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Topographical variation in patellar subarticular calcified tissue density

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INTRODUCTION

Degenerative lesions of patellar articular cartilage are a normal finding in older subjects (Emery & Meachim, 1973), especially women (Meachim, Bentley & Baker, 1977). The initiation and evolution of the lesions, and their potential to progress to osteoarthritic bone exposure, differ in different regions of the patellar surface (Emery & Meachim, 1973). It has recently been suggested that this topographical variation in degeneration of the uncalcified articular cartilage on the patella may be related to topographical variation in the density and texture of the subarticular calcified tissue (Townsend, Rose, Radin & Raux, 1977). Subarticular density must influence the resilience of the articular cartilage base, and so affect the ability of the uncalcified cartilage to withstand damage during compressive loading (Freeman & Meachim, 1973).

Darracott & Vernon-Roberts (1971) measured bone density in six control patellae during an investigation of the bony changes in patients with chondromalacia patellae. However, their data were presented as averages for the whole patella, and did not include an analysis for possible topographical variation nor any specific analysis of the subarticular zone. In the present study, 25 left patellae from adult necropsies were used for quantitation of calcified tissue density in an immediately subarticular zone at a 'lateral' and a 'medial' sampling site. Values were obtained both for the bone and the calcified cartilage, since both contribute to the resilience of the articular cartilage base. The original 25 patellae, together with a further 60, were then used for a topographical study of the comparative frequency of macroscopically apparent articular cartilage degeneration, and bone exposure, on a 'lateral' and a 'medial' region of the patellar surface.

MATERIAL AND METHODS

Quantitation of subarticular calcified tissue density

Topographical variation in subarticular calcified tissue density was studied quantitatively by point counting photomicrographs taken at $\times 60$ from histological sections cut vertical to the articular surface. This part of the study used patellae from the left knee joint of 25 adult white European subjects (14 men; 11 women), aged 18–79 years, obtained from necropsies in Liverpool. Specimens with osteoarthritic bone exposure were excluded from this part of the study, since in such patellae some of the tissue needed for point counting had been destroyed. The specimens were fixed in formal saline, and then sawn vertical to the articular surface to give a transverse (i.e. lateral to medial) slab, 4 mm thick, in the vicinity of the transverse ridge.

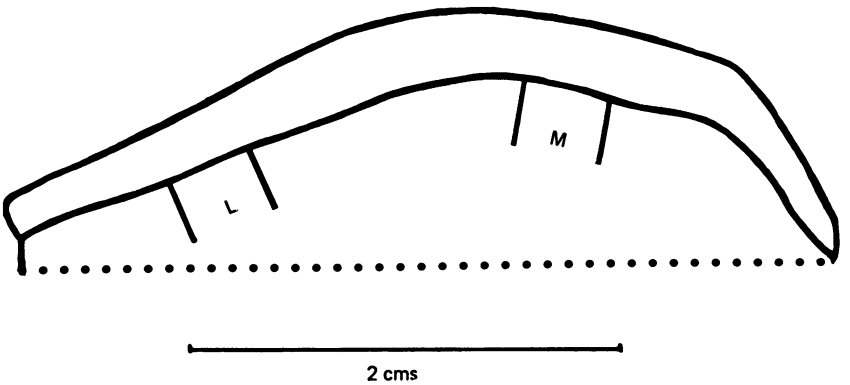

Fig. 1. Transverse slab of patella showing, in the vertical plane, the positions of the sites L ('lateral') and M ('medial') used for quantitation of subarticular calcified tissue density. It will be noted that the articular surface area at these two sites is less than that at the regions L and M used in the study of degenerative changes (Fig. 4).

