Sveriges lantbruksuniversitet
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Faculty of Veterinary Medicine and Animal Science

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- a comparison of diagnostic imaging and histologic findings

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– en jämförelse mellan bilddiagnostiska och histologiska fynd

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SUMMARY

Osteoarthritis (OA) is a progressive, low-grade inflammatory disease of synovial joints. It is characterized by the degradation of articular cartilage but involves the whole joint including the subchondral bone and soft tissues. In cats, it is a frequent disease of appendicular joints and the coxofemoral joint is amongst the most frequently affected. Feline OA may be challenging to diagnose, as cats may not show typical clinical signs of joint-associated pain. There is a lack of knowledge regarding which mild joint changes indicate coxofemoral OA compared to normal anatomical variations in coxofemoral joint shape, margins and density. As computed tomography (CT) has several advantages over radiography (e.g. capacity to detect mild changes in density, providing a three-dimensional image and thus the ability to reconstruct the image in any plane), it would be of interest to know whether CT is a useful tool in diagnosing feline OA.

The aims of this study were to investigate whether osseous changes in feline coxofemoral joints detected by CT are associated with histologic findings of OA, as well as to describe the changes seen with CT and histology. An additional aim was to compare the frequency of detection of feline coxofemoral joint OA by CT and radiography.

The study material included whole-body CT studies and histological samples from 21 euthanized cats (29 coxofemoral joints). Eight cats also had radiographs. Some of the samples had been collected previously but all histologic sampling done in 2018 was CT-guided (20 joints from 12 cats), i.e. areas of specific interest in CT images were sampled using subjective guidance from CT images. CT and radiography images of joints were graded for lesions suspected to indicate OA. Histologically, a system based on Pritzker *et al*. (2006) for grading cartilage lesions was used and other lesions such as osteophytes were recorded. After grading of the joints, statistical analysis of association between classifications of joints as either OA positive or OA negative (between histology and CT) was performed using Fisher's exact test. To investigate associations between grades (Pritzker and CT) and age, and between CT grades and radiography grades Spearman rho was used.

The result of this study shows that osseous changes detected by CT are associated to histologic OA lesions in feline coxofemoral joints. However, the results need to be interpreted with care, as the majority of joints were histologically OA positive (27 of 29 joints) and the statistical analysis of the data was limited. Of the 27 histological OA positive joints, 23 joints (85%) were diagnosed as OA positive by CT. The frequency of detection of coxofemoral joint OA for CT and radiography is about the same (9/14 joints for radiography, 10/14 joints for CT). No certain conclusion could be made about CT's ability to detect histologically normal joints, as there were few joints without histologic cartilage lesions in the study.

There were eleven joints that were diagnosed as OA positive by CT based on the presence of osteophytes, where osteophytes could not be verified histologically. Whether there were no osteophytes in the joints (false positive CT diagnosis) or whether the suspected osteophyte was not included in the histology sample is not possible to determine. Nevertheless, the majority of these eleven joints had other bone shape features seen histologically which were suspected to explain the 'osteophyte' suspected in the CT image. Further research is needed to determine whether these shapes are normal variation or osseous features associated to OA.

CONTENT

INTRODUCTION

Osteoarthritis (OA) is a progressive, low-grade inflammatory joint disease that involves degradation of all tissues of synovial joints (Robinson *et al*., 2016). In cats, it is a frequent disease with prevalence from radiographic studies being as high as 61% in middle aged to elderly cats (Slingerland *et al*., 2011). Of the appendicular joints affected by OA, the coxofemoral joint is reported to represent between 17 and 51%, making it one of the most frequently affected joints (Clarke *et al*., 2005; Godfrey, 2005; Lascelles *et al*., 2007).

The diagnosis of feline OA is complicated by the fact that radiographic and post-mortem findings (macroscopic and histologic findings in joints) do not always correlate with clinical findings (Clarke and Bennett, 2006; Lascelles *et al*., 2007; Freire *et al*., 2011). Some authors argue that radiographic signs such as osteophytes are not as frequent or severe in feline joints as they are in canine joints, and that it may not be appropriate to apply the same methods in diagnosing the disease in those two different species (Hardie *et al*., 2002; Clarke and Bennett, 2006; Lascelles *et al*., 2010). Furthermore, there is still a lack of detailed knowledge concerning the appearance of normal anatomic features compared to lesions indicative of OA in the feline coxofemoral joint, and few studies verify the result of clinical and/or radiographic findings by post-mortem and histological examination.

Computed tomography (CT) has several advantages over radiography. Compared to radiography there is more contrast between tissues in a CT image so that milder changes in density can be detected. Furthermore, the information in a CT image is three-dimensional (3D) and structures can be viewed in any plane, thus there is no superimposition of adjacent structures (Schwarz and Saunders, 2011; Mahoney, 2012). These properties, in combination with the increasing availability of CT in veterinary medicine make it of great interest to know if CT could be a useful tool to diagnose OA in cats and if it is better than radiography for OA detection. There are only a very limited number of reports regarding the use of CT for the diagnosis of OA in veterinary medicine.

The aims of this study were to:

- Investigate whether osseous changes in feline coxofemoral joints detected by CT are associated with OA diagnosed by histology and thus whether CT is a useful tool in the diagnosis of OA.
- Describe osseous features of coxofemoral feline joints found in CT images and histology.
- Compare the frequency of detection of feline coxofemoral joint OA by CT and radiography.

LITERATURE REVIEW

The synovial joint

General architecture

A synovial joint consists of two bones which are separated by a fluid-filled cavity (Fig. 1). This generally allows a large range of motion between these bones. The joint cavity is surrounded by a synovial membrane that inserts on the periphery of the articular surfaces and in most synovial joints, a fibrous capsule (the joint capsule) strengthens the synovial membrane externally. In certain joints, ligaments (i.e. additional strong fibrous bands) are localized in strategical sites to add to the joint's stability or restrict movement in certain directions (Dyce et al., 2010).

The articular surface consists of a thin layer of hyaline cartilage on the synovial cavity side of the bone surfaces, in order to reduce friction between opposing joint surfaces and protect the bone ends from traumatic injury (Sjaastad *et al*., 2010). Macroscopically, the articular cartilage is translucent and glassy, generally white with a blue or pink shade in young animals. A yellowish color implies degenerative change, i.e. loss of elasticity (Dyce *et al*., 2010). Adult articular cartilage is softer and smoother compared to bone tissue, and it does not contain blood vessels or nerves (Sjaastad *et al*., 2010).

The synovial membrane produces synovial fluid that fills the joint cavity and provides lubrication to the articular surfaces, as well as nutrients to cells in the cartilage layer (chondrocytes) (Sjaastad *et al*., 2010). In normal joints, the synovial membrane is glistening pink (Dyce *et al*., 2010).

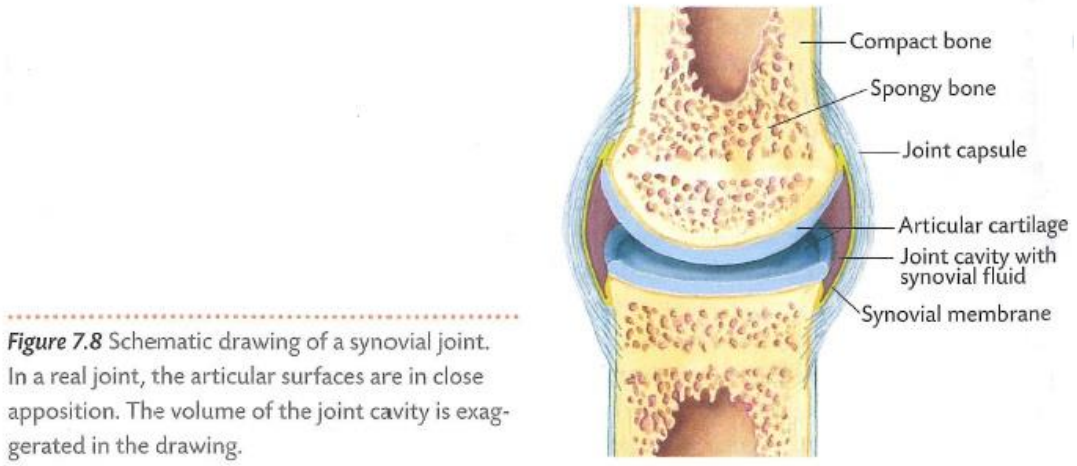

Figure 7.8 Schematic drawing of a synovial joint. In a real joint, the articular surfaces are in close apposition. The volume of the joint cavity is exaggerated in the drawing.

Fig. 1. *Synovial joint architecture. Printed with permission from Sjaastad et al. (2010). Oslo: Scandinavian Veterinary Press.*

Normal histology

The articular cartilage of synovial joints is avascular (Kierszenbaum and Tres, 2016) and its architecture is well-arranged with a superficial, a middle and a deep zone of uncalcified hyaline cartilage (Fig. 2) (Pritzker, 2003). The so-called tidemark divides this cartilage layer from the calcified cartilage beneath it. The superficial zone contains elongated, flattened chondrocytes,

while chondrocytes in the middle and deep zones are more ovoid in shape. In the deep zone, the chondrocytes are vertically aligned (Pritzker, 2003).

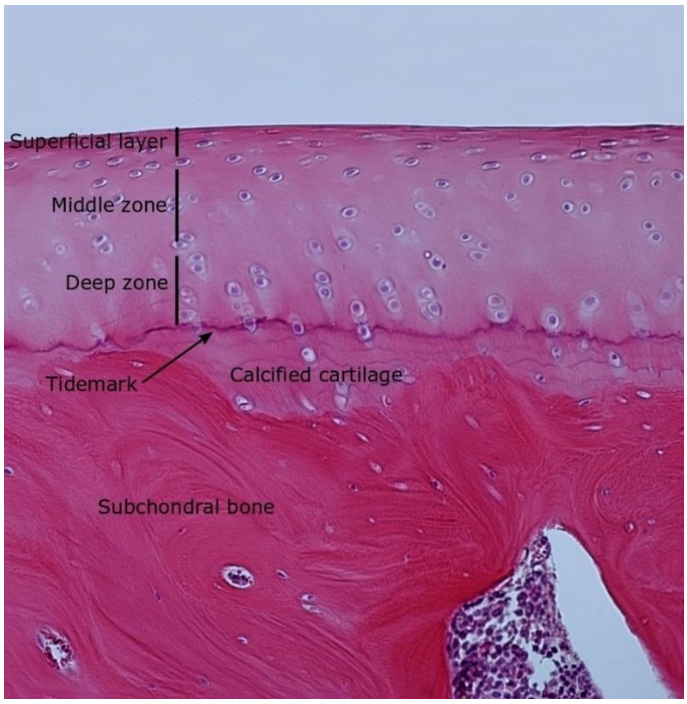

Fig. 2. *Histologic appearance of normal feline articular cartilage. Hematoxylin and eosin stain x20.*

Chondrocytes produce an extracellular matrix (ECM), which consists of mainly type II collagen fibers, the proteoglycan aggrecan, hyaluronic acid, structural non-collagenous proteins, glycoproteins, ions and water (Pritzker, 2003; Kierszenbaum and Tres, 2016). In the superficial zone, collagen fibers are arranged parallel to the cartilage surface, while the collagen fibers in the middle zone change direction and are aligned perpendicular to the cartilage surface in the deep zone (Pritzker, 2003).

Below the deep zone and tidemark lies a zone of calcified cartilage in which the chondrocytes are embedded in a matrix containing calcium apatite crystals, and deep to this cartilage layer lies the subchondral bone (Fig. 2) (Pritzker, 2003).

The joint capsule has a synovial lining that surrounds the joint cavity. The synovial lining comprises one or two discontinuous cell layers of synoviocytes, including macrophage-like synovial cells (type A) and fibroblast-like synovial cells (type B), without basal lamina but with some underlying fibrous connective tissue and/or adipose tissue. The joint capsule consists of dense type I collagen fiber bundles in-between which elongated fibrocytes are embedded (Pritzker, 2003; Kierszenbaum and Tres, 2016).

Anatomy of the feline coxofemoral joint

The coxofemoral joint is a ball-and-socket joint allowing a large range of motion (Sjaastad *et al*., 2010). It consists of the acetabulum (the lateral depression of the *os coxae*) and the femoral head, surrounded by a fibrous joint capsule (McClure *et al*., 1973).

The acetabulum consists of parts of all three bones of the *os coxae – ischium*, *ilium* and *pubis* (Dyce *et al*., 2010). The lunate articular surface composes the cranial, dorsal and caudal parts of the acetabular joint part (Fig. 3A). In its ventromedial part, the articular surface is interrupted by the acetabular notch. Across this notch extends the transverse acetabular ligament (McClure *et al*., 1973). The transverse ligament is continuous with the acetabular labrum, which is a fibrocartilaginous structure that attaches to the acetabular rim and by that enlarges the lunate articular surface (Fig. 4) (Seldes *et al*., 2001; Dyce *et al*., 2010). The deepest part of the acetabulum is the acetabular fossa, in which the femoral head ligament (i.e. round ligament) has its insertion. This ligament attaches in the fovea of the head (*fovea capitis*), which is a depression on the medial aspect of the head (Fig. 3B) (McClure *et al*., 1973).

The joint capsule attaches around the acetabular margin and around the femoral neck. The joint capsule contains three minor thickenings, the ischiofemoral, iliofemoral and pubofemoral ligaments (McClure *et al*., 1973).

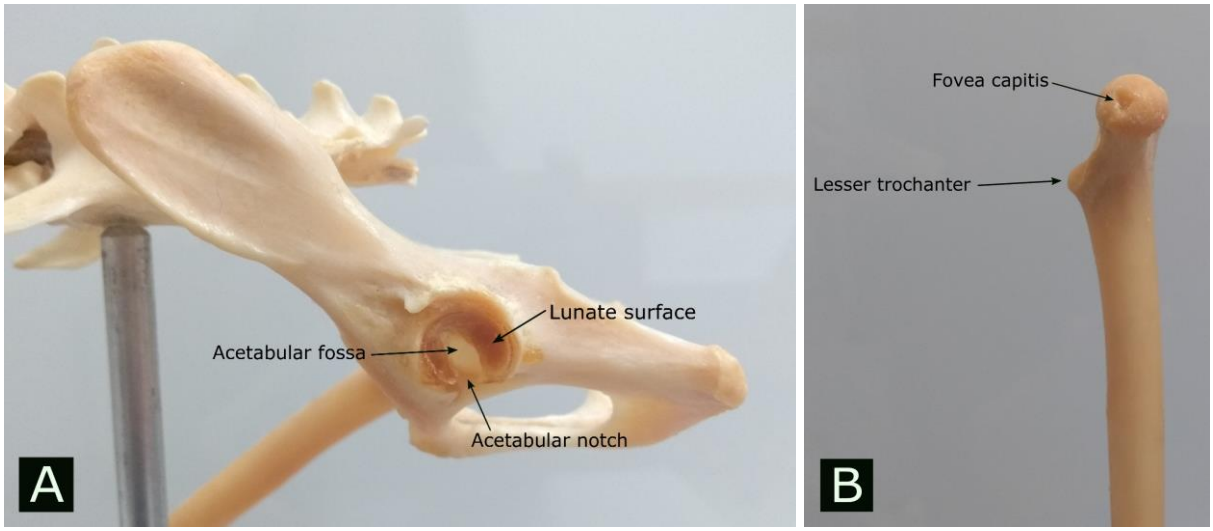

Fig. 3. *Photographs of bone specimens showing the feline acetabulum in lateral view (A) and femur in medial view (B.)*

Fig. 4. *Histologic image (hematoxylin and eosin stain, x4) of normal acetabular labrum (arrows) on the caudoventral acetabular margin. Note also the insertion of the joint capsule a short distance from the articular surface*.

