Histological features of the distal third metacarpal bone in Thoroughbred racehorses, with and without lateral condylar fractures.

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SUMMARY

A detailed histopathological study of the distal third metacarpal bone of Thoroughbred racehorses was undertaken to characterise lesions previously observed on magnetic resonance imaging (MRI). The bones were selected and grouped on the basis of MRI features. Representative sections in different planes were obtained and processed for histopathology. All lesions observed in the articular cartilage (AC) and subchondral bone (SCB) were recorded and graded with a specific scoring system, partially based on the Osteoarthritis Research Society International system. The scoring system included the severity of the lesion. Descriptive statistics and linear mixed effects models were performed. A positive correlation was observed between the severity of histopathological changes in the superficial and deeper osteochondral tissues, and between the number of race starts and AC score. Age was not correlated with AC or SCB score. A moderate variation in AC and SCB scores was observed between the groups; however there were differences within individual bones. Bones with focal palmar necrosis (FPN) showed significant differences in the histologic scoring of the AC compared with bones without FPN. Bones with incomplete fractures or larger areas of bone remodelling showed significant differences in SCB pathology when compared with bones with FPN. Haematoidin was detected in areas with excessive SCB and cancellous bone sclerosis and/or irregular bone density. This finding is suggestive of poor blood perfusion in these areas.

Keywords: horse, distal third metacarpal bone, lateral condylar fracture, histology, haematoidin.

INTRODUCTION

Fractures of the lateral condyle (LC) of the third metacarpal bone (MC3) are the most common reason for euthanasia of horses on UK racecourses (Parkin *et al*., 2004a, b). These fractures pose both welfare and economic issues for the racing industry. Consequently, research has been undertaken to investigate aspects of the equine distal MC3 bone structure (Martin *et al*., 1996; Riggs, 1999; Boyde *et al*., 2004), nutrition and vascularization (Kawcak *et al*., 2001), mineralization (Boyde and Firth., 2005), mechanical properties (Rubio-Martinez et al., 2008), response of bone to exercise (Boyde, 2003; Muir *at al*., 2008) and arthroscopic description of the articular surfaces of distal MC3 (Vandeperren *et al*., 2009).

In studies comparing Thoroughbred racehorses in training with non-athletic horses there are clear differences in the osteochondral unit, in particular subchondral bone (SCB) density, tidemarks in the calcified cartilage (CC) and thickness of the hyaline cartilage (HC) (Muir *et al*., 2008). Remodelling of the osteochondral unit appears to happen naturally as an adaptation to exercise (Murray *et al.*, 2001; Muir *et al*., 2008; Santschi, 2008). However, it has been highlighted that in some cases modelling and remodelling of the distal MC3 under exercise is associated with SCB/CC/HC pathology (Muir *et al*., 2008), and even condylar fractures (Whitton *et al*., 2010; Tranquille *et al*., 2012). Similar studies in distal limb bones of rats suggest that development of extensive remodelling is associated with specific regional adaptive changes, bone fatigue, hyperaemia and associated decreased lacuno-canalicular interstitial fluid (Muir *et al*., 2007). However, little detailed histopathological study of the equine distal MC3 has been undertaken. Previous investigations have suggested that pre-existing pathological defects and bone microcracking occurs prior to fractures (Muir *et al*., 2006, 2008; Kristofferson *et al*., 2010). A number of studies have suggested that LC fractures are the result of two different processes: overload arthrosis with microfractures and failure in

SCB (Norrdin *et al*., 1998; Cruz and Hurtig, 2008), or that they are the end stage of a series of fatigue-related events (Kawcak *et al*., 1995; Riggs, 1999).

Focal palmar necrosis (FPN), or palmar osteochondral disease, has previously been described on MRI and post-mortem examination (Boyde and Firth, 2005; Parkin *et al.*, 2006; Barr *et al.*, 2009; Riggs, 2009; Richardson and Dyson, 2010; Powell, 2011, 2012; Tranquille *et al.*, 2012). It has been described as a lesion that could encompass abnormalities such as bone necrosis, proteinaceous fluid accumulation, cartilage thickening and infolding into the SCB. Findings from Tranquille *et al.* (2012) indicated that this type of lesion could be protective against LC fracture by preventing the horse exercising to maximal capacity.

The objective of the study was to describe the spectrum of histological features in the distal MC3 of Thoroughbred racehorses, with and without LC fractures, to provide an insight into the aetiopathogenesis of LC fracture. It was hypothesized that: 1) There would be a positive relationship in the severity of histopathological features between superficial and deeper osteochondral tissues; 2) There would be a positive relationship between numbers of race starts, age, and pathology observed in HC and SCB; 3) Changes would be consistent within each group and within each bone section from a single bone; 4) The degree of SCB sclerosis would increase with age and/or number of starts; 5) Bones with FPN observed on MRI would score higher in HC pathology than bones without FPN; whilst those with no FPN but SCB changes would score higher in SCB histology.