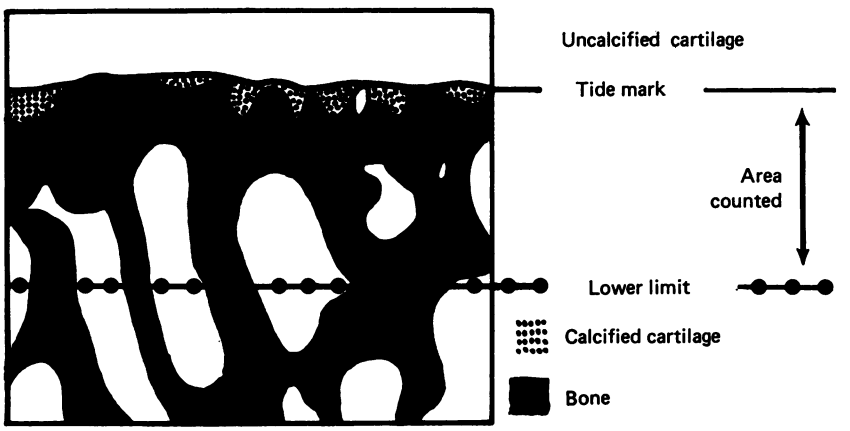

Fig. 2. Diagram of the area used for point counting the photomicrographs. The lower limit of the area counted was taken 5 cm below the tide mark position on the photomicrographic prints at $\times 60$, and thus corresponds to a vertical distance of 0·8 mm (i.e. 50/60 mm) in the actual sections. The marrow is shown in the diagram as the clear spaces between the bone trabeculae.

The slab was divided into tissue blocks which were decalcified in sulphosalicylic acid and then processed to obtain paraffin sections stained with haematoxylin and eosin.

Subarticular calcified tissue density (referring to proportion of space occupied by calcified cartilage and bone, not to amount of mineral matter) was sampled at a 'lateral' (L) and a 'medial' (M) site, situated as shown in Figure 1. In the sections each site occupied a tangential distance of 4 mm along the tide mark of the calcified–uncalcified cartilage interface (Fig. 2), and a vertical distance of 0·8 mm from the tide mark to the lower limit of the area counted (Fig. 2). Thus, in the photographic prints at $\times 60$, a total print area of 24×5 cm was available for counting. Two photomicrographs, slightly overlapping, were used for each site.

For point counting, a grid with the points arrayed at the corners of squares, 5 mm^2 in size, was superimposed over the photographic print. All the counts were made by one observer, and good agreement was obtained between counts, which were made in triplicate or duplicate for each print. The results were calculated by

first applying the following formulae separately to each print, and then taking the final value for the site as the mean of the results obtained from its two prints.

Subarticular *total calcified tissue density* was expressed, as a percentage of the area counted (Fig. 2), by the formula:

$$\left(\frac{\text{bone points} + \text{calcified cartilage points}}{\text{bone points} + \text{calcified cartilage points} + \text{marrow points}}\right) \times 100\,\%.$$

Points over vascular spaces were included with the marrow points.

Subarticular *bone density* was expressed as a percentage of the points which remained after excluding those for the calcified cartilage, by the formula:

$$\left(\frac{\text{bone points}}{\text{bone points} + \text{marrow points}}\right) \times 100\,\%.$$

The *amount of calcified cartilage* was expressed not as a percentage but as the mean number of calcified cartilage points counted beneath standard lengths of 12 cm along the tide mark (Fig. 2) in the photographic prints. This expression was selected since it reflects the actual thickness of the calcified zone cartilage.

The statistical analyses were made using Student's *t*-tests except where indicated otherwise.

Assessment for articular cartilage degeneration

The state of the uncalcified articular cartilage was observed in the 25 patellae used for the subarticular density study, and in a further 60 adult left patellae obtained from necropsies. Specimens with osteoarthritic bone exposure were included in this part of the study. The 85 subjects (45 men; 40 women) were aged from 18 to 96 years. The state of the patellar articular surface was assessed by one or more of the following methods: macroscopic inspection of the patella *en face* and of the transverse slab as seen in the vertical plane; India ink staining of the patella *en face* (Meachim, 1972) before fixation and sawing of the specimen; histology of vertically cut paraffin sections. The terminology used to describe the histological appearance of the cartilage surface has been given previously (Meachim, 1972; Emery & Meachim, 1973). A note was made if a cartilage lesion was macroscopically obvious *en face* before India ink staining. The distribution of macroscopically apparent cartilage lesions and areas of bone exposure was mapped on a drawing of the patella *en face*, and also observed in photographs at $\times 3$ of a transverse slab as seen in the vertical plane. The findings, together with those from histology, and from India ink staining when pertinent, were analysed for each of the patellar articular surface regions L, C, M and D (see Fig. 4). Articular regions L ('lateral') and M ('medial') each occupied a larger patellar *en face* surface area than that immediately over their corresponding subarticular sampling sites.