Osteoarthritis

Definition of OA

OA is a progressive, low-grade inflammatory disease that involves destruction of all tissues in a synovial joint. It leads eventually to the structural and functional destruction of the joint (Pritzker, 2003; Poulet and Staines, 2016; Robinson *et al*., 2016; Deveza and Loeser, 2018). In human literature, some authors describe OA as a complex syndrome with several subtypes (based on underlying etiology or disease mechanisms) rather than a single disease (Deveza and Loeser, 2018).

The Osteoarthritis Research Society International (OARSI) is a medical society dedicated to advancing the understanding, early detection, treatment and prevention of OA through research (OARSI 2018). Their current definition of OA is as follows:

"Osteoarthritis is a disorder involving movable joints characterized by cell stress and extracellular matrix degradation initiated by micro- and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal joint tissue metabolism) followed by anatomic, and/or physiologic derangements (characterized by cartilage degradation, bone remodeling, osteophyte formation, joint inflammation and loss of normal joint function), that can culminate in illness." (OARSI 2018)

This means that OA can be defined and diagnosed on several levels including a molecular, microscopic, macroscopic, radiologic and clinical level.

It is important to distinguish the term OA from degenerative joint disease (DJD), which includes OA but also other lesions of joints such as isolated degenerative changes (e.g. enthesiophytes and degenerative soft tissue mineralization which might be a result of joint inflammation), spondylosis deformans of intervertebral disc joints and traumatic arthritis (Pritzker, 2003; Bennett *et al*., 2012).

Etiology

The exact mechanisms in the development of OA are still largely unknown (Gao *et al*., 2013; Poulet and Staines, 2016). Earlier, OA has been classified as either idiopathic/primary or secondary in both human and veterinary medicine literature. This classification system is now questionable, as etiologies have been identified for most of idiopathic cases of OA in humans (Deveza and Loeser, 2018).

Many gene expression studies have been made in humans and those studies have found a great variety of gene expression patterns which makes it difficult to draw any apparent conclusion regarding genetic predisposition (Kumarasinghe *et al*., 2010; den Hollander *et al*., 2018; Skarp and Kiviranta, 2018). On the other hand, many risk factors or contributing factors have been linked to OA in humans including ageing, metabolic disturbances, obesity, joint injury, biomechanical factors, (i.e. joint shape and alignment) and hormonal changes such as oestrogen deficiency (Poulet and Staines, 2016; Deveza and Loeser, 2018).

Etiology of feline OA

In cats, some causes for OA are well established such as osteochondrodysplasia in the Scottish Fold breed and metabolic disturbances such as mucopolysacharidosis (Gao *et al*., 2013; Godfrey, 2005). Other causes for OA in cats are hip dysplasia (HD), traumatic injuries such as fractures and articular cartilage injuries, systemic diseases such as acromegaly, nutritional disorders, non-infectious polyarthropathy, infectious arthropathy and osteomyelitis (Pritzker, 2003; Godfrey, 2005; Clarke and Bennett, 2006; Gao *et al*., 2013).

The cause of OA in cats is most often unknown (Godfrey, 2005; Clarke *et al*., 2005; Clarke and Bennett, 2006), which indicates that there still is a lack of knowledge concerning the etiology for feline OA (Bennett *et al*., 2012; Frye *et al*., 2016).

Most prevalence studies show a correlation between appendicular joint OA and age (Godfrey, 2005; Clarke and Bennett, 2006; Lascelles *et al*., 2010; Slingerland *et al*., 2011; Gao *et al*., 2013) but no correlation with breed (Hardie *et al*., 2002; Clarke *et al*., 2005) or gender (Hardie *et al*., 2002; Clarke *et al*., 2005; Clarke and Bennett, 2006; Lascelles *et al*., 2010). Gao *et al*. (2013) suggest that feline OA could be a form of 'accelerated ageing'.

Feline HD

Perry (2016) argues that cats normally have a shallower acetabulum than dogs, and that HD would be overdiagnosed if the criterion was less than 50 per cent coverage of the femoral head. According to some authors, HD accounts for 20% of feline hip OA cases (Bennett *et al*., 2012) and there are studies that show a radiologic prevalence of OA between 60 and 95.6% amongst cats with HD (Clarke *et al*., 2005).

The etiology for HD is mostly genetic with some breeds such as Maine Coon, Himalayan and Persian breeds having the highest frequency (Clarke *et al*., 2005; Perry, 2016).

The exact pathogenesis for HD leading to OA in cats is not fully established but one contributing factor is a shallow acetabulum and subsequent incongruence of the joint in these individuals (Clarke and Bennett, 2006; Perry, 2016). In dogs, HD features such as joint laxity with following subluxation of the femur is accepted as an important factor in OA pathogenesis. In cats though, subluxation of the femur is not consistently seen as a feature of OA, thus the role of joint laxity is more uncertain (Perry, 2016). One study done during 1998 showed a correlation between joint laxity and DJD in cats (Langenbach *et al*., 1998).

Pathogenesis

Lesions in OA may involve all the different tissues of the joint as the disease progresses, including articular cartilage, subchondral bone, synovial membrane and joint capsule (Pritzker *et al*., 2006; Deveza and Loeser, 2018). Although many aspects of its pathogenesis still are unknown, there is consensus that the earliest changes of the disease are molecular (Pritzker, 2003; Poulet and Staines, 2016; Kempf, 2018). Recent scientific research regarding the pathogenesis of OA shows that each of the different risk or predisposing factors may activate different pathways for the promotion of OA lesions and there may be some pathways that involve a wider range of inflammatory processes than others (Deveza and Loeser, 2018).

Different subsets of the disease may have different target tissues for primary lesions, such as the articular cartilage, bone, soft tissue or even a combination of those (Pritzker *et al*., 2006).

It is believed that OA lesions evolve as a result of an abnormal or inappropriate reparative process in the joint tissue (Pritzker, 2003) and it has been shown that oxidative stress is a main contributor to the development of these lesions (Poulet and Staines, 2016). Exactly how this process starts is not known but once initiated, pro-inflammatory cytokines such as different growth factors and matrix-degrading enzymes promote tissue destruction (Pritzker, 2003; Deveza and Loeser, 2018). Some of the suggested mediators of the process are insulin-like growth factor-1 (IGF-1), transforming growth factor-α (TGF-α) and TGF-β, interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α) and nitric oxide amongst others (Pritzker, 2003; Poulet and Staines, 2016; Frye *et al*., 2016). Other possible molecular alterations may include decreased/altered production of type II collagen and other supportive collagens, cyclo-oxygenase 2, matrix metalloprotease or other ECM component expression (Frye *et al*., 2016).

The initial morphologic reaction to these molecular alterations is edema in the cartilage ECM. Edema develops as a result of chondrocytes secreting enzymes which promote degradation of ECM proteoglycans. Without these proteoglycans, the ECM loses its ability to maintain normal water concentration. Adjacent chondrocytes will start producing proteoglycans in excess, leading to absorption of water and thus hypertrophy. The edema leads to debilitation of the weight bearing hyaline cartilage, as the meshwork of collagen fibers is stretched and thinned, leading to even greater susceptibility to mechanical injury such as cracks in the superficial matrix layer (called superficial fibrillation) or even matrix fragment delamination. At the base of this fibrillation, chondrocyte apoptosis and even necrosis begins to occur (Pritzker, 2003).

Once the ECM components have started to degrade, the process risks to go on as degradation products have been shown to promote further tissue degeneration (Poulet and Staines, 2016). An inflammatory response, which involves the synovial membrane and surrounding soft tissue occurs, as immune cells react to ECM components, plasma proteins, calcium phosphate crystals and alarmins (proteins/peptides derived from damaged cells) (Frye *et al*., 2016).

Simultaneously, the articular cartilage, which now has fewer viable chondrocytes, progressively loses the ability to restore and maintain ECM components such as proteoglycans and collagen. The susceptibility to injury is thus enhanced, with possible vertical crack propagation (called fissures) into deeper layers of cartilage and the degrading process then continues in chondrocytes adjacent to these fissures (Pritzker, 2003).

With progressing lesions in articular cartilage, its weight bearing capacity decreases and the calcified cartilage advances into the hyaline cartilage (Pritzker, 2003). Vascularization of the calcified cartilage layer has been shown to occur in feline joints (Leijon *et al*., 2017) and this increases the risk for microfractures through the cartilage (Pritzker, 2003). In these sites, reparative processes mediated by fibroblasts occur (Pritzker, 2003). Those fibroblasts produce a more fibrous matrix containing type I collagen. Hence, this repair tissue is more fibrocartilaginous and its fibers are organized perpendicular to the joint surface (Pritzker, 2003).

Additionally to developing fissures, the hyaline cartilage becomes subject to erosion such as delamination (loss of superficial zone fragments) and excavation (cavity formation with matrix loss in deeper layers). Further progression of the disease may lead to denudation, which means complete erosion of hyaline cartilage to the level of either calcified cartilage or subchondral bone. The subchondral bone becomes less mineralized than deeper trabecular bone and more metabolically active. There may also be fibrocartilaginous repair tissue on the subchondral surface and even above its level, which represents the earliest stage of remodeling and deformation of the articular surface. This process is, as mentioned above, triggered by microfractures and might continue in adjacent sites, developing into bone remodeling at cartilage/bone interfaces (mostly at the joint margin but sometimes more centrally in the joint) with activation of connective tissue at the joint margin. This activation triggers fibrocartilaginous proliferation and osseous metaplasia may evolve to form an osteophyte (Pritzker *et al*., 2006). Remodeling leads to alterations in mechanical load, and as a result, osteosclerosis and osteoporosis may develop. These changes are traditionally believed to be features of an advanced disease process but research has been able to demonstrate that this type of alterations in subchondral bone also occur earlier in the disease process (Turmezei and Poole, 2011).

Inflammatory processes in the soft tissue around the joint (synovitis) have been suggested to be related to more severe cartilage lesions in cats, as synovitis is found with increasing frequency when more severe OA lesions are seen (Leijon *et al*., 2017). This could imply that synovitis – at least in cats - rather is a result of OA than preceding the changes (Leijon *et al*., 2017), although Robinson *et al*. (2016) state that in humans, inflammation is present early in the disease mechanism and even preceding structural changes. This chronic, low-grade inflammation is mainly mediated by innate immune defense mechanisms and characterized by overexpression of inflammatory mediators in the synovial membrane (Robinson *et al*., 2016).

Osteophyte development

The pathophysiology of osteophyte formation is not completely understood (Kaneko *et al*., 2013; Junker *et al*., 2016).

Osteophyte formation tends to develop first on the joint margins but osteophytes may also develop centrally in the articular parts of the joint, so-called central osteophytes. Central osteophytes are believed to evolve as a result of repair of cartilage damage such as fissures, microfractures or excavation (Pritzker *et al*., 2006). Early osteophyte development is believed to be triggered by activation of connective tissue in which cells undergo chondrogenesis and osseous metaplasia. This occurs by mature chondrocytes subsequently being replaced by osteoblasts mediating new bone formation by endochondral ossification, deposition of bone and formation of bone marrow cavities (Pritzker *et al*., 2006; van der Kraan and van den Berg, 2007; Kaneko *et al*., 2013).

Several factors have been suggested to trigger osteophyte development, such as mechanical factors, growth factors and cytokines that stimulate mesenchymal stem cells in the periosteum to proliferate (van der Kraan and van den Berg, 2007; Junker *et al*., 2016). One cytokine suggested to be involved in osteophyte formation is TGF-β. It has been shown that macrophages, under the influence of TGF-β, produce factors that contribute to the stimulation

of mesenchymal cells. Amongst other factors, macrophage-derived bone morphogenetic proteins (BMP-2 and -4) are believed to play an important role (van Lent *et al*., 2004). The removal of synovial lining macrophages induced a drastic reduction of osteophyte formation in a study in mice, emphasizing the role of these cells in the pathogenesis (Blom *et al*., 2004).

Another factor which recently has been shown to be necessary for osteophyte formation in mice is perlecan, a type of proteoglycan expressed in cartilage and in the synovium. Perlecan is involved in cell growth, differentiation and signaling. Synovial cells upregulate the expression of perlecan in response to TGF-β. Mice who lacked perlecan expression had significantly reduced osteophyte size and maturation (Kaneko *et al*., 2013).

A study in humans from 2016 showed that marginal osteophyte size was not related to its development grade. Instead, size was influenced by intraarticular space; with limited space the osteophytes remained smaller. The study also showed that age and gender were not related to the number of osteophytes (Junker *et al*., 2016).

Features of OA

Macroscopic features of OA

Tissue changes related to OA may be seen more or less evident in all the different joint tissues depending on the progression of the disease.

Cartilage fibrillation or erosion may be seen macroscopically as roughened articular surface. In some joints, grooves (wear lines) and ridges or other surface irregularities may be prominent (Freire *et al*., 2011; Bennett *et al*., 2012). In joints with total cartilage loss, bone eburnation of the subchondral bone can be seen as smooth, shiny surface (Bennett *et al*., 2012).

In feline coxofemoral joints, cartilage damage has been shown to be most frequently located on the craniodorsal surface of the femoral head as well as the corresponding surface on the acetabulum (Freire *et al*., 2011).

Protruding new bone formation, i.e. osteophytes and joint surface remodeling may be seen macroscopically (Freire *et al*., 2011; Bennett *et al*., 2012). Osteophytes are identified by their irregular surface and lighter cartilage compared to normal articular cartilage (Junker *et al*., 2016).

The joint capsule may be thickened or contain calcified plaques. The synovium may contain osteocartilaginous bodies, so-called osteochondromas. These may be free within the joint cavity, attached to or embedded within the synovial membrane (Bennett *et al*., 2012).

Histologic OA features

Early morphologic OA changes in hyaline cartilage may present with cartilage swelling, i.e. edema (condensation of collagen fibers in the superficial or upper mid zone) or variable matrix cationic staining. There may be some proliferation of chondrocytes, viewed as chondrocyte clusters, cellular hypertrophy, disorganization of cellular arrangement, as well as chondrocyte death with cell membrane "ghosts" (Pritzker *et al*., 2006). These changes may appear mainly

superficially at the initial stage of the disease, but may extend into mid and deep zones of the cartilage with disease progression (Pritzker, 2003).

