Original research

Accuracy of computed tomography in diagnosing temporomandibular joint osteoarthritis relative to histopathological findings – an ex–vivo study of 41 horses

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Abstract

Computed tomography (CT) is commonly used for imaging the equine head, particularly when diseases of the temporomandibular joint (TMJ), including osteoarthritis (OA), are suspected. However, the accuracy of CT–based diagnosis of TMJ OA remains uncertain. This prospective descriptive cross–sectional study aimed to describe the CT and histopathological findings of TMJ OA concerning horse age, assess the correlation between the CT and histopathological findings, and evaluate the accuracy of CT–based diagnosis of TMJ OA. CT images and tissue samples from 41 heads (82 TMJs) were independently and simultaneously reviewed by two observers. Descriptive statistics on both findings, their age– and TMJ OA–related frequency distribution, and the accuracy of TMJ OA diagnosis were calculated. Based on CT findings, TMJ OA was recognized in 50% of joints in horses aged 1–4 years and 100% in horses aged 5–15 and >15 years. However, based on histopathological findings, TMJ OA was confirmed in 18% of joints in horses aged 1–4 years, 63% in horses aged 5–15 years, and 83% in horses >15 years. The occurrence of both findings differed among horses of different ages. Consequently, the CT–based diagnosis of TMJ OA showed moderate accuracy with a 0.79 sensitivity and 0.50 specificity. However, accuracy metrics also differed among horses of different ages. Equine TMJ may undergo both age– and disease–related remodeling, detectable through CT and histopathology. However, the sensitivity and specificity of CT–based TMJ OA diagnosis may be affected by the consideration of 'CT anatomical variations'.

KEY WORDS

temporomandibular joint disease, degenerative joint disease, anatomical variation, inflammation, equine

1 INTRODUCTION

Diseases of the temporomandibular joint (TMJ) in horses have been suggested to be rare or rarely reported;[1–3] however, their prevalence in the equine population has not yet been estimated. The TMJ facilitates movement of the mandibular condyle relative to the temporal bone;[4] therefore, diseases–related dysfunctions may lead to various clinical symptoms, ranging in severity from mild behavioural issues[5–7] to moderate masticatory difficulties[8,9] and severe inability to open[10,11] or close[12,13] the mouth.

Among TMJ diseases, osteoarthritis (OA) may develop as a primary condition[5–7,11,14,15] or secondarily to fractures[16] or septic arthritis.[1,10,17] Increasingly, horses presented to veterinarians due to milder symptoms – such as poor performance,[6,7] aversive behaviours,[5,6] head–shaking syndrome,[5,18] and resistance or reluctance to work on the bit[6,7] – are suspected of having TMJ OA. Consequently, TMJ OA is discussed more frequently[3,19] as a potential cause of nonspecific clinical symptoms associated with decreased performance in equine athletes. [3,5–7,11,14,15] However, initial diagnosis of primary TMJ OA – typically based on detailed history and clinical symptoms[3] – requires confirmation using diagnostic imaging modalities[5–7,11,14–16,20] and functional tests when possible.[6,7]

Among diagnostic imaging modalities, ultrasonography,[5,11] conventional radiography,[5,11,16] and/or computed tomography (CT)[5–7,14,15,20] are used clinically for the diagnosis of TMJ OA. Although many clinicians emphasize the advantages of CT over conventional radiography,[1,10,16,17,21] even when specific TMJ projections are employed,[22–24] radiography remains limited by significant superimposition of anatomical structures, which reduces image clarity. Moreover, with recent advances in performing CT imaging of the equine head under standing sedation,[25,26] CT examinations in horses are expected to increase. Consequently, CT is expected to be widely utilized in assessing TMJ OA, as it has been the case for research[15,18,20] and clinical[5–7,14] purposes. The increased availability and safety of CT imaging also align with the growing number of case reports of primary TMJ OA,[3,5–7,11,14,15] which have shown responses to intra–articular medication[3,5–7] and have been associated with head–shaking syndrome[5,15] or more subtle riding problems. [3,6,7] Although almost all of these horses were diagnosed using CT,[5–7,14,15] histopathological confirmation of TMJ OA has been reported in only two cases,[5,14] and the diagnostic accuracy of CT for TMJ OA remains uncertain.

It should be noted that primary TMJ OA is thought to result from age–related ('wear and tear') lesions,[5,6,20,27] leading to damage and degeneration of the articular cartilage.[28] Unlike appendicular joints, where the articular surface is covered by hyaline cartilage,[29] the TMJ is lined with fibrocartilage,[30] making histopathological findings of degeneration more specific to this joint.[5,14,27,31,32] Degeneration of the articular cartilage is typically accompanied by inflammation and remodeling of the surrounding tissue, beginning in the subchondral bone and progressively involving the synovium[28] and the fibrocartilaginous intra–articular disc.[20,33] Consequently – because articular cartilage is poorly visualized radiographically – irregular subchondral bone contours, subchondral bone cysts, and mineralization of the intra–articular disc are the most commonly reported CT findings in primary TMJ OA. [5–7,14,15,20]

However, considering that degenerative changes accumulate with age[27,32] and that TMJ OA is associated with synovitis and capsulitis,[1,28] the histopathological confirmation of inflammatory changes in

the joint capsule remains the reference for confirming TMJ OA. This is particularly important in the context of diagnostic imaging, as CT findings in the TMJ may be incidental and may not manifest clinically in particular horses.[20] These findings, referred to as 'CT anatomical variation',[20] differ from normal anatomy[34] but occur in horses without moderate to severe clinical symptoms of TMJ dysfunction.[20] Therefore, inclusion of such findings in CT–based assessment may affect the diagnostic accuracy for TMJ OA. Moreover, recent case reports have linked certain 'CT anatomical variations' – such as subchondral bone cysts in the mandibular condyle[6,7] and mineralization of the intra–articular disc[5,6] – with mild performance–related clinical symptoms of TMJ diseases, thereby increasing interest in the radiological manifestations of TMJ OA and renewing discussion on their clinical significance.[3,19]

To address the gap in understanding the role of CT findings in equine TMJ OA, we hypothesize the age–related occurrence of CT and histopathological findings as well as their correlation with horses' age. We also hypothesize a correlation between the occurrence of CT and histopathological findings. Finally, we hypothesize that the accuracy of CT–based diagnosis of TMJ OA varies depending on the horse's age. The study aims to: (1) describe the CT and histopathological findings of TMJ OA in horses in relation to age; (2) assess the correlation between the occurrence of CT and histopathological findings; and (3) evaluate the accuracy of CT–based diagnosis of TMJ OA in relation to histopathology.

2 METHODS

2.1 Selection and description of subjects

This prospective descriptive cross–sectional study was conducted on 50 equine cadaver heads collected from horses at a commercial slaughterhouse in Poland. The sample size was calculated through a power analysis for the qualitative scale, using the formula suggested by Charan and Kantharia for basic statistical tests[35]. Previous study indicates that 'CT anatomical variations' occurred in 29% of TMJ from asymptotic horses (0.29 proportions)[20]; thus, the occurrence in both asymptotic and symptomatic horses is expected to be twice higher (0.58 proportions). Using a Type I error rate fixed at 5% (P = 0.05) and a power of 80%, the sample size was estimated to be 45.4. The sample size was then adjusted by 10% to account for attrition related to the eligibility criteria, resulting in an estimated corrected sample size of 50.4.

The study was conducted between May and June 2023. Horse heads were collected post–mortem, which does not fall under the legislation for the protection of animals used for scientific purposes, according to national law (Dz. U. 2015 poz. 266) and EU law (2010–63–EU directive). Therefore, no Ethical Committee approval was required. At the time of slaughter, the age of each horse was recorded.

The cadaver heads underwent TMJ palpation, CT examination, and tissue sample collection for histopathological assessment. Results from the TMJ palpation, CT examination, and histopathological assessment were used for joint inclusion/exclusion and data grouping. The eligibility criteria required the presence of a macroscopically intact joint capsule, a complete CT scan of the TMJ, and complete histopathological slides without signs of autolysis. The exclusion criteria included the presence of a wound in the orbit area, the joint capsule disruption, fracture of the coronoid process, mandibular condyle or

temporal bone, TMJ luxation, the presence of dentigerous cysts in the TMJ area, radiological signs of TMJ neoplasia, and missing or poor–quality tissue samples on histopathology slides.

2.2 CT image collection and analysis

CT examination was performed using a multi–slice scanner (Revolution CT, 64–slice, GE Healthcare). Cadaver heads were scanned in ventral recumbency with the following acquisition parameters: helical scan mode, current 275 mA; voltage range 70–140 kV using GSI–QC mode (Dual Energy); gantry rotation 0.08/s/HE+; table travel 39.4 mm/rotation; pitch 0.984:1; slice thickness 2.5 mm. The scan length was adjusted from the rostral aspect of the lips to the caudal aspect of the occipital bone, with the number of slices tailored to the size of each head. The CT scans were processed using the AW workstation (GE Healthcare) and VolumeShare 7 software (GE Healthcare) to generated a detailed reconstruction with a mono voltage of 70 keV and a slice thickness of 0.625 mm. The reconstructed CT images were saved in DICOM format and reviewed in both bone (window ±1500, level 300) and soft tissue (window ±300, level 50) windows using multiplanar reconstruction. The images were independently, simultaneously, and randomly reviewed using Osirix MD software version 12.0 (Pixmeo SARL) by Polish board–certified veterinary diagnostic imaging (PCVDI) radiologists (T.J.) and (M.D.). Both observers were blinded to the horse data and histopathological findings. For statistical analysis, any disagreements were resolved by a Polish board–certified equine disease specialist (PCED) (B.T.), and the final assessment was considered.

A CT findings extraction template, supported by reference images from existing studies, [5–7,14,15,20] was prepared to assist in the CT images review. The template, available in Table 1, records the presence (1) or absence (0) of the CT findings for each joint including bone surface changes (flattening / irregularity – irregular subchondral bone contour), subchondral bone changes (scattered, spherical or linear regions of hypodensity / with or without surrounding hyperdensity (sclerosis)), bone margins changes (enthesiophytes / marginal osteophytes), and joint space and soft tissue changes (narrowing / widening / hyperdensity). The localization of specific CT findings to the mandibular condyle, zygomatic process of temporal bone, intra–articular disc, and soft tissue was also recorded.

Irregularity was defined as irregular subchondral bone contour, while subchondral bone cysts were categorized into two groups: those with surrounding hyperdensity (sclerosis) and those with little to no surrounding hyperdensity (sclerosis).[20] For both cyst groups, communication with joint space (open or not open) was noted.[20] Enthesiophytes were defined as irregular medial margin of the mandibular condyle at the insertion of the lateral pterygoid muscle,[20] while osteophytes referred to irregular lateral or rostral margins of the mandibular condyle or zygomatic process of temporal bone.[16,20] Scattered and spherical regions of hypodensity (with or without surrounding hyperdensity), linear regions of hypodensity (mandibular cleft), enthesiophytes on the medial aspect of the mandibular condyle, and regions of hypodensity in the intra–articular disc (mineralization of the intra–articular disc) were considered as potential 'CT anatomical variations'[20] and were marked in tables with asterisks.

2.3 Tissue samples collection and analysis

From each joint, four tissue samples were collected: the articular cartilage of the mandibular condyle, the articular cartilage of the zygomatic process of the temporal bone, the intra–articular disc, and the joint capsule including the synovium. The samples were immersed in 10% neutral buffered formalin (NBF solution, Sigma–Aldrich) and fixed for 48 hours at room temperature. Samples were then embedded in paraffin (Paraffin wax, Sigma–Aldrich) for standard histological staining using a hematoxylin–eosin (HE) protocol (hematoxylin, 3801520E, Leica; eosin, HT1103128; Sigma–Aldrich). The HE–stained slides were scanned using a Pannoramic 250 Flash III scanner (3DHistech) and evaluated with Pannoramic Viewer version 1.15.4 (3DHistech, magnification 40×–400×). The slides were reviewed independently, simultaneously, and randomly by two observers (Ł.Z. and M.D.) – both holding Master's degree in animal science and trained in histopathology assessment. Both observers were blinded to the horse data and CT findings. Any disagreements were resolved by a third party (B.P.), and the final assessment was considered.

