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 temporomandibular joint (TMJ), including osteoarthritis (OA), are suspected. However, certain CT findings have been proposed as 'anatomical variations', and their clinical significance remains unknown. The objectives of this prospective descriptive cross-sectional study were to describe the CT and histopathological findings of TMJ OA in relation to horse age, evaluate the accuracy of TMJ OA diagnosis with regard to 'CT anatomical variations', and explore the correlations between specific CT and histopathological findings. 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–related frequency, and accuracy of TMJ OA diagnosis were calculated. Based on histopathological assessment, TMJ OA was confirmed in 18% of joints in horses aged 1–4 years, 63% of joints in horses aged 5–15 years, and 83% of joints in horses over 15 years. Considering entire CT findings, TMJ OA was radiologically recognized in 76% of joints, with a sensitivity of 0.95 and a specificity of 0.37. When 'CT anatomical variations' were excluded, TMJ OA was recognized in 30% of joints, with a sensitivity of 0.51 and a specificity of 0.88. Findings suggest that the equine TMJ may undergo both age–related and disease–related remodeling, detectable through CT and histopathology, and that the inclusion or exclusion of certain 'CT anatomical variations' in the diagnostic protocol affects the sensitivity and specificity of CT–based TMJ OA recognition.

KEY WORDS

temporomandibular joint disease, degenerative joint disease, subchondral bone cyst, cartilage degeneration, equine

1 INTRODUCTION

Diseases of the temporomandibular joint (TMJ) in horses has been suggested to be rare or rarely reported;[1–3] however, their prevalence in the equine population has not yet been estimated. The TMJ functions to facilitate movement of the condylar process of the mandible relative to the zygomatic process of the temporal bone.[4] Therefore, TMJ diseases can cause joint dysfunctions that lead to clinical symptoms varying in severity, from mild behavioural issues[5,6,7] to moderate masticatory difficulties,[8,9] and, in severe cases, an inability to open[10,11] or close[12,13] the mouth.

Among TMJ diseases, osteoarthritis (OA) may develop as a primary disease[5–7,11,14,15] or secondarily to fractures[16] or septic arthritis.[1,10,17] Primary TMJ OA is rarely accompanied by severe symptoms such as an inability or reluctance to open the mouth, which can lead to significant weight loss.[11] More commonly, moderate symptoms of TMJ dysfunction are reported, including masticatory problems such as difficulty eating,[14] clunking or clicking sounds during mastication,[5,6] or quidding.[5,11] Increasingly, horses suspected of having TMJ OA are presented to veterinarians due to milder symptoms, such as performance issues, aversive behaviours,[5,6] head–shaking syndrome,[5,18] and resistance or reluctance to work on the bit.[6,7] As a result, TMJ OA is increasingly discussed[3,19] as a potential cause of nonspecific clinical symptoms related to decreased performance in equine athletes. [3,5–7,11,14,15]

Primary TMJ OA is thought to result from age–related ('wear and tear') lesions,[5,6,20,21] leading to damage and degeneration of the articular cartilage.[22] Unlike appendicular joints, where the articular surface is covered by hyaline cartilage,[23] the TMJ is lined with fibrocartilage,[24] making histopathological findings of degeneration more specific to this joint.[5,14,20,25.26] Degeneration of the articular cartilage is typically accompanied by inflammation and remodeling of the surrounding tissue, starting with the subchondral bone and progressively involving the synovium[22] and the fibrocartilaginous intra–articular disc.[21,27] Consequently, subchondral bone cysts, irregular subchondral bone contours, and mineralization of the intra–articular disc are the most commonly reported computed tomography (CT) findings in primary TMJ OA. [5–7,14,15,21]

In horses, CT imaging of the TMJ is frequently used to confirm an initial diagnosis of primary TMJ OA, typically based on detailed history, clinical symptoms,[3] and supported by functional tests,[6,7] radiography,[5,11,16] and/or ultrasonography.[5,11] Many clinicians emphasize the advantages of CT over radiography,[1,10,16,17,28] even when specific TMJ projections are used,[29–31] due to the significant superimposition of structures in radiographic imaging of the TMJ, which limits the radiographic clarity.

As a result, CT has been widely utilized in assessing primary TMJ OA for both clinical[5–7,14] and research[15,18,21] purposes. With the recent advantages in performing CT imaging of the horse's head under standing sedation,[32,33] the number of head CT examinations and the detection rate of TMJ OA cases are expected to increase. This increased availability and safety of CT imaging aligns with the growing number of case reports of primary TMJ OA,[3,5–7,11,14,15] which have responded 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 findings of TMJ OA have been described in only two cases.[5,14]

Interestingly, some CT finding in the TMJ are suspected to be incidental and may not manifest clinically in certain horses.[21] These findings, proposed as 'CT anatomical variation', differ from normal anatomy[34] but appear in horses without moderate to severe clinical symptoms of TMJ dysfunction.[21] However, later case reports have linked some 'CT anatomical variations', such as subchondral bone cysts in the mandibular condyle[6,7] and mineralization of the intra–articular disc,[5,6] to mild clinical symptoms of TMJ dysfunction, particularly in horses with poor performance. To address the gap in understanding the role of CT findings in equine TMJ OA, we hypothesize that the accuracy of radiological diagnosis of TMJ OA for individual joints varies depending on the specific set of CT findings considered. Additionally, we hypothesize a relationship between the occurrence of specific CT and histopathological findings.

The study aims to: (1) describe the CT and histopathological findings of TMJ OA in horses in relation to age; (2) evaluate the accuracy of radiological diagnosis of TMJ OA in relation to histopathology, particularly concerning 'CT anatomical variations'; and (3) describe the correlation between specific CT and histopathological findings.

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)[21]; 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. Biological sample collection was performed 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. The cadaver heads underwent TMJ palpation, CT examination, and tissue sample collection for histopathological assessment between May and June 2023. The age of each horse at the time of slaughter was recorded. Results from the TMJ palpation, CT examination, and histopathological evaluation were used to include or exclude the respective head. 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 a Polish board–certified veterinary diagnostic imaging (PCVDI) radiologist (T.J.) and a third–year PCVDI resident (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, [5–7,14,15,21] 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).[21] For both cyst groups, communication with joint space (open or not open) was noted.[21] Enthesiophytes were defined as irregular medial margin of the mandibular condyle at the insertion of the lateral pterygoid muscle,[21] while osteophytes referred to irregular lateral or rostral margins of the mandibular condyle or zygomatic process of temporal bone.[16,21] 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[21] (AV) and were marked accordingly in tables with asterisks.