The ECM may present with superficial fibrillation, i.e. cracks which arise parallel to the cartilage surface in the superficial layer (Fig. 5A) (Pritzker, 2003). As more severe lesions evolve, vertical, oblique and branched fissures into mid and deep zones are seen (Fig. 5B). Matrix loss is seen as discontinuity of the surface and may involve only the superficial zone (abrasion and delamination) or even deeper zones (excavation when a bigger cartilage fragment is lost, denudation when matrix is lost to the level of subchondral bone) (Pritzker *et al*., 2006).

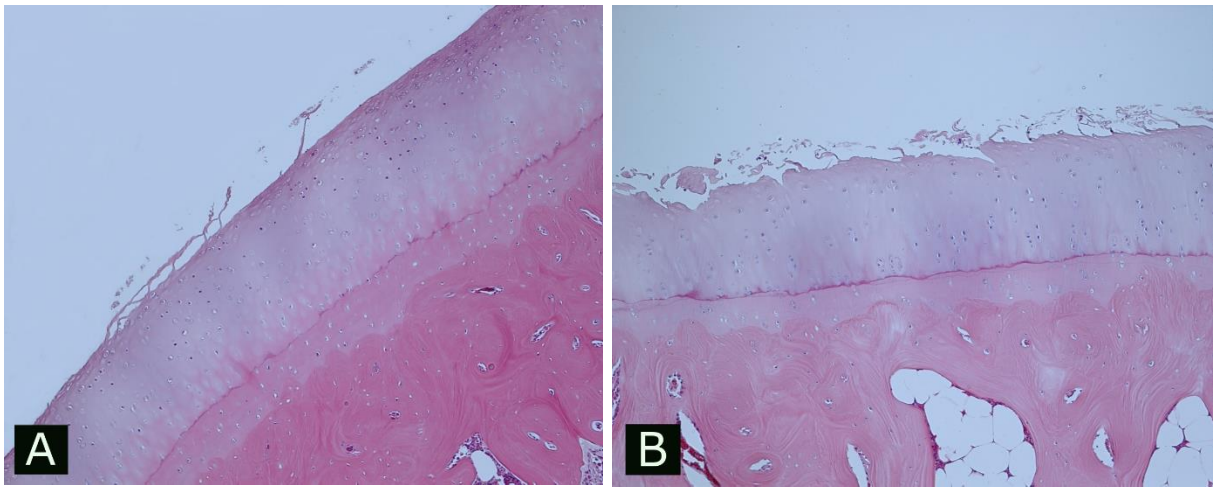

Fig. 5. *Examples of microscopic appearance of superficial fibrillation (A) and fissures into upper mid zone with superficial matrix loss (B). Hematoxylin and eosin stain x10.*

The calcified cartilage may become vascularized at the osteochondral junction and as calcification progresses focally or more diffusely, the tidemark may be disrupted by vascular structures (Pritzker, 2003; Leijon *et al*., 2017). Reduplication of the tidemark on the other hand is suggested to be a normal variation according to Leijon *et al*., 2017 in their study of feline stifle joints.

Subchondral bone is subject to bone resorption and reparative processes seen as fibrocartilaginous tissue or new bone formation. The subchondral bone plate becomes thickened. As the remodeling progresses, mechanical forces on the subchondral plate are altered and an adaption of the subjacent bone occurs; increased mechanical load leads to osteosclerosis while decreased mechanical load leads to osteoporosis. Disruption of the articular plate by microfractures may occur as a result of the remodeling process. Below the articular surface, bone necrosis with resorption, repair and apposition of new bone may follow (Pritzker, 2003).

Typical for OA is also the formation of osteophytes (van der Kraan and van den Berg, 2007; Bennett *et al*., 2012; Junker *et al*., 2016; Poulet and Staines, 2016), which are defined as "fibrocartilage-capped bony outgrowths" (van der Kraan and van den Berg, 2007; Junker *et al*., 2016). According to Junker *et al*. (2016) there are several grades of osteophytes in human patients, depending on their histological and immunohistochemical features. They also differ in size and location in the joint.

An osteophyte may consist of a cap of mesenchymal connective tissue, a cartilage area (in non-ossified osteophytes) and, in advanced osteophytes, an ossified area that may contain trabecular bone and bone marrow spaces. In contrast, a less developed osteophyte may consist of only connective tissue and cartilage areas (Junker *et al*., 2016).

Hyperplasia of the synovial lining, inflammatory lymphocytic infiltrates and stromal cell hypercellularity may be present (Pritzker, 2003; Leijon *et al*., 2017). If osteochondromas are present in the synovial membrane, these may be seen as areas of demarcated trabecular bone formation with a cartilaginous surface (Bennett *et al*., 2012).

Diagnosing feline OA

One of the major symptoms of OA in humans is pain with subjacent locomotion problems, joint stiffness and sleep disturbances. It is however well known that symptoms and radiologic signs may differ with some individuals experiencing pain without having radiologic signs of OA and the other way around (Deveza and Loeser, 2018). The same issue exists in veterinary medicine (Clarke and Bennett, 2006; Lascelles *et al*., 2007; Klinck *et al*., 2012).

In cats, assessing the full clinical picture of locomotion problems can be a challenge for several reasons. When it comes to assessment of pain, Clarke and Bennett (2006) showed that pain/OA may be over-diagnosed as a result of cats' resentment to orthopedic examination and handling (Clarke and Bennett, 2006). On the other hand, they argue that cats are less demonstrative of pain than dogs when it comes to signs such as lameness. In their study, there were few cats with radiologic OA who had a reduced range of motion upon orthopedic examination and none who had crepitus in any of the affected appendicular joints (Clarke and Bennett, 2006).

With the problematic assessment of cats in the clinical situation comes an increased importance of the anamnesis and signs reported by the owner. A study of owner-perceived signs related to OA showed that gait changes (e.g. lameness, stiffness, changes in limb carriage) were the most common signs reported by owners (Klinck *et al*., 2012). Other locomotor signs included unwillingness to jump and a reduced height of jump (Clarke and Bennett, 2006). Altered behavior, such as decreased grooming, changes in litter box use, alterations in claw-sharpening behavior or reduction of playing and hunting, and general signs of illness may be present but could be difficult to relate directly to OA pain (Slingerland *et al*., 2011; Klinck *et al*., 2012).

Radiography

Imaging is considered a crucial part of OA diagnosis and radiography is currently the most frequently used method to diagnose OA in veterinary medicine.

Radiographic signs of OA are periarticular new bone formation (i.e. osteophytes and enthesiophytes), subchondral bone sclerosis and cyst-like lesions, remodeling of articular surfaces, perichondral bone erosion and joint-associated soft tissue mineralization. In some appendicular joints such as the stifle, joint effusion and thickened periarticular soft tissue may be present (Allan, 2000; Hardie *et al*., 2002; Clarke *et al*., 2005; Godfrey, 2005; Lascelles *et al*., 2010).

Some authors argue that certain lesions such as enthesiophytes and soft tissue mineralization, although indicative of OA, may occur independently (Clarke *et al*., 2005). Another difficulty in the assessment of lesions is that subchondral sclerosis may be more readily identified in joints with osteophytes, which may either be a result of superimposition of the osteophytes on the subchondral region or reflect that osteophytes and sclerosis develop concurrently (Clarke *et al*., 2005).

Standard radiographic projections for assessing the pelvis region are lateral and extended ventrodorsal (VD) projections centered at the level of the coxofemoral joints. A flexed (frog-legged) VD projection may be used to define regions that are subject to superimposition on standard views (Mahoney, 2012).

There is a lack of detailed information concerning normal anatomic appearance of feline coxofemoral joints as seen in various diagnostic imaging methods. In some cases, it may therefore be difficult to determine whether mildly pointed margins of acetabulum and femur are normal or if these pointed margins may be signs of osteophytes related to OA.

There is also a lack of information concerning lesions indicative of feline OA as there are few studies investigating consensus between radiologic and histologic findings (Lascelles *et al*., 2010; Freire *et al*. 2011; Leijon *et al*., 2017). It has been suggested that typical features of feline OA may differ from canine OA with some authors arguing that osteophyte formation may be less extensive and smaller in cats (Hardie *et al*., 2002; Clarke *et al*., 2005). However, intra- and periarticular mineralizations (other than osteophytes) are believed to be more common in cats (Allan, 2000; Lascelles *et al*., 2010; Freire *et al*., 2011). Nevertheless, Freire *et al*. (2011) showed that in the coxofemoral joint, osteophyte formation is more frequent than articular mineralizations and found that 86% of feline coxofemoral OA joints displayed osteophytes. Other reported differences compared to dogs are less synovial effusion or thickened periarticular soft tissue, less remodeling of the femoral head and neck and less subchondral bone erosions or cysts (Allan, 2000; Clarke and Bennett, 2006; Freire *et al*., 2011).

Regarding the general anatomy of the feline coxofemoral joint, there is consensus that the feline acetabulum normally is shallower compared to the dog (Allan, 2000; Perry, 2016). Cats do have a lower mean Norberg angle (NA) than dogs but a similar distraction index (DI) (Langenbach *et al*., 1998). In cats with OA compared to healthy cats, the NA is lower and the DI is higher (Langenbach *et al*., 1998).

Consensus between radiography and clinical picture?

Lascelles *et al*. (2007) found that only one third of feline joints with radiologic signs of OA elicited pain on physical examination (18 of 55 joints), in another study (Klinck *et al*., 2012) it was 38% (12 of 21 cats). Clarke and Bennett (2006) found that some cats who had both anamnestic and radiologic signs of OA did not show signs of pain on physical examination. On the other hand, 34% (Clarke and Bennett, 2006) and 74% (Lascelles *et al*., 2007) respectively of joints which elicited pain reactions on clinical examination did not have any radiologic signs of OA.

When it comes to lameness, only 4% (Hardie *et al*., 2002), 16.7% (Clarke *et al*., 2005) 17.5% (Godfrey, 2005) and 43% (Clarke and Bennett, 2006) respectively of cats with radiologic signs of OA had signs of lameness. Other locomotor signs such as stiff gain, difficulty jumping and inactivity, as well as hind limb weakness and shuffling forelimb are generally seen among only a small number of cats with radiologic OA (Godfrey, 2005).

There is agreement that one reason for low grade of consensus between different diagnostic methods is the cats' body constitution and great agility, enabling them to hide signs of pain and lameness even at late stages of the disease (Clarke and Bennett, 2006; Hardie *et al*., 2002). Another reason may be that early changes in joint degeneration mainly comprise various levels of cartilage damage, a feature that is not radiographically detectable. In many cases, osteophytosis and subchondral bone sclerosis are features of a more advanced disease process (Freire *et al*., 2011).

In veterinary practice, it is difficult to know which diagnostic method provides the true picture and it is therefore important to take all parts of anamnesis, thorough physical examination and radiologic methods into account (Klinck *et al*., 2012).

CT

Compared to radiography, CT allows improved visualization of musculoskeletal structures for several reasons: it eliminates the effect of superimposition of adjacent structures by giving the possibility of slice-by-slice assessment, it allows to correct the viewed image for positioning artefacts by adjusting the planes and it produces an image with more contrast, i.e. more shades of grey (Schwarz and Saunders, 2011; Mahoney, 2012).

Currently, there is no validated system to grade OA changes in CT in either human or veterinary medicine (Turmezei and Poole, 2011; Gold *et al*., 2015).

For more detailed information about CT technique, see the 'Veterinary CT' section.

Other diagnostic imaging techniques

Magnetic resonance imaging (MRI) is an important imaging technique for diagnosing OA in research. It is mainly used in human medicine but also to some extent in veterinary medicine (D'Anjou *et al*., 2008; Guillot *et al*., 2012; Glodek *et al*., 2015). Its value compared to other methods lies in the ability to assess cartilage and articular/peri-articular soft tissue details such as subchondral bone marrow lesions found in OA (Turmezei and Poole, 2011; Mahoney, 2012). On the other hand, some OA features can be difficult to assess with MRI such as the full extent of osteophytes (Turmezei and Poole, 2011). In veterinary medicine, another disadvantage of MRI compared to CT is the prolonged scan time, which may require general anesthesia of the animal while the shorter CT scan is possible to perform in a sedated animal (Mahoney, 2012).

Scintigraphy for musculoskeletal diagnosis is mainly used in equine practice (Daniel *et al*., 2012; Graham *et al*., 2015), and is used occasionally in dogs and rarely in cats. It is useful for localizing pathological changes to a joint or region but it is poor at depicting anatomical details compared to other imaging methods (Daniel *et al*., 2012) and therefore not very useful in OA diagnosis.

Ultrasound is mainly used in equine practice, and occasionally in dogs for joint disease diagnosis (Tomlinson *et al*., 2000; De Lasalle *et al*., 2016). Its advantage is the possibility of providing information about soft tissues, articular cartilage and the subchondral bone surface but only joint surfaces that can be reached by the ultrasound beam can be evaluated (Tomlinson *et al*., 2000).

Veterinary CT

General principles

In contrast to radiography, which produces two-dimensional images of three-dimensional (3D) body parts, CT offers the possibility to produce 3D data using x-rays (Thrall, 2013). The image is produced in a donut-shaped rotating gantry which contains the x-ray tube on one side and the detector array on the other side. The patient lies on a movable table positioned in the central tunnel. With the gantry rotating (producing the X and Y axes) and the patient moving forward along its own axis (the Z axis), data along three axes of a certain area of the body can be collected (Schwarz and Saunders, 2011). The tomographic nature allows production of data in slices (i.e. thin sections), which can be varied in thickness and superimposition of adjacent structures is thereby eliminated (Thrall, 2013).

Data is produced by sending x-rays from the x-ray tube to the detector and the x-ray attenuation of the tissue is measured. The various tissues' differences in attenuation gives the contrast resolution that allows different tissues to be defined in the image. CT has a higher contrast resolution than radiography, for example, liquids can be differentiated from soft tissue in CT but not in radiography (Thrall, 2013).

Each slice that is produced consists of a matrix of voxels (volume elements) that are small cubic elements and each voxel is displayed as a pixel (picture element) on a computer screen. Each voxel represents a level of x-ray attenuation for that cubic volume of tissue. For tissue with higher attenuation, the voxel will be displayed as a brighter pixel on the screen, while areas with lack of or low x-ray attenuation (such as air) will appear as dark pixels. If a voxel contains a mixture of tissues then different attenuation levels will occur within the same voxel, but it is the average attenuation value that will be shown by the pixel brightness (Thrall, 2013). Thus, a tissue structure smaller in size than the voxel in size cannot be defined independently and this effect is called partial volume averaging and results in partial volume averaging artifacts. This also means that smaller voxels (dependent on matrix size and slice thickness) result in higher spatial resolution in the image. However, smaller voxels will also result in higher 'image noise', which in turn reduces spatial resolution. There is thus a balance between these two factors for giving an image with optimal spatial resolution (Schwarz and Saunders, 2011; Thrall, 2013).