RESULTS

Topographical variation in subarticular density

The values from the 25 adult left patellae used for subarticular quantitation by point counting indicated that total calcified tissue density (Table 1) was significantly greater at the 'lateral' (L in Fig. 1) than at the 'medial' (M) sampling sites. A topographical difference in terms of bone density, after excluding the points for calcified cartilage (Fig. 2), was also statistically significant (Table 2). Comparison of the

Table 1. *Subarticular total calcified tissue density sampled at sites L ('lateral') and M ('medial') as shown in Fig. 1*

Results from all the 25 patellae used for the density study.

$$\left(\frac{\text{Bone points} + \text{calcified cartilage points}}{\text{Bone points} + \text{calcified cartilage points} + \text{marrow points}}\right) \text{ as \%.}$$

Sex	Number of patellae	'Lateral' site Mean	'Lateral' site Standard error	'Lateral' site Range	'Medial' site Mean	'Medial' site Standard error	'Medial' site Range	'Lateral'/'medial' comparison
Men	14	79%	2·3	68–91%	63%	2·3	40–72%	$P < 0{\cdot}001$
Women	11	82%	2·1	70–92%	63%	2·1	53–73%	$P < 0{\cdot}001$
Men/women comparison			$P > 0{\cdot}1$			$P > 0{\cdot}1$		

Table 2. *Subarticular bone density*

Results from all the 25 patellae used for the density study.

$$\left(\frac{\text{Bone points}}{\text{Bone points} + \text{marrow points}}\right) \text{ as \%.}$$

Sex	Number of patellae	'Lateral' site Mean	'Lateral' site Standard error	'Medial' site Mean	'Medial' site Standard error	'Lateral'/'medial' comparison
Men	14	75%	2·7	58%	2·7	$P < 0{\cdot}001$
Women	11	78%	2·1	57%	2·6	$P < 0{\cdot}001$

Table 3. *Amount of calcified cartilage*

Results from all the 25 patellae used for the density study. Mean number of calcified cartilage points as total points beneath standard lengths of tide mark.

Sex	Number of patellae	'Lateral' site Mean	'Lateral' site Standard error	'Medial' site Mean	'Medial' site Standard error	'Lateral/'medial' comparison
Men	14	41	2·9	26	2·1	$P < 0{\cdot}001$
Women	11	41	4·7	32	4·2	$P > 0{\cdot}1$

amount (i.e. thickness) of calcified cartilage at the two sites (Table 3) showed a difference which was statistically significant in the men, although not in the women. For both sites the mean values for total density (Table 1), bone density (Table 2) and calcified cartilage (Table 3) were similar in the men and women.

Inspection of subarticular bone texture, as seen in slab radiographs (Fig. 3) and photomicrographs, showed that there was often a qualitative difference between the two sampling sites. At the 'medial' site the subarticular trabeculae often had a predominantly vertical orientation with respect to the articular surface, whereas at the 'lateral' site there was often a more even mixture of vertical and tangential trabeculae, giving a less striated but more radiodense appearance at this site (Fig. 3). This difference was already apparent before the age of 20 years (Fig. 3).

In eight of the 25 patellae used for subarticular quantitation the surface of the

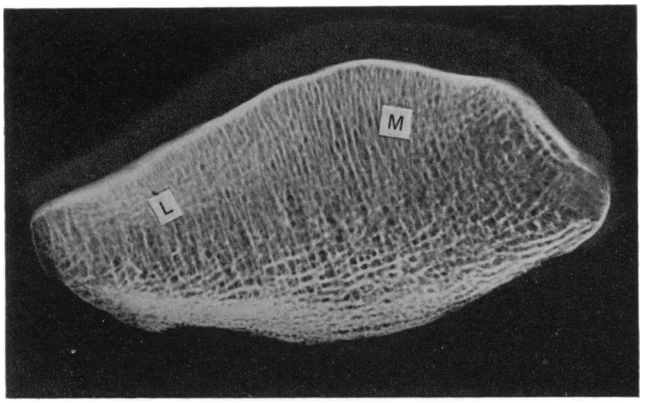

Fig. 3. Transverse slab radiograph of patella from a woman aged 18 years. Compare the subarticular bone architecture at the lateral site (above L) with that at the medial sampling site (above M).