TMJ OA was CT–based recognized using three thresholds: (1) entire CT findings group: TMJ was considered as OA–affected if any CT finding was present; (2) CT findings without AV group (CT findings - AV): TMJ was considered OA–affected if any non–AV CT finding was present; (3) CT findings without AV

but with cyst group (C) (CT findings - AV / + C): TMJ was considered OA–affected if any non–AV CT finding or subchondral bone cyst was present.

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. [5,14,20,22,25,26] 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.

Given TMJ OA is linked to degenerative and inflammatory lesions leading to synovitis and capsulitis,[1,22] and degenerative changes accumulate with age,[20,26] the criterion for confirming TMJ OA was the presence of inflammatory signs in the joint capsule. TMJ OA was histopatologically confirmed when the presence of the inflammatory signs in joint capsule was observed. If signs of articular cartilage degeneration were present without inflammatory signs, the TMJ was classified as normal.

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.). Based on age records, the horse´s heads were categorized into one of three age categories for analyses: (1) 1–4 years, (2) 5–15 years, and (3) >15 years.

Descriptive statistics (median, range, and percentage) of age and number of affected joints were performed for total data set and each age category. Continuous variables are presented as median (range). The presence (1) or absence (0) of the CT and histopathological findings were presented in binary format. The frequency of CT findings and histopathological findings between age categories was compared using a Chi–square test. Differences were considered significant for $P < 0.05$.

The results of the CT–based determination of TMJ OA were then compiled with the histopathologically confirmed disease. The accuracy of CT–based recognition of TMJ OA was calculated in relation to the histopathological diagnosis using three data sets: (1) entire CT findings, (2) CT findings – AV (anatomical variation group), and (3) CT findings – AV / + C (cyst group). Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) for TMJ OA recognition were estimated. The values of Se, Sp, PPV, and NPV were calculated across a range from 0.1 to 1.0 using standard formulae.[36]

Data series were tested for normality using the Shapiro–Wilk test. Since none of the data series followed a normal distribution, Spearman's rank correlation coefficient (ρ) was calculated to assess the correlation between the occurrence of CT and histopathological findings. Correlations were calculated both for the total data set and for each age category separately. 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).[37]

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).

CT findings of TMJ OA were detected in 76% of joints when entire CT findings were considered, in 37% of joints when AV were excluded (CT findings - AV), and in 41% of joints when AV excepting subchondral bone cysts (CT findings - AV / + C) were excluded. These findings were partially dichotomously associated with age, with significant differences in the frequency of specific CT findings differed between horses aged 1–4 years and those aged 5–15 years as well as between horses aged 1–4 years and those older than 15 years. However, there was no significant difference between horses aged 5–15 years and those older than 15 years. A summary of the CT findings is shown in Table 2.

In 1–4 years old horses, TMJ OA was recognized 50% of joints when entire CT findings were considered, in 6% of joints when CT findings - AV were considered, and in 15% of joints when CT findings - AV / + C were considered. In this age–related category, 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). Of these horses, 6% of the entire CT findings were limited to the right joint, 9% to the left joint, and 35% were bilateral. When CT findings - AV were considered, 3% of CT findings were limited to each side. When CT findings - AV / + C were considered, per 6% changes were bilateral and limited to the left joint, while 3% limited to the right joint.

In 5–15 years old horses, TMJ OA was recognized in 100% of joints when entire CT findings were considered, in 46% of joints when CT findings - AV were considered, and in 58% of joints when CT findings - AV / + C were considered. In this age–related category, 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 when entire CT findings were considered. When CT findings - AV were considered, 42% of findings remained bilateral and in 4% – 8% CT findings were limited to left and right side, respectively. When CT findings - AV / + C were considered, 42% of findings were bilateral and the findings were limited to the left and right side in two and three joints, respectively.

In >15 years old horses, TMJ OA was recognized in 100% of joints when entire CT findings were considered, in 54% of joints when CT findings - AV were considered, and in 63% of joints when CT findings - AV / + C were considered. In this age–related category, 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). Bilateral findings were present in all joints when entire CT findings were considered. When CT findings - AV were considered, 33% findings were bilateral, and 4%–8% were limited to left and right side, respectively. When CT findings - AV / + C were considered, 50% of findings were bilateral, 8% were limited to the left joint.

Histopathological findings of TMJ OA were detected in 50% of joints and showed a clear dichotomous age–related association. The frequency of specific histopathological findings differed between horses aged 1–4 years and 5–15 years, 1–4 years and horses older than 15 years, as well as horses aged 5–15 years and those older than 15 years. A summary of histopathological findings is shown in Table 3.

In 1–4 years old horses, 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 old horses, 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 old horses, 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.

A total of 39 of 65 (60%) joints diagnosed with TMJ OA based on the entire CT findings were histopathologically confirmed. When using histopathology as the reference standard, the sensitivity of this CT–based recognition was 0.95, and the specificity was 0.37. However, many joints were falsely classified as positive, as indicated by the low PPV of 0.60, while few joints were falsely classified as negative, as reflected by the high NPV of 0.88. For TMJ OA diagnoses based on CT findings - AV, 21 out of 26 (80%) joints were histopathologically confirmed. The sensitivity was 0.51, while the specificity was 0.88. Despite a lower rate of false positives (indicated by a high PPV of 0.81), there were more false negatives, as reflected in the low NPV of 0.64. For TMJ OA diagnoses based on CT findings - AV / + C, 26 of 34 (76%) joints were histopathologically confirmed. The sensitivity was 0.63, and the specificity was 0.80. A moderate number of joints were falsely classified as either positive or negative, as indicated by moderate PPV of 0.76 and NPV of 0.69. A summary of the histopathological confirmation of TMJ OA, including a breakdown by age categories, is shown in Table 4.