MATERIALS AND METHODS

Thirty-eight MC3 bones were selected from an archive of bones collected from horses that were euthanased at UK racecourses between 1999 and 2005, as part of a Horserace Betting Levy Board funded study, conducted at the University of Liverpool (Parkin *et al*., 2004a, b).

The bones were divided into eight groups as defined by lesions observed on MRI in a previous study (Tranquille *et al.,* 2012):

Group 1: Bones with incomplete LC fractures;
Group 2: Non-fractured bones with mild FPN;
Group 3: Non-fractured bones with severe FPN;
Group 4: Non-fractured bones without FPN with round bone shaped reaction in SCB;
Group 5: Non-fractured bones without FPN with triangular bone shaped reaction in SCB;
Group 6: Non-fractured bones without FPN with bone reaction covering both condyles;
Group 7: Non-fractured boned with no MRI detected changes;
Group 8: Bones with complete LC fractures.

Histologic preparation

Sagittal sections of the lateral and medial condyles and sagittal ridge (Fig 1a), and dorso-palmar sections with an angle of 35° to the longitudinal axis, were taken (Fig 1b). Eleven bones only had sagittal sections and 27 bones had both sagittal and dorso-palmar sections.

Gross specimens 4-5mm thick were sectioned with a large band saw (Biro UK, London, England) and fixed in buffered 10% formalin for seven days. The samples were then decalcified in rapid decalcifier (CellPath PLC, Mochdre, Newtown, Powys, Wales) for two days and washed in running tap water for three hours. Histological processing was

performed on an automatic tissue processor (Bayer VIP, Newbury, Berkshire, England) using alcohol, xylene and paraffin wax (Ralwax, VWR International Ltd, Poole, Dorset, England). The samples were then blocked out in paraffin wax in a large mould and two sections were cut from each block on a base sledge microtome at a thickness of 3 to 5µm. The sections were mounted on gelatin-coated slides and dried overnight at 50ºC. One section was stained with Harris' haematoxylin and 1% eosin in 70% ethanol and the other with 1% toluidine blue. Slides were then dehydrated in alcohol, cleared in xylene and mounted in DPX (DPX, Raymond A Lamb Ltd, Eastbourne, East Sussex, England).

Histological assessment

The HC, CC and SCB/trabecular bone were examined under a light microscope (Olympus DP12 microscope, Olympus UK Ltd, London, England). A scoring system was adapted from one previously used by the group (Tranquille *et al.*, 2009), which included some aspects of the Osteoarthritis Research Society International grading system (McIlwraith *et al*., 2010). The scoring system defined histological features and attributed a grade based on the presence and the severity of the lesion with 0 representing no change/lesion present and 1-3 representing progressively increased lesion severity for HC (Table 1a), CC (Table 1b) and SCB/trabecular bone (Table 1c). A total score for each section was calculated to provide an overall assessment of the degree of pathology at that site, within each bone.

Interobserver repeatability between two anatomical pathologists was assessed by use of five repeated scorings for 10 sections. Final assessment was carried out when the coefficient of variation was < 2%. Maria-Jose Pinilla carried out all histologic interpretations.

Statistical analysis

Item analysis was used to investigate the degree of correlation between different pathologies in each tissue of a section. Cronbach's Alpha was calculated to identify particular groups of pathologies that were most highly correlated. Linear mixed effects models were used, to account for repeated measures, to assess the effects of MRI grouping, section orientation, horse age and total number of race starts on the outcomes (total HC and CC score, from now on referred to as AC score, or SCB score). Linear contrast was used to compare the average effect of groups 2 and 3 and the average effect of groups 4, 5 and 6. Weighted Pearson's correlation (Bland and Altman, 1995) was used to assess the correlation between AC score and SCB score. All analyses were performed with statistical analysis software (SPSS Statistics 20, SPSS Inc, Chicago, Illinois, USA). Significance was defined as a value of $P < 0.05$.

RESULTS

The 38 bones came from 34 horses with a mean age of 6.1±2.3 years. The mean number of race starts was 20 (range: 1-74).

Group 1: Bones with incomplete LC fractures (n=4)

Severe SCB sclerosis was seen in two bones while three bones showed moderate sclerosis; in this area microscopic incomplete fractures and SCB collapse were identified in five bones. There was heavy remodelling in all bones. Degenerative changes within the HC were observed in two bones. More severe HC, CC and SCB changes were observed in LC and the lateral sagittal groove compared to the medial aspect of distal MC3.

Group 2: Non-fractured bones with mild FPN (n=5)

Moderate to severe degenerative changes in the HC, accompanied with chondrocyte loss were present in all bones. Concurrent moderate sclerosis was also observed in three bones. Moderate HC fibrillation was detected in two bones. Greater pathological scores were observed in LC compared to the medial condyle.