Creating the CT image

Due to the fixed direction of the x-rays in the gantry, data can only be collected parallel to the X-Y-plane which generally represents the transverse plane of the patient lying on the table. But since data is collected in multiple slices, a whole set of voxels, i.e. several matrixes of tiny cubes, is rendered. This makes it possible for the computer to rearrange the voxels in different planes than the original one and display a matrix of pixels along another plane of the body. It is this process that is called multiplanar reformatting or multiplanar reconstruction (MPR)

(Thrall, 2013). Another method of reconstruction is 3D volumetric reconstruction (3D VR) which gives a realistic 3D appearance of body parts by representing each voxel with a certain color and opacity. However, it comes with the disadvantages of superimposition and decreased resolution. (Schwarz and Saunders, 2011).

The extent of x-ray attenuation is presented in Hounsfield Units (HU) or CT numbers and its values for different tissues are distributed relative to the attenuation of water and air. Water is assigned 0 HU and air, which lacks x-ray attenuation -1000 HU. This results in CT numbers for different tissues being relatively stable, as they become independent of other physical aspects such as x-ray spectrum (Schwarz and Saunders, 2011).

A typical CT scanner has the capacity to measure HU values from approximately -1000 to +3095 HU, rendering a total of 4096 shades of gray. This amount of shades cannot be resolved by the human eye, making it necessary to adjust the image before the assessment. This is done by adjusting the window width (WW) and window level (WL) which correspond to median and range of HU shown in the image. Window level thus determines which HU is at the center of the window (i.e. brightness of the image) and WW determines the extent of grey shades displayed from minimum to maximum (i.e. the image contrast). Choice of WW and WL depend of the type of tissue, i.e. its density, to be assessed in the image (Thrall, 2013).

The standard planes for reconstruction of the CT image are transverse plane, sagittal plane and dorsal or frontal plane for axial and appendicular skeleton, respectively (Schwarz and Saunders, 2011).

MATERIAL AND METHODS

Study material

The study used material based on 21 cats presented for post-mortem examination for educational/scientific purposes at the Department of Biomedical Sciences and Veterinary Public Health (BVF), Swedish University of Agricultural Sciences (SLU).

Twenty-one whole-body CT studies, eight radiographic studies and tissues from 29 coxofemoral joints were examined in this study. The material from fourteen of these cats had been collected previously (for studies of feline obesity and OA), while seven cats were recruited for the study during 2018. The reasons for euthanasia were unrelated to this study.

The inclusion criteria were an age of more than 1.5 years and the possibility of assessment within 48 hours (24 hours for cats recruited during 2018) post-mortem. Exclusion criteria were known trauma to the coxofemoral joints, neoplasia in the coxofemoral joints and Scottish fold breed.

Procedure for collecting samples

Radiology

All cats included had CT of the coxofemoral joint region within 48 hours post-mortem. The CT images were acquired using a 64 slice multidetector CT scanner (Definition, Siemens Medical Systems, Erlangen, Germany), 250 kV, 160 mAs, slice thickness 0.6 mm, slice increment 0.3 mm, focal spot 1.2 mm and a high-resolution kernel (B70s). The cats were placed in ventral recumbency with extremities extended.

All cats collected during 2018 and one cat collected previously also had radiographs within 24 hours post-mortem. Extended VD and flexed VD projection radiographs (Adora RF CPI, Canon, Tokyo, Japan) were taken of the coxofemoral joints using a computed radiography system (Fujifilm FCR XG-1, Tokyo, Japan) with exposure settings 70 kVp and 3.2 mAs. Cats were positioned using sandbags and bandaging tape.

Histologic samples

One or both coxofemoral joints were removed from each cadaver and fixed in 10% neutral buffered formalin. After seven or eight days of formalin fixation, the coxofemoral joints were transferred to a decalcifying solution (Kristensen Urkalkningslösning, Solveco, Rosersberg, Sweden). When the degree of tissue decalcification was suitable for further processing, osteochondral tissue from the acetabulum and the proximal femur were sampled. Joints collected during 2018 were sampled with subjective image guidance by a pathologist (CL) and a radiologist (CJL). Image guidance using MPR and 3D VR of the CT images aimed to include lesions of primary interest detected in CT images in the tissue sample. Radiographs were not used for image guidance. In cases that did not show any changes in CT or on macroscopic examination, samples from standard locations (sections in an oblique dorsal plane, passing from the craniodorsal to the caudoventral aspect of the acetabulum and femoral head) were collected. Each joint had a minimum of one sample from the acetabulum and one from the femoral head with an attempt to obtain samples from apposing joint surfaces. These samples were then

embedded in paraffin blocks, cut in approximately 4 um sections and stained with hematoxylin and eosin (H&E) and toluidine blue.

For nine joints (nine cats) collected prior to 2018, there were prepared slides available for microscopic examination and from others (seven joints from five cats), coxofemoral joints that had been stored in formalin were sampled for paraffin embedding using the same image guidance techniques as described above for the cats collected in 2018.

Assessment of material

Grading system for CT and radiology

All CT images and radiographs were graded individually during a period of four days by a final year veterinary student (GR) and a board certified veterinary radiologist (CJL), using a 4 grade system that was developed for the study (see 'Grading of CT detected lesions'). All images were anonymized using a random number picker (numbergenerator.org) and readers did not have knowledge of the histological joint grading. Each cat had different codes for CT and radiography and images were viewed in a randomized order. Image viewing was done using digital imaging and communications in medicine (DICOM) viewing software (OsiriX v 4.1.2, Pixmeo, Geneva, Switzerland). CT images were viewed using 3D multiplanar reconstructions in a bone window (window level 700 HU and window width 4000 HU). When grades differed, the final grade was decided by consensus.

Alignment in CT

To achieve accurate CT standard locations, the alignment of each individual was done as follows (see Fig. 6-9):

Acetabulum (Fig. 6-8, A.-F.): the sagittal plane was adjusted first by aligning it parallel to and through the center of the spine (in dorsal plane images) as well as through the center of the sacrum and pelvic symphysis (in transverse plane images), then the transverse plane was aligned perpendicular to the length axis of the body and a symmetrical view of the left and right coxofemoral joint was created. Finally, the dorsal plane was adjusted (in sagittal plane images) so it was parallel to the sacrum.

Femur (Fig. 9, G.-H.): The sagittal and dorsal planes were aligned parallel to the epiphysis, then the transverse plane was aligned perpendicular to the long-axis of the femoral epiphysis. The transverse plane was aligned through fovea capitis (in dorsal plane images), then the dorsal plane was adjusted (in sagittal and transverse plane images) so it passed through the fovea capitis and center of the femoral neck.

Standard locations in CT

In CT images, in addition of assessing the joint as a whole, several standard locations were assessed to investigate the locations of changes detected on the acetabulum and femoral head and neck (see Fig. 7-10):

A. craniodorsal aspect of acetabulum in transverse and dorsal plane (Fig. 6-7)
B. cranioventral aspect of acetabulum in transverse and dorsal plane (Fig. 6)
C. caudodorsal aspect of acetabulum in transverse and dorsal plane (Fig. 7)

D. caudoventral aspect of acetabulum in transverse plane (Fig. 7)
E. lateral aspects (dorsal and ventral) of acetabulum at the level of fovea capitis' caudal margin in transverse plane (Fig. 8)
F. mid cranial and caudal aspects of acetabulum at the level of fovea capitis' dorsal margin in dorsal plane (Fig. 8)
G. proximal and distal aspects of femoral head and neck in dorsal plane (Fig. 9)
H. cranial and caudal aspects of femoral head and neck in transverse plane (Fig. 9)

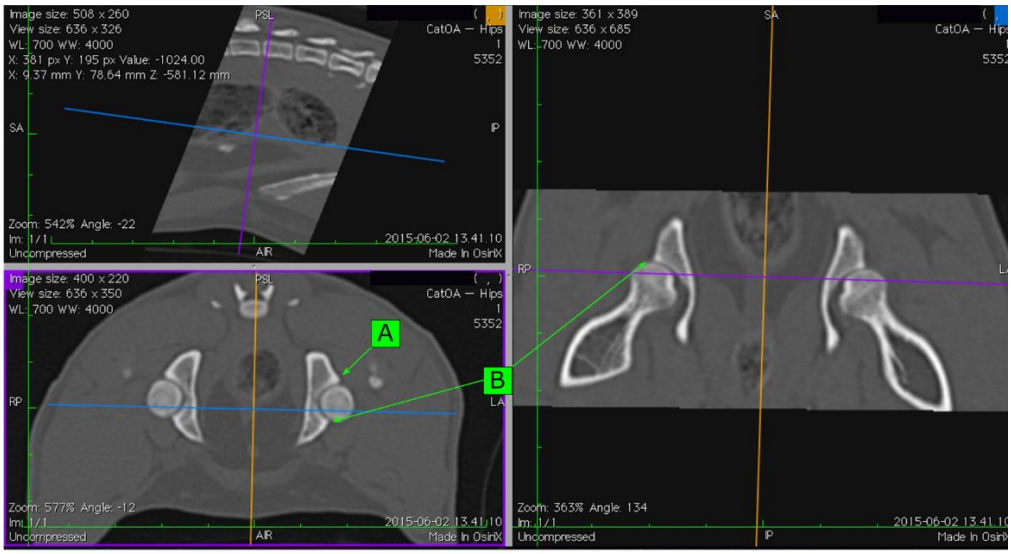

Fig. 6. *Computed tomography images using multiplanar reconstruction showing the alignment to assess the craniodorsal (A) and cranioventral (B) aspects of acetabulum. To get a view of A and B, the transverse plane was aligned through the cranial margin of fovea capitis on femur and the dorsal plane through the most ventral quarter of the acetabulum.*

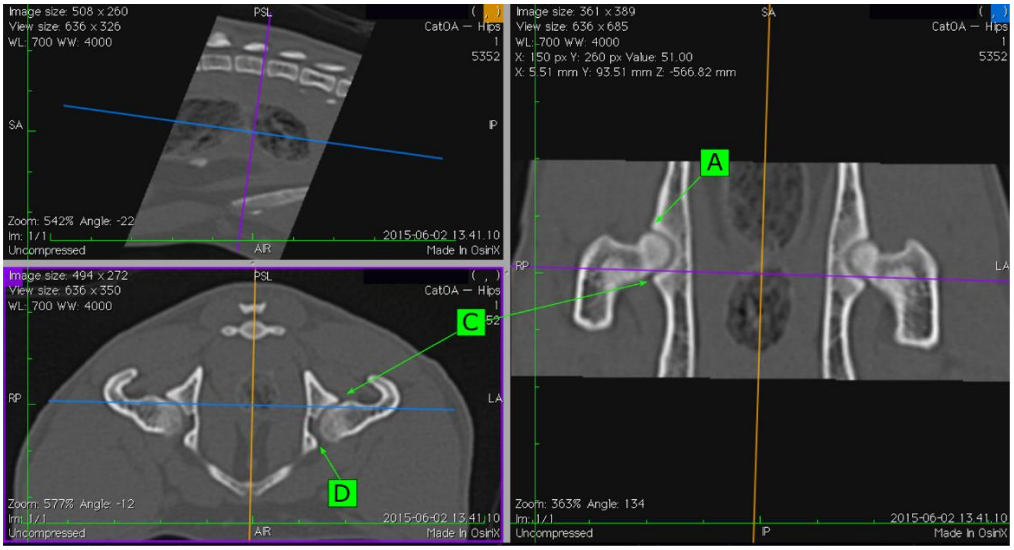

Fig. 7. *Computed tomography images using multiplanar reconstruction showing the alignment for assessment of craniodorsal (A), caudodorsal (C) and caudoventral (D) aspects of acetabulum. For A (dorsal plane), C and D, the transverse plane was aligned through the caudal quarter of the acetabulum and the dorsal plane through the dorsal quarter.*

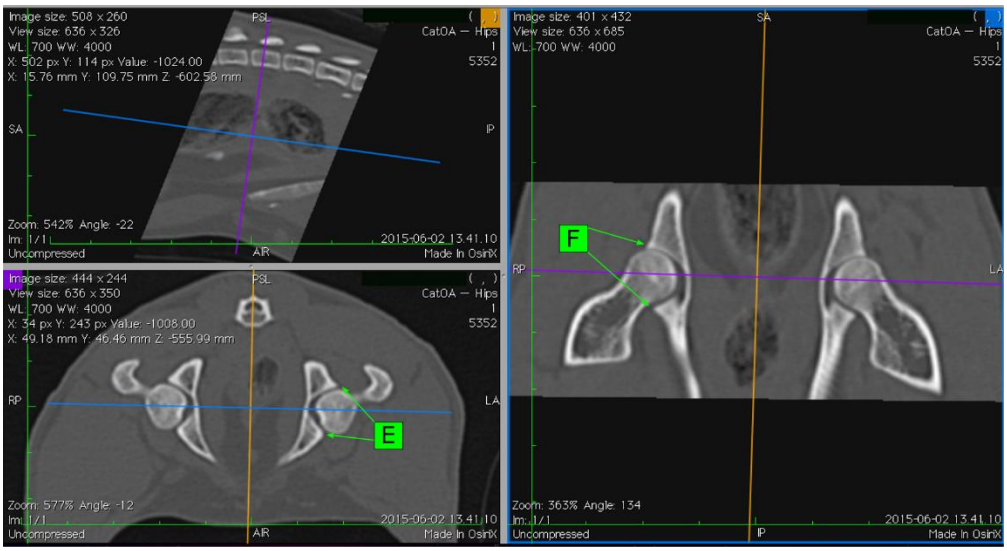

Fig. 8. *Computed tomography images using multiplanar reconstruction showing the alignment for assessment of lateral aspects (dorsal and ventral) (E) and mid cranial and caudal aspects (F) of acetabulum. E and F was viewed by aligning the dorsal plane through the dorsal margin of fovea capitis on femur and the transverse plane through the center of the femoral head.*

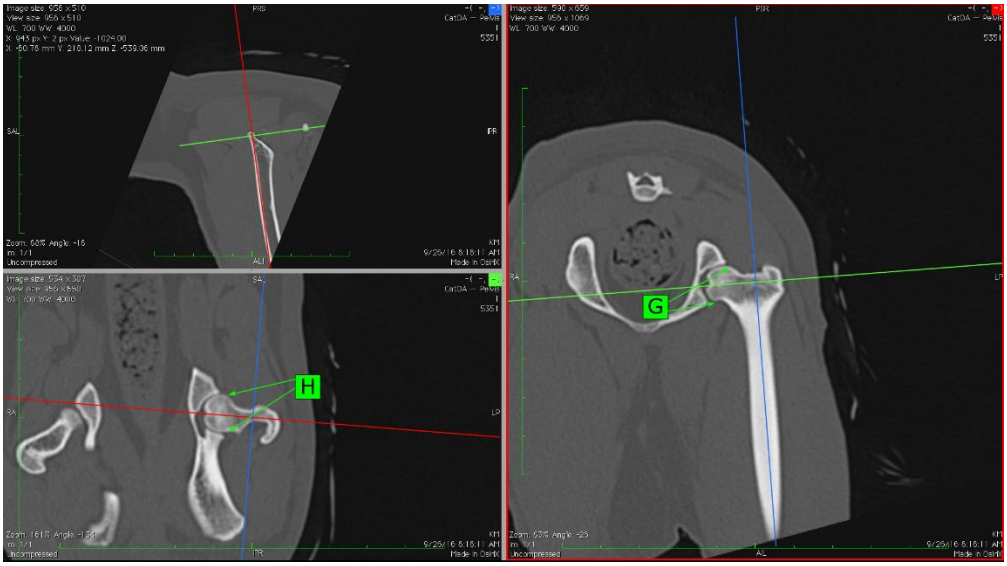

Fig. 9. *Computed tomography images using multiplanar reconstruction showing the alignment for assessment of proximal and distal (G) and cranial and caudal (H) aspects of femoral head and neck.*

Grading of CT detected lesions

The following lesions were graded for each joint; osteophytes and entheseophytes, subchondral sclerosis, subchondral lysis (including cyst-like lesions and subchondral bone defects), thickened periarticular soft tissue/synovial effusion and joint-associated (intra-/extraarticular) mineralization. Lesions were assigned to one of the following grades; 0 = normal, 1 = suspected but uncertain lesion, 2 = definite mild lesion, 3 = definite moderate to severe lesion.