To assist with the review of HE–stained slides, histopathological findings of TMJ OA were extracted from existing studies. [5,14,27,28,31,32] The presence (1) or absence (0) of histopathological findings was recorded for each joint for the articular cartilage of the mandibular condyle and temporal bone, the intra–articular disc, and the joint capsule. For the articular cartilage, cartilage degeneration, chondrocyte apoptosis, chondrocyte hypertrophy, endochondral ossification, and the presence of chondrones, inflammatory cells, and angiogenesis were scored. For the intra–articular disc, chondroid fibrosis, chondroid metaplasia, chondro–osseous metaplasia, and the presence of inflammatory cells were scored. In the joint capsule, inflammatory signs such as intimal hyperplasia, subintimal edema, subintimal fibrosis and the presence of inflammatory cells and angiogenesis were also scored.

2.4 Statistical analysis

The selection and execution of statistical tests were performed by two observers (Ł.Z. and M.D.), both of whom hold Master's degrees in animal science and have completed training in biostatistics. Data sets were prepared using commercially available software Microsoft Excel (Microsoft Excel 2020, Microsoft Corp) and statistical analysis was carried out using GraphPad Prism software (Version 6, GraphPad Software Inc.).

2.4.1 CT and histopathological findings of TMJ OA across age groups

The horse heads and thus all TMJs, regardless of the presence/absence of CT and histopathological findings of TMJ OA, were grouped based on age records. Consequently, three age–related groups were created: (1) 1–4 years group, (2) 5–15 years group, and (3) >15 years group. Descriptive statistics (median, range, and percentage) of age and number of affected joints were calculated for each age–related group.

For each age–related group, the frequency distribution of occurrence of CT findings and histopathological findings was calculated separately. Then, the frequency distribution was compared between age–related groups using a Chi–square test. Differences were considered significant for $P < 0.05$.

Additionally, the correlation between the horse's age and the occurrence of CT and histopathological findings was calculated separately. The Spearman's rank correlation coefficient (ρ) was used because the CT

and histopathological findings represented by the binary data. Correlations were considered significant for $P < 0.05$. The strength of the correlations was interpreted as weak (≤ 0.40), moderate (0.41–0.70), strong (0.71–0.90), or very strong (≥ 0.91). The direction of the correlation was interpreted as positive (> 0) or negative (< 0).[36]

2.4.2 Correlation between CT and histopathological findings of TMJ OA

For all TMJs, with no additional grouping, the correlation between the CT and histopathological findings was then calculated. The Spearman's rank correlation coefficient (ρ) was used because the CT and histopathological findings represented by the binary data. Correlations were considered significant for $P < 0.05$. The strength of the correlations was interpreted as weak (≤ 0.40), moderate (0.41–0.70), strong (0.71–0.90), or very strong (≥ 0.91). The direction of the correlation was interpreted as positive (> 0) or negative (< 0).[36]

2.4.3 Accuracy of CT in diagnosing TMJ OA compared to histopathology

TMJs were grouped into the comparison group (TMJs with OA) and control group (TMJs without OA) based on the histopathological findings. The criterion for confirming TMJ OA was the presence of inflammatory changes in the joint capsule, including presence of inflammatory cells, edema or fibrosis.[1,22] The comparison group – including the histopathologically confirmed TMJ OA – met the criterion of the joint capsule inflammation, regardless of other histopathological and CT findings. The control group – including the histopathologically confirmed OA–free TMJs – met the lack of the joint capsule inflammation criterion, regardless of other histopathological and CT findings. Consequently, the TMJ was classified as normal if the articular cartilage degeneration was present without inflammatory signs.

For each TMJ OA–related group, the frequency distribution of occurrence of CT findings was calculated. Then, the frequency distribution was compared between the comparison and control group using a Chi–square test. Differences were considered significant for $P < 0.05$.

Finally, the accuracy of CT–based recognition of TMJ OA was calculated for a total data set (all TMJs) and each age–related group, separately. The histopathologically confirmed TMJ OA (the comparison group) was used as a reference. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were estimated were calculated across a range from 0.1 to 1.0 using standard formulae.[37] Additionally, the area under curve (AUC) represented area under the ROC (receiver operating characteristic) curve was calculated. The confidence interval (95% CI) was calculated for Se, SP, and AUC.

3 RESULTS

Of the 50 equine cadaver heads (100 TMJs) collected, nine heads were excluded due to various reasons: wound penetrating into the TMJ (n = 2), disruption of the joint capsule (n = 4), fracture of the mandibular condyle (n = 1), and missing of the intra–articular disc sample in the histopathology slides (n = 2). A total of 41 heads (82 TMJs) met the inclusion criteria. These included 23 females and 18 males, all of which were warmblood horses. The median age for the entire horses was 11 years (range 1–25). The age, gender, and

sample size for each age category were as follows: (1) 1–4 years (horses n = 17; TMJs n = 34; median age of 2 years (range 1–4); 6 females, 11 males), (2) 5–15 years (horses n = 12; TMJs n = 24; median age of 11.5 years (range 7–15); 8 females, 4 males), and >15 years (horses n = 12; TMJs n = 24; median age of 20 years (range 16–25); 9 females, 3 males).

3.1 CT findings of TMJ OA across age groups

CT findings of TMJ OA were detected in 79% of joints. These findings were partially dichotomously associated with age, with significant differences in the frequency distribution of CT findings between 1–4 years group and 5–15 years group ($P < 0.0001$) as well as between 1–4 years group and >15 years group ($P < 0.0001$). However, no significant difference between 5–15 years group and >15 years group ($P = 0.84$) was observed. A summary of the CT findings is shown in Table 2.

In 1–4 years group, TMJ OA was radiologically recognized 50% of joints. In this age–related group, regions of hypodensity in the subchondral bone of the mandibular condyle, both scattered without surrounding sclerosis (54% of joints) and spherical with surrounding sclerosis (9% of joints) were observed, along with irregularity of the bone surface of the mandibular condyle only in 6% of joints. Spherical regions of hypodensity in two joints were noted without intra–articular communication, and in one joint with communication (Figure 1). In this age–related group, 6% of the entire CT findings were limited to the right joint, 9% to the left joint, and 35% were bilateral.

In 5–15 years group, TMJ OA was radiologically recognized in 100% of joints. In this age–related group, spherical regions of hypodensity in the mandibular condyle were found in 29% of joints with surrounding sclerosis, and in 13% of joints without. Flattening (75% of joints) and irregularity (42% of joints) of the mandibular condyle were also observed. Spherical regions of hypodensity in seven joints were noted without an intra–articular communication, and in three joints with communication. Medial enthesiophytes (100% of joints) and lateral osteophytes (4% of joints) were observed on the mandibular condyle margins, and joint space narrowing was detected (21% of joints) (Figure 2). All findings were bilateral.

In >15 years group, TMJ OA was radiologically recognized in 100% of joints. In this age–related group, spherical regions of hypodensity in the mandibular condyle were found in 29% of joints without surrounding sclerosis and in 8% of joints without. Flattening (100% of joints) and irregularity (33% of joints) of the mandibular condyle were observed. Spherical regions of hypodensity in five joints demonstrated intra–articular communication; in four joints, they did not. Medial enthesiophytes were noted in all joints, and joint space narrowing was detected in 21% of joints (Figure 3). All findings were bilateral.

Considering significant correlations between horses' age and CT findings across all studied joints, all age–correlated CT findings were observed in the mandibular condyle. A strong positive correlation was found between age and its flattening as well as between age and medial enthesiophytes on its margin. A moderate negative correlation was found between age and scattered hypodensity without surrounding sclerosis. A weak positive correlation was noted between age and irregularity of the mandibular condyle as well as age and spherical regions of hypodensity with surrounding sclerosis.

3.2 Histopathological findings of TMJ OA across age groups

Histopathological findings of TMJ OA were detected in 50% of joints and showed a clear dichotomous age–related association. The frequency distribution of histopathological findings differed between 1–4 years group and 5–15 years group ($P < 0.0001$), 1–4 years group and >15 years group ($P < 0.0001$), as well as 5–15 years group and >15 years group ($P = 0.012$). A summary of histopathological findings is shown in Table 3.

In 1–4 years group, TMJ OA was histopathologically confirmed in 18% of joints, with inflammatory cells present in joint capsule in all cases (Figure 4). Of those horses, 3% of findings were limited to the right joint and 3% to the left joint, while 12% were bilateral.

In 5–15 years group, TMJ OA was histopathologically confirmed in 63% of joints. The diagnosis was based on the following findings in the joint capsule: inflammatory cells (58% of joints), angiogenesis (38% of joints), subintimal edema (25% of joints), subintimal fibrosis (13% of joints), and intimal hyperplasia (8% of joints). Additionally, degeneration of the articular cartilage was observed in 83% of mandibular condyle samples and 71% of temporal bone samples. Chondrocyte apoptosis was noted in 54% of mandibular condyle samples and 38% of temporal bone samples (Figure 5). Histopathological findings were bilateral in 58% of joints, and in 4% were limited to the left joint.

In >15 years group, TMJ OA was histopathologically confirmed in 83% of joints. This was based on a combination of findings in the joint capsule, including the presence of inflammatory cells (71% of joints), subintimal edema (54% of joints), angiogenesis (42% of joints), and subintimal fibrosis (13% of joints). Additionally, articular cartilage degeneration was observed in all mandibular condyle samples and 92% of temporal bone samples, with chondrocyte apoptosis seen in 42% of mandibular condyle samples and 46% of temporal bone samples (Figure 6). Bilateral histopathological findings were present in 75% of joints, and 8% were limited to the right joint.

Considering significant correlations between horses' age and histopathological findings across all studied joints, a strong positive correlation was found between age and cartilage degeneration both in the mandibular condyle and in the temporal bone. A moderate positive correlation was found between age and chondrocyte apoptosis – similarly both in the mandibular condyle and in the temporal bone. A moderate positive correlation was also noted between age histopathological findings observed in the joint capsule – such as subintimal fibrosis, presence of the inflammatory cells, and angiogenesis. A weak positive correlation was noted between age and presence of chondrones in the mandibular condyle as well as age and angiogenesis in the temporal bone.

3.3 Correlation between CT and histopathological findings of TMJ OA

Considering the significant correlations between CT and histopathological findings across all studied joints, very strong and strong positive correlations were observed between flattening of the mandibular condyle and articular cartilage degeneration in both the mandibular condyle and the temporal bone, as well as between medial enthesophyte and articular cartilage degeneration in both the mandibular condyle and the temporal bone. Moderate positive correlations were noted between flattening of the mandibulat condyle and

chondrocyte apoptosis in both the mandibular condyle and the temporal bone, as well as between medial enthesophyte and articular cartilage degeneration in both the mandibular condyle and the temporal bone. Week to moderate positive correlations were observed between irregularity of the bone surface of the mandibular condyle and spherical regions of hypodensity with surrounding sclerosis in the mandibular condyle in relation to cartilages degeneration and apoptosis in both articular cartilages. Conversely, week to moderate negative correlations were found between scattered regions of hypodensity without surrounding sclerosis in the mandibular condyle in relation to both articular cartilage degeneration and apoptosis, as well as inflammatory cell infiltration and angiogenesis in the joint capsule. Additionally, week to moderate positive correlations were observed between specific signs of joint capsule inflammation and CT findings in the mandibular condyle such as flattening, irregularity, spherical regions of hypodensity with surrounding sclerosis, and medial enthesophyte. A summary of all Spearman correlation coefficients for the total of 82 joints is shown in Table 4, while corresponding summaries for subsequent age categories are provided in supplementary Table S1–S3.

3.4 Accuracy of CT in diagnosing TMJ OA compared to histopathology

CT findings were noted in 95% of TMJs with histopathologically confirmed OA and in 63% of TMJs histopathologically confirmed as OA–free showing significant differences in the frequency distribution of CT findings between the comparison and control group ($P < 0.0001$). A summary of the CT findings is shown in Table 5.

In the comparison group, flattening (78% of joints) and irregularity (44% of joints) of the mandibular condyle were observed. In the subchondral bone of the mandibular condyle, regions of hypodensity were noted – predominately spherical with surrounding sclerosis (29% of joints), but also spherical (7% of joints) and scattered (5% of joints) without surrounding sclerosis. Medial enthesiophytes (85% of joints) and lateral osteophytes (2% of joints) were observed on the mandibular condyle margins, and joint space narrowing (12% of joints) was detected.

In the control group, flattening (24% of joints) and irregularity (5% of joints) of the mandibular condyle were also noted. In the subchondral bone of the mandibular condyle, regions of hypodensity were noted – predominately scattered without surrounding sclerosis (24% of joints), but also spherical without (12% of joints) and with surrounding sclerosis (5% of joints). Medial enthesiophytes of the mandibular condyle (32% of joints) and joint space narrowing (7% of joints) were also observed.

