Figure 2).

With regard to the age groups, specificity was generally higher than the sensitivity of scintigraphic examination. Sensitivity declined from 16 years of age onwards regardless of performance type, whilst specificity rose steadily from younger to older horses.

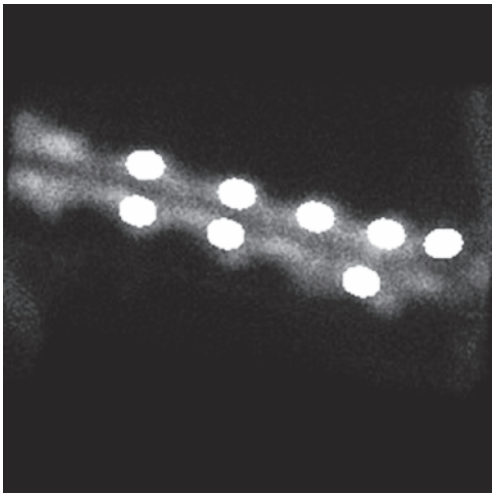

Fig. 1 Scintigraphic image of a left quine cervical spine. Masks defining for regions of interests and reference areas of the facet joints from C3/4 to C6/7, vertebral body of C3, C4 and C6 are outlined. | *Szintigramm einer Halswirbelsäule von links, alle Masken für die Interessen- und Referenzareale der Facettengelenke C 3/4 bis C 6/7 und der Wirbelkörper C 3, C 4 und C 6 sind eingezeichnet.*

The highest positive predictive values were seen in age group 2 (11–15 years) ($P < 0.001$) whilst the lowest values were seen in group 3 (>16 years). Scintigraphy correlated the best with clinical findings as compared to radiography. Scintigraphy correlated poorly with radiography; a high variation in sensitivity and specificity was seen.

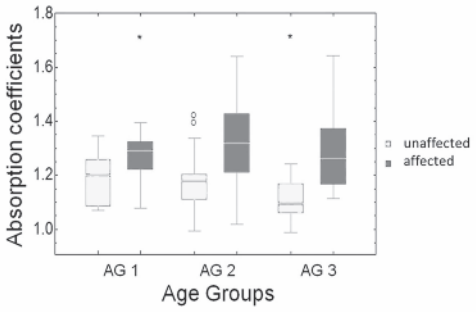

Fig. 2 Box-and-whisker plot of Absorption coefficients of C6/7 in three age groups (AG 1 to AG 3) in clinically unaffected and affected horses. Each box represents the values from the 25th to 75th percentiles, the horizontal line in each box represents the median, whiskers represent the minimum to maximum values. Circles and asterisks represent outliers and extreme values, respectively. There was a significant difference between affected and unaffected animals in AG 2 ($P<0.001$). | *Box-Whisker-Plots der Speicherquotienten der Facettengelenke von C 6/7 bei drei Altersgruppen (AG 1 bis AG 3) bei klinisch unauffälligen und klinisch auffälligen Pferden. Jeder Kasten repräsentiert die Werte vom 25. bis zum 75. Perzentil, die horizontale Linie repräsentiert den Medianwert, die Antennen repräsentieren Minimium und Maximum. Kreise und Asterisks repräsentieren Ausreißer und Extremwerte. Es besteht ein signifikanter Unterschied zwischen klinisch auffälligen und unauffälligen Pferden der Altersgruppe von 11 bis 15 Jahren (AG 2 ($P<0.001$)).*