Only one grade was given per type of lesion in each location, i.e. the grade for the most severe lesion. Moderate and severe lesions were combined in one grade. For each lesion, its location

was noted as a comment. The overall highest grade among all lesions later determined the joint's OA classification.

Figures 10-12 show CT images with examples of lesions for some of the grades and aim to show examples at the lower end of the scale for the grade, e.g. a joint with grade 2 had either the same or greater extent of lesions as the example (but not as great as grade 3).

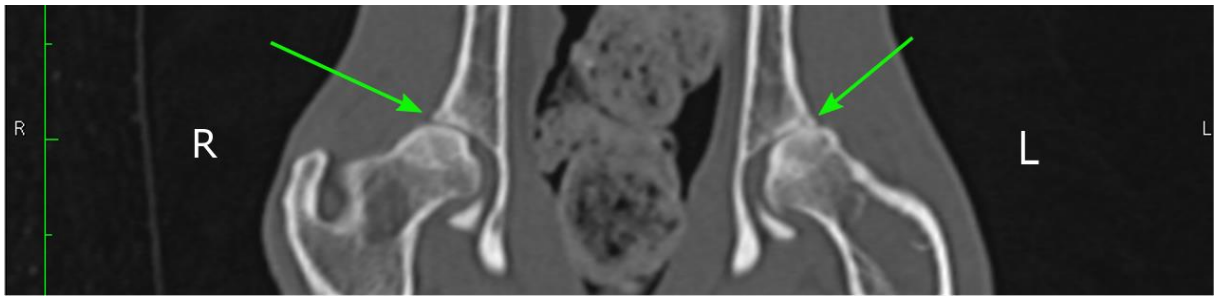

Fig. 10. *Computed tomography images using multiplanar reconstruction showing osteophytes (arrows) on craniodorsal margins of acetabulum, grade 1 on cat's right side and grade 2 on cat's left side.*

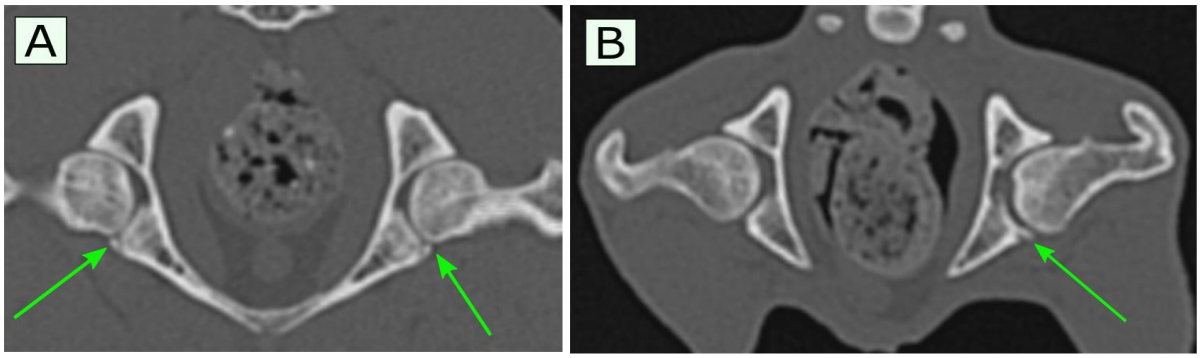

Fig. 11. *Computed tomography images using multiplanar reconstruction showing grade 2 osteophytes (arrows) on cranioventral (A) and grade 3 osteophyte (arrow) on mid ventral (B) aspects of acetabulum.*

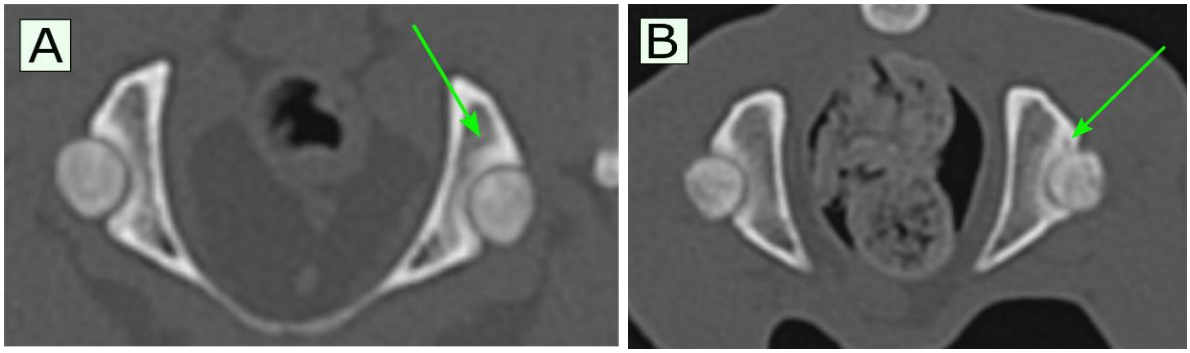

Fig. 12. *Computed tomography images using multiplanar reconstruction showing grade 2 sclerosis (A) and grade 2 lysis (B), indicated by arrows.*

The same system was used for grading of lesions in CT and radiography.

Classification 0-3 from CT

According to the most severe lesion grade each joint was classified from CT images as normal, joint with possible mild OA, joint with definite mild OA or joint with moderate/severe OA (Table 1). Joints were then grouped in either OA positive (OA+) or OA negative (OA-) joints with suspected joints classified as OA+.

Table 1. *Classification of joints based on computed tomography grading of lesions.*

Classification criteria	**Classification**
No lesion graded higher than 0	Normal joint, no lesions (0) – **OA negative**
At least one lesion graded 1 but no lesions graded higher	Possible mild OA (1) – **OA positive**
At least one lesion graded 2 but no lesions graded higher	Definite mild OA (2) – **OA positive**
At least one lesion graded 3	Moderate/severe OA (3) – **OA positive**

Grading system for histology

All histologic samples were assessed and graded for cartilage lesions by a final year veterinary student (GR) according to a protocol with grades 0-6.5, modified from a grading system by Pritzker *et al*. (2006), see Table 2. A subset of sections from joints collected prior to 2018 had been previously graded by a board certified veterinary pathologist (CL) and a resident in veterinary pathology (AL) and these sections were used as training materials for the veterinary student. Following this training GR graded all the joints. All samples were anonymized using a random number picker (excel for some samples prepared prior to 2018, numbergenerator.org for 2018 samples) and each sample, i.e. each acetabulum or femur, got its own code number. The student did the histology grading after the CT and radiography grading but the sample number randomization meant that the grades from CT and radiography could not be derived by the student from the code number.

The microscopic morphology of the joint samples was described.

Grades 1-5 involve articular cartilage lesions only, whereas grades 5.5-6.5 also take changes in the subchondral bone into account. Grades 6-6.5 involve deformation of the joint surface but peripheral osteophytes as such are not included in the purpose of defining the grade, as they may be present even in earlier grades where deformation is absent (Pritzker *et al*., 2006). Peripheral lesions, located at the margins between hyaline cartilage and fibrocartilage were included in the grading and the depth of the lesion estimated from where the cartilage zones of adjacent hyaline cartilage were clearly seen.

Table 2. *Brief outline of histology grading system for determination of cartilage lesions*[a]

Lesion grade	Histological criteria
0	Normal; no subgrade
1	Slight fibrillation may be present, no cellular changes
1.5	Slight fibrillation of superficial layer may be present, proliferation, hypertrophy and/or loss of chondrocytes in superficial zone
2	Fibrillation extends through superficial zone; cellular lesions as above
2.5	As grade 2, but with matrix loss in superficial zone
3	As above, but with vertical or oblique simple fissures and cellular changes extending into mid zone
3.5	As above, but with vertical or oblique branched fissures
4	Superficial zone focally or multifocally delaminated; cellular lesions as above
4.5	Mid zone excavated; cellular lesions as above
5	Complete erosion of hyaline cartilage to calcified cartilage or subchondral bone
5.5	As above, but with repair tissue extending to the level of the eroded surface
6	Bone remodeling with deformation of articular contour, repair tissue extending above denuded surface or extensive eburnation of calcified cartilage or subchondral bone
6.5	As above, but with pronounced reparative process with ongoing ossification (central osteophytes) or remodeling reminiscent of anchylosis

[a]Modified from Pritzker *et al.,* 2006.

After grading, the joints were classified as either OA+ or OA- in regard to presence of cartilage lesions. For statistical analysis of comparison of the number of OA+ and OA- joints between imaging techniques and histology, it was decided to classify Pritzker grade 0-1 = normal, Pritzker grades 1.5-2.5 = minimal OA, Pritzker grades 3-3.5 = mild OA and Pritzker grades 4-6.5 = moderate/severe OA. Normal joints were classified as OA-, whereas joints with Pritzker grade 1.5 or higher were classified as OA+.

The joints were also assessed regarding the presence of marginal osteophytes. Osteophytes were defined as fibrocartilage-associated bony outgrowths at the joint margins. In order to be defined as osteophyte, the outgrowth had to clearly disturb the contour of the bone and/or other

mineralized tissue. Samples were classified as either positive (present) or negative (not present). Uncertain cases were classified as negative and described as comments.

Subjective comparison of radiology and histology

After grading both radiologic and histologic material the results were matched for each joint by decoding the anonymized material. A comparison of diagnostic imaging and histologic findings was done for each joint. When possible (in regard to the exact location of histologic sampling), radiologic and histologic features or lesions at the same location were compared. Observations were noted as comments.

Statistical analysis

All statistical analyses were performed using the Minitab 18 software. For all analyses which involved Pritzker grades, the highest grade for each joint (i.e. either acetabulum or femur) was used. *P*-values ≤ 0.05 were considered significant.

Spearman rho was used in order to investigate association between Pritzker grades and age and CT grades and age, as well as between CT grades and radiography grades.

To evaluate association between CT OA+/- and histology OA+/- joints, Fisher's exact test was performed. Pritzker grades 0-1 and CT grade 0 were categorized as OA- and Pritzker grades $\geq$ 1.5 and CT grades 1-3 were categorized as OA+.

RESULTS

Study population

There were 21 cats for a total of 29 histologic coxofemoral joint samples included in the study. In CT, all 42 joints could be assessed. Only eight of the cats had radiographs taken giving a total of 16 assessable coxofemoral joints of which 14 were available for histologic assessment. Although three histologic samples had some preparation artefacts involving the articular cartilage, the majority of the joint surface was visible in all samples and it was thus decided to still include all samples.

The age of the cats ranged from eighteen months to nineteen years (median = 9 years, mean = 10.2 years +/- SD = 4.6). Two thirds (14/21) were domestic shorthair cats and one third (7/21) were pure breed (one of each of the following: Bengal, Ragdoll, Cornish rex, Norwegian forest cat, Burmese, British shorthair and European shorthair). Gender was evenly distributed with ten neutered males, nine neutered females and two intact females.

Age was found to be significantly associated to Pritzker grade (Spearman rho = 0.538, p = 0.003) but not to OA severity in CT classification (Spearman rho = 0,222, p = 0.246).

CT and histology findings

The results for the classification of joints based on CT and histologic assessment of the articular cartilage (Pritzker grades) are listed in Table 3 and 4 respectively. In CT, for all except one joint it was the presence and extent of osteophytes that determined the joints' classification (i.e. was given the highest score of assessed parameters on CT). Only two joints (one cat) were found to have no cartilage changes. Of the four joints with suspected mild OA on CT (grade 1), one joint had minimal OA histologically, the remaining three suspected joints were graded moderate/severe OA in histology. Of the joints that were classified as OA+, the majority of histologically OA+ were in the moderate/severe group, whereas in the CT OA+ group the proportions of the groups were more evenly distributed (Tables 3 and 4).

Table 3. *Classification of joints based on computed tomography*.

CT classification	No. of joints	Percent
Moderate/severe (3)	8	27%
Mild (2)	11	38%
Suspected (1)	4	14%
Normal (0)	6	21%
Total	**29**	**100**

Table 4. *Classification of joints based on histology.*

Histology classification	No. of joints	Percent
Moderate/severe (4-6.5)	16	55%
Mild (3-3.5)	5	17%
Minimal (1.5-2.5)	6	21%
Normal (0-1)	2	7%
Total	**29**	**100**

For twenty-five of 29 joints (86%) there was perfect agreement of classification as either OA+ or OA- in both CT and histology (See Table 5). Of all the joints that were histologically OA+, 23 of 27 (85%) were also classified OA+ on CT. Fisher's exact test showed a statistically significant association between CT and histology regarding classification of joints as OA+ and OA-, respectively (p=0.037). See Fig. 13 for an overview of Pritzker grade distribution within each CT classification. Only two joints were negative for histologic cartilage lesions and these were also classified OA- in CT. Those two joints belonged to the same 18 month old cat.