Group 3: Non-fractured bones with severe FPN (n=5)

Lesions observed in this group were similar to those seen in Group 2 but were more severe; particularly in HC and CC. Chronic degenerative changes (HC fibrillation and chondrocyte loss) combined with SCB sclerosis, collapse, necrosis and haemorrhage was observed in only one bone. More severe lesions were observed in LC and sagittal ridge compared to the medial condyle.

Group 4: Non-fractured bones without FPN with round bone shaped reaction in SCB (n=7)

Mild degenerative HC changes (irregular articular surface and chondrocyte clustering) were seen in three bones. Focal, more severe HC changes are seen in two bones combined with mild to moderate SCB sclerosis with reduction of the medullary spaces was observed in four bones. One bone showed SCB collapse and disruption of CC/SCB tidemark. More severe lesions were observed in LC compared to the medial condyle.

Group 5: Non-fractured bones without FPN with triangular bone shaped reaction in SCB (n=5)

SCB plate sclerosis and thickening of the trabeculae were detected in all bones in this group. Chondrocyte loss with focal fibrillation in HC was observed in 2 bones. More severe lesions were observed in LC and lateral sagittal groove compared to the medial condyle and medial sagittal groove.

Group 6: Non-fractured bones without FPN with bone reaction covering both condyles (n=5)

Lesions observed in this group were similar to those seen in Group 5. Moderate to severe SCB sclerosis was observed in all bones, including the sagittal ridge area in one of the bones. Mild degenerative changes in HC were observed in three bones. The severity of the lesions observed was equal between the lateral and medial condyles.

Group 7: Non-fractured boned with no MRI detected changes (n=4)

Mild HC fibrillation, mild matrix pallor and focal areas with fewer/increased numbers of chondrocytes in HC and CC were seen in three bones in LC. Mild to moderate focal sclerosis of the SCB plate was seen in all bones.

Group 8: Bones with complete LC fractures (n=3)

There was total HC loss, resulting in the exposure of the underlying SCB (eburnation). There was evidence of chronic degenerative changes (chondrocyte loss and HC fibrillation) in all bones. Bones with comminuted LC fractures had scant bone remodelling and mild areas of SCB sclerosis.

Histological patterns between the groups

Proportions of lesion severity for different features in the HC, CC and SCB tissues were graphically presented in Figure 2. Across the groups the most common lesions in HC were chondrocyte and glycosaminoglycan loss and HC fibrillation (Figure 3). The most common lesions in CC were disruptions of the layer and discontinuity of the tidemark associated with SCB pathology (Figure 4). The most common lesions in SCB were excessive sclerosis beyond the SCB plate (Figure 5) and irregular thickening of the trabeculae (Figure 6). Severe

lesions were identified in eight bones with SCB collapse with severe HC changes (Figure 7). Incomplete fractures were observed in heavily or irregularly modelled bones with substantial sclerosis of the cancellous bone.

Item analysis showed that in HC, the comparison between different pathologies in each section, revealed Cronbach's Alpha greater than 0.8, indicating a good degree of consistency within the HC pathologies.

There was a positive correlation between AC and SCB score (weighted Pearson Correlation 0.58; P= 0.0004). The number of starts was associated with increased AC score (P=0.04) but not associated with SCB score. There was no association between age and AC or SCB score.

The adjusted mean AC and SCB scores for different lesion group and sections were given in Table 2. No significant difference between groups (P=0.206) or between sections (P=0.303) in AC was identified. However, bones with FPN (groups 2 & 3) showed slightly higher histologic scoring in the AC (4.9±2.1, P=0.02) compared with bones without FPN (groups 4, 5 & 6).

There was an overall difference in SCB scores between groups (P=0.024). Group 4 had lower SCB scores compared to groups 1, 2, 3 or 6 (P ranged from 0.006 to 0.04), and group 5 had significantly lower SCB score compared to groups 1 and 6 (P=0.02 for both comparisons).

There was also a significant difference in SCB scores between sections (P=0.005). Lateral condyle section had significantly higher score compared to dorso-palmar medial section

(*P*=0.001), sagittal ridge section (*P*=0.002) and medial condyle section (*P*=0.02); there was also difference between dorso-palmar lateral and medial sections (*P*=0.03).

DISCUSSION

The results from this study support hypothesis 1 as there is a positive relationship in the severity of histopathological features between the superficial and deeper osteochondral layers. The results partially support hypothesis 2 as there was an association between number of race starts and AC score but there was no association with SCB score. The results partially support hypothesis 3 as there was no significant variation between groups. However there were significant differences within individual bones. The results do not support hypothesis 4 as SCB scores were not associated with age or number of starts. The results also support hypothesis 5 as bone specimens with FPN observed on MRI scored higher in HC pathology, while those with no FPN but SCB changes on MRI scored higher in SCB histology.