Considering the most important significant correlations between CT and histopathological findings across all studied joints, very strong and strong positive correlations were observed for flattening of the mandibular condyle and articular cartilage degeneration in both the mandibular condyle and the temporal bone, as well as for medial enthesophyte and articular cartilage degeneration in both the mandibular condyle and the temporal bone. Moderate positive correlations were noted for flattening of the mandibulat condyle and chondrocyte apoptosis in both the mandibular condyle and the temporal bone, as well as for medial enthesophyte and articular cartilage degeneration in both the mandibular condyle and the temporal bone. Week to moderate positive correlations were observed for 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 for 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 changes 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 5, while corresponding summaries for subsequent age categories are provided in supplementary Table S1–S3.

4 DISCUSSION

The detectability of TMJ OA based on CT findings varied depending on the specific features considered. Including the entire potential CT findings resulted in the recognition of more affected joints, with high sensitivity but low specificity. Of the total TMJ OA cases recognized, only 60% were confirmed histologically, although this percentage increased with the age of the horses. When all proposed 'CT anatomical variations' were excluded from the CT–based recognition of TMJ OA, fewer affected joints were identified, leading to a decrease in sensitivity. However, specificity improved, with 81% of histologically confirmed TMJ OA cases being identified, and again, this percentage increased with the age of the horses. Including CT findings typically described in horses with mild clinical symptoms of TMJ OA[6,7] produced intermediate results in terms of both the number of affected joints and the specificity and sensitivity of the diagnosis. In this total dataset, 71% of TMJ OA cases were confirmed histologically, with the percentage increasing with age. Notably, in this last group of CT findings, the presence of subchondral bone cysts in the mandibular condyle showed only moderate positive correlation with degeneration and apoptosis in the articular cartilage, while mineralization of the intra–articular disc, reported in previous studies,[5,6] was not observed in this study. However, the flattening of mandibular condyle and the presence of medial enthesiophytes on the mandibular condyle – both previously suggested as 'CT anatomical variations'[21] – were strongly positively correlated with articular cartilage degeneration and moderately positively correlated with chondrocyte apoptosis and inflammatory signs in the joint capsule. These findings suggest that more attention should be given to these CT markers in future studies on both age–related and OA–related changes in equine TMJ. Thus, the author's hypothesis that the accuracy of radiological diagnosis of TMJ OA varies by individual joint, depending on the specific CT findings considered, has been confirmed. Additionally, the authors hypothesized a relationship between the occurrence of specific CT and histopathological findings. The results of this study support that hypothesis, identifying four CT findings in the mandibular condyle – flattening, irregularity, subchondral bone cyst, and medial – as being positively associated with joint degeneration and inflammation. Conversely, spherical region of hypodensity in the mandibular condyle were not found to be negatively related to TMJ OA.

The CT study of the equine TMJ has shown that CT findings related to age and/or OA are relatively common, but their pathological significance remains unclear.[21] To date, only five case reports described the

radiologic and histopathological findings simultaneously 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] Although several research studies have examined tissues from equine TMJ histopathologically, only one study specifically addressed TMJ OA, however, without considering CT imaging.[25] The other four studies described normal articular cartilage[20,24,26,41] and the intra–articular disc[25] with only two linking their findings to μCT.[26,41] Few more additional case reports[6,7] and research studies[15,18,21,34] have address CT imaging of both normal[21,34] and OA–affected[6,7,15,18,21] TMJs, however, they not include histological analysis. Therefore, this is the first study to assess the accuracy of radiological diagnosis of TMJ OA relative to histopathological findings and relationship between both types of findings, aiming to help bridge the gap in understanding the pathophysiology of TMJ OA.

Pimentel et al.[14] reported a case of 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)[21], 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] Smyth et al.[5] reported the case of a 18–year–old mare with unilateral CT findings and bilateral histopathological findings of TMJ OA. CT findings included subchondral bone sclerosis of the mandibular condyle, medial osteophytes of the mandibular condyles (referred to as enthesophytes)[21], lateral osteophytes of the temporal bone, and mineralization of the intra–articular disc. Histopathological analysis showed 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 signs in the joint capsule. However, these findings appeared only in horses over 5 years old, similar to the elder mare report by Smyth et al.[5], and not in younger fillies, as reported by Pimentel et al.[14] Additionally, in this study, lateral osteophytes were found on the mandibular condyle, in contrast to Smyth et al.[5], where they were present on the temporal bone. The lateral osteophytes showed only a weak positive correlation with subintimal edema in the joint capsule. Other findings described by Pimentel et al.[14], aside from mineralization of the intra–articular disc, may be related to the osteochondrosis observed in this filly, but none of these were observed in the current study.

Similar CT findings involving the mandibular condyle, intra–articular disc, and occasionally the temporal bone have been reported in other cases of TMJ OA, though these were not corroborated by histopathological assessments. 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 clinical symptoms of TMJ dysfunction.[21] Among 'CT anatomical variation', scattered regions of hypodensity in the mandibular

condyle were associated with young horses, while the occurrence of subchondral bone cysts, flattening, and medial enthesiophytes on the mandibular condyle increased with age.[21] Our findings align with those of Carmalt et al.[21] and further suggest that CT findings typical of young horses are moderately negatively correlated with cartilage degeneration, chondrocyte apoptosis and signs of joint capsule inflammation. These features do not co–occur with TMJ OA and are unlikely to contribute to the disease. Similarly, some histopathological findings have been described in clinically normal horses of various ages.[20] Based on the total TMJ score (a sum of histopathological findings), horses under 10 years old showed fewer histopathological changes in both articular cartilages and intra–articular disc compared to horses over 20 years old.[20] However, specific histopathological findings were not described, particularly inflammatory signs in the joint capsule. Age–related differences in the appearance of articular cartilage, as described by Smyth et al.[20] were well–illustrated but not specifically described by Mirahmadi et al.[26]. Mirahmadi et al.[26] 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, changes occurring in the aging articular cartilage of the mandibular condyle are different from those seen in hyaline cartilage.[26] Since the articular cartilage is unable to completely heal, damage accumulates and becomes more frequent in older horses.[20] However, these studies evaluated CT[21] and histopathological[5,26] findings independently, without a reference. Only Guerrero Cota et al.[25] used gross evidence as a reference to assess the presence and severity of OA. In previous studies, gross examination of the TMJs was not conducted.[5,21,26] Interestingly, none of the horses in Guerrero Cota et al. study[25] had a history of clinical symptoms of TMJ disease, yet 12 of the 32 TMJ analyzed showed gross evidence of mild OA, and 9 had severe OA, which led to a reduction in the tensile properties of the intra–articular disc.[25]