Table 4. *Subarticular total calcified tissue density*

Results from eight patellae in which the uncalcified cartilage at L, C and M (Fig. 4) in the transverse slab was all histologically intact or showed only minimal change.

$$\left(\frac{\text{Bone points} + \text{calcified points}}{\text{Bone points} + \text{calcified cartilage points} + \text{marrow points}}\right) \text{ as } \%.$$

	Number of patellae	'Lateral' site			'Medial' site			'Lateral'/'medial' comparison
		Mean	Standard error	Range	Mean	Standard error	Range	
Both sexes	8	77%	3·0	70–91%	62%	2·8	51–72%	$P < 0{\cdot}01$

uncalcified articular cartilage on regions L, C and M (Fig. 4) was histologically intact or else showed only minimal changes. Statistical analysis of the values from these eight patellae, considered alone, again showed that subarticular total calcified tissue density (Table 4) was significantly greater at the 'lateral' as compared to the 'medial' sampling sites; moreover, the mean values were similar to those for all 25 specimens (Table 1).

From all the specimens used for subarticular quantitation the values for the 25 subarticular 'lateral' sites were analysed according to the state of the uncalcified articular cartilage on region L of the patellar surface (Fig. 4), while the values for the 25 subarticular 'medial' sites were analysed according to the state of the articular cartilage on regions C and M (Fig. 4) considered together as one segment. There were no statistically significant differences when the subarticular density values were compared for differing states of the articular cartilage (Table 5). It must, however, be emphasised that this analysis does not discount the possibility of significant changes in subarticular values in specimens with osteoarthritic bone exposure, since, as previous explained, such specimens were excluded from the 25 used for subarticular point counting.

Comparison of the subarticular density values from eight subjects aged from 18 to 39 years, with those from 17 subjects aged from 45 to 79 years, showed no significant differences between the younger and older group (Table 6). Again it should be noted that in this part of the study the older patellae did not include any specimens with osteoarthritic bone exposure.

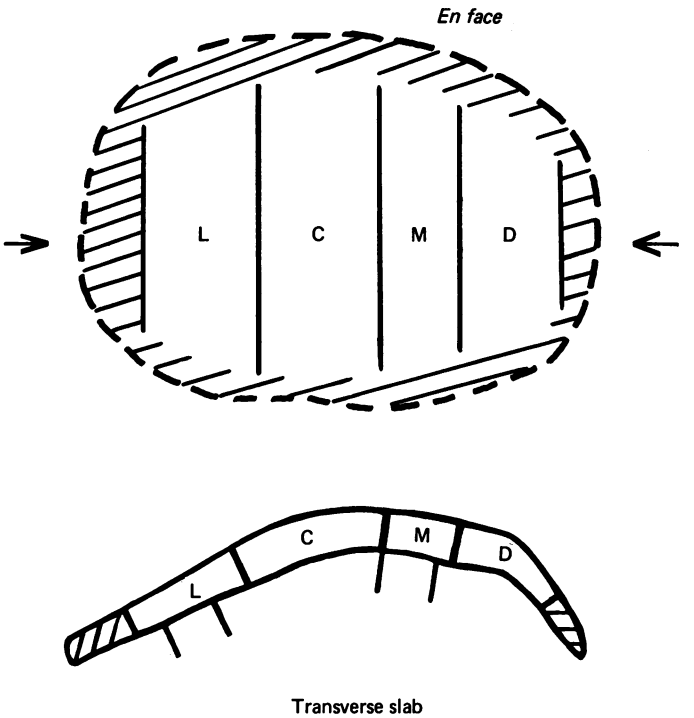

Fig. 4. Positions, as seen *en face* and in vertical section, of the divisions into patellar articular surface regions (L, C, M, D) used in the study of degeneration of uncalcified cartilage and of bone exposure; the lateral (left) and medial (right) peripheries (cross-hatched) were excluded from the study. Articular regions L ('lateral') and M ('medial') each occupied a larger patellar *en face* surface area than that immediately over their corresponding subarticular sampling sites L and M (Fig. 1).