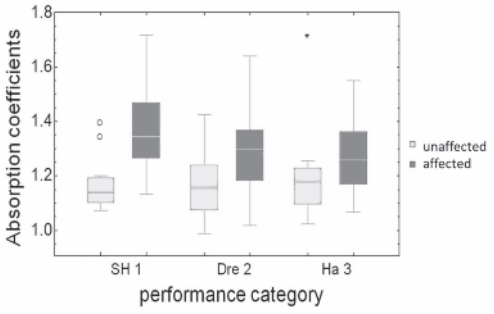

Fig. 3 Box-and-whisker plot of Absorption coefficients of C6/7 articular facet absorption coefficient based on the performance category [show-jumping (SH 1), dressage (Dre 2), or hacking (Ha 3)] of clinically affected and unaffected horses. Each box represents the values from the 25th to 75th percentiles, the horizontal line in each box represents the median, whiskers represent the minimum to maximum values (excluding outliers). Circles and asterisks represent outliers and extreme values, respectively. There was a significant difference between affected and unaffected animals in the show-jumping ($P<0.001$). | *Box-Whisker-Plots der Speicherquotienten der Facettengelenke von C 6/7 bei drei verschiedenen Nutzungsrichtungen (SH 1: Springpferde, Dre 2: Dressurpferde, Ha 3: Freizeitpferde) bei klinisch unauffälligen und klinisch auffälligen Pferden. Jeder Kasten repräsentiert die Werte vom 25. bis zum 75. Perzentil, die horizontale Linie repräsentiert den Medianwert, die Antennen repräsentieren Minimium und Maximum. Kreise und Asterisks repräsentieren Ausreißer und Extremwerte. Es besteht ein signifikanter Unterschied zwischen klinisch auffälligen und unauffälligen Pferden bei den Springpferden (SH 1 ($P<0.001$))*

Differences were less obvious in the performance categories, although sensitivity and specificity were slightly lower in the hack category. Positive predictive values were highest in correlating scintigraphy with clinical findings.

Discussion

An absorption coefficient of 1.243 at the C6/7 level in Warmblood horses was found to reliably separate clinically affected from clinically normal horses. This is in agreement with a previously published article that documents a reference range of 0.75 to 1.23 for clinically normal horses, although that study's equine population and evaluation methods differed from those used in this study (*Keyl* et al. 2009). Values were determined for the C6/7 articular facets only, as it is impossible to differentiate between the different facet joints based on clinical symptoms alone. The C6/7 of the spine has been reported as a vulnerable site in the cervical spine of horses (*Hett* et al. 2006).

The AC value was highest in the clinically normal age group of 11 to 15 years and steadily declined with rising age. This trend was also noted in the clinically affected group and can be explained by the higher rate of bone metabolism in this age group of horses. In a previous study (*Didierlaurent* et al. 2009) no age-related differences were seen in scintigraphic findings in younger horses of the cervical spine in a group af racing thoroughbreds and trotters. The highest AC values were observed in the show jumping and dressage horses at the peaks of their performance careers. This was not observed in the hack category, where a clear-cut difference between AC values in affected and unaffected horses could not be identified.

The clinically affected group had more variablility in AC values than the unaffected group. A possible explanation for this includes the fact that exact clinical criteria could not be established. Another possibility is that unilateral articular facet pathology may be present with only mild regional uptake seen on scintigraphy due to the combination of counts with the unaffected side when recorded on a lateral projection.
If no or minimal uptake is seen on scintigraphy in older horses, this can be considered unaffected. The higher specificity found in this group reliably confirmed pathology, whereas specificity was considerably lower in younger horses due to more frequently seen higher AC values in this group. The higher AC value observed in the middle aged group was consistent with pathology as substantiated by a higher positive predictive value, which indicates how many high risk patients actually have the pathology in question.

In regard to performance categories, the most apparent difference of the facet absorption coefficient of the facet joint C6/7 between affected and unaffected groups was found in the show jumping group, less so in the dressage group, and least in the hack group. This suggests that performance type affects bone metabolism in the cervical spine. In a previous study, it was noted that racing throughbreds also had much higher radiopharmaceutical uptake in the C6/7 articular facets, possibly due to stretching of the neck and thus increased motion of the lower cervical spine during racing (*Didier-*

Table 1 Sensitivity, specificity, positive and negative predictive values in the various age group/performance category combinations correlated with clinical, scintigraphic and radiographic evaluation of the equine facet joints C6/7. / *Sensitivität, Spezifität und Positive und Negative Vorhersagewerte bei Pferden unterschiedlicher Altersgruppen/Nutzungsrichtungen der Facettengelenke C 6/7 in Abhängigkeit der klinischen, szintigraphischen und radiologischen Befunde*