Bones showing FPN on MRI had higher histological scores for HC and CC, indicating that there was an association between MRI findings and histological AC score for bones in groups 2 and 3. The existence of a direct correlation between histological SCB features and articular tissues of the MC3 suggests that they act as a unit, supporting previous research (Van de Harst *et al.*, 2005).

When comparing the differences in SCB scores between the different sections it was observed that LC scored higher than the medial condyle or sagittal ridge in seven of the eight groups assessed. This indicates more extensive remodelling, and potentially more pathological changes, in LC compared to the medial condyle or sagittal ridge. Osteonal structure studies (Martin *et al.*, 1996) showed evidence of regional variations in osteonal size

and structure, which are generated during remodelling, and suggests a biomechanical aetiology. This supports the findings of Parkin *et al.*, (2006) who observed that LC fractures are more likely to occur than medial condylar fractures.

The histopathology score of HC and the MRI observations of the HC supported the designation of the groups according to MRI appearance. However, there was some disparity in the scoring within the same group, which is likely to reflect the high level of detail generated on microscopic analysis when compared to the MRI findings.

Results indicate that the evenness of the articular surface, fibrillation, matrix disruption and chondrocytes/chondrones distribution in HC are positively correlated with each other. This result reinforces the idea of the existence of coordinated, progressive changes along the same pathological spectrum in HC in response to adaptation and pathology.

Approximately half of the bones (51%) showed a grade 2 SCB plate sclerosis, which was interpreted as an adaptive change as sclerosis happens physiologically as an adaption to exercise (Murray *et al*., 2001; Boyde and Firth, 2005; Firth, 2006). However, there does not appear to be a direct correlation between SCB plate sclerosis and increased age or numbers of race starts. This result supports the previous findings of Parkin *et al.* (2006) that stated that the degree of gross pathology in distal MC3 was not associated with horse age, career length or number of race starts. The age range and number of race starts in these horses along with the relatively low number of samples included made it difficult to establish a clear association between excessive sclerosis and pathology. The lack of correlation observed between age and SCB plate sclerosis may be attributed to the potentially false assumption

that older horses would have undergone a more prolonged and intense training/racing regimen and therefore will have more sclerosis.

There was no association between age, number of race starts or study group when bones with similar SCB pathology were assessed. The fact that not all bones with SCB collapse/haemorrhage had visible HC erosions (although expansion of the HC layer was commonly observed), suggested that this pathology originated within the SCB and that the cartilage alterations observed were adaptive changes to remedy the loss in bone volume and to keep the articular surface even. The majority of these lesions were observed in areas with excessive SCB and cancellous bone sclerosis and/or irregular bone density. The consistent, repeated, findings of haemorrhage and haematoidin formation were suggestive of poor blood perfusion in these areas. Haematoidin crystals are reddish brown, highly birefringent and represent the breakdown product of haemoglobin, formed in the tissues from haemoglobin, particularly under conditions of reduced oxygen tension (Rosca *et al.*, 2006). Bright yellow material was present in many of the haemorrhage areas and polarised light demonstrated refringency in some of them. The presence of haematoidin indicates a slow, indolent process of absorption of the haemorrhage. Knowing that the SCB receives oxygen/nutrition by diffusion from the medullary areas (Kawcak *et al.*, 2001), it is not unreasonable to think that diminution in the area occupied by marrow due to sclerosis may result in chronic bone hypoxia potentially leading to devitalisation and bone collapse.

Subchondral bone remodelling has been demonstrated by increased vascularisation and also acute loading of cortical bone elicits a hyperaemic response (Kawcak *et al.*, 2001). In this context, the study of the non-fractured bones suggested that excessive sclerosis may lead to poor bone perfusion and progressive ischaemic changes accompanied with SBC collapse.

These lesions may occur independently of HC changes or associated with them; it was difficult to establish whether or not these lesions progress invariably to incomplete fractures. Tranquille *et al.* (2012) showed that these bones can be identified by changes in signal intensity on MRI. These results suggest that it is potentially possible to identify horses at risk of LC fracture and do a follow up on the clinical progression, evaluating at the same time treatment effectiveness.

Clefting of the CC was observed in bones in which the SCB density was increased but without evidence of any inflammation, necrosis or other signs of disease; it was considered that the clefts within the CC layer represented a retraction of the sclerotic tissue during processing. This clefting is a different process from *in-vivo* microcracks, which shows evidence of tissue reaction such as haemorrhage and bone necrosis.

Histological assessment of bones with comminuted fractures showed scant bone remodelling and mild sclerosis. These findings suggest that these fractures were not likely to be fatigue-related. In contrast, the incomplete fractures appear within heavily remodelled areas suggesting these incomplete fractures may be fatigue related when forces exceed the weight bearing/shock absorption capacities of the bone. However, these observations cannot be substantiated statistically due to lack of sufficient numbers; a larger histological study with larger number of fractured bones would be desirable to confirm these preliminary findings. A recent study by Jacklin and Wright (2012) support a varied aetiopathogenesis of distal MC3 fractures and statistically indicate that in approximately half of the fractured bones studied, the fractures did not arise from areas typically associated with cumulative fatigue.