One limitation of this study is that gross examination of the TMJs was not performed. However, the reference for confirming TMJ OA was based on both, the connection between degenerative and inflammatory changes leading to synovitis and capsulitis,[1,22] as well as degenerative changes accumulated with aging in horses.[20,26] A second limitation is the absence of clinical data of the horses whose heads were studied. Since the heads were collected post–mortem at the slaughterhouse, no clinical symptoms could be assessed. Similarly, in Carmalt et al.'s study[21] on large sample of asymptomatic horses, follow–up data and information on the horses' behavior during eating or riding were unavailable, making it difficult to fully determine the absence of mild symptoms of TMJ dysfunction. The authors suggested that some horses with 'CT anatomical variations' may have had subclinical TMJ disease, without showing moderate or severe clinical symptoms of pain or dysphagia.[21] Under specific conditions,[6,7] these horses may exhibit aversive behaviours, decreased performance, or mild masticatory problems. Experimental studies have shown that horses with TMJ pain induced by inflammation can adjust their eating habits to maintain weight and body condition.[42] Other experimental studies have indicated that horses with acute TMJ inflammation tend to avoid pressure from the bit on the affected side, although the handler may counterbalance this by increasing rein tension on the unaffected side, masking any objective differences.[43] Additionally, acute inflammation responses differ 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 account for the limited

publications on TMJ OA related to 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 vague clinical symptoms, TMJ OA cases may be missed or misdiagnosed.[5,6,7] This hypothesis is supported by the results of current study, which show that the sensitivity and specificity of TMJ OA diagnosis vary depending on which CT findings are considered, leading to differing numbers of histologically confirmed cases.

Despite these limitations, the results of this study suggest that not only clinically normal horses,[20] but also horses with active inflammatory processes in the TMJ, accumulate changes in the joint with age. These changes can be detected through both CT imaging[21] and histopathology[20]. While histopathology of the TMJ provides definitive evidence of degeneration of the articular cartilage and intra–articular disc,[5] it requires post–mortem sampling. However, synovial fluid, which can be collected in live horse via arthrocentesis, has been used in research[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. Moreover, synovial fluid may be collected during the same procedure as local analgesia[6,7,13] and intra–articular medication,[5,7] which does not require additional arthrocentesis. Smyth et al.[20] highlighted that their study was limited by the lack of correlation between histopathological findings and inflammatory cytokine concentrations. Future research should, therefore, focus on a broader range of TMJ OA biomarkers[22] to better understand the significance of histopathological findings and support clinical trials targeting mild clinical symptoms. Since CT imaging does not allow for direct visualization of articular cartilage, magnetic resonance imaging (MRI) would enhance further investigation. Following the suggestion of Carmalt et al.,[21] MRI could help distinguish between degenerative and developmental changes in the mandibular condyle. Therefore, work is currently underway to further investigate the MRI findings and synovial fluid biomarkers for equine TMJ OA. We hope that this study and future research will help answer the question posed by Carmalt et al.[21] “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 in this study and were 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', their inclusion or exclusion in diagnostic protocols affects the sensitivity and specificity of CT–based TMJ OA recognition. However, the clinical significance of these results remains unknown.

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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[5–7,14,15,21] 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[14,21]	2	ScHD+Scl MC* Figs. 2[15],7[21]	scattered regions of hypodensity in MC with surrounding hyperdensity[15,21]	2	Medial Ent MC* Fig. 4[21]	enthesiophytes on the medial aspect of MC[21]	2	Narrow JS Fig. 1[15]	narrowing of the joint space[15]
3	Irr MC Fig. 1[15]	irregularity of MC[15,21]	3	ScHD-Scl MC* Fig. 2[21]	scattered regions of hypodensity in MC without surrounding hyperdensity[21]	3	Lateral Ost MC	marginal osteophytes on the lateral aspect of MC[21]	3	Wide JS Fig. 2[15]	widening of the joint space[15]
4	Irr TB	irregularity of TB[21]	4	ScHD+Scl TB* Fig. 2[15]	scattered regions of hypodensity in TB with surrounding hyperdensity[15,21]	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[21]	hyperdensity of the intra–articular disc[5,6,21]
			5	ScHD-Scl TB*	scattered regions of hypodensity in TB without surrounding hyperdensity[21]	5	Lateral Ost TB	marginal osteophytes on the lateral aspect of TB[21]	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[21]	spherical region of hypodensity in MC with surrounding hyperdensity [6,7,15,21]				6	Min Soft	hyperdensity of soft tissues of the periarticular region[21]
			7	SphHD-Scl MC* (group 2 of cyst) Fig. 3[21]	spherical region of hypodensity in MC without surrounding hyperdensity[21]						
			8	SphHD+Scl TB* (group 1 of cyst)	spherical region of hypodensity in TB with surrounding hyperdensity[21]						
			9	SphHD-Scl TB* (group 2 of cyst)	spherical region of hypodensity in TB without surrounding hyperdensity[21]						
			10	Cleft MC*	linear region of hypodensity in MC with surrounding hyperdensity[21]						

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 62/82 (76%), 30/82 (37%), or 34/82 (41%) joints.

CT findings	**Age**		
	1–4 years (*n* = 34)	**5–15 years (*n* = 24)**	**>15 years (*n* = 24)**
Bone surface			
Normal	32 (94%)	2 (8%)	0
Flatt MC*	0	18 (75%)	24 (100%)
Irr MC	2 (6%)	10 (42%)	8 (33%)
Subchondral bone			
Normal	19 (54%)	14 (58%)	15 (63%)
ScHD-Scl MC*	12 (35%)	0	0
SphHD+Scl MC*	3 (9%)	7 (29%)	7 (29%)
SphHD-Scl MC*	0	3 (13%)	2 (8%)
Bone margins			
Normal	34 (100%)	0	0
Medial Ent MC*	0	24 (100%)	24 (100%)
Lateral Ost MC	0	1 (4%)	0
Joint space and soft tissue			
Normal	34 (100%)	19 (79%)	21 (88%)
Narrow JS	0	5 (21%)	3 (13%)
Entire CT findings	17 (50%)	24 (100%)	24 (100%)
CT findings - AV	2 (6%)	13 (54%)	11 (46%)
CT findings - AV / + C	5 (15%)	15 (63%)	14 (58%)
Chi–square test	$P < 0.0001$	$P < 0.0001$	$P = 0.84$

Note: AV = anatomical variations; C = cyst; 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; + = with; - = without; % = percentage of affected joints from each age category; * = CT anatomical variations. The differences were considered significant for *P*–value < 0.05.