Considering all studied TMJ, 39 of 65 (60%) joints diagnosed with TMJ OA based on the CT findings were histopathologically confirmed, resulting in 0.79 sensitivity and 0.50 specificity of CT–based diagnosis. In 1–4 years group, only 4 of 17 (24%) joints diagnosed based on the CT findings were histopathologically confirmed, resulting in 0.50 sensitivity and 0.82 specificity. In 5–15 years group, 15 of 24 (58%) CT–based OA diagnoses were histopathologically confirmed, resulting in 1.00 sensitivity and 0.37 specificity. In >15 years group, as many as only 20 of 24 (83%) CT–based OA diagnoses were histopathologically confirmed, resulting in 1.00 sensitivity but 0.17 specificity. In all groups – a total TMJ

data set and subsequent age–related groups – AUC was comparable ranging from 0.58 for >15 years group to 0.69 for 5–15 years group. A summary of the accuracy metrics is shown in Table 6.

4 DISCUSSION

The accuracy of CT–based diagnosis of equine TMJ OA is moderate when all CT findings are considered and varies among age–related groups. Sensitivity of CT–based diagnosis was lowest in the 1–4 years group; however, specificity was highest, and PPV was moderate. This indicates the presence of many true positive and false positive results as well as some false negatives. This may be due to the inclusion in CT assessment of the scattered regions of hypodensity in the mandibular condyle, which have previously been suggested as 'CT anatomical variations'.[20] These scattered hypodense regions occurred only in the 1–4 years group and showed a moderate negative correlation with horse age, as well as weak to moderate negative correlations with cartilage degeneration, chondrocyte apoptosis and changes in the joint capsule – such as inflammatory infiltration and angiogenesis. However, this CT finding has not yet been described in any other study that also reported clinical sympthoms.

In contrast, sensitivity of CT–based diagnosis was highest in the 5–15 and >15 years groups, while specificity decreased with increasing age. This indicates a lack of false negative results but the inclusion of many false positive results. This may be related to the inclusion in CT assessment of flattening of mandibular condyle and the presence of medial enthesiophytes on the mandibular condyle, which have also been suggested as 'CT anatomical variations'.[20] These CT findings were strongly positively correlated with horse age and articular cartilage degeneration, as well as moderately positively correlated with chondrocyte apoptosis and inflammatory signs in the joint capsule. In case of potential overdiagnosis, less attention appears to have been given to the presence of the spherical regions of hypodensity in the mandibular condyle with surrounding sclerosis in the mandibular condyle. These potential 'CT anatomical variations'[20] showed a moderate positive correlation with horse age, cartilage degeneration, and chondrocyte apoptosis. However, such subchondral bone cysts have also been reported as clinically significant and have been surgically treated with long–term follow–up.[15] Finally, another potential 'CT anatomical variations'[20] – mineralization of the intra–articular disc – was not observed in this study, although it has been suggested to be clinically significant in previous studies.[5,6] These findings suggest that greater attention should be given to such CT findings in future studies addressing both age–related and OA–related changes in equine TMJ. Therefore, the author's hypothesis – that the accuracy of CT–based diagnosis of TMJ OA varies with age, likely due to the incorporation of CT findings regarded as 'CT anatomical variations'[20] – is supported.

CT study of the equine TMJ has shown that CT findings related to age and/or OA are relatively common; however, their pathological significance remains unclear.[20] To date, only five case reports have simultaneously described radiologic and histopathological findings in individual horses: two with TMJ OA,[5,14] one with septic TMJ arthritis,[38] one with squamous cell carcinoma,[39] and one with a dentigerous cyst.[40] Moreover, among research studies that have examined equine TMJ tissues histopathologically, only one specifically addressed TMJ OA, although without incorporation CT imaging.[31] The remaining four studies described normal articular cartilage[27,30,32,41] and the intra–articular disc,[31] while only two linking

their findings with micro–CT (μCT).[32,41] A few additional case reports[6,7] and research studies[15,18,20,34] have addressed CT imaging of both normal[20,34] and OA–affected[6,7,15,18,20] TMJs; however, they did not include histopathological analysis. Therefore, to the best of the authors' knowledge, this is the first cross–sectional study to investigate the correlation between CT and histopathological findings in equine TMJ OA.

In the first of these two case reports, Pimentel et al.[14] described unilateral TMJ dysplasia with osteochondrosis–like lesions and associated OA in 15–month–old filly. CT findings included flattening of the mandibular condyle, osseous fragments within the joint space, medial osteophytes (referred to as enthesophytes)[20], and mineralization of the intra–articular disc. Histopathology revealed foci of necrosis, chondrocyte hypertrophy, endochondral ossificatio, multiple cyst–like defects, and fibrosis in the articular cartilage of the mandibular condyle.[14] In the second case reports, Smyth et al.[5] described an 18–year–old mare with unilateral CT findings and bilateral histopathological evidence of TMJ OA. CT findings included subchondral bone sclerosis of the mandibular condyle, medial osteophytes of the mandibular condyles (referred to as enthesophytes)[20], lateral osteophytes of the temporal bone, and mineralization of the intra–articular disc. Histopathological analysis revealed cartilage degeneration and chondrones in both the mandidular condyle and temporal bone, as well as chondroid and chondro–osseous metaplasia in the intra–articular disc.[5]

In the present cross–sectional study, flattening and medial enthesiophytes of the mandibular condyle were strongly positively correlated with articular cartilage degeneration and moderately positively correlated with chondrocyte apoptosis and chondrones in the articular cartilage, as well as inflammatory changes in the joint capsule. However, these findings were observed only in horses older than 5 years, similar to the older mare report by Smyth et al.[5], and not in younger filly described by Pimentel et al.[14] Additionally, in the current study, lateral osteophytes were found on the mandibular condyle, whereas Smyth et al.[5] reported them on the temporal bone. The lateral osteophytes demonstrated only a weak positive correlation with subintimal edema in the joint capsule. Other findings described by Pimentel et al.[14] – apart from mineralization of the intra–articular disc – may have been related to the osteochondrosis observed in that filly, but none of these were identified in the present study. The results obtained in this study support the hypothesis that certain CT and histopathological findings are correlated in TMJ OA, helping to bridge the current gap in understanding the pathophysiology of TMJ OA.

Similar CT findings involving the mandibular condyle, intra–articular disc, and occasionally the temporal bone have been reported in other cases of TMJ OA, although these were not corroborated by histopathological analysis. Findings such as flattening[14] and irregularity[15] of the mandibular condyle, subchondral bone cysts (spherical region of hypodensity with surrounding hyperdensity) in the mandibular condyle,[6,7,15] narrowing or widening of the joint space[15], and mineralization of the intra–articular disc[5,6] have been observed in horses with clinical symptoms of TMJ dysfunction. However, many of these CT findings have also been described as age–related 'CT anatomical variation' in horses without moderate to severe clinical symptoms of TMJ dysfunction.[20]

Among the 'CT anatomical variation', the occurrence of the scattered regions of hypodensity in the mandibular condyle decreased with increasing age, whereas the subchondral bone cysts, flattening, and

medial enthesiophytes on the mandibular condyle increased with age.[20] Our findings confirm those of Carmalt et al.,[20] demonstrating corresponding negative and positive correlations, respectively. Furthermore, CT findings typical of younger horses were moderately negatively correlated with cartilage degeneration, chondrocyte apoptosis and signs of joint capsule inflammation. These features do not co–occurred with TMJ OA and are unlikely to contribute to the disease process. Similarly, some histopathological findings have been described in clinically normal horses of various ages.[27] Based on the total TMJ score (a sum of histopathological findings), horses under 10 years of age showed fewer histopathological changes in both the articular cartilages and intra–articular disc compared to horses over 20 years of age. However, specific histopathological findings – particularly inflammatory changes in the joint capsule – were not reported.[27] Age–related differences in the appearance of articular cartilage, as described by Smyth et al.,[27] were well illustrated but not specifically described by Mirahmadi et al.[32]. Mirahmadi et al.[32] demonstrated that, with age, the articular cartilage of the mandibular condyle becomes thinner, while the content of collagen, glycosaminoglycan, and pentosidine increases, and water content decreases. Therefore, the structural changes occurring in the aging articular cartilage of the mandibular condyle differ from those seen in hyaline cartilage.[32] Because articular cartilage is incapable to completely regeneration, damage accumulates over time and becomes more frequent in older horses.[27] However, these studies evaluated CT[20] and histopathological[5,32] findings independently, without reference and direct correlation. Only Guerrero Cota et al.[31] used gross anatomical evidence as a reference to assess the presence and severity of OA; while in most previous studies, gross examination of TMJs was not performed.[5,20,32] The results obtained in present study support the hypothesis that both CT and histopathological findings exhibit age–related patterns and are correlated with the horse's age.

One limitation of this study is that gross examination of the TMJs was not performed. However, the reference standard for confirming TMJ OA was based on the inflammatory changes leading to synovitis and capsulitis.[1,28] A second limitation is the absence of clinical data for the horses whose heads were examined. Because the heads were collected post–mortem from the slaughterhouse, clinical symptoms could not be assessed. Similarly, in the study by Carmalt et al.'s[20] involving a large cohort of asymptomatic horses, follow–up data and information regarding behavior during eating or riding were unavailable, making it difficult to definitively fully exclude the presence of mild symptoms of TMJ dysfunction. The authors suggested that some horses with 'CT anatomical variations' may have had subclinical TMJ disease without exhibiting moderate or severe clinical symptoms of pain or dysphagia.[20] Under certain conditions,[6,7] such horses may display aversive behaviours, reduced performance, or mild masticatory problems. Experimental study has demonstrated that horses with TMJ pain induced by inflammation can modify their eating habits to maintain body weight and condition.[42] Other experimental study has shown that horses with acute TMJ inflammation tend to avoid bit pressure on the affected side; however, handlers may counterbalance this by increasing rein tension on the unaffected side, thereby masking objective signs of asymmetry.[43] Additionally, acute inflammation response differs between the TMJ and peripheral joints,[44] suggesting that the equine TMJ may be less sensitive to pain,[45] likely due to its distinct immune response.[44] This could also explain the limited number of publications on TMJ OA associated with mild clinical symptoms, such as

yawning when seeing the bridle,[7] standing at rest with the tongue protruded,[6] and aversive behaviour during riding.[5–7]

Given the subtle clinical symptoms, it has been postulated that TMJ OA cases may be clinically missed or misdiagnosed.[5–7] In those studies, TMJ OA was confirmed radiologically using CT,[5–7] consequently mild clinical symptoms were linked with CT findings. However, this hypothesis is not supported by the results of current study. Although, the sensitivity and specificity of CT for diagnosing TMJ OA were moderate, the high number of false positive results indicates that many CT findings suggestive of TMJ OA are not corroborated histopathology, introducing a potential bias toward overdiagnosis rather than underdiagnosis. This was particularly evident in the two age–related groups representing horses over 5 years of age, in which no false negative CT–based diagnoses were found. Only in the 1–4 year–old group were there cases of CT–based underdiagnosis accompanied by histopathologically confirmed inflammatory changes in the joint capsule.

Unfortunately, histopathology – which provides definitive evidence of degeneration of the articular cartilage and intra–articular disc[5] – requires post–mortem sampling, limiting its clinical application. A potential clinical alternative may be synovial fluid analysis, as synovial fluid can be collected from live horse via arthrocentesis, often during the same procedure as local analgesia[6,7,13] and intra–articular medication.[5,7] Synovial fluid analysis has recently been employed in both research studies [44–47] to investigate the mechanisms of TMJ OA and in clinical cases[3,5,8,10,48] to support the identification of underlying TMJ disease. However, the correlation between histopathological findings and inflammatory cytokine concentrations has not yet been investigated.[27] Therefore, further clinical studies should focus on a broader range of TMJ OA biomarkers[28] to better elucidate the relevance of histopathological findings and support clinical trials targeting mild clinical symptoms. It is hope that this study, together and future research, will help address the question posed by Carmalt et al.[20] “When do CT findings become evidence of TMJ disease in an apparently clinically normal horse?”

In conclusion, the results of this study indicate that the equine TMJ may undergo age–related and disease–related remodeling, detectable through both CT and histopathological findings. Scattered regions of hypodensity in the mandibular condyle were specific for young horses and negatively correlated with inflammatory signs in the joint capsule. In contrast, irregularity, flattening, and medial enthesiophytes in the mandibular condyle were specific to middle–aged and older horses and were positively correlated with articular cartilage degeneration, chondrocyte apoptosis, and chondrones in the articular cartilage, as well as inflammatory signs in the joint capsule. Since some of these CT findings are considered 'CT anatomical variations', they may affects the sensitivity and specificity of CT–based TMJ OA diagnosis, which is moderate if all CT findings are considered. However, the clinical significance of these results remains unknown.