Limitations of this study related to the relatively small numbers of horses analysed, which did not permit a robust statistical analysis. However the level of detail in the characterisation of lesions in each horse was excellent. Additional problems in this study were the lack of complete set of sections for every bone due the presence of fractures, which would have facilitated the comparison between sections.

CONCLUSIONS

A positive correlation was observed between the severity of histopathological changes in the superficial and deeper osteochondral tissues, and between the number of race starts and AC score. Age was not correlated with AC or SCB score. A moderate variation in AC and SCB scores was observed between the groups; however there were differences within individual bones. More changes were observed in LC compared with the medial condyle and sagittal ridge areas. Bones with FPN showed significant differences in the histologic scoring of the AC compared with bones without FPN. Bones with incomplete fractures or larger areas of bone remodelling showed significant differences in SCB pathology when compared with bones with FPN. The findings suggest that SCB sclerosis may be associated with progressive ischaemic damage. Haematoidin was detected in areas with excessive SCB and cancellous bone sclerosis and/or irregular bone density. This finding is suggestive of poor blood perfusion in these areas.

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CONFLICT OF INTEREST STATEMENT

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of this paper.

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FIGURE LEGENDS

Figure 1. A = Dorso-palmar photo of a distal aspect of the third metacarpal bone showing where the sagittal sections were taken. Medial is to the left and lateral is to the right. B = Sagittal photo of a distal aspect of the third metacarpal showing where the dorso-palmar section with an angle of 35° to the longitudinal axis were taken. Dorsal is to the left and palmar to the right.

Figure 2. Series of bar charts showing the proportions of scores for different features in the hyaline cartilage (HC), calcified cartilage (CC) and subchondral bone (SCB) tissues for each of the eight lesion groups.

Figure 3. Photomicograph of a tissue section obtained from the medial condyle of the distal third metacarpal bone from a Group 2 bone showing loss of chondrocytes [C] and fibrillation (arrow) of the hyaline cartilage. Toluidine blue stain; bar = 200μm.

Figure 4. Photomicograph of a tissue section obtained from the lateral condyle of the distal third metacarpal bone from a Group 4 bone showing disruption and discontinuity of the calcified cartilage layer associated with marked subchondral bone pathology (arrow). Harris's haematoxylin and eosin stain; bar = 200μm.

Figure 5. Photomicograph of a tissue section obtained from the lateral condyle of the distal third metacarpal bone from a Group 5 bone showing marked sclerosis extending below the subchondral plate (arrows) in the palmar and dorsal aspects. Toluidine blue stain; bar = 1cm.

Figure 6. Photomicograph of a tissue section obtained from the lateral condyle of the distal third metacarpal bone from a Group 4 bone showing loss of medullary spaces (arrow) and irregular thickening of the trabeculae (circle) in the subchondral bone. Harris's haematoxylin and eosin stain; bar = 200μm.

Figure 7. Photomicograph of a tissue section obtained from the lateral condyle of the distal third metacarpal bone from a Group 3 bone showing severe lesion with collapse of the subchondral bone and associated disruption of hyaline cartilage and calcified cartilage layers. Harris's haematoxylin and eosin stain; bar = 200μm.

TABLES

TABLE 1a. Hyaline cartilage grading scheme.

HYALINE CARTILAGE			
B	Regularity of articular surface	0 1 2 3	Normal Mildly Moderate Severe
C	Fibrillation	0 1 2 3	No fibrillation seen Mild Moderate Severe
D	Articular cartilage thickness variation	0 1 2 3	Expected thickness for the area Mild variations Moderate variations Severe, extensive or focal variations (e.g. cartilage plugs)
E	Alteration of matrix structure	0 1 2 3	Normal staining for glycosaminoglycans and matrix structure Mild matrix pallor and separation of fibres Moderate matrix pallor and separation of fibres Marked matrix pallor and separation of fibres
F	Chondrocyte clustering	0 1 2 3	Normal appearance of chondrocytes and lacunae Formation of double chondrocytes Presence of triplet chondrocytes and loss of the linearity Large numbers of chondrocytes clustered together within single supersized lacunae
G	Irregular distribution of chondrocytes	0 1 2 3	Orderly distribution of chondrocytes Focal areas with fewer or increased numbers of chondrocytes than expected Focal areas with moderate variations in numbers Extensive and severe alterations in chondrocyte distribution
H	Chondrocyte loss/necrosis	0 1 2 3	Presence of chondrocytes in the lacunae Occasional foci with empty lacunae Moderate loss of chondrocytes Severe loss of chondrocytes

TABLE 1b. Calcified cartilage grading scheme.