Table 5. *Comparison of classification of joints as OA positive (OA+) or OA negative (OA-) in computed tomography (CT) and histology*

		Histology		
		OA +	OA-	**Total**
CT	OA +	23	0	23
	OA -	4	2	6
	Total	27	2	29

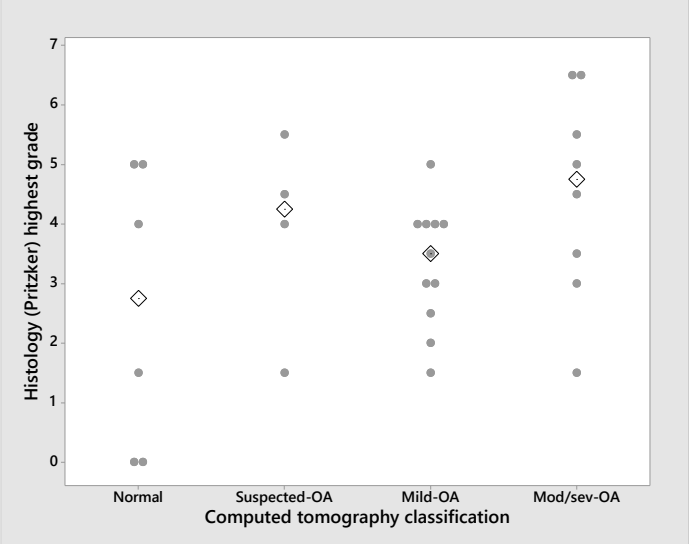

Fig. 13. *Distribution of histological Pritzker grades (0-6.5, highest grade for either femur or acetabular cartilage for each joint) for each computed tomography (CT) classification. Diamonds represent the median Pritzker grade within each CT classification group.*

Only two joints were negative for histologic cartilage lesions and these were also classified OA- in CT. Those two joints belonged to the same 18 month old cat.

Four joints that were histologically OA+ were not diagnosed as OA+ by CT. The Pritzker grades for these four joints ranged from 1.5 to 5. Three of these joints did not have osteophytes on histologic examination. The joint that did have an osteophyte histologically was not detected by CT (CT classification 0). The osteophyte comprised a small but distinct hook-shaped bone proliferation in the margin of the articular surface of the femoral head with Pritzker grade 0 for femur and Pritzker grade 1.5 for acetabulum (i.e. minimal OA histologically).

Twelve of the 23 joints that were graded as OA+ both histologically and in CT were assessed as having osteophytes in both methods.

The remaining eleven of 23 OA+ joints that were detected in both CT and histology showed no osteophytes on histological examination. Four of these were classified as suspected joints on CT and four of these eleven joints did not have tissue samples collected with CT-guidance. In several of these eleven joints, histology revealed an uneven shape or tissue architecture in the acetabulum which could not be defined as osteophyte but which also was uncertain whether it represented normal anatomic variation.

Acetabular margination not assessed as osteophytes

Some features of acetabular shape and tissue architecture at acetabular margins were not assessed as osteophytes in histological sections and it was thus uncertain if those should be defined as features of feline OA or normal anatomic variation. These included cats that had an uneven mature bone shape in acetabular margins histologically (examples given in Fig. 14 to 16) and lesions with subchondral bone sclerosis with questionable disturbance of the bone/mineralized tissue contour (Fig. 17). In a few cases, depending on the exact location, the bone was covered only by a thin layer of loose connective tissue and thus was not defined as osteophyte.

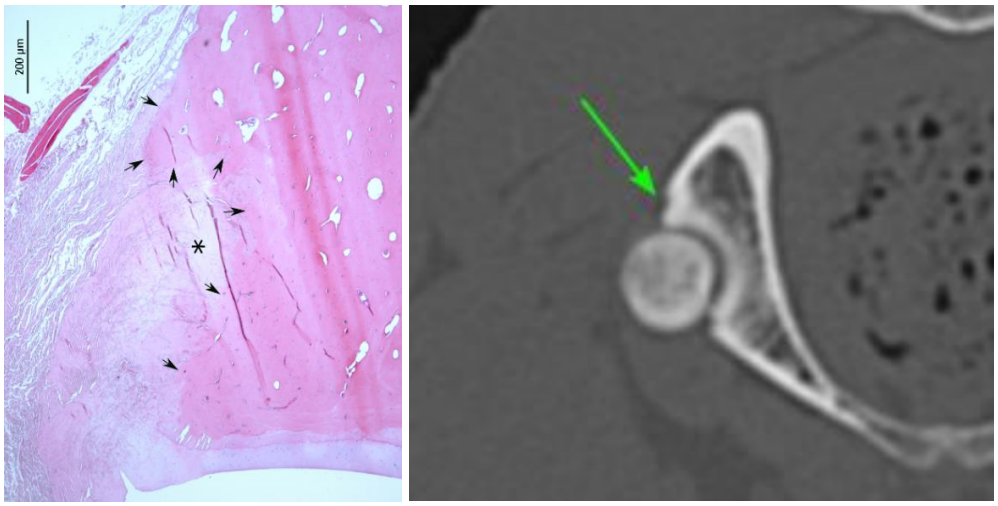

Fig. 14. *Left craniodorsal acetabular margin in six year old cat, histological sample (left panel, hematoxylin and eosin stain, x4) and computed tomography (CT) image (right panel). In CT, the feature was assessed as a mild osteophyte (grade 2). Histology reveals that the bone tissue follows the outer margination of the ilium smoothly and does not appear like an outgrowth. However, there is an area lacking lamellar bone (*) which gives an irregular contour of the bone. Pritzker grades for this joint were 2 for acetabulum and 3.5 for femur.*

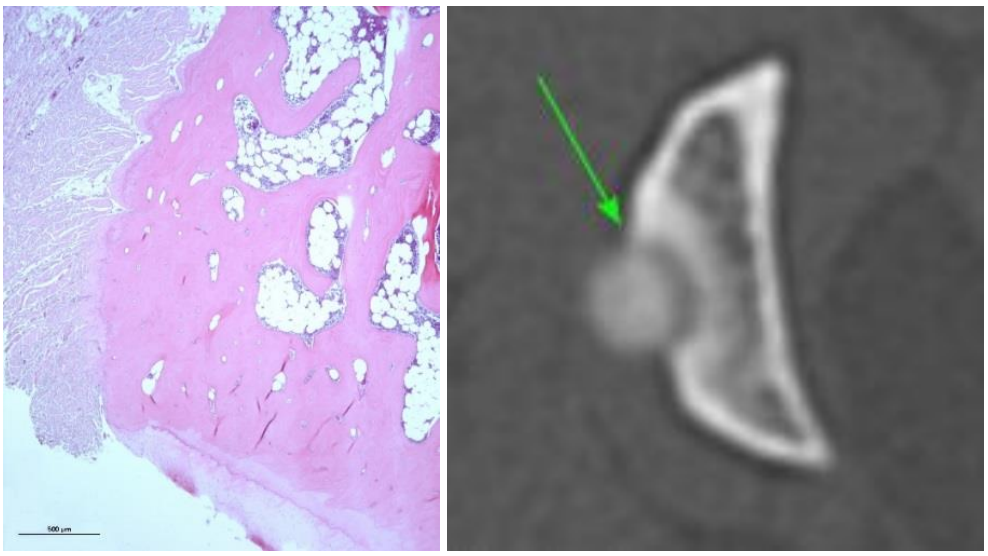

Fig. 15. *Right craniodorsal margin of acetabulum of an eleven year old cat, histologic sample (left panel, hematoxylin and eosin stain, x4) and computed tomography (CT) image (right panel). The margination of the non-articular surface is wavy but smooth and not capped with fibrocartilage. In CT, the feature was assessed as suspected osteophyte (grade 1, arrow). Pritzker grades for this joint were 1.5 for acetabulum and 0 for femur.*

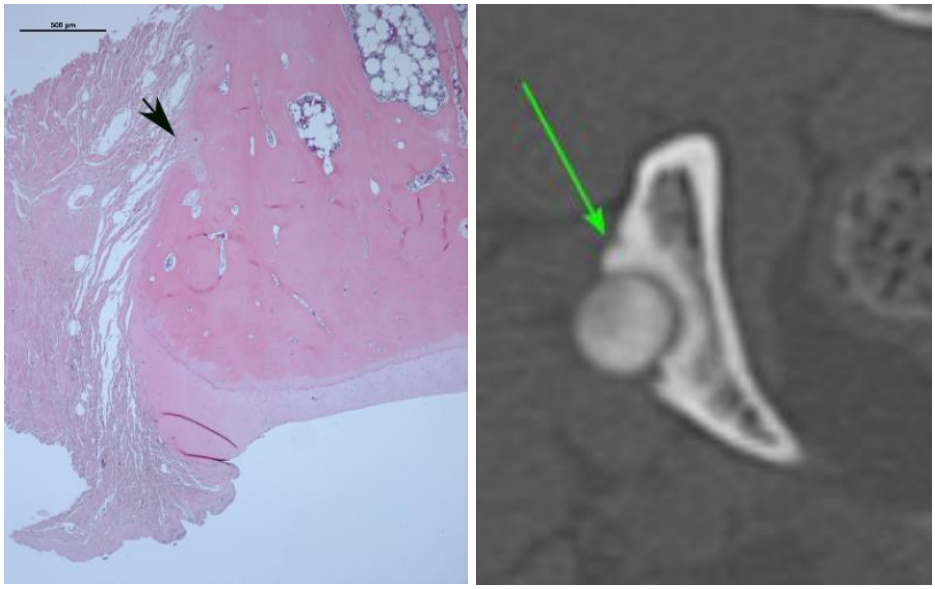

Fig. 16. *Right craniodorsal acetabular margin of a seven year old cat, histologic sample (left panel, hematoxylin and eosin stain, x4) and computed tomography (CT) image (right panel). In CT, the feature was assessed as mild osteophyte (grade 2, arrow). In histology, the feature was not assessed as osteophyte, as it was not a distinct outgrowth. Note the area where the bone tissue is interrupted by non-osseous connective tissue (arrowhead), which explains the shape visible in the CT image. The Pritzker grades for this joint were 4 for acetabulum and 3.5 for femur.*

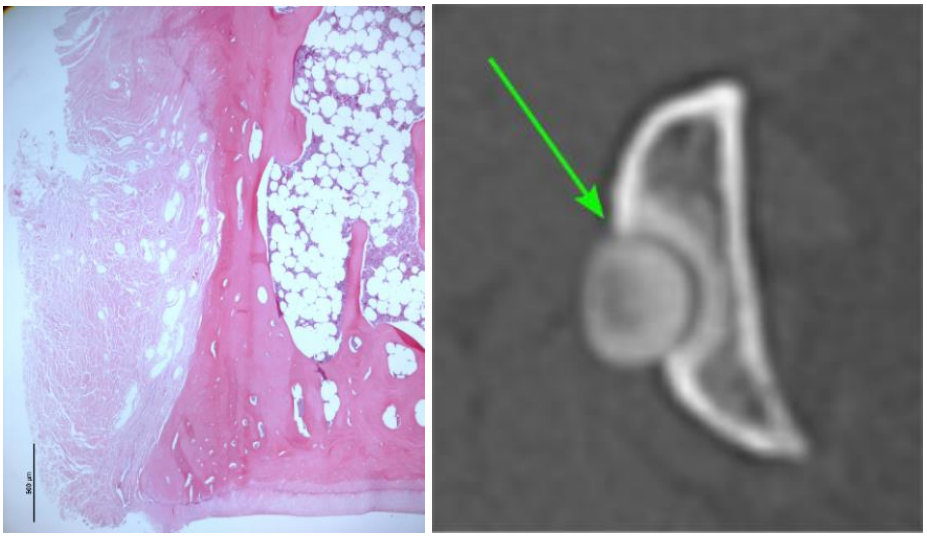

Fig. 17. *Microscopic image of the left acetabular margin of a thirteen year old cat (left panel, hematoxylin & esosin stain, x4) which was assessed normal on computed tomography (grade 0, arrow in right panel). The Pritzker grade was 5 for this acetabulum and 5 for femur. The image shows an example of a relatively pointy marginal shape which was not assessed as osteophyte in neither CT nor histology and thus could represent normal anatomic variation.*

Osteophytes in histology

Thirteen of 29 joints had osteophytes on histological examination. Only three of these joints had osteophytes on the femur, of which two had Pritzker grade 6.5 with osteophytes on both the acetabulum and femur. The third joint had Pritzker grade 0 for the femur and 1.5 for the acetabulum. The other ten joints had osteophytes only on the acetabulum and the Pritzker grades for these varied greatly. Only four femurs/acetabula with osteophytes had Pritzker grades over 2.5 (i.e. mild to moderate/severe stages of OA), while the remaining nine femurs/acetabula had grades between 1.5 and 2, i.e. minimal OA. Worth to note is that in many of these joints (8 of 13), osteophytes were localized at the part of the joint (i.e. acetabulum or femur) with the less severe cartilage lesions. When taking into account the Pritzker grade for the whole joint, i.e. the highest Pritzker grades for either acetabulum or femur for each joint, only two joints with osteophytes had a highest Pritzker grade between 1.5 and 2 and the remaining eleven joints had grades 3 or higher.

Several acetabula classified as having osteophytes showed a similar appearance. In these joints the acetabular margin showed a relatively thin bony outgrowth adjacent to the outer margin of the bone, similar to an extension of the non-articular surface (Fig. 18 and 19). These osteophytes had a thin base that connected them to the underlying bone.