REFERENCES

1. Balducci J, Ruby J, Hall C, Williams J. Arthrotomy, curettage and medical management of septic arthritis and osteomyelitis of the temporomandibular joint in a horse. *Equine Vet Educ.* 2021;33:e5–e11. https://doi.org/10.1111/eve.13156
2. Reisbig NA, Pifko J, Lanovaz JL, Weishaupt MA, Carmalt JL. The effect of acute equine temporomandibular joint inflammation on response to rein–tension and kinematics. *Front Vet Sci.* 2023;10:1213423. https://doi.org/10.3389/fvets.2023.1213423
3. Carmalt JL. Equine poor performance: the logical, progressive, diagnostic approach to determining the role of the temporomandibular joint. *J Am Vet Med Assoc.* 2024;262:397–404. https://doi.org/10.2460/javma.23.09.0513
4. Bonin SJ, Clayton HM, Lanovaz JL, Johnson TJ. Kinematics of the equine temporomandibular joint. *Am J Vet Res.* 2006;67:423–428. https://doi.org/10.2460/ajvr.67.3.423
5. Smyth T, Allen AL., Carmalt JL. Clinically significant, nontraumatic, degenerative joint disease of the temporomandibular joints in a horse. *Equine Vet Educ.* 2017;29:72–77. https://doi.org/10.1111/eve.12382
6. Carmalt JL, Reisbig NA. Arthroscopic treatment of bilateral mandibular condylar cysts and associated osteoarthritis of the temporomandibular joints in a horse. *Equine Vet Educ.* 2022;34:e352–358. https://doi.org/10.1111/eve.13602
7. Jørgensen E, Christophersen MT, Kristoffersen M, Puchalski S, Verwilghen D. Does temporomandibular joint pathology affect performance in an equine athlete? *Equine Vet Educ.* 2015;27:126–130. https://doi.org/10.1111/eve.12268
8. Frietman SK, van Proosdij ER, Veraa S, de Heer N, Ter Braake F. A minimally invasive partial condylectomy and temporal bone resection for the treatment of a suspected chronic synovial sepsis of the temporomandibular joint in a 3.5–year–old paint horse gelding. *Vet Q.* 2018;38:118–124. https://doi.org/10.1080/01652176.2018.1535216
9. Lean NE, Young A, Ahern BJ. Fusobacterium necrophorum septic arthritis of the temporomandibular joint in an Australian Stockhorse mare. *Equine Vet Educ.* 2021;33:e429–432. https://doi.org/10.1111/eve.13362
10. Elzer EJ, Wulster KB, Richardson DW, Ortved KF. Standing arthroscopic treatment of temporomandibular joint sepsis in a horse. *J Vet Dent.* 2020;37:94–99. https://doi.org/10.1177/0898756420948269
11. Sanders RE, Schumacher J, Brama PAJ, Zarelli M, Kearney CM. Mandibular condylectomy in a standing horse for treatment for osteoarthritis of the temporomandibular joint. *Equine Vet Educ.* 2014;26:624–628. https://doi.org/10.1111/eve.12235
12. Luedke LK, Nixon AJ, Whitty JA, Irby NL, Ducharme NG. Bilateral rostral temporomandibular luxation with bilateral coronoid fracture in a Welsh pony. *Vet Rec Case Rep.* 2020;8:e000785. https://doi.org/10.1136/vetreccr–2018–000785

13. Souza APA, Torres RVS, Oliveira CJ, Silva FWS, Franco FA, Florence CO, Cabral JPMS. Reduction of temporomandibular joint (TMJ) luxation in a horse–case report. *Arq Bras Med Vet Zootec.* 2023;75:947–951. https://doi.org/10.1590/1678–4162–12985

14. Pimentel KL, Allen AL, Carmalt JL. Developmental orthopaedic disease and early osteoarthritis of the temporomandibular joint in a 15-month-old Quarter Horse filly. *Equine Vet Educ.* 2022;34:e203–210. https://doi.org/10.1111/eve.13578

15. White SA, Carmalt JL, Schumacher J, Amitrano FN, Ortved K, Henry TJ, Arnold CE. Long–Term Outcome of Horses Undergoing Unilateral Mandibular Condylectomy and Meniscectomy for Temporomandibular Joint Disease. *Front Vet Sci.* 2022;9:898096.

16. Magri C, Schramme M, Nottrott K, Schweizer-Gorgas D, Segard E, Kelly G, Schumacher J. Coronoidectomy as a treatment for mandibular immobility caused by fracture of the coronoid process in three horses. *Equine Vet Educ.* 2021;33:e331–337. https://doi.org/10.1111/eve.13396

17. Barnett TP, Powell SE, Head MJ, Marr CM, Steven WN, Payne RJ. Partial mandibular condylectomy and temporal bone resection for chronic, destructive, septic arthritis of the temporomandibular joint in a horse. *Equine Vet Educ.* 2014;26:59–63. https://doi.org/10.1111/eve.12053

18. Perrier M, Manso-Díaz G, Dunkel B. Computed tomography findings in horses presented with signs of head-shaking. *Equine Vet J.* 2023;55:649–655. https://doi.org/10.1111/evj.13889

19. Witte TH. TMJ pathology: is it real? *Equine Vet Educ.* 2016;28:173–174. http://doi.org/10.1111/eve.12340

20. Carmalt JL, Kneissl S, Rawlinson JE, Zwick T, Zekas L, Ohlerth S, Bienert-Zeit A. Computed tomographic appearance of the temporomandibular joint in 1018 asymptomatic horses: a multi-institution study. *Vet Radiol Ultrasound.* 2016;57:237–245. https://doi.org/10.1111/vru.12334

21. Crijns CP, Weller R, Vlaminck L, Verschooten F, Schauvliege S, Powell SE, Gielen IMVL. Comparison between radiography and computed tomography for diagnosis of equine skull fractures. *Equine Vet Educ.* 2019;31:543–550. https://doi.org/10.1111/eve.12863

22. Ramzan PHL, Marr CM, Meehan J, Thompson A. Novel oblique radiographic projection of the temporomandibular articulation of horses. *Vet Rec.* 2008;162:714–716. https://doi.org/10.1136/vr.162.22.714

23. Ebling AJ, McKnight AL, Seiler G, Kircher PR. A complementary radiographic projection of the equine temporomandibular joint. *Vet Radiol Ultrasound.* 2009;50:385–391. https://doi.org/10.1111/j.1740–8261.2009.01554.x

24. Townsend NB, Cotton JC, Barakzai SZ. A tangential radiographic projection for investigation of the equine temporomandibular joint. *Vet Surg.* 2009;38:601–606. https://doi.org/10.1111/j.1532–950X.2009.00536.x

25. Brounts SH, Henry T, Lund JR, Whitton RC, Ergun DL, Muir P. Use of a novel helical fan beam imaging system for computed tomography of the head and neck in sedated standing horses: 120 cases (2019–2020). *JAVMA* 2022;260:1361–1368. https://doi.org/10.2460/javma.21.10.0471

26. Stieger–Vanegas SM, Hanna AL. The role of computed tomography in imaging non–neurologic disorders of the head in equine patients. *Front Vet Sci.* 2022;9:798216. https://doi.org/10.3389/fvets.2022.798216

27. Smyth TT, Allen AL, Carmalt JL. Histologic assessment of age–related changes in the temporomandibular joints of horses. *Am J Vet Res.* 2019;80:1107–1113. https://doi.org/10.2460/ajvr.80.12.1107

28. Jasiński T, Turek B, Kaczorowski M, Brehm W, Skierbiszewska K, Bonecka J, Domino M. Equine Models of Temporomandibular Joint Osteoarthritis: A Review of Feasibility, Biomarkers, and Molecular Signaling. *Biomedicines* 2024;12:542. https://doi.org/10.3390/biomedicines12030542

29. Bertone AL, Bramlage LR, McIlwraith CW, Malemud CL. Comparison of proteoglycan and collagen in articular cartilage of horses with naturally developing osteochondrosis and healing osteochondral fragments of experimentally induced fractures. *Am J Vet Res.* 2005;66:1881–1890. https://doi.org/10.2460/ajvr.2005.66.1881

30. Adams K, Schulz–Kornas E, Arzi B, Failing K, Vogelsberg J, Staszyk C. Functional anatomy of the equine temporomandibular joint: Histological characteristics of the articular surfaces and underlying tissues. *Vet J.* 2018;239:35–41. https://doi.org/10.1016/j.tvjl.2018.08.003

31. Guerrero Cota JMG, Leale DM, Arzi B, Cissell DD. Regional and disease–related differences in properties of the equine temporomandibular joint disc. *J Biomech.* 2019;82:54–61. https://doi.org/10.1016/j.jbiomech.2018.10.017

32. Mirahmadi F, Koolstra JH, Fazaeli S, Lobbezoo F, van Lenthe GH, Snabel J, Everts, V. Aging does not change the compressive stiffness of mandibular condylar cartilage in horses. *Osteoarthr Cartil.* 2018;26:1744–1752. https://doi.org/10.1016/j.joca.2018.08.007

33. Carmalt JL, Tucker ML. Arthroscopic approach and intra-articular anatomy of the equine discomandibular joint compartment of the temporomandibular joint. *Vet Surg.* 2020;49:1326–1333. https://doi.org/10.1111/vsu.13487

34. Rodríguez MJ, Latorre R, Lopez-Albors O, Soler M, Aguirre C, Vázquez J, Agut A. Computed tomographic anatomy of the temporomandibular joint in the young horse. *Equine Vet J.* 2008;40:566–571. https://doi.org/10.2746/042516408X322166

35. Charan J, Kantharia N. How to calculate sample size in animal studies? *J Pharmacol Pharmacother*. 2013;4:303–306. https://doi.org/10.4103/0976–500X.119726

36. Schober P, Boer C, Schwarte LA. Correlation coefficients: appropriate use and interpretation. *Anesth Analg*. 2018;126:1763–1768. https://doi.org/10.1213/ANE.0000000000002864

37. Dohoo I, Martin W, Stryhn H. *Veterinary Epidemiologic Research.* 2nd ed. VER Inc., 2009.

38. Weller R, Taylor S, Maierl J, Cauvin ERJ, May SA. Ultrasonographic anatomy of the equine temporomandibular joint. *Equine Vet J.* 1999;31:529–532. https://doi.org/10.1111/j.2042–3306.1999.tb03863

39. Perrier M, Schwarz T, Gonzalez O, Brounts S. Squamous cell carcinoma invading the right temporomandibular joint in a Belgian mare. *Can Vet J.* 2010;51:885.

40. Howell A, Panizzi L, Simpson E. Surgical treatment of a five-structure dentigerous cyst in a Warmblood filly. *Equine Vet Educ.* 2023;35:e372–377. https://doi.org/10.1111/eve.13746

41. Mirahmadi F, Koolstra JH, Fazaeli S, Lobbezoo F, van Lenthe GH, Snabel J, Everts V. Diffusion of charged and uncharged contrast agents in equine mandibular condylar cartilage is not affected by an increased level of sugar–induced collagen crosslinking. *J Mech Behav Biomed Mater.* 2019;90:133–139. https://doi.org/10.1016/j.jmbbm.2018.10.022

42. Smyth TT, Carmalt JL, Treen TT, Lanovaz JL. The effect of acute unilateral inflammation of the equine temporomandibular joint on the kinematics of mastication. *Equine Vet J.* 2016;48:523–527. https://doi.org/10.1111/evj.12452

43. Reisbig NA, Pifko J, Lanovaz JL, Weishaupt MA, Carmalt JL. The effect of acute equine temporomandibular joint inflammation on response to rein–tension and kinematics. *Front Vet Sci.* 2023;10:1213423. https://doi.org/10.3389/fvets.2023.1213423

44. Carmalt JL, Bell CD, Tatarniuk DM, Suri SS, Singh B, Waldner C. Comparison of the response to experimentally induced short–term inflammation in the temporomandibular and metacarpophalangeal joints of horses. *Am J Vet Res.* 2011;72:1586–1591. https://doi.org/10.2460/ajvr.72.12.1586

45. Pereira TP, Staut FT, Machado TS, Brossi PM, Baccarin RY, Michelotto PV. Effects of the oral examination on the equine temporomandibular joint. *J Equine Vet Sci.* 2016;43:48–54. https://doi.org/10.1016/j.jevs.2016.04.091

46. Norvall A, Guerrero Cota J, Pusterla N, Cissell D. Ultrasound–guided arthrocentesis of the temporomandibular joint in healthy adult horses is equivalent to blind arthrocentesis. *Vet Radiol Ultrasound.* 2020;61:346–352. https://doi.org/10.1111/vru.12836

47. Carmalt JL, Gordon JR, Allen AL. Temporomandibular joint cytokine profiles in the horse. *J Vet Dent.* 2006;23:83–88. https://doi.org/10.1177/08987564060230020

48. Carmalt JL, Wilson DG. Arthroscopic treatment of temporomandibular joint sepsis in a horse. *Vet Surg.* 2005;34:55–58. https://doi.org/10.1111/j.1532–950x.2005.00010.x

Tables

Table 1. Computed tomography (CT) findings of temporomandibular joint osteoarthritis (TMJ OA) localized in the mandibular condyles (MC), the temporal bone (TB), the joint space, and the soft tissue. Each finding is supported by reference[5–7,14,15,20] and figure in referred study.