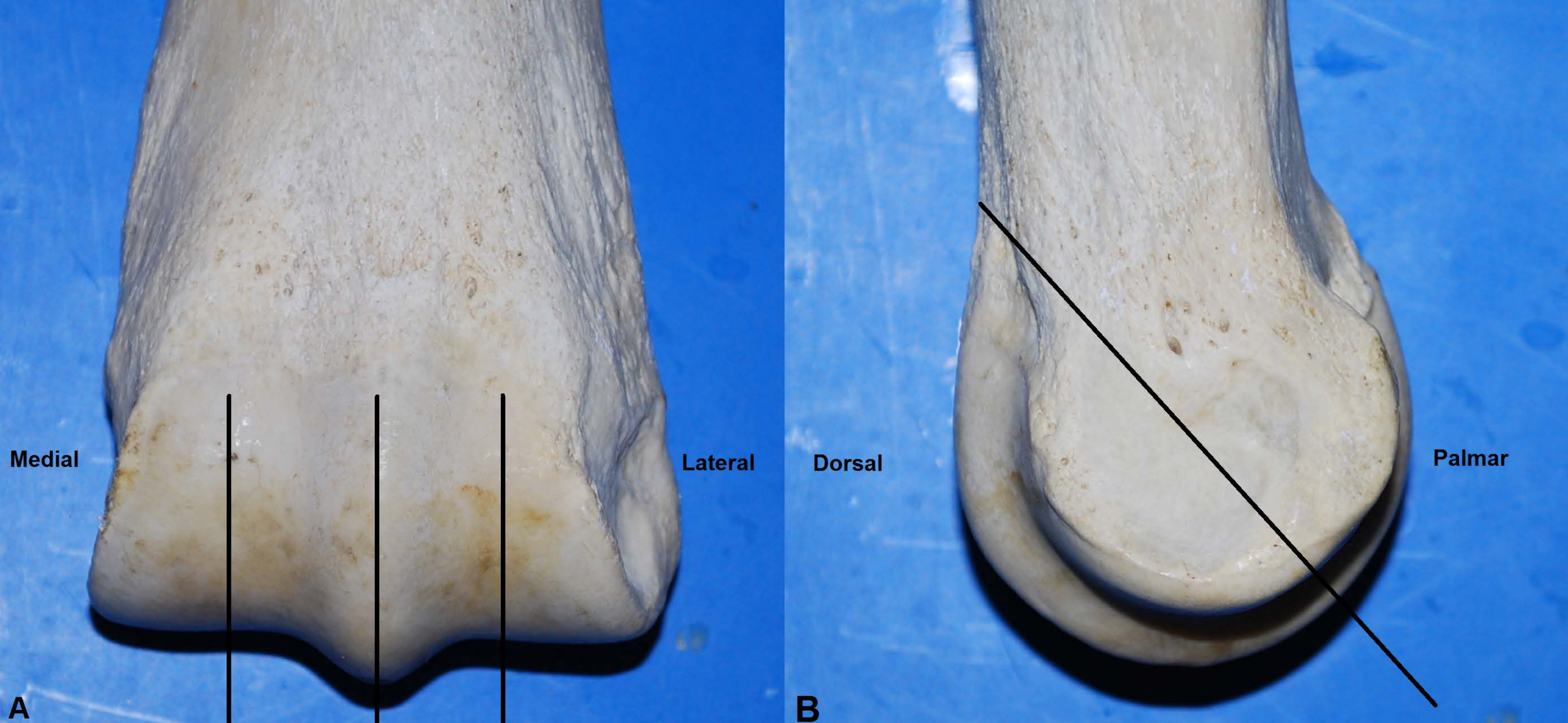
CALCIFIED CARTILAGE			
J	Clefts	0 1 2 3	Absence of clefts Occasional focal clefts Moderate numbers of clefts Large numbers of clefts
K	Variations in depth	0 1 2 3	Expected variations in depth Minimal variation Moderate variation Severe variations
L	Tidemark incongruences	0 1 2 3	Expected pattern of the tidemark Lack of parallelism Reduplication Absence
SCB-CC Interface			
M	Vascular incursions	0 1 2 3	No incursion Occasional Moderate numbers Very frequent
N	Island of hyaline cartilage present in the SCB plate	0 1 2 3	No islands Occasional, small islands Large single cartilage islands of CC in the SCB Numerous islands of CC in the SCB