	Group	Sensitivity %	Specificity %	PPV	95% CI PPV	NPV	Significance
Radio and Clinic	AG 1	28.6	90.9	85.7	42.0-99.2	40.0	
Radio and Clinic	AG 2	53.1	84.4	87.2	71.8-95.2	47.4	P<0.0001
Radio and Clinic	AG 3	33.3	70.6	37.5	10.2-74.1	66.7	
Radio and Clinic	SH 1	56.0	77.8	77.8	51.9-92.6	56.0	P<0.05
Radio and Clinic	Dre 2	42.2	83.9	79.2	57.3-92.1	50.0	P<0.05
Radio and Clinic	Ha 3	41.7	81.8	83.3	50.9-97.1	39.1	
Radio and Scinti	AG 1	25.0	83.3	71.4	30.3-94.9	40.0	
Radio and Scinti	AG 2	61.5	84.1	82.0	65.9-91.9	64.9	P<0.0001
Radio and Scinti	AG 3	57.1	78.9	50.0	17.4-82.5	83.3	
Radio and Scinti	SH 1	65.2	85.0	83.3	57.7-95.6	68.0	P<0.005
Radio and Scinti	Dre 2	44.7	81.6	70.8	48.7-86.6	59.6	P<0.05
Radio and Scinti	Ha 3	50.0	82.3	75.0	42.8-93.3	60.9	
Scinti and Clinic	AG 1	71.4	54.5	75.0	50.6-90.4	50.0	
Scinti and Clinic	AG 2	71.9	81.2	88.5	75.9-95.2	59.1	P<0.0001
Scinti and Clinic	AG 3	55.6	88.2	71.4	30.3-94.9	78.9	
Scinti and Clinic	SH 1	84.0	88.9	91.3	70.5-98.5	80.0	P<0.0001
Scinti and Clinic	Dre 2	66.7	74.2	78.9	62.2-89.7	60.5	P<0.001
Scinti and Clinic	Ha 3	62.5	72.7	83.3	57.7-95.6	47.1	

PPV: Positive Predictive Value, NPV: Negative Predictive Value, Radio: Radiographic examination, AG: Age Group, Show Jumping: SH 1, dressage: Dre 2, Hacking: Ha 3, Scinti: Scintigraphic examination, CI: Confidence interval, PPV: Positiver Vorhersagewert, NPV: Negativer Vorhersagewert, Radio: Röntgenuntersuchung, AG: Altersgruppe, SH 1: Springpferde, Dre 2: Dressurpferde, Ha 3: Freizeitpferde, Scinti: Szintigraphische Untersuchung, CI: Konfidenzintervall

laurent et al. 2009). Further studies attempted to show that rounding of the neck in dressage horses and show jumpers predisposed these horses to pathology of the lower cervical spine; however, this could not be confirmed on radiographs (*Down* and *Henson* 2009).

The equine cervical spine is generally difficult to evaluate. While enlarged articular facets correlated better with age than with clinical findings, reported and commonly used measurements on radiographic images are regarded as being too inaccurate for a definitive antemortal diagnosis of compressive cervical spinal cord disease (*Engel* 2010, *Down* and *Henson* 2009). Inter- and intraobserver variability was greater than the difference between pathological and non-pathological findings (*Lischer* et al. 2010). Therefore, radiographic findings should be interpreted with caution. In this study, we attempted to achieve more accuracy by combining radiographic and scintigraphic findings. This work has already been done in the thoracic spine (*Gillen* et al. 2009). Our findings differ significantly from those seen in the prior study. Unlike the cervical spine, a high sensitivity of radiographic findings and good correlation of radiographic to scintigraphic findings were documented for the thoracic spine. This may be due to the fact that clinically insignificant radiographic changes are commonly seen in the thoracic spine. In contrast to the thoracic spine, the presence of changes on scintigraphy in the cervical spine were found to be more significant when combined with clinical findings than changes observed on radiography and scintigraphy alone.

When scintigraphic findings were correlated with clinical findings, the sensitivity and specificity of the cervical spine evaluation were more accurate than those of the thoracic spine. Show jumpers had much higher predictive values than their dressage and hacking counterparts. The most obvious difference between affected and non-affected horses was observed in this particular group. The explanation for increased uptake at C 6/7 in the study of racing thoroughbreds cannot account for the findings seen in our performance categories. Thus, the biomechanical mechanisms that determine the AC values recorded in our horses remain uncertain at this time (*Didierlaurent* et al. 2009).

Conclusion

In conclusion our study revealed that absorption coefficients in the equine cervical spine differ between age groups and performance categories, although larger numbers of horses are need to confirm this finding. The established AC value of 1.243 may serve as reference level to suggest pathology, but must be correlated with clinical findings. Scintigraphic findings are more predictive of clinical pathology than radiographic changes.

Conflict of interest statement

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

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