Bone surface			Subchondral bone			Bone margins			Joint space and soft tissue		
1	Normal Fig. 1[7]	no flattening and irregularity[7]	1	Normal Fig. 1[7]	no hypodensity and hyperdensity[7]	1	Normal Fig. 1[7]	lack of osteophytes and enthesiophytes[7]	1	Normal Fig. 1[7]	no hyperdensity and narrowing/widening[7]
2	Flatt MC* Fig. 3[14]	flattening of MC[14,20]	2	ScHD+Scl MC* Figs. 2[15],7[20]	scattered regions of hypodensity in MC with surrounding hyperdensity[15,20]	2	Medial Ent MC* Fig. 4[20]	enthesiophytes on the medial aspect of MC[20]	2	Narrow JS Fig. 1[15]	narrowing of the joint space[15]
3	Irr MC Fig. 1[15]	irregularity of MC[15,20]	3	ScHD-Scl MC* Fig. 2[20]	scattered regions of hypodensity in MC without surrounding hyperdensity[20]	3	Lateral Ost MC	marginal osteophytes on the lateral aspect of MC[20]	3	Wide JS Fig. 2[15]	widening of the joint space[15]
4	Irr TB	irregularity of TB[20]	4	ScHD+Scl TB* Fig. 2[15]	scattered regions of hypodensity in TB with surrounding hyperdensity[15,20]	4	Rostral Ost MC Fig. 3[14]	marginal osteophytes on the rostral aspect of MC[14]	4	Min Disc* Figs. 1[5], 2[6],8[20]	hyperdensity of the intra–articular disc[5,6,20]
			5	ScHD-Scl TB*	scattered regions of hypodensity in TB without surrounding hyperdensity[20]	5	Lateral Ost TB	marginal osteophytes on the lateral aspect of TB[20]	5	Osseous JS Figs. 1–2[15], 3[14]	hyperdense osseous fragments in the joint space[14,15]
			6	SphHD+Scl MC* (group 1 of cyst) Figs. 2[6], 4[6], 1[7], 1[15], 5–6[20]	spherical region of hypodensity in MC with surrounding hyperdensity [6,7,15,20]				6	Min Soft	hyperdensity of soft tissues of the periarticular region[20]
			7	SphHD-Scl MC* (group 2 of cyst) Fig. 3[20]	spherical region of hypodensity in MC without surrounding hyperdensity[20]						
			8	SphHD+Scl TB* (group 1 of cyst)	spherical region of hypodensity in TB with surrounding hyperdensity[20]						
			9	SphHD-Scl TB* (group 2 of cyst)	spherical region of hypodensity in TB without surrounding hyperdensity[20]						
			10	Cleft MC*	linear region of hypodensity in MC with surrounding hyperdensity[20]						

Note: Cleft = linear region of hypodensity; Disc = intra–articular disc; Ent = enthesiophytes; Flatt = flattening; Irr = irregularity; JS = joint space; MC = mandibular condyle; Min = mineralization (hyperdensity); Ost = osteophytes; ScHD = scattered regions of hypodensity; Scl = sclerosis (surrounding hyperdensity); SphHD = spherical region of hypodensity; Soft = soft tissues; TB = temporal bone; + = with; - = without; * = CT anatomical variations.

Table 2. Summary of CT findings in 41 horses (82 TMJs) with radiologically recognized temporomandibular joint osteoarthritis (TMJ OA) affecting a total of 65/82 (79%) joints.

CT findings	Age–related groups			
	1–4 years (*n* = 34)	**5–15 years (*n* = 24)**	**>15 years (*n* = 24)**	**Correlation (ρ; *P*)**
Bone surface				
Normal	32 (94%)	2 (8%)	0	**ρ = -0.81; *P* < 0.0001**
Flatt MC*	0	18 (75%)	24 (100%)	**ρ = 0.85; *P* < 0.0001**
Irr MC	2 (6%)	10 (42%)	8 (33%)	**ρ = 0.35; *P* = 0.001**
Subchondral bone				
Normal	19 (54%)	14 (58%)	15 (63%)	ρ = 0.10; *P* = 0.38
ScHD-Scl MC*	12 (35%)	0	0	**ρ = -0.51; *P* < 0.0001**
SphHD+Scl MC*	3 (9%)	7 (29%)	7 (29%)	**ρ = 0.36; *P* = 0.001**
SphHD-Scl MC*	0	3 (13%)	2 (8%)	ρ = -0.02; *P* = 0.89
Bone margins				
Normal	34 (100%)	0	0	**ρ = -0.86; *P* < 0.0001**
Medial Ent MC*	0	24 (100%)	24 (100%)	**ρ = 0.86; *P* < 0.0001**
Lateral Ost MC	0	1 (4%)	0	ρ = 0.07; *P* = 0.52
Joint space and soft tissue				
Normal	34 (100%)	19 (79%)	21 (88%)	ρ = -0.17; *P* = 0.12
Narrow JS	0	5 (21%)	3 (13%)	ρ = 0.17; *P* = 0.12
Affected joints	17 (50%)	24 (100%)	24 (100%)	
Chi–square test	*P* < 0.0001	*P* < 0.0001	*P* = 0.84	

Note: CT = computed tomography; Ent = enthesiophytes; Flatt = flattening; Irr = irregularity; JS = joint space; MC = mandibular condyle; *n* = number of affected joints; Ost = osteophytes; ScHD = scattered regions of hypodensity; Scl = sclerosis (surrounding hyperdensity); SphHD = spherical region of hypodensity; % = percentage of affected joints from each age–related group; * = CT anatomical variations. The differences in the frequency distribution of CT findings between age–related groups were considered significant for *P*–value < 0.05. The Spearman correlation coefficient (ρ) was considered significant for *P*–value < 0.05 and then highlighted with bold font.

Table 3. Summary of histopathological findings in 41 horses (82 TMJs) with histopathologically confirmed temporomandibular joint osteoarthritis (TMJ OA) affecting a total of 41/82 (50%) joints.

Histopathological findings	**Age–related groups**			
	1–4 years (*n* = 34)	**5–15 years (*n* = 24)**	**>15 years (*n* = 24)**	**Correlation (ρ; *P*)**
Articular cartilage of the mandibular condyle				
Normal	34 (100%)	3 (13%)	0	**ρ = -0.85; *P* < 0.0001**
Cartilage degeneration	0	20 (83%)	24 (100%)	**ρ = 0.84; *P* < 0.0001**
Chondrocyte apoptosis	0	13 (54%)	10 (42%)	**ρ = 0.45; *P* < 0.0001**
Chondrones	0	0	6 (25%)	**ρ = 0.29; *P* = 0.008**
Angiogenesis	0	3 (13%)	1 (4%)	ρ = 0.14; *P* = 0.20
Articular cartilage of the temporal bone				
Normal	34 (100%)	6 (25%)	2 (8%)	**ρ = -0.80; *P* < 0.0001**
Cartilage degeneration	0	17 (71%)	22 (92%)	**ρ = 0.79; *P* < 0.0001**
Chondrocyte apoptosis	0	9 (38%)	11 (46%)	**ρ = 0.46; *P* < 0.0001**
Angiogenesis	0	0	3 (13%)	**ρ = 0.32; *P* = 0.004**
Intra–articular disc				
Normal	34 (100%)	24 (100%)	24 (100%)	–
Joint capsule				
Normal	27 (79%)	9 (38%)	4 (17%)	**ρ = -0.57; *P* < 0.0001**
Intimal hyperplasia	0	2 (8%)	0	ρ = 0.01; P = 0.90
Subintimal edema	0	6 (25%)	3 (13%)	ρ = 0.21; P = 0.06
Subintimal fibrosis	0	3 (13%)	13 (54%)	**ρ = 0.59; *P* < 0.0001**
Inflammatory cells	6 (18%)	14 (58%)	17 (71%)	**ρ = 0.46; *P* < 0.0001**
Angiogenesis	0	9 (38%)	10 (42%)	**ρ = 0.42; *P* < 0.0001**
Affected joints	6 (18%)	15 (63%)	20 (83%)	
Chi–square test	*P* < 0.0001	*P* < 0.0001	*P* = 0.02	

n = number of affected joints; % = percentage of affected joints from each age–related group. The differences in the frequency distribution of histopathological findings between age–related groups were considered significant for *P*–value < 0.05. The Spearman correlation coefficient (ρ) was considered significant for *P*–value < 0.05 and then highlighted with bold font.

Table 4. The Spearman correlation coefficient (ρ) between CT findings and histopathological findings detected in a total of 82 joints in 41 horses.

CT findings / histopathological findings	Bone surface		Subchondral bone			Bone margins		Joint space
	Flatt MC*	Irr MC	ScHD-Scl MC*	SphHD+Scl MC*	SphHD-Scl MC*	Medial Ent MC*	Lateral Ost MC	Narrow JS
Articular cartilage of the mandibular condyle								
Cartilage degeneration	**ρ = 0.95; *P* < 0.0001**	**ρ = 0.30; *P* = 0.01**	**ρ = –0.45; *P* < 0.0001**	**ρ = 0.36; *P* = 0.001**	ρ = 0.06; *P* = 0.60	**ρ = 0.91; *P* < 0.0001**	ρ = 0.10; *P* = 0.36	**ρ = 0.22; *P* = 0.04**
Chondrocyte apoptosis	**ρ = 0.45; *P* < 0.0001**	**ρ = 0.40; *P* = 0.0002**	**ρ = -0.26; *P* = 0.02**	**ρ = 0.37; *P* = 0.0007**	ρ = -0.11; *P* = 0.31	**ρ = 0.53; *P* < 0.0001**	ρ = 0.18; *P* = 0.11	ρ = 0.16; *P* = 0.15
Chondrones	**ρ = 0.27; *P* = 0.01**	ρ = 0.06; *P* = 0.60	ρ = -0.12; *P* = 0.30	**ρ = 0.25; *P* = 0.03**	ρ = -0.09; *P* = 0.41	**ρ = 0.24; *P* = 0.03**	ρ = -0.03; *P* = 0.78	ρ = 0.07; *P* = 0.56
Angiogenesis	ρ = 0.11; *P* = 0.34	ρ = 0.00; *P* = 0.98	ρ = -0.09; *P* = 0.40	ρ = 0.20; *P* = 0.07	ρ = -0.07; *P* = 0.51	ρ = 0.19; *P* = 0.09	ρ = -0.03; *P* = 0.82	ρ = -0.07; *P* = 0.51
Articular cartilage of the temporal bone								
Cartilage degeneration	**ρ = 0.88; *P* < 0.0001**	**ρ = 0.31; *P* = 0.004**	**ρ = -0.39; *P* = 0.002**	**ρ = 0.41; *P* = 0.0001**	ρ = 0.10; *P* = 0.38	**ρ = 0.80; *P* < 0.0001**	ρ = 0.12; *P* = 0.30	**ρ = 0.26; *P* = 0.02**
Chondrocyte apoptosis	**ρ = 0.44; *P* < 0.0001**	**ρ = 0.34; *P* = 0.002**	**ρ = -0.24; *P* = 0.03**	**ρ = 0.35; *P* = 0.001**	ρ = -0.09; *P* = 0.42	**ρ = 0.48; *P* < 0.0001**	ρ = 0.20; *P* = 0.08	ρ = 0.10; *P* = 0.37
Angiogenesis	ρ = 0.19; *P* = 0.09	ρ = 0.19; *P* = 0.08	ρ = -0.08; *P* = 0.47	**ρ = 0.26; *P* = 0.02**	ρ = -0.06; *P* = 0.57	ρ = 0.16; *P* = 0.14	ρ = -0.02; *P* = 0.85	ρ = -0.06; *P* = 0.57
Joint capsule								
Intimal hyperplasia	ρ = 0.00; *P* = 0.97	**ρ = 0.28; *P* = 0.01**	ρ = -0.07; *P* = 0.56	ρ = 0.14; *P* = 0.22	ρ = -0.05; *P* = 0.64	ρ = 0.13; *P* = 0.23	ρ = -0.02; *P* = 0.88	ρ = -0.05; *P* = 0.64
Subintimal edema	**ρ = 0.34; *P* = 0.002**	**ρ = 0.25; *P* = 0.02**	ρ = -0.15; *P* = 0.19	ρ = 0.15; *P* = 0.17	ρ = 0.02; *P* = 0.89	**ρ = 0.30; *P* = 0.01**	**ρ = 0.32; *P* = 0.004**	**ρ = 0.28; *P* = 0.01**
Subintimal fibrosis	**ρ = 0.36; *P* = 0.001**	**ρ = 0.37; *P* = 0.0007**	ρ = -0.20; *P* = 0.07	**ρ = 0.35; *P* = 0.001**	ρ = -0.06; *P* = 0.60	**ρ = 0.41; *P* = 0.0001**	ρ = -0.05; *P* = 0.63	ρ = -0.06; *P* = 0.60
Inflammatory cells	**ρ = 0.44; *P* < 0.0001**	**ρ = 0.46; *P* < 0.0001**	**ρ = -0.24; *P* = 0.03**	**ρ = 0.31; *P* = 0.01**	ρ = -0.05; *P* = 0.65	**ρ = 0.46; *P* < 0.0001**	ρ = 0.12; *P* = 0.27	ρ = 0.11; *P* = 0.30
Angiogenesis	**ρ = 0.48; *P* < 0.0001**	ρ = 0.16; *P* = 0.15	**ρ = -0.23; *P* = 0.04**	**ρ = 0.29; *P* = 0.01**	ρ = -0.18; *P* = 0.10	**ρ = 0.46; *P* < 0.0001**	ρ = 0.20; *P* = 0.07	ρ = 0.01; *P* = 0.90