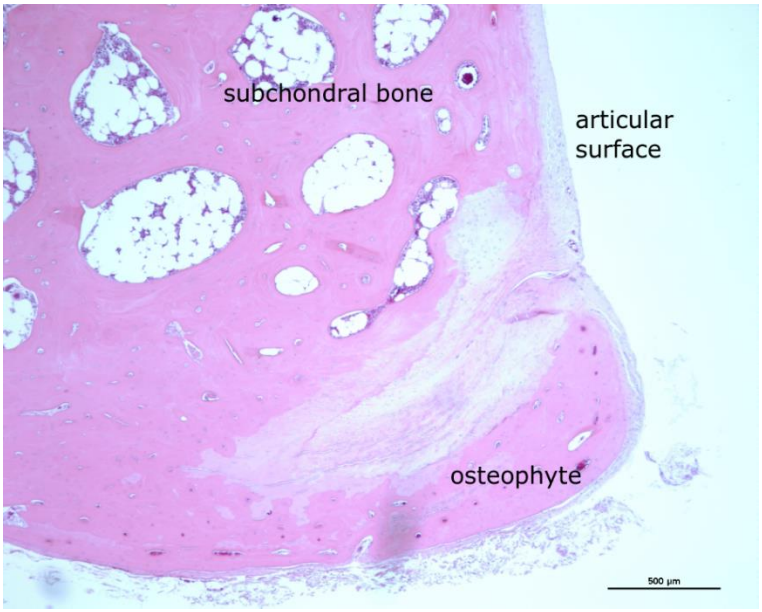

Fig. 18. *Histologic image of osteophyte on mid ventral acetabular margin in six year old cat. Pritzker grades for this joint were 2 for acetabulum and 5 for femur. Hematoxylin and eosin stain, x10. In CT, the feature was assessed as grade 2 (definite mild) osteophyte.*

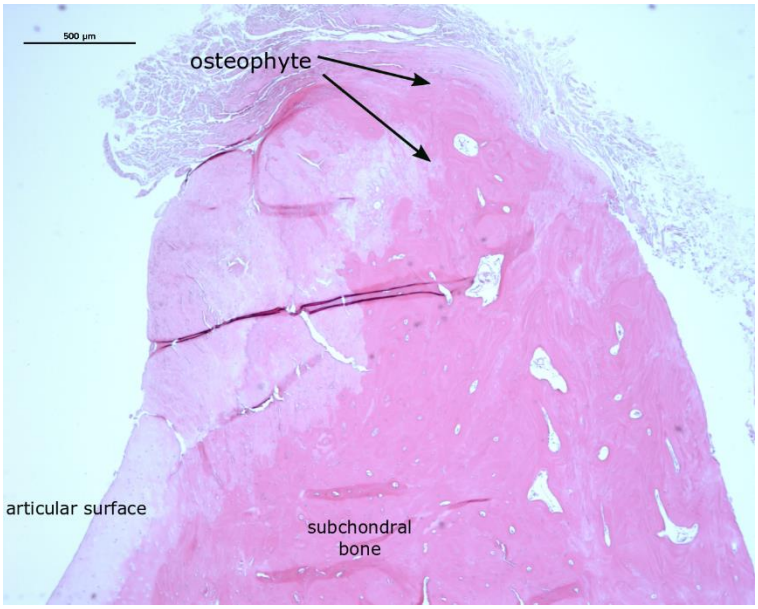

Fig. 19. *Histologic image of osteophyte on craniodorsal acetabular margin. Note the osseous tissue extending towards the articular surface peripherally (arrows) altering the normal smooth contour of the mineralized tissue. Pritzker grades for this joint were 2.5 for acetabulum and 5 for femur. Hematoxylin and eosin stain, x4. In CT, the feature was assessed as grade 3 (moderate/severe) osteophyte.*

Other acetabular margination findings

On histological examination, five acetabula presented with fissure-like lesions in the coxofemoral labra. Four of these were localized in the transition zone between articular cartilage and labrum, whereas one tear was within the labrum. Pritzker grades for four of these acetabula were 2-3.5 and for one acetabulum 5. CT classification for these ranged from 0 to 3

(1 acetabulum grade 0, 3 acetabula grade 2 and 1 acetabulum grade 3) See Fig. 20 for an example of a labral fissure.

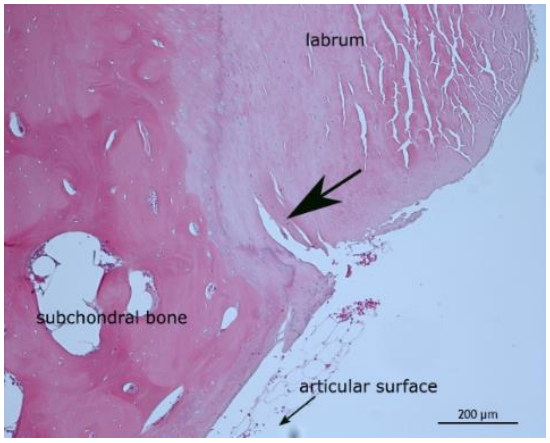

Fig. 19. *Microscopic appearance of a joint with acetabular labral fissure-like lesion (arrow). Pritzker grade for this acetabulum was 1.5 and for the femur 5, Computed tomography (CT) grade was 0. Hematoxylin and eosin stain x10.*

Osteophytes on CT

The most frequent location for osteophytes detected by CT was the craniodorsal margin of the acetabulum (location A) with 28 of 42 joints (67%) affected, followed by the cranioventral (location B, 17/42 joints (40%)), mid ventral (location Ev, 16/42 joints (38%)) and mid dorsal (location Ed, 11/42 joints (26%)) margins (see Figs. 6-9 for reference positions of locations). Regarding the severity of osteophytes, the craniodorsal, mid ventral and mid dorsal margins had more severe lesions than the cranioventral margin, where the majority of osteophytes (10 of 17) were suspected but uncertain lesions (grade 1). See Table 6 for an overview of the total number of joints with osteophytes in the various locations on the acetabulum. Note that a joint may have had osteophytes in several locations, meaning the total number of osteophytes exceeded the number of joints.

Table 6. *Localization and severity of acetabular osteophytes detected in CT images*

Joint margin location	Total	Mod/sev	Mild	Suspected
Craniodorsal (A)	**28**	7	12	9
Cranioventral (B)	**17**	2	5	10
Mid ventral (Ev)	**16**	3	7	6
Mid dorsal (Ed)	**11**	2	7	2
Mid cranial (Fcr)	**8**	-	2	6
Caudodorsal (C)	**3**	-	1	2
Mid caudal (Fcd)	**2**	-	-	2
Caudoventral (D)	**1**	1	-	-

Regarding the femoral head and neck, osteophytes were rarely detected in CT. Only one joint had definite mild lesions and this joint had osteophytes both on proximal and caudal head/neck. Remaining osteophytes were noted as suspected uncertain lesions (Table 7).

Table 7. *Localization and severity of femoral osteophytes detected in CT images*

Joint margin location	Total	Mod/sev	Mild	Suspected
Proximal (Gpr)	4	-	1	3
Caudal (Hcd)	4	-	1	3
Distal (Gd)	3	-	-	3
Cranial (Hcr)	-	-	-	-

Radiography

With radiography, 9 of 14 histologically OA+ joints (64%) were diagnosed as OA+ (2/9 graded as suspected OA in radiography), while the remaining five joints that were OA+ histologically were not detected by radiography (i.e. grade 0). Of the five joints that were not detected, two joints had osteophytes on histology. None of the 14 assessed joints were graded normal in histology, thus it was not possible to perform Fisher's exact test for association of histology and radiography classification (OA+/-). For an overview of distribution between histological and radiographic grades for these 14 joints, see Fig. 21.

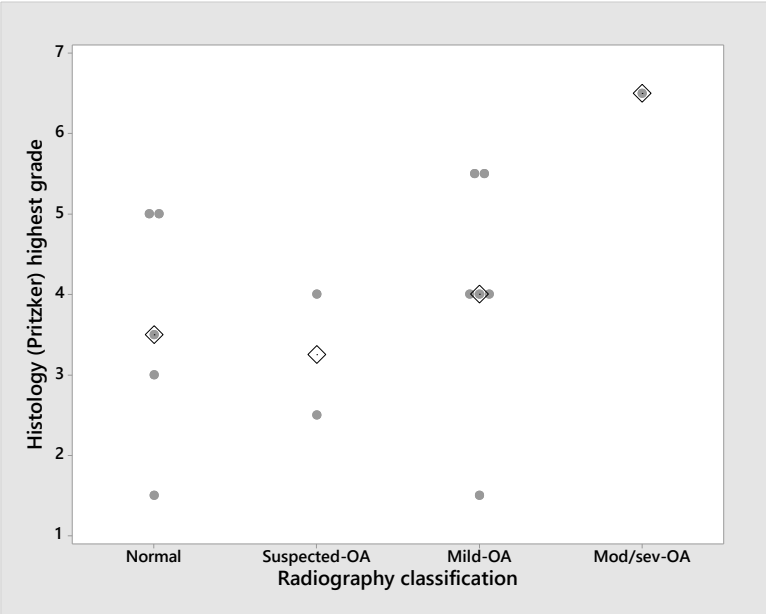

Figure 20. *Distribution of histologic (Pritzker) and radiographic grades for the fourteen joints graded in radiography. Diamonds represent the median Pritzker grade within each radiography classification.*

Five joints that did not have osteophytes on histology were still diagnosed as OA+ in radiography (2 of 5 as suspected mild OA joints). Their radiographic classification was determined by the bony acetabular margination assessed as osteophytes which could not be verified by histology. All of these five histologic joint samples were collected with CT-guidance. See Fig. 22 and 23 for an example of a cat with grade 2 for osteophytes in both joints on radiographs but only an osteophyte on the left acetabulum was verified histologically. The right acetabular margin of this cat (without osteophyte) is shown in Fig. 16. Worth to note is that histologic sampling was done without image guidance from radiographs.

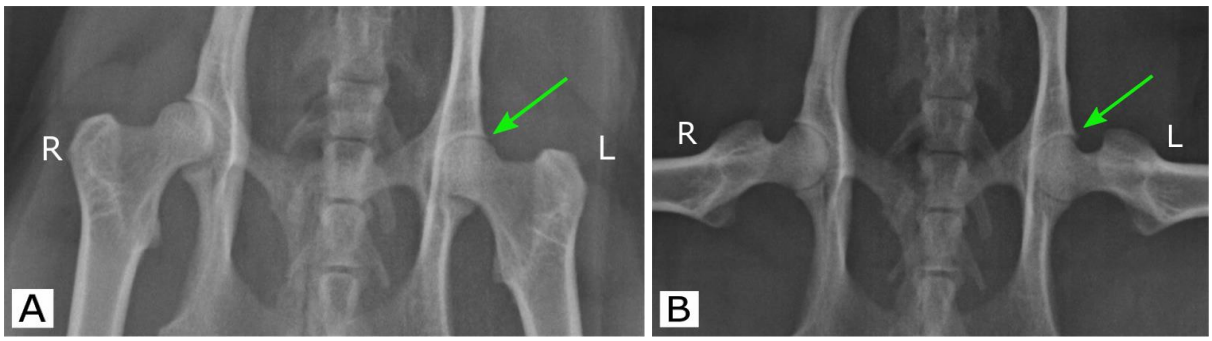

Fig. 21. *Radiographs of seven year old cat, extended ventrodorsal (A) and flexed ventrodorsal (B) projections. Both joints were given grade 2 for osteophytes on radiographs but only an osteophyte on the left side (arrows) could be verified histologically. Pritzker grades for the right joint were 4 for acetabulum and 3.5 for femur, for the left joint they were 2.5 for acetabulum and 4 for femur.*

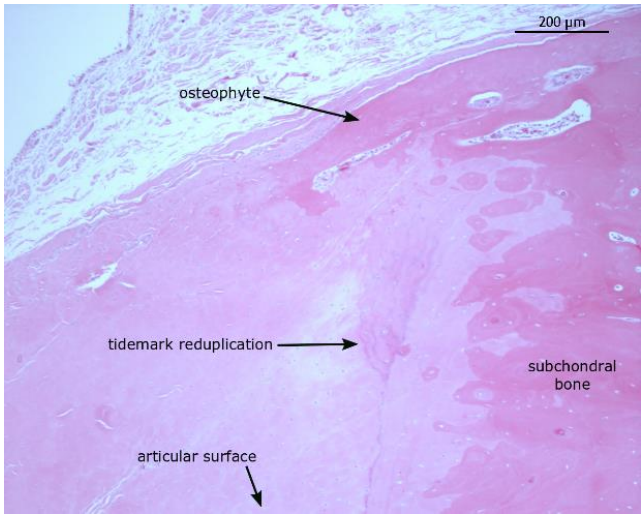

Fig. 22. *Peripheral margin of the left acetabulum of the cat shown in Fig. 22. An osteophyte could be verified histologically. It should however be noted that, as histologic samples were not collected with guidance from radiographs, the location for this osteophyte may not agree perfectly with the location which is pointed out in Fig. 22. Hematoxylin and eosin stain, x10.*

The comparison of OA grades in CT and radiography is shown in Fig. 24. For the same 14 joints radiography classified 9/14 joints as OA+ and CT classified 10/14 as OA+, however there was not perfect agreement of classification as OA+ (i.e. one of the four joints that was classified OA- in CT was classified as suspected OA in radiography). There was a statistically significant association between grades in CT and radiography for these 14 joints (Spearman rho = 0.573, p = 0.032).

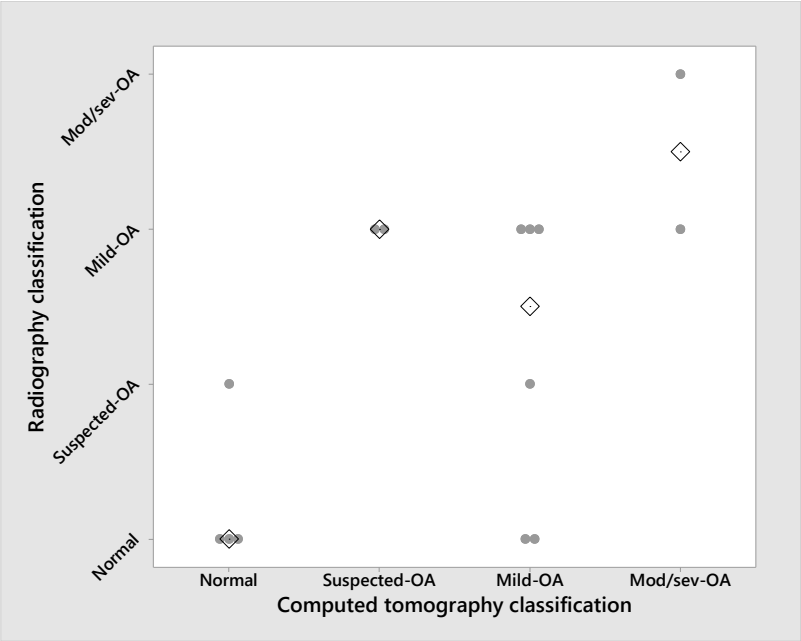

Figure 23. *Distribution of radiography and computed tomography (CT) grades for the fourteen joints graded in radiography. Diamonds represent the median radiography grade within each CT classification.*

DISCUSSION

Cartilage damage as seen in OA can be graded by the histologic method developed by Pritzker *et al*. (2006). In this study, the method was used to compare histologic cartilage lesions with osseous changes found in CT and radiography and to classify joints as either OA+ or OA-. It may be argued that it is problematic to compare cartilage damage with osseous changes. Even if Pritzker grades reflect increasing OA lesions (such as increasing depth of cartilage lesions), osseous changes are not primarily taken into account with this method. However, the overview of grades distribution in Fig. 13 gives the impression that there is a trend towards increased grade of CT lesions with increasing grade of histologic cartilage lesions.

In order to fully evaluate what is seen in CT images, a histologic assessment of recognized OA features such as osteophytes and sclerosis/lysis is needed and the Pritzker grading does not provide a complete assessment of those features. An alternative approach is to collect CT-guided histological samples, as these give the most relevant answers as to what is seen in the CT image and make comparisons of these samples regarding not only cartilage lesions but also bone remodeling. There are other histologic OA grading systems which consider not only cartilage structure lesions and chondrocyte pathology but also proteoglycan staining of the cartilage layer, collagen integrity, tidemark alterations, thickness of the subchondral bone plate and synovial pathology (Cook *et al*., 2010). Toluidine stained sections, used for assessment of proteoglycan content, were available in this study but not evaluated in regard to staining intensity (reflecting amounts of proteoglycans). Such grading systems require more time than available for this study. Furthermore, in this study, not all samples were collected with CT-guidance and in the samples that had not been collected in that manner, areas of interest may not have been included. In future research, it would be of great value to collect all histology samples with CT-guidance. The image guidance method used in the current study was subjective and sampling was guided by macroscopic identification of the feature of interest, and by assessing the local anatomy in the region of the joint to localize the area where the feature was expected to be located. There was no attempt made in the current study to verify the correct location of the samples taken by doing CT of the samples taken and comparing to the original CT image. Further, processing of the samples by embedding in wax blocks and cutting those in varying depths and angles can contribute to not capturing small features of interest. Thus, even the CT-guidance used in the current study is not a guarantee for getting samples of the exact location with features of interest (e.g. osteophytes, particularly if the features are very small). Although, it would be expected to be better than using standard sampling for histology.