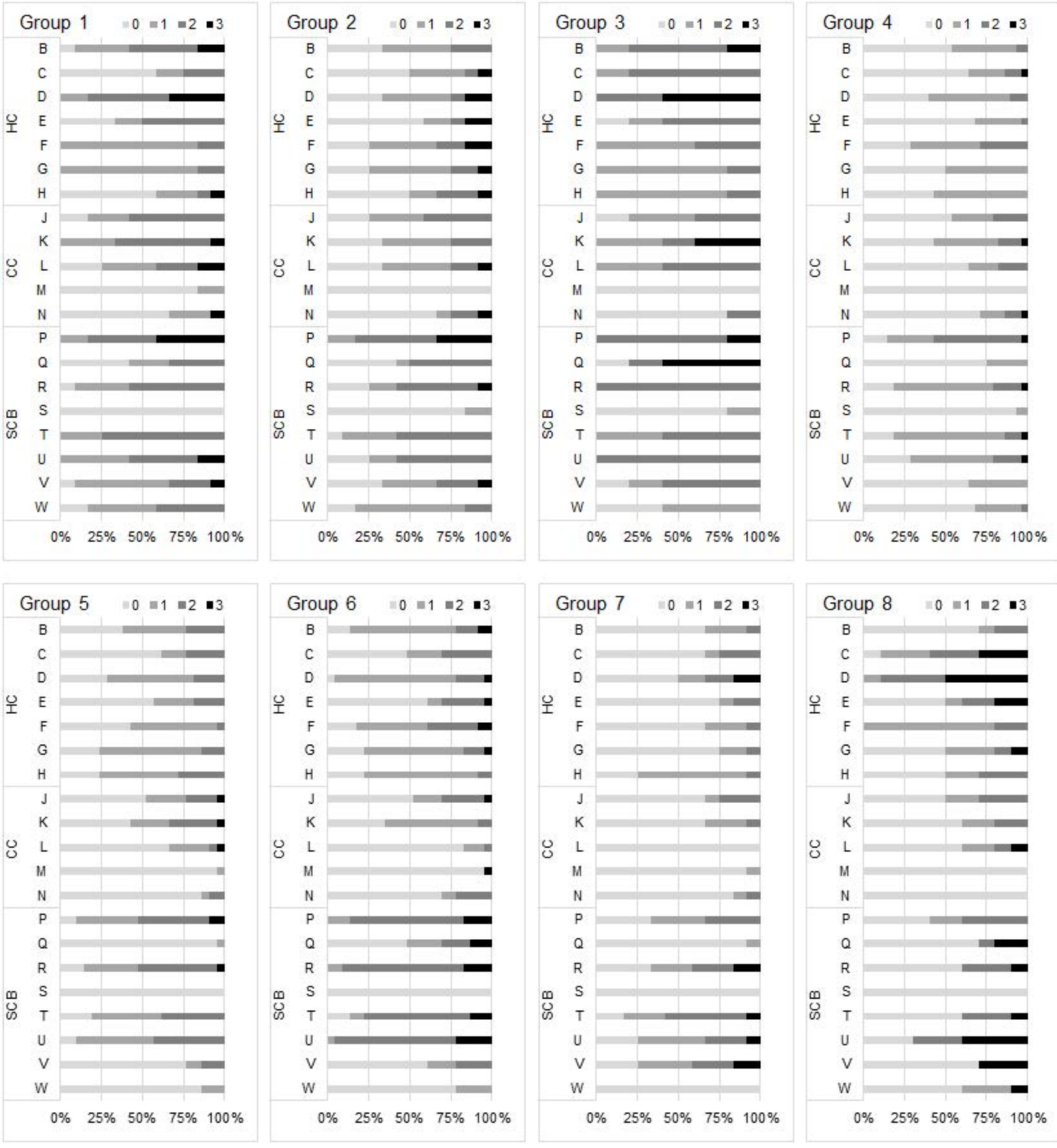
TABLE 1c. Subchondral bone grading scheme.