Note: CT = computed tomography; Ent = enthesiophytes; Flatt = flattening; Irr = irregularity; JS = joint space; MC = mandibular condyle; Ost = osteophytes; ScHD = scattered regions of hypodensity; Scl = sclerosis (surrounding hyperdensity); SphHD = spherical region of hypodensity; + = with; - = without; * = CT anatomical variations. The correlations were considered significant for *P*-value < 0.05 and then highlighted with bold font.

Table 5. Summary of CT findings in 41 horses (82 TMJs) with histopathologically confirmed temporomandibular joint osteoarthritis (TMJ OA) (comparison group) and OA–free TMJs (control group).

CT findings	TMJ OA–related groups	
	Comparison group (*n* = 41)	**Control group (*n* = 41)**
Bone surface		
Normal	5 (12%)	29 (71%)
Flatt MC*	32 (78%)	10 (24%)
Irr MC	18 (44%)	2 (5%)
Subchondral bone		
Normal	23 (56%)	25 (61%)
ScHD-Scl MC*	2 (5%)	10 (24%)
SphHD+Scl MC*	12 (29%)	2 (5%)
SphHD-Scl MC*	3 (7%)	5 (12%)
Bone margins		
Normal	6 (15%)	28 (68%)
Medial Ent MC*	35 (85%)	13 (32%)
Lateral Ost MC	1 (2%)	0
Joint space and soft tissue		
Normal	36 (88%)	38 (93%)
Narrow JS	5 (12%)	3 (7%)
Affected joints	39 (95%)	26 (63%)
Chi–square test	$P < 0.0001$	

Note: CT = computed tomography; Ent = enthesiophytes; Flatt = flattening; Irr = irregularity; JS = joint space; MC = mandibular condyle; *n* = number of affected joints; Ost = osteophytes; ScHD = scattered regions of hypodensity; Scl = sclerosis (surrounding hyperdensity); SphHD = spherical region of hypodensity; % = percentage of affected joints from each TMJ OA–related group; * = CT anatomical variations. The differences in the frequency distribution of CT findings between TMJ OA–related groups were considered significant for *P*–value < 0.05.

Table 6. The accuracy of computed tomography (CT) in diagnosing temporomandibular joint osteoarthritis (TMJ OA) compared to histopathological reference confirmation in 41 horses (82 TMJs).

Accuracy metrics	All TMJs	Age–related groups		
		1–4 years	**5–15 years**	**>15 years**
Recognized / Confirmed; *n* / *n* (%)	65 / 39 (60%)	17 / 4 (24%)	24 / 15 (58%)	24 / 20 (83%)
Se	0.79	0.50	1.00	1.00
95% CI	0.69 to 0.87	0.32 to 0.68	0.86 to 1.00	0.86 to 1.00
Sp	0.50	0.82	0.37	0.17
95% CI	0.39 to0.61	0.65 to 0.93	0.19 to 0.59	0.05 to 0.37
AUC	0.65	0.66	0.69	0.58
95% CI	0.56 to 0.73	0.53 to 0.79	0.53 to 0.84	0.42 to 0.74
PPV	0.60	0.24	0.63	0.83
NPV	0.88	0.88	–	–

Note: AUC = area under curve; NPV = negative predictive value; PPV = positive predictive value; Se = sensitivity; Sp = specificity; *n* / *n* = number of TMJ OA radiologically recognized and histopathologically confirmed; % = percent of histopathologically confirmed TMJ OA; 95% CI = 95% confidence interval.

Figures

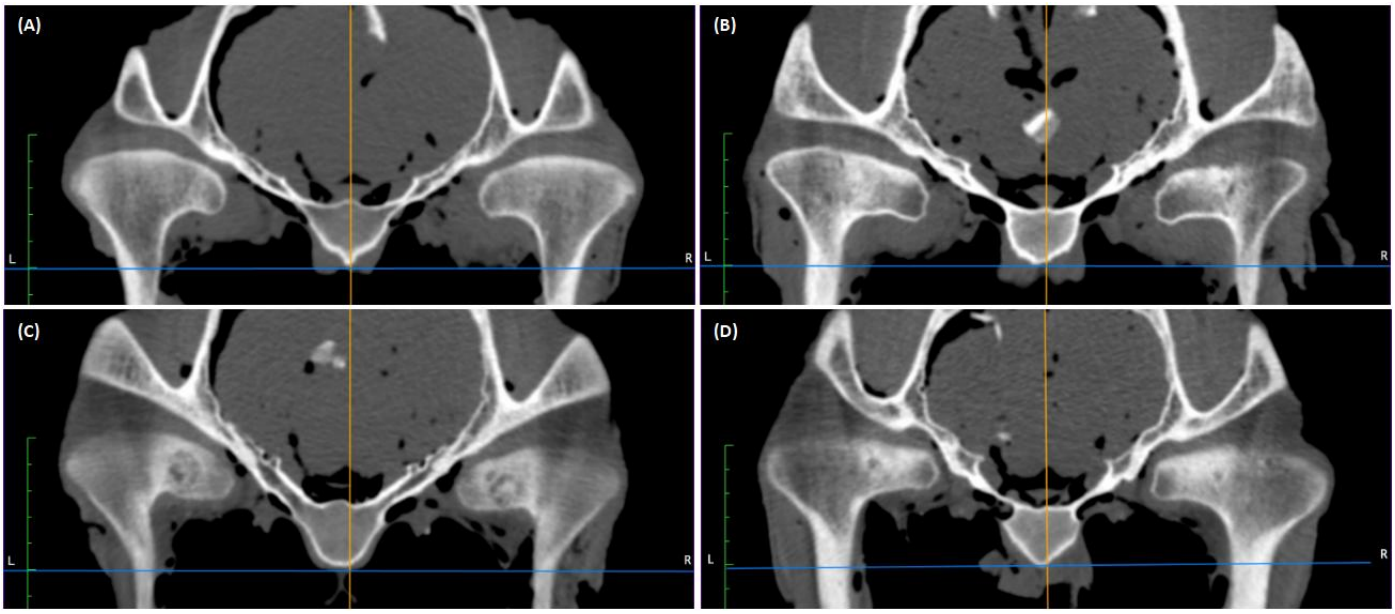

FIGURE 1 Examples of transverse CT images of temporomandibular joints (TMJs) of 1–4 years old horses. A, Globose shape and homogenous mandibular condyles in normal joints. B, Irregularity of the bone surface of the right mandibular condyles. C, Scattered regions of hypodensity in the right and left mandibular condyles. D, Small spherical region of hypodensity (subchondral bone cysts) with surrounding hyperdensity (sclerosis) in the right and left mandibular condyles.

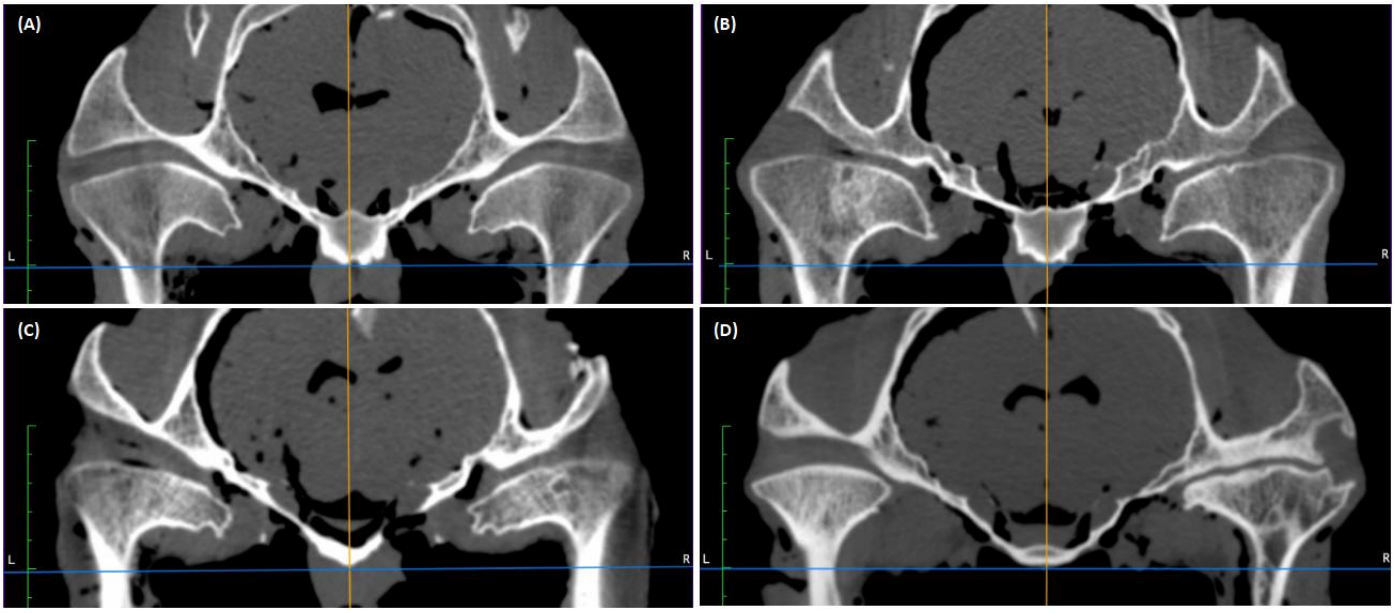

FIGURE 2 Examples of transverse CT images of temporomandibular joints (TMJs) of 5–15 years old horses. A, Flattening and medial enthesiophytes of both mandibular condyles. B, Irregularity of the bone surface of the right mandibular condyle and spherical region of hypodensity (subchondral bone cysts) with surrounding hyperdensity (sclerosis) in the left mandibular condyle. Flattening and medial enthesiophytes of both mandibular condyles. C, Spherical region of hypodensity (subchondral bone cysts) with surrounding hyperdensity (sclerosis) in the caudal aspect of right mandibular condyle. Flattening and medial enthesiophytes of both mandibular condyles. D, Irregularity of the bone surface, medial enthesiophytes, and lateral osteophytes of the right mandibular condyle. Narrowing of the right joint space.