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**		
	1–4 years (*n* = 34)	**5–15 years (*n* = 24)**	**>15 years (*n* = 24)**
Articular cartilage of the mandibular condyle			
Normal	34 (100%)	3 (13%)	0
Cartilage degeneration	0	20 (83%)	24 (100%)
Chondrocyte apoptosis	0	13 (54%)	10 (42%)
Chondrones	0	0	6 (25%)
Angiogenesis	0	3 (13%)	1 (4%)
Articular cartilage of the temporal bone			
Normal	34 (100%)	6 (25%)	2 (8%)
Cartilage degeneration	0	17 (71%)	22 (92%)
Chondrocyte apoptosis	0	9 (38%)	11 (46%)
Angiogenesis	0	0	3 (13%)
Intra–articular disc			
Normal	34 (100%)	24 (100%)	24 (100%)
Joint capsule			
Normal	27 (79%)	9 (38%)	4 (17%)
Intimal hyperplasia	0	2 (8%)	0
Subintimal edema	0	6 (25%)	3 (13%)
Subintimal fibrosis	0	3 (13%)	13 (54%)
Inflammatory cells	6 (18%)	14 (58%)	17 (71%)
Angiogenesis	0	9 (38%)	10 (42%)
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 category. The differences were considered significant for *P*–value < 0.05.

Table 4. The CT–based recognized and histopathologically confirmed temporomandibular joint osteoarthritis (TMJ OA) in 41 horses (82 TMJs) supported with assessment of accuracy of TMJ OA recognition.

Age	Criteries	Recognized / Confirmed; *n* / *n* (%)	Se	Sp	PPV	NPV
1–4 years	Entire CT findings	17 / 4 (24%)	0.67	0.54	0.24	0.88
	CT findings - AV	2 / 1 (50%)	0.17	0.96	0.50	0.84
	CT findings - AV / + C	5 / 2 (40%)	0.33	0.89	0.40	0.86
5–15 years	Entire CT findings	24 / 15 (63%)	1.00	0.00	0.63	–
	CT findings - AV	13 / 10 (77%)	0.67	0.67	0.77	0.55
	CT findings - AV / + C	15 / 11 (73%)	0.73	0.56	0.73	0.56
>15 years	Entire CT findings	24 / 20 (83%)	1.00	0.00	0.83	–
	CT findings - AV	11 / 10 (91%)	0.50	0.75	0.91	0.23
	CT findings - AV / + C	14 / 13 (93%)	0.65	0.75	0.93	0.30
Total	Entire CT findings	65 / 39 (60%)	0.95	0.37	0.60	0.88
	CT findings - AV	26 / 21 (81%)	0.51	0.88	0.81	0.64
	CT findings -AV / + C	34 / 26 (76%)	0.63	0.80	0.76	0.69

Note: AV = anatomical variations; C = cyst; Cleft = linear region of hypodensity; CT = computed tomography; + = with; - = without; *n* / *n* = number of CT–based recognized and histopathologically confirmed OA; % = percent of histopathologically confirmed OA from CT–based recognized OA in studied horses. Se = sensitivity, Sp = specificity, PPV = positive predictive value, NPV = negative predictive value.

Table 5. 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 highlighted with bold font.

Figures

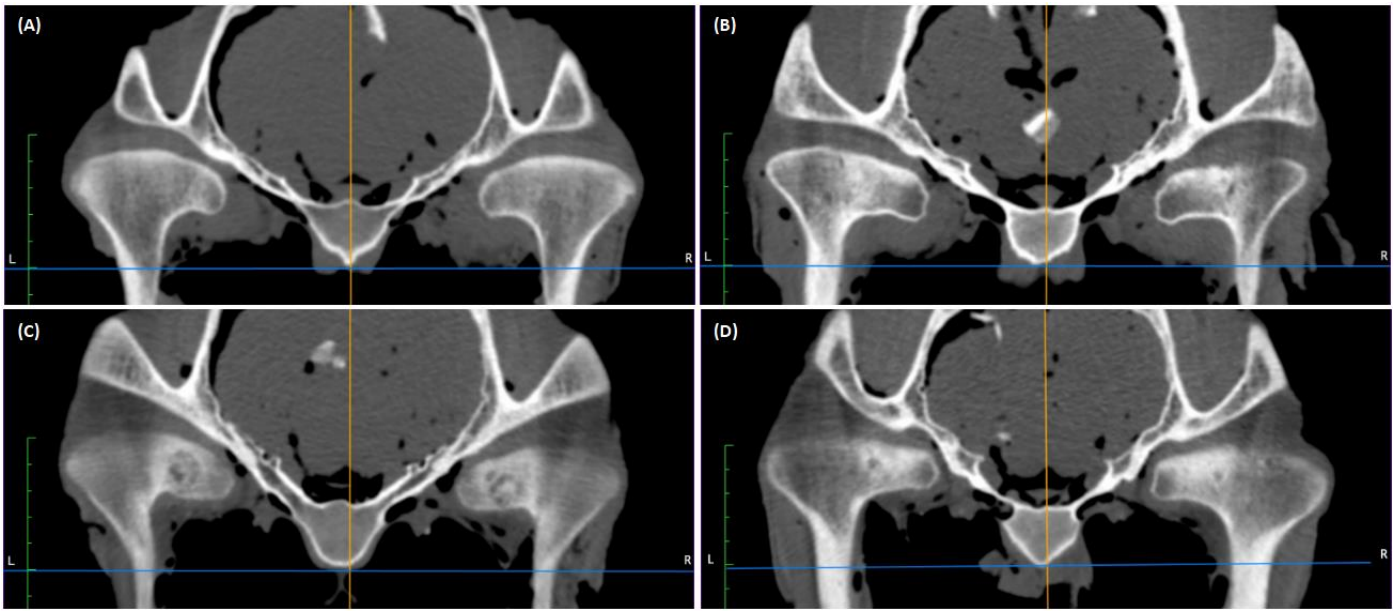

FIGURE 1 Examples of transverse CT images of temporomandibular joints (TMJs) of 1–4 years old horses. A, Globose shape and homogenous mandibular condyles in normal joints. B, Irregularity of the bone surface of the right mandibular condyles. C, Scattered regions of hypodensity in the right and left mandibular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