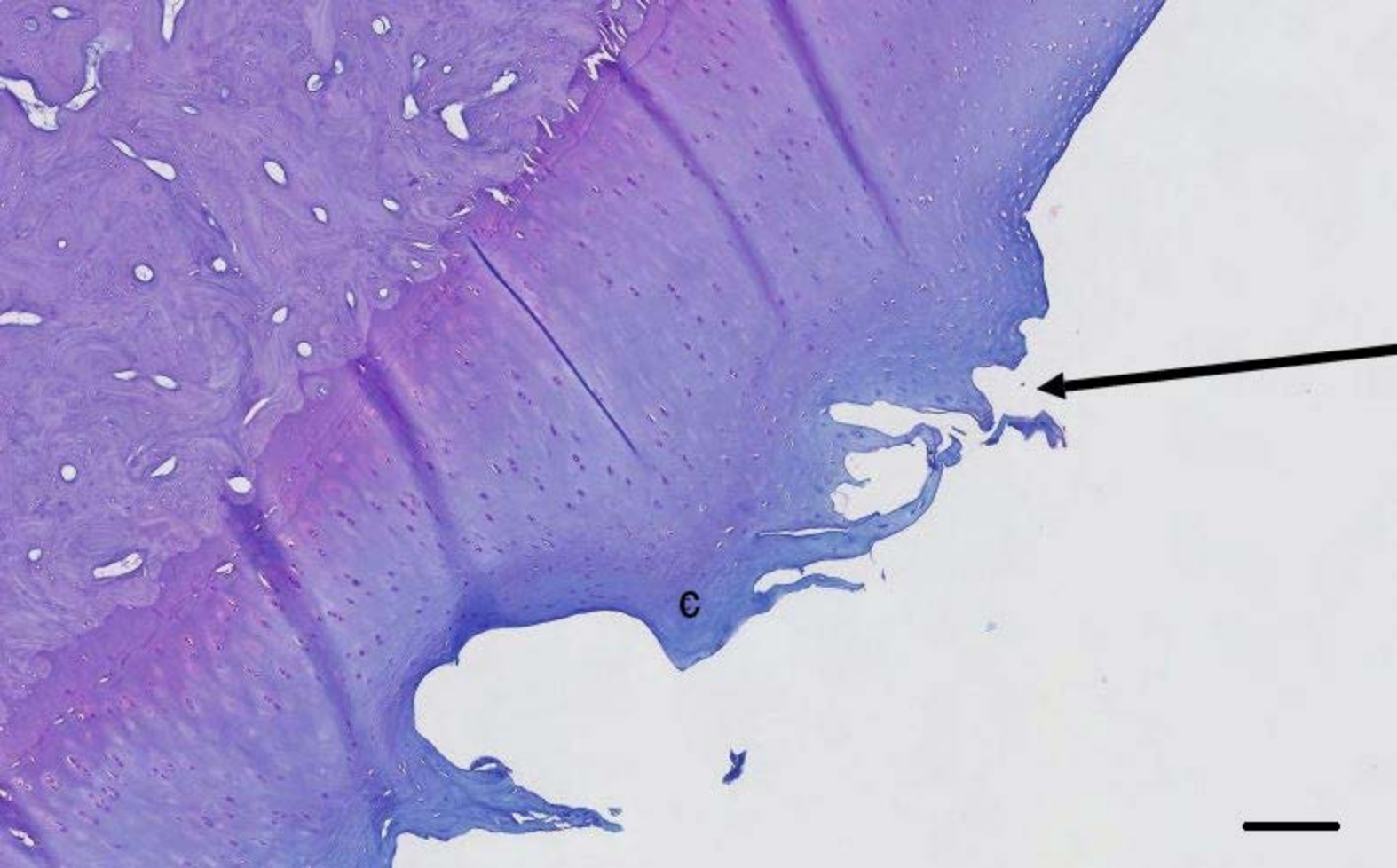
SUBCHONDRAL BONE			
P	Sclerosis of the subchondral plate and adjacent cancellous bone	0 1 2 3	No sclerosis Mild, focal Moderate, focal to more extensive sclerosis Severe sclerosis, extending into non-weight bearing areas
Q	Areas of subchondral bone collapse	0 1 2 3	No collapse Small, discrete areas of collapse Focal necrosis of the SCB plate and minor haemorrhage Collapse of the SC plate with haemorrhage/haematoidin
R	Obliteration of cancellous areas with compact bone	0 1 2 3	Expected width of the cancellous areas Focal and minimal Moderate Marked and/or in areas that bear no weight
S	Replacement with woven bone	0 1 2 3	No presence of woven bone Occasional discrete areas Moderate, Focal to more extensive replacement Marked replacement
T	Replacement with osteonal bone	0 1 2 3	No lamellar bone deposit Minimal Moderate, focal to more extensive Marked deposition of lamellar bone
U	Increase in trabecular width with reduction of marrow spaces	0 1 2 3	No thickening of trabeculae Focal and minimal Moderate thickening Marked thickening and thickening in areas that bear no weight
V	Presence of microcracks in the cancellous bone	0 1 2 3	No microcracks Localised Moderate numbers Large numbers of microcracks in cancellous bone
W	Presence of Howship's lacunae with or without osteoclasts	0 1 2 3	No lacunae Discrete and few Multifocal Numerous resorption lacunae

Table 2. Adjusted articular cartilage (AC) and subchondral bone (SCB) scores (mean ± standard error) for different lesion groups and sections.

Factor	Category	AC scores	SCB scores
Lesion group	1	12.75±2.31	11.03±1.49
	2	11.92±2.11	9.99±1.36
	3	15.43±2.93	10.32±1.88
	4	7.63±1.59	5.60±1.03
	5	8.33±1.82	6.29±1.18
	6	10.39±1.85	10.34±1.20
	7	5.85±2.19	6.58±1.41
	8	9.81±2.49	7.33±1.61
Section	Dorsopalmar lateral	10.06±1.07	8.93±0.68
	Dorsopalmar medial	9.79±1.07	7.34±0.68
	Sagittal ridge	11.88±1.06	7.56±0.67
	Lateral condyle	10.10±1.14	10.25±0.73
	Medial condyle	9.49±1.29	8.11±0.82


Medial
Lateral
A
Dorsal
Palmar
B


Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
Group 8
0 1 2 3
HC
CC
SCB
B C D E F G H
J K L M N
P Q R S T U V W
0% 25% 50% 75% 100%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