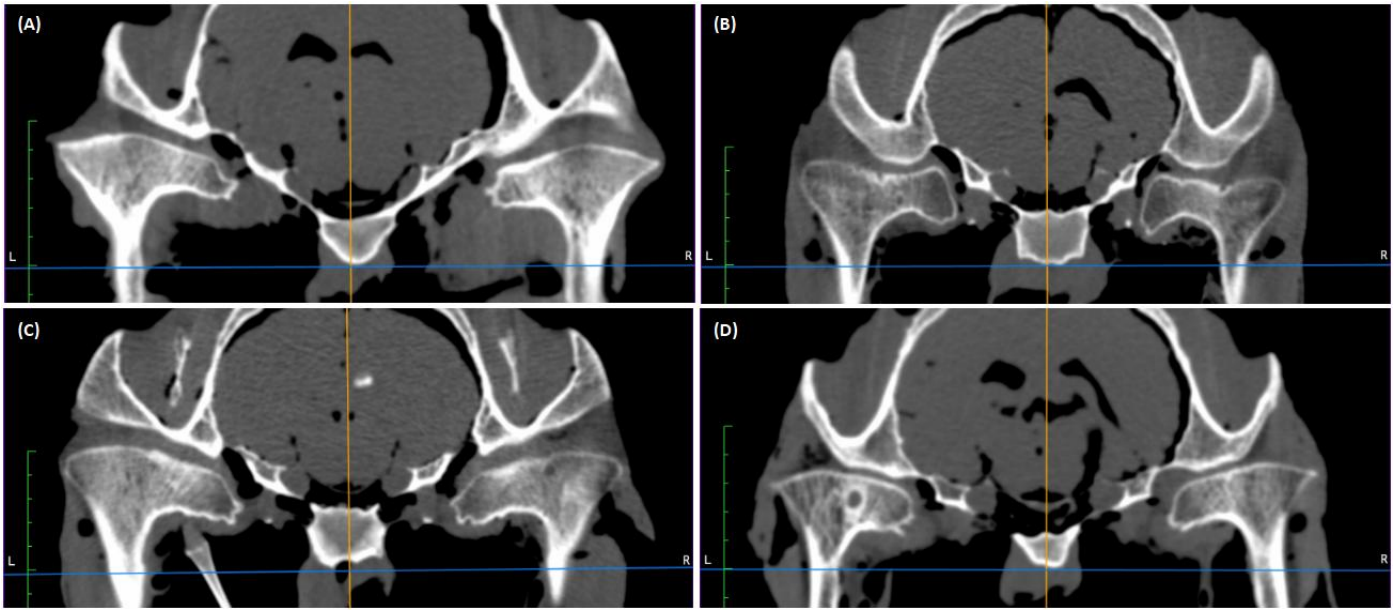

FIGURE 3 Examples of transverse CT images of temporomandibular joints (TMJs) of >15 years old horses. A, Flattening and medial enthesiophytes of both mandibular condyles. B, Irregularity of the bone surface of both mandibular condyles. Flattening and medial enthesiophytes of both mandibular condyles. C, Spherical region of hypodensity (subchondral bone cysts) without surrounding hyperdensity (sclerosis) in the right mandibular condyle. Flattening and medial enthesiophytes of both mandibular condyles. D, Spherical region of hypodensity (subchondral bone cysts) with surrounding hyperdensity (sclerosis) in the left mandibular condyle. Flattening and medial enthesiophytes of both mandibular condyles. Narrowing of both joint spaces.

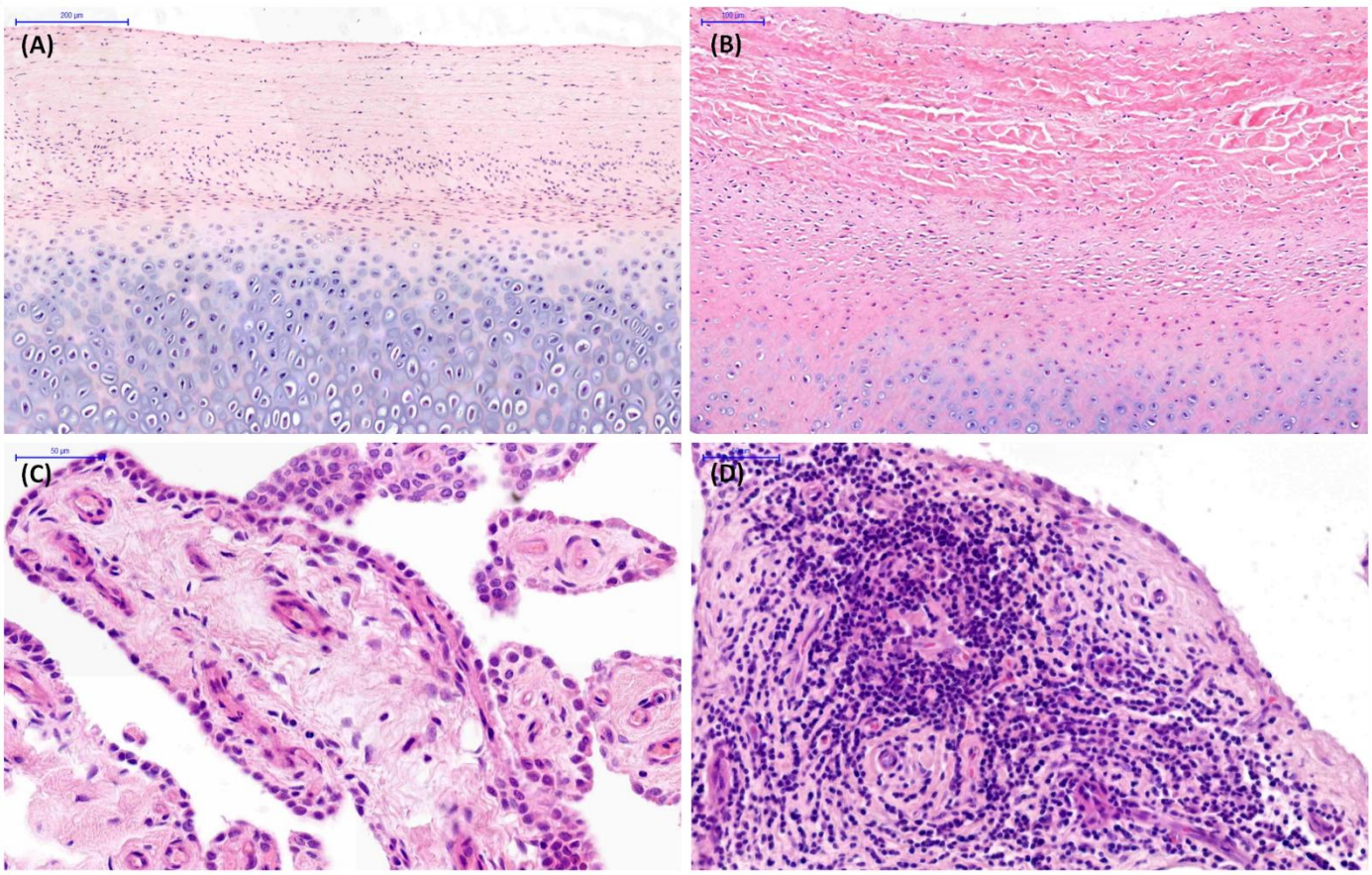

FIGURE 4 Examples of histopathological images of H–E stained temporomandibular joint (TMJ) tissue sections of 1–4 years old horses. A, Normal articular cartilage of the mandibular condyle. Scale bare = 200 µm. B, Normal articular cartilage of the temporal bone. Scale bare = 100 µm. C, Normal joint capsule. Scale bare = 50 µm. D, Joint capsule with an infiltration of inflammatory cells. Scale bare = 50 µm.

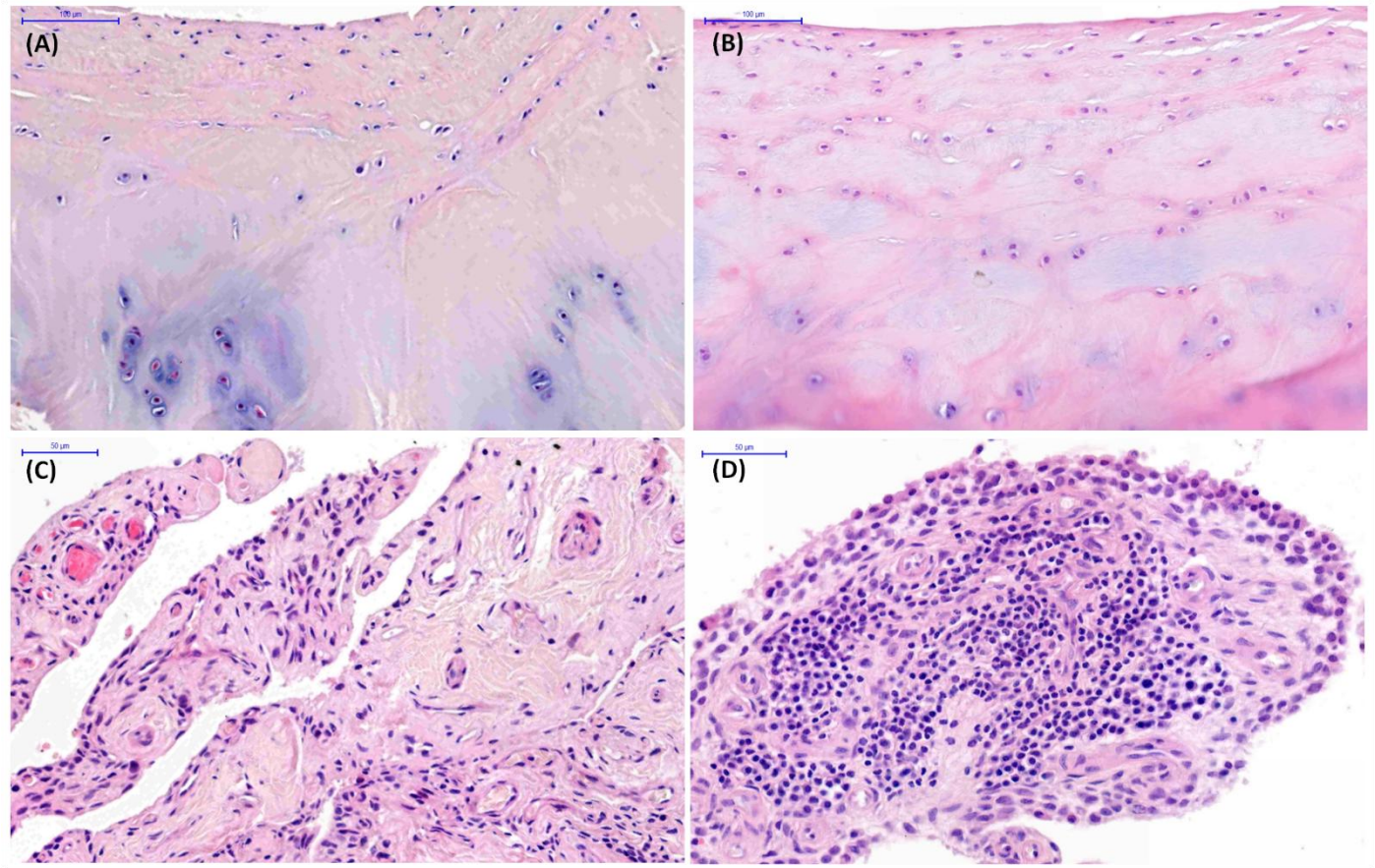

FIGURE 5 Examples of histopathological images of H–E stained temporomandibular joint (TMJ) tissue sections of 5–15 years old horses. A, Cartilage degeneration and chondrocyte apoptosis of articular cartilage of the mandibular condyle. Scale bare = 100 µm. B, Cartilage degeneration and chondrocyte apoptosis of articular cartilage of the temporal bone. Scale bare = 100 µm. C, Subintimal edema and angiogenesis in the joint capsule. Scale bare = 50 µm. D, Joint capsule with an infiltration of inflammatory cells. Scale bare = 50 µm.