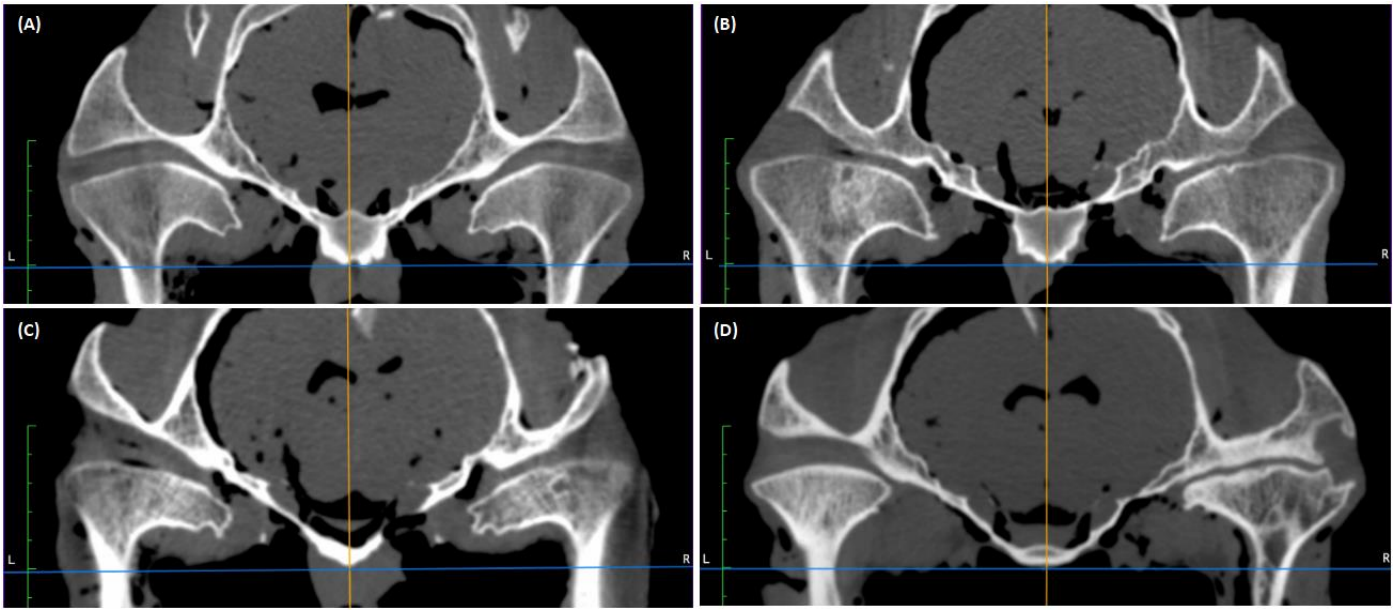

FIGURE 2 Examples of transverse CT images of temporomandibular joints (TMJs) of 5–15 years old horses. A, Flattening and medial enthesiophytes of both mandibular condyles. B, Irregularity of the bone surface of the right mandibular condyle and spherical region of hypodensity (subchondral bone cysts) 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.

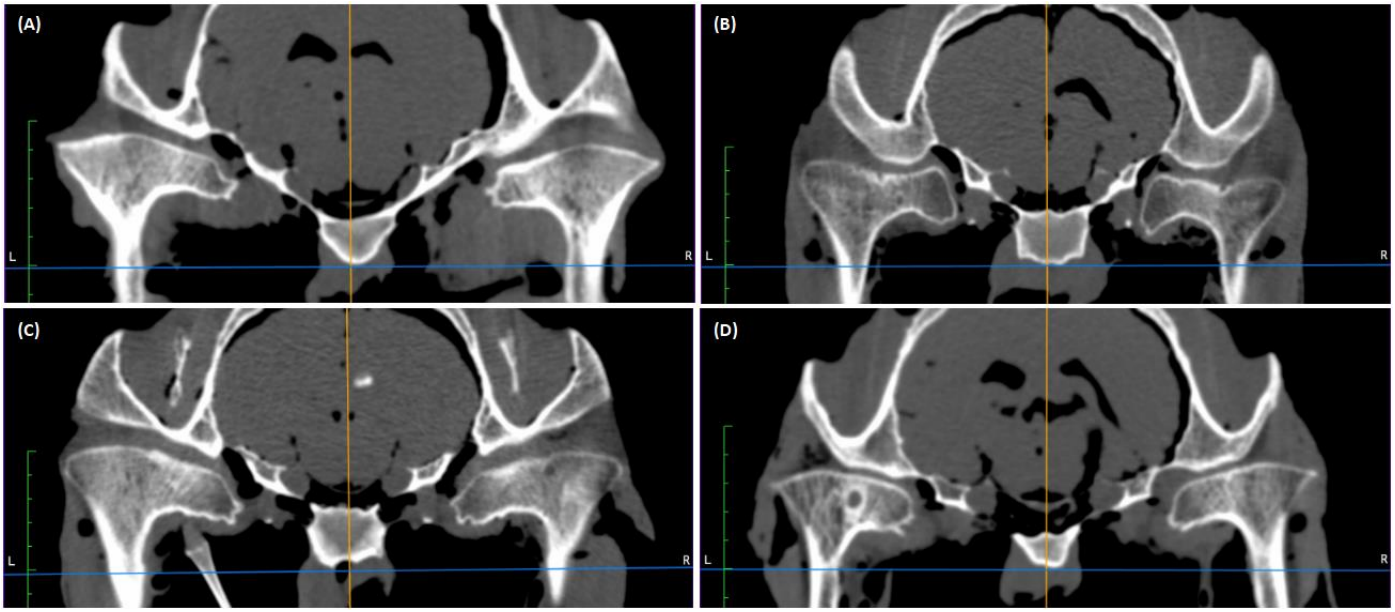

FIGURE 3 Examples of transverse CT images of temporomandibular joints (TMJs) of >15 years old horses. A, Flattening and medial enthesiophytes of both mandibular condyles. B, Irregularity of the bone surface of both mandibular condyles. Flattening and medial enthesiophytes of both mandibular condyles. 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.