The Pritzker grading system can be used in a more advanced way, by also ‘staging’ cartilage lesions, i.e. assessing the horizontal extent of cartilage involved. This is done without regard of the underlying stage. Grade and stage are then combined to an OA score (Pritzker *et al*., 2006). The advantage with this more comprehensive method is that joints with the same depth of cartilage lesions but different extent get different OA scores. In this study, due to time limitations the extent of lesions has not been taken into account neither in histologic nor in image assessment.

Another aspect to comparing early histologic lesions to later osseous OA features is the difficulty of estimating the clinical relevance of very low Pritzker grades, e.g. a joint with

histological grade 1.5 may present with only cellular changes. It is not certain that this individual will develop more severe OA lesions and by that, clinical OA.

In this study, it was decided to classify joints with Pritzker grades 0-1 as normal and grades 1.5-2 as minimal OA. It is however not obvious that this cut-off was the most appropriate and that joints with Pritzker grade 2.5 or more should be classified as definite OA. Joints with minimal OA were then classified as OA+ in the same manner as joints with suspected OA from CT classification (i.e. grade 1). This classification of suspected/minimal OA cases as OA+ contributed to the low number of joints that were histologically OA- and it also had a strong impact on statistical results. If suspected/minimal joints would have been classified as OA- instead, Fisher's exact test for association would not have been statistically significant ($p=0.11$ instead of 0.037). Furthermore, the fact that only one cat (two joints) were classified as OA- made it inappropriate to evaluate the ability of CT/radiography to detect normal joints.

Another contributing factor to the low number of joints being histologically OA- was the sampling of the study population. While cases collected during 2018 were collected randomly as cats were presented for post-mortem examination, joints collected earlier were mostly included due to macroscopic or radiologic changes. It would be of value to do a similar study with more evenly distributed groups to evaluate which imaging features most likely are associated to cartilage lesions in feline coxofemoral joints, as well as the frequency of detection of joints with cartilage lesions. However, due to the high frequency of histological OA changes detected in cat joints (Leijon *et al.*, 2017) it is likely to be problematic to identify cats that have normal articular cartilage that are age matched to cats with articular cartilage OA changes.

As there is no validated grading system of OA lesions on CT images, a grading system had to be developed for this study. The system used was relatively basic, as the different lesion types were not added to a final joint score but rather it was the most severe lesion that determined each joint's classification. A weakness with this system was that it did not include information regarding joints with multiple lesion types since these may have been be grouped together with joints with single lesion types, as long as they received the same grade for any lesion. Also, the system grouped moderate and severe lesions together, since this was felt to be compatible with the study's aim of investigating mild OA changes in the CT images. In future research it could be valuable to have separate groups for moderate and severe lesions. Another difficulty in the grading system was to determine a distinct cut-off between different lesion grades, as the grades of lesions often were not distinct. A way to minimize this issue in the current study was to illustrate the different grades with examples prior to grading, and to apply a final consensus between the two assessors.

Joints with mild and moderate Pritzker grades (up to grade 5) cannot be expected to be diagnosed with OA on CT and radiography, as they, according to Pritzker *et al*. (2006) do not present with osseous changes such as bone remodeling and osteophytes, and it is just these changes that CT and radiography rely on to detect OA. In this study however, several joints with Pritzker grades 1.5-4.5 were assessed to have osteophytes histologically. It raises the question whether the definition of osteophyte in this study did not correspond to the definition of earlier research (van der Kraan and van den Berg, 2007; Junker *et al.*, 2016), whether earlier studies have overlooked osteophytes or whether these OA features may in fact develop early in

feline coxofemoral joint OA. Another explanation could also be that locations with focal, more severe cartilage lesions were not included in the histological sample. The osteophyte present in the joint with lowest Pritzker grades (1.5 for acetabulum and grade 0 for femur) was assessed to be on the femoral head. This osteophyte matched with the definition of "fibrocartilage-capped bony outgrowth" (Junker *et al.*, 2016) but it was in a location where small irregularities in bone formation were seen in several cats. It can therefore be argued that the histological classification of this particular osteophyte may have been incorrect. This also implies that protruding bone on the ventral articular margin of the femur cannot be interpreted as a certain sign of OA, as many cats had varying types of bone formation here that did not fit the definition of an osteophyte.

Only one of the seven joints with CT classification 1 based on suspected osteophytes was sampled for histology with CT-guidance (see Fig. 15). The other joints had either been sampled without CT-guidance, in other locations or not at all. This resulted in few answers in this study whether the suspicions could be confirmed or not. A study that either focuses on these small lesions or has a bigger study population with increased chance of including cats with only small lesions is needed to further evaluate these small suspected lesions.

About half of the joints that were graded as OA+ in both histology and CT had osseous lesions classified as osteophytes with both histology and CT (12 of 23 joints). This implies that in CT the presence of osteophytes is the most important feature for being able to diagnose coxofemoral OA in cats. The remaining 11 of the 23 joints OA+ had no osteophytes on histological examination and the CT classification thus was based on osteophytes that could not be confirmed histologically. Three of these joints were sampled without CT-guidance, which could be the reason for these particular joints. Another reason for the OA+ classification could be that several of these cats had a rough acetabular margin shape that was interpreted as representative of osteophytes in CT although based on some of the histology sections from this study that shape may be within normal variation for the feline acetabulum. In some cases, it was difficult to determine whether the histologic features should be assessed as osteophytes or not. For the histological assessment for osteophytes, uncertain cases were classified as negative and so some osteophytes could have been classified negative. One explanation for the difficulties in the determination may be that there were early stages of osteophyte development. However, this raises the need for increased information about the normal histologic features of the feline acetabulum. The fact remains that most joints with this type of osseous changes had histologic cartilage lesions and it needs therefore to be investigated whether these marginal features are directly associated to the OA disease process. Only four joints that were histologically OA+ were not detected by CT. Three of these had only cartilage lesions and no osteophytes, which is a likely explanation for why they could not be detected on CT.

Most of the acetabular lesions classified as osteophytes in the histology sections were similar to each other regarding their hook-shape and thin connection to underlying tissue, looking like an extension of the non-articular surface. There were few osteophytes that were more rounded in shape. One reason could however be that this rounded type of osteophyte is more difficult to identify (and that they thus not were classified as osteophytes in this study), as these may not protrude as distinctly from the original bone as the hook-shaped osteophytes do.

Although cats are reported to develop osteophytes in OA affected joints to a lesser degree than dogs (Hardie *et al*., 2002; Clarke *et al*., 2005), 13 of 29 joints in this study were assessed to have osteophytes histologically. This implies that the coxofemoral joints may be an exception to the theory suggested by Freire *et al*. (2011). However, since there was some selection of cats with clinically suspected coxofemoral OA in this study it could also be a result of selection bias.

Several joints with labral fissures or suspected labral fissures were detected in this study. This was an unexpected and interesting finding. Labral tears are a recognized cause of hip pain in human patients and their description in humans (Seldes *et al*., 2001) fits with histologic findings in this study. They have also been suggested to be predisposing for OA in humans (Seldes *et al*., 2001). Further research may investigate the prevalence of labral tears amongst cats and their possible importance and association to OA.

When interpreting the results of association in this study, it should be noted that the data used was not entirely independent, as two coxofemoral joints were sampled from some cats while only one joint was sampled from others. The used statistical tests did not take this lack of independence into account and due to time constraints there was no possibility to process the data with more complicated statistical methods, which would have been necessary to fully evaluate the association.

Regarding radiography, it is difficult to draw certain conclusions from this study, as only a small number of joints were examined and none of the 14 joints were histologically OA-. Still, the results show that CT and radiography have about the same frequency of detection of osseous changes associated to coxofemoral joint OA in cats in this study. On the other hand, no conclusion can be made as to the imaging methods' ability to detect normal joints. The study also shows that there is an association between grading of the severity of OA lesions in CT and in radiography.

In this study, osseous changes detected by CT were associated to cartilage lesions in feline coxofemoral joints. This result should though be interpreted with care; as most cats in the study population had histologic cartilage lesions and there only was a small number of joints without such lesions it is difficult to determine whether this is a true association. Also, the statistical analysis of the data in the study was limited. Further research is needed to investigate whether protruding bone tissue and a rough shape in acetabular margins represent normal variation or early stages of new bone formation and whether these features are associated to feline coxofemoral joint OA.

POPULAR SCIENCE SUMMARY

Osteoarthritis (OA) is a joint disease that leads to tissue changes including cartilage, bone and soft tissue. Articular cartilage is the thin layer of flexible, smooth tissue covering the top of each bone within the joint and it has an important shock absorbing function to protect the underlying bone. The destruction of cartilage and underlying bone, as well as changes in the soft tissues may lead to joint pain and subsequent stiffness and/or lameness.

Articular cartilage consists of cells called chondrocytes embedded in abundant ground substance (so called extracellular matrix). The chondrocytes produce and secrete components of the ground substance that maintain its flexible, shock absorbing properties. Among the most important components are proteoglycans, which contribute to maintaining water content in the cartilage, and collagen fibers which are organized in a certain direction for best shock absorbing properties. As OA progresses, the chondrocyte capacity to produce and maintain cartilage substances is affected. Due to this, the cartilage loses its flexible properties and becomes susceptible to mechanical injury with lesions emerging, such as cracks and loss of tissue. The cartilage may disappear completely with the underlying bone becoming exposed. Beyond cartilage lesions, the underlying bone tissue may be subject to remodeling and injury such as microfractures and increased density (osteosclerosis). Injuries may heal with repair tissue. One type of remodeling which is typical for OA is osteophyte formation. Osteophytes are bony outgrowths usually found on the joint margin (they may also be central in advanced cases) that alter the bone's outer contour. The lesions caused by OA may best be seen microscopically as both cellular changes and tissue lesions in the matrix of the cartilage and underlying bone are visible.

The causes for some individuals being affected of OA are still not completely understood but some factors have been pointed out as contributing to an increased risk. These are genetic factors, obesity, ageing, metabolic disturbances, joint injury, hormonal changes, systemic disease and biomechanical factors such as joint shape and alignment.

In cats, OA is a relatively frequent disease. There are studies which have shown the occurrence amongst cats to be in between 16.5 and 61%, somewhat depending on the age distribution in the study population. Research has also shown that the cats' hips are amongst the most frequently affected joints in the body, therefore this study was focused on hip joints.

OA is to this day mainly diagnosed with radiography. One problem with that is that cartilage tissue cannot be seen on x-ray images and the diagnosis is therefore possible only in a more advanced disease progress with bone remodeling and/or osteophytes.

Computed tomography (CT) uses x-rays but in contrast to traditional radiography, it collects three-dimensional information of the examined body part. That makes it possible to reconstruct the image information in any plane on the computer screen and to assess body parts from any point of view. Another advantage with CT over radiography is that different tissues are easier to distinguish from each other. But still, cartilage tissue is not visible in CT and CT provides limited possibility to assess soft tissue. As CT currently is not used frequently to diagnose hip joint OA lesions in cats, there is a lack of information concerning CT features of hip joints with OA and normal hip joints, respectively.

The aim of this study was to investigate whether various bony features in cats' hip joints detected by CT are associated with microscopic findings of OA, as well as to describe these features found in CT and microscopy. Another aim was to compare the frequency of detection of OA in cats' hip joints by CT and radiography.

The study material used was whole-body CT studies of 21 cats euthanized for reasons unrelated to this study. Eight cats had also radiographs. Twenty-nine hip joints from these cats were sampled for microscopic assessment. Some of these samples had been collected in previous years for other studies but all sampling for microscopy done during 2018 was done with CT-guidance, i.e. samples were taken from areas of the hips where interesting bony features were seen in the CT images. Joints were graded in CT images and radiographs regarding a number of recognized OA-lesions (osteophytes and osteosclerosis, amongst others), using a grading system developed for this study as no such grading system existed previously. The grading of anonymized images was done individually by a final year veterinary student and a veterinary radiologist and when grades differed, the final grade was decided by consensus.

For microscopic assessment, a grading system modified from a system developed by Pritzker *et al*. (2006) for cartilage lesions was used and other lesions such as osteophytes and other bone shape abnormalities were noted as a comment as these are not taken into account in the grading system. Regarding cartilage lesions, joints were graded by a final year veterinary student after training by a veterinary pathologist.

After grading all joints, they were classified into OA positive and OA negative groups for each assessment method. Some statistical analyses were performed to evaluate agreement between grades of lesions seen in imaging and microscopically, and some comparisons were made in a descriptive manner.

The age of cats included in the study ranged from 18 months to nineteen years and age was found to be significantly associated to the extent of microscopic cartilage lesions. Microscopically, only two joints (one cat, aged 18 months) of 29 joints were found to be negative regarding cartilage lesions and these were also assessed as OA negative in CT. There was a statistically significant association between classification in histology and CT concerning classification of joints as either OA positive or OA negative. Also, there is a trend towards higher grades for lesions on CT with increasing cartilage lesions.

An important feature for the detection of OA positive joints in CT were osteophytes. Most of the joints that were found to display osteophytes on microscopy were also classified as OA positive on CT (12 of 13 joints with osteophytes). The majority of joints (3 of 4 joints = 75%) which CT did not detect (although OA positive microscopically) did not have osteophytes.

An interesting result of this study was that several joints with mild cartilage lesions were found to have osteophytes, which is in line with literature that suggests that osteophytes can develop early in the OA disease process.

Some joints were classified as OA positive in CT based on features such as uneven bone shape (indicative of osteophytes) which could not be verified as osteophytes microscopically. Instead,

these features raised the question whether they represent normal anatomic variation in cats' hip joints or if they still could be features directly associated to OA. Further research is needed to determine the answer to that question.

In this study, bony changes detected by CT were associated to microscopic OA-cartilage lesions in cats' hip joints. The result should though be interpreted with care, as the majority of examined joints were microscopically OA positive (27 of 29 joints) and only two joints OA negative. Of the 27 positive joints, 23 joints (85%) were diagnosed as OA positive by CT as well. Regarding radiography, the study shows that the frequency of detection of bony features associated to hip joint OA in CT and radiography is about the same (9/14 joints in radiography, 10/14 joints in CT). As none of these 14 joints were OA negative microscopically, no conclusion can be made about the imaging methods' ability to detect normal joints.

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