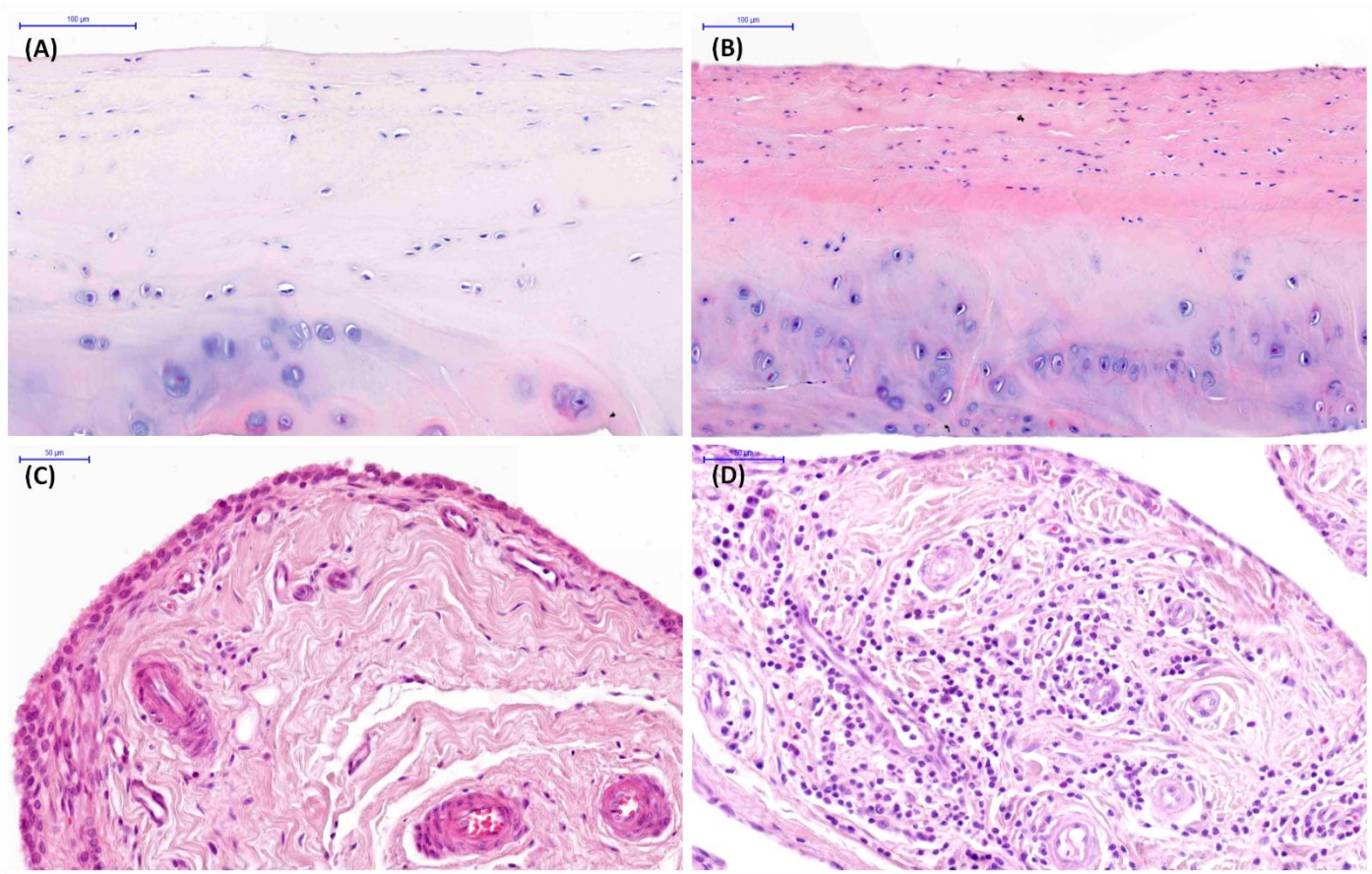

FIGURE 6 Examples of histopathological images of H–E stained temporomandibular joint (TMJ) tissue sections of >15 years old horses. A, Cartilage degeneration, chondrocyte apoptosis, and chondrones in articular cartilage of the mandibular condyle. Scale bare = 100 μm. B, Cartilage degeneration and chondrocyte apoptosis of articular cartilage of the temporal bone. Scale bare = 100 μm. C, Subintimal fibrosis in the joint capsule. Scale bare = 50 μm. D, Joint capsule with an infiltration of inflammatory cells. Scale bare = 50 μm.

Appendix

Supplementary Table S1–S3.

Table S1. The Spearman correlation coefficient (ρ) between CT findings and histopathological findings detected in 34 joints in 1-4 years horses.

CT findings / histopathological findings	Bone surface		Subchondral bone			Margins of bone		Joint space
	Flatt MC*	Irr MC	ScHD-Scl MC*	SphHD+Scl MC*	SphHD-Scl MC*	Medial Ent MC*	Lateral Ost MC	Narrow JS
Articular cartilage of the mandibular condyle								
Cartilage degeneration	X	X	X	X	X	X	X	X
Chondrocyte apoptosis	X	X	X	X	X	X	X	X
Chondrones	X	X	X	X	X	X	X	X
Angiogenesis	X	X	X	X	X	X	X	X
Articular cartilage of the temporal bone								
Cartilage degeneration	X	X	X	X	X	X	X	X
Chondrocyte apoptosis	X	X	X	X	X	X	X	X
Angiogenesis	X	X	X	X	X	X	X	X
Joint capsule								
Intimal hyperplasia	X	X	X	X	X	X	X	X
Subintimal edema	X	X	X	X	X	X	X	X
Subintimal fibrosis	X	X	X	X	X	X	X	X
Inflammatory cells	X	ρ = 0.21; *P* = 0.23	ρ = -0.02; *P* = 0.92	ρ = 0.12; *P* = 0.47	X	X	X	X
Angiogenesis	X	X	X	X	X	X	X	X

Note: CT = computed tomography; Ent = enthesiophytes; Flatt = flattening; Irr = irregularity; JS = joint space; MC = mandibular condyle; Ost = osteophytes; ScHD = scattered regions of hypodensity; Scl = sclerosis (surrounding hyperdensity); SphHD = spherical region of hypodensity; + = with; - = without; * = CT anatomical variations; X = finding not present in this age class. The correlations were considered significant for *P*-value < 0.05.

Table S2. The Spearman correlation coefficient (ρ) between CT findings and histopathological findings detected in 24 joints in 5-15 years horses.

CT findings / histopathological findings	Bone surface		Subchondral bone			Margins of bone		Joint space
	Flatt MC*	Irr MC	ScHD-Scl MC*	SphHD+Scl MC*	SphHD-Scl MC*	Medial Ent MC*	Lateral Ost MC	Narrow JS
Articular cartilage of the mandibular condyle								
Cartilage degeneration	**ρ = 0.77; *P* < 0.0001**	ρ = -0.08; *P* = 0.73	X	ρ = 0.04; *P* = 0.85	ρ = 0.17; *P* = 0.43	no variability	ρ = 0.09; *P* = 0.66	ρ = -0.05; *P* = 0.83
Chondrocyte apoptosis	ρ = 0.05; *P* = 0.82	**ρ = 0.44; *P* = 0.03**	X	ρ = 0.04; *P* = 0.86	ρ = -0.16; *P* = 0.46	no variability	ρ = 0.19; *P* = 0.37	ρ = 0.06; *P* = 0.78
Chondrones	X	X	X	X	X	X	X	X
Angiogenesis	ρ = -0.07; *P* = 0.74	ρ = -0.06; *P* = 0.77	X	ρ = 0.03; *P* = 0.87	ρ = -0.14; *P* = 0.51	no variability	ρ = -0.08; *P* = 0.71	ρ = -0.19; *P* = 0.36
Articular cartilage of the temporal bone								
Cartilage degeneration	**ρ = 0.69; *P* = 0.0002**	ρ = -0.02; *P* = 0.94	X	ρ = 0.21; *P* = 0.32	ρ = 0.24; *P* = 0.25	no variability	ρ = 0.13; *P* = 0.53	ρ = 0.10; *P* = 0.63
Chondrocyte apoptosis	ρ = 0.05; *P* = 0.82	ρ = 0.39; *P* = 0.06	X	ρ = 0.07; *P* = 0.74	ρ = -0.03; *P* = 0.88	no variability	ρ = 0.27; *P* = 0.20	ρ = 0.24; *P* = 0.26
Angiogenesis	X	X	X	X	X	X	X	X
Joint capsule								
Intimal hyperplasia	ρ = -0.17; *P* = 0.42	ρ = 0.36; *P* = 0.09	X	ρ = 0.14; *P* = 0.52	ρ = -0.11; *P* = 0.60	no variability	ρ = -0.06; *P* = 0.77	ρ = -0.15; *P* = 0.47
Subintimal edema	ρ = 0.33; *P* = 0.11	ρ = 0.29; *P* = 0.17	X	ρ = 0.05; *P* = 0.81	ρ = 0.07; *P* = 0.74	no variability	ρ = 0.36; *P* = 0.08	ρ = 0.18; *P* = 0.41
Subintimal fibrosis	ρ = -0.36; *P* = 0.08	**ρ = 0.45; *P* = 0.03**	X	ρ = 0.03; *P* = 0.87	ρ = -0.14; *P* = 0.51	no variability	ρ = -0.08; *P* = 0.71	ρ = 0.12; *P* = 0.59
Inflammatory cells	ρ = 0.10; *P* = 0.65	**ρ = 0.54; *P* = 0.01**	X	ρ = 0.17; *P* = 0.43	ρ = -0.19; *P* = 0.37	no variability	ρ = 0.18; *P* = 0.41	ρ = 0.02; *P* = 0.94
Angiogenesis	ρ = 0.25; *P* = 0.24	ρ = 0.04; *P* = 0.84	X	ρ = -0.12; *P* = 0.58	ρ = -0.29; *P* = 0.17	no variability	ρ = 0.27; *P* = 0.20	ρ = -0.19; *P* = 0.39

Note: CT = computed tomography; Ent = enthesiophytes; Flatt = flattening; Irr = irregularity; JS = joint space; MC = mandibular condyle; no variability = finding presents in all joints in this age class (all realizations = 1); Ost = osteophytes; ScHD = scattered regions of hypodensity; Scl = sclerosis (surrounding hyperdensity); SphHD = spherical region of hypodensity; + = with; - = without; * = CT anatomical variations; X = finding not present in this age class (all realizations = 0). The correlations were considered significant for *P*-value < 0.05 and then highlighted with bold font.

Table S3. The Spearman correlation coefficient (ρ) between CT findings and histopathological findings detected in 24 joints in >15 years horses.

CT findings / histopathological findings	Bone surface		Subchondral bone			Margins of bone		Joint space
	Flatt MC*	Irr MC	ScHD-Scl MC*	SphHD+Scl MC*	SphHD-Scl MC*	Medial Ent MC*	Lateral Ost MC	Narrow JS
Articular cartilage of the mandibular condyle								
Cartilage degeneration	no variability	no variability	X	no variability	no variability	no variability	X	no variability
Chondrocyte apoptosis	no variability	ρ = 0.12; *P* = 0.58	X	ρ = 0.39; *P* = 0.06	ρ = -0.25; *P* = 0.23	no variability	X	ρ = -0.06; *P* = 0.77
Chondrones	no variability	ρ = 0.00; *P* = 1.00	X	ρ = 0.26; *P* = 0.21	ρ = -0.17; *P* = 0.42	no variability	X	ρ = 0.07; *P* = 0.74
Angiogenesis	no variability	ρ = -0.15; *P* = 0.49	X	ρ = 0.32; *P* = 0.12	ρ = -0.06; *P* = 0.77	no variability	X	ρ = -0.08; *P* = 0.71
Articular cartilage of the temporal bone								
Cartilage degeneration	no variability	ρ = 0.21; *P* = 0.32	X	ρ = 0.19; *P* = 0.37	ρ = 0.09; *P* = 0.67	no variability	X	ρ = 0.11; *P* = 0.60
Chondrocyte apoptosis	no variability	ρ = 0.06; *P* = 0.78	X	ρ = 0.33; *P* = 0.12	ρ = -0.28; *P* = 0.19	no variability	X	ρ = -0.35; *P* = 0.10
Angiogenesis	no variability	ρ = 0.27; *P* = 0.21	X	ρ = 0.31; *P* = 0.14	ρ = -0.11; *P* = 0.60	no variability	X	ρ = -0.14; *P* = 0.51
Joint capsule								
Intimal hyperplasia	no variability	X	X	X	X	no variability	X	X
Subintimal edema	no variability	ρ = 0.00; *P* = 1.00	X	ρ = 0.03; *P* = 0.87	ρ = -0.11; *P* = 0.06	no variability	X	ρ = 0.24; *P* = 0.26
Subintimal fibrosis	no variability	ρ = 0.30; *P* = 0.16	X	**ρ = 0.41; *P* = 0.049**	ρ = -0.03; *P* = 0.91	no variability	X	**ρ = -0.41; *P* = 0.046**
Inflammatory cells	no variability	ρ = 0.26; *P* = 0.22	X	ρ = 0.31; *P* = 0.32	ρ = -0.14; *P* = 0.52	no variability	X	ρ = -0.03; *P* = 0.87
Angiogenesis	no variability	ρ = -0.06; *P* = 0.78	X	ρ = 0.21; *P* = 0.06	ρ = -0.25; *P* = 0.23	no variability	X	ρ = -0.06; *P* = 0.77

Note: CT = computed tomography; Ent = enthesiophytes; Flatt = flattening; Irr = irregularity; JS = joint space; MC = mandibular condyle; no variability = finding presents in all joints in this age class (all realizations = 1); Ost = osteophytes; ScHD = scattered regions of hypodensity; Scl = sclerosis (surrounding hyperdensity); SphHD = spherical region of hypodensity; + = with; - = without; * = CT anatomical variations; X = finding not present in this age class (all realizations = 0). The correlations were considered significant for *P*-value < 0.05 and then highlighted with bold font.