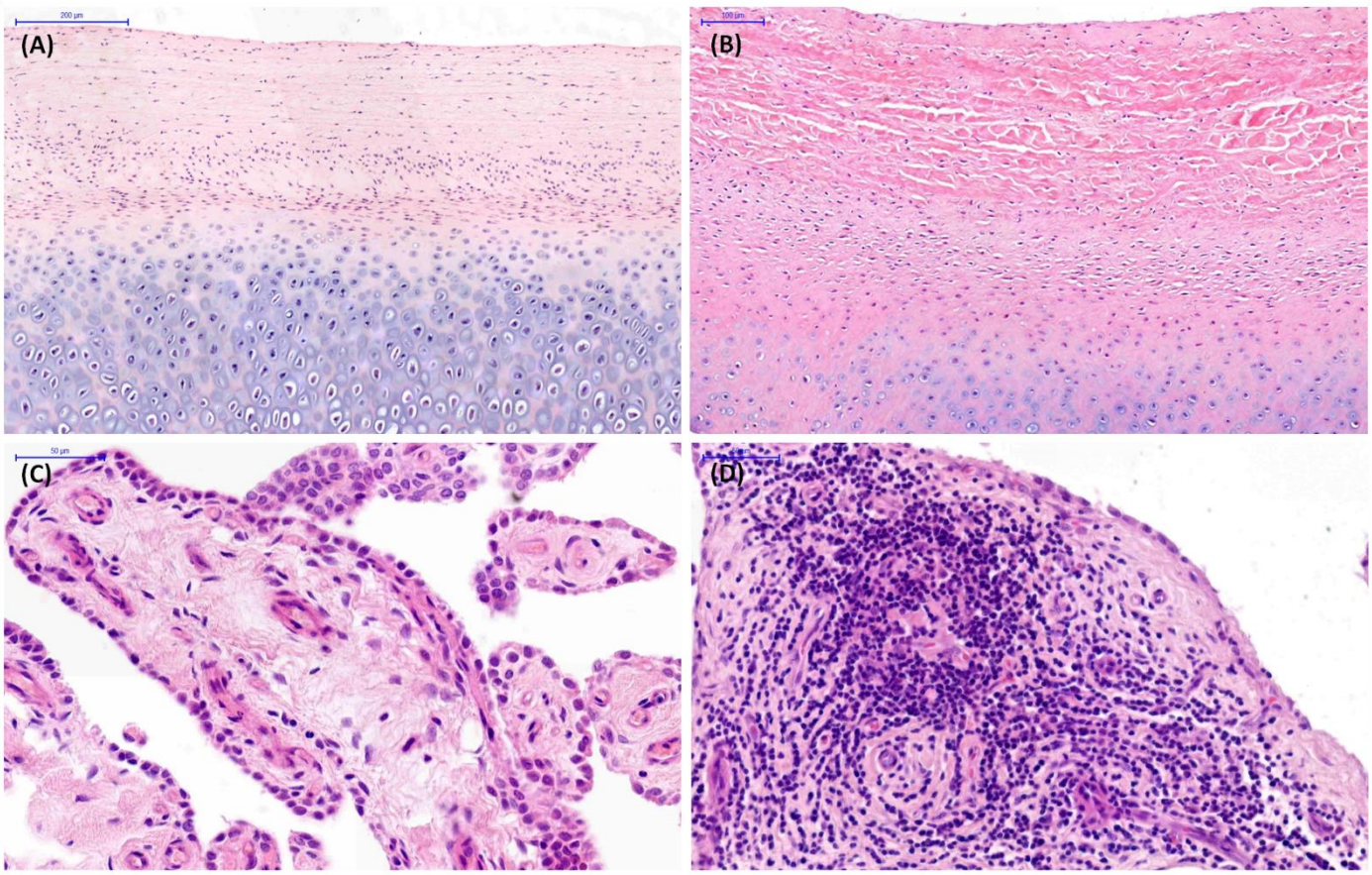

FIGURE 4 Examples of histopathological images of H–E stained temporomandibular joint (TMJ) tissue sections of 1–4 years old horses. A, Normal articular cartilage of the mandibular condyle. Scale bare = 200 µm. B, Normal articular cartilage of the temporal bone. Scale bare = 100 µm. C, Normal joint capsule.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

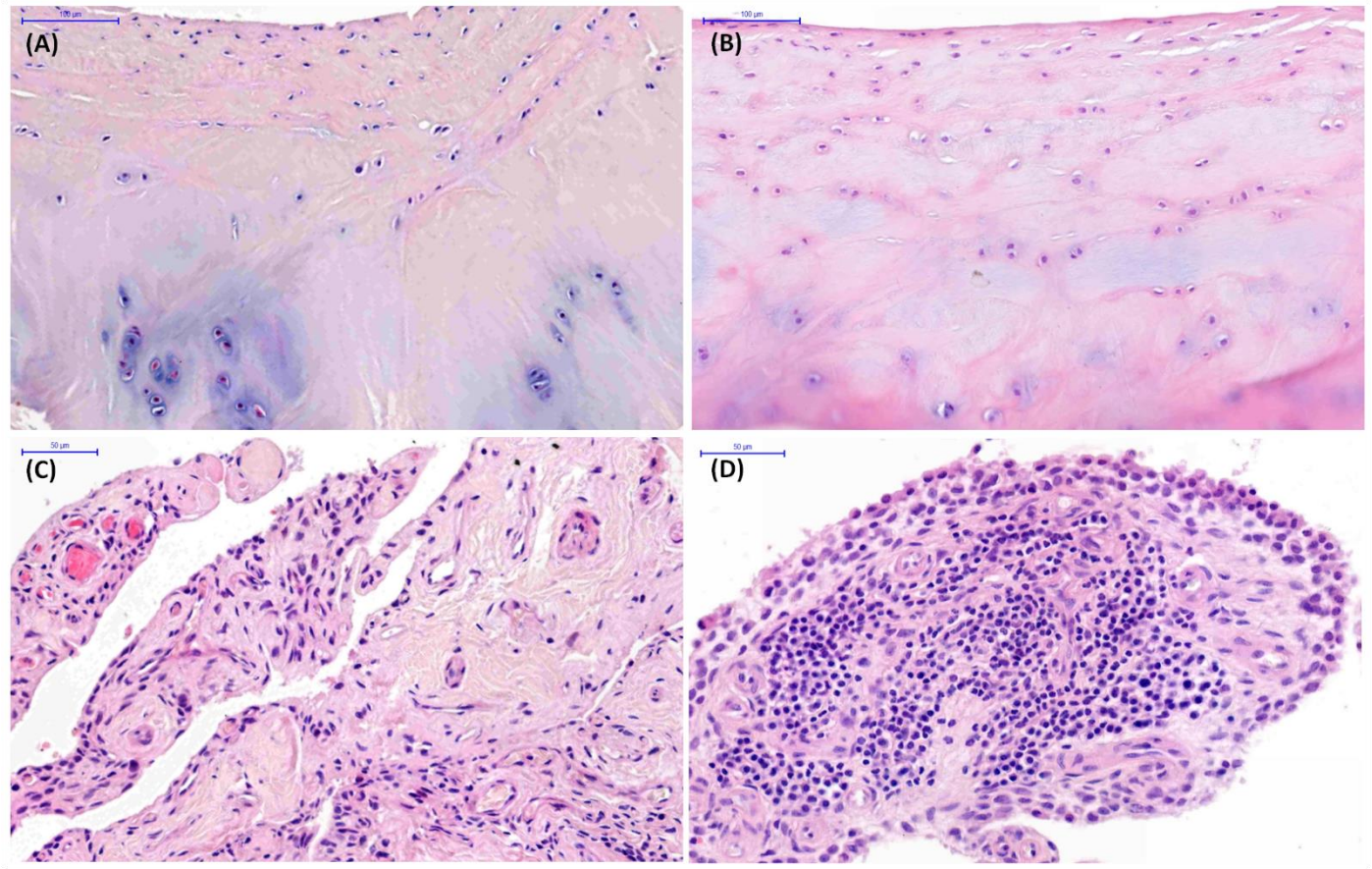

FIGURE 5 Examples of histopathological images of H–E stained temporomandibular joint (TMJ) tissue sections of 5–15 years old horses. A, Cartilage degeneration and chondrocyte apoptosis of articular cartilage of the mandibular condyle. Scale bare = 100 µm. B, Cartilage degeneration and chondrocyte apoptosis 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.

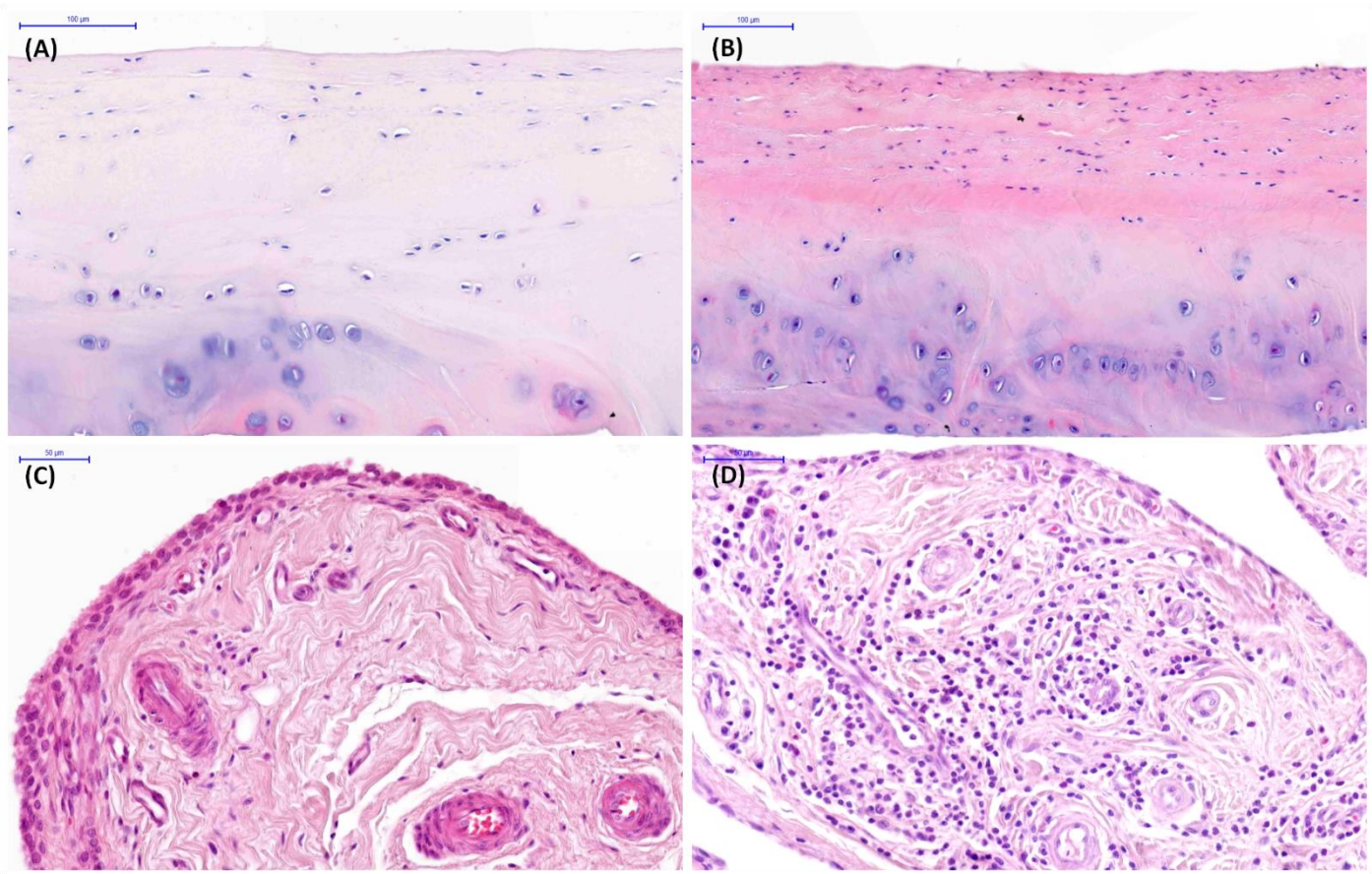

FIGURE 6 Examples of histopathological images of H–E stained temporomandibular joint (TMJ) tissue sections of >15 years old horses. A, Cartilage degeneration, chondrocyte apoptosis, and chondrones in articular cartilage of the mandibular condyle. Scale bare = 100 μm. B, Cartilage degeneration and chondrocyte 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 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 highlighted with bold font.