Table 5. *Subarticular bone density (as %) and amount of calcified cartilage (as total points beneath a standard length of tide mark)*

Results for the 25 subarticular 'lateral' sites analysed according to the state of the uncalcified cartilage at L in Fig. 4; results for the 25 subarticular 'medial' sites analysed according to the state of the uncalcified cartilage at C and M considered together as one segment. It should be noted that specimens with osteoarthritic bone exposure could not be included in the density study; with their exclusion from it, no statistically significant differences were found (all P values $> 0{\cdot}1$ or $> 0{\cdot}05$).

State of uncalcified cartilage at 'L' or at 'C plus M' (Fig. 4)	'Lateral' subarticular site			'Medial' subarticular site		
	Number of sites	Bone as % (mean value)	Calcified cartilage as points (mean value)	Number of sites	Bone as % (mean value)	Calcified cartilage as points (mean value)
Histologically intact or only minimal changes	12	76	39	9	58	26
Histologically overt fibrillation, not macroscopically apparent	11	77	44	9	61	27
Macroscopically apparent lesion, without bone exposure	2	82	41	7	53	35

Table 6. *Comparison of eight patellae from subjects aged* 18–39 *years with* 17 *from subjects aged* 45–79 *years*

Subarticular total calcified tissue density calculated as shown in Table 1, subarticular bone density as in Table 2, and amount of calcified cartilage as in Table 3.

		'Lateral' site			'Medial' site		
Age range (years)	Number of patellae	Total density as % (mean value)	Bone density as % (mean value)	Calcified cartilage as points (mean value)	Total density as % (mean value)	Bone density as % (mean value)	Calcified cartilage as points (mean value)
18–39	8	80	75	45	64	59	30
45–79	17	82	77	39	62	57	28
Young/old comparison		$P > 0{\cdot}1$	$P > 0{\cdot}1$	$P > 0{\cdot}1$	$P > 0{\cdot}1$	$P > 0{\cdot}1$	$P > 0{\cdot}1$

Topographical variation in articular cartilage degeneration

In 85 left patellae (the 25 used for subarticular quantitation, and a further 60) from subjects aged from 18 to 96 years, articular cartilage degeneration of a degree macroscopically apparent *en face* on the unstained surface was present, with or without bone exposure, on articular region L (Fig. 4) in 26 instances, and on articular region M (Fig. 4) in 31 instances. However, 10 of the 26 lesions on surface region L ('lateral') had progressed to bone exposure, as compared with bone exposure in only four of the 31 lesions on surface region M ('medial'). This difference between the 'lateral' (L) and 'medial' (M) regions in progression to bone exposure was statistically significant on Fisher's Exact Test ($P = 0{\cdot}034$).

DISCUSSION

The difference in subarticular calcified tissue density found between the 'lateral' and the less dense 'medial' patellar sampling sites (Fig. 1) cannot be attributed to osteoarthritic bone exposure, since specimens with bone exposure were excluded from the subarticular quantitation part of the study. Likewise, the topographical variation in subarticular density cannot be attributable to degenerative changes in the overlying uncalcified articular cartilage, since differences between the 'lateral' and 'medial' sites were found whatever the state of the articular surface (Tables 4, 5). These comments in no way discount the possibility that degenerative changes in the uncalcified cartilage, and osteoarthritic bone exposure, may in themselves, when present, lead to changes in calcified cartilage thickness and in bone density. For example, a subarticular pathological osteosclerosis, often interspersed with osteolytic foci, occurs on the zenith of osteoarthritic femoral heads (Meachim, Hardinge & Williams, 1972).

It seems unlikely that the topographical variation found in subarticular density is due primarily to a differing frequency of trabecular microfractures in the subarticular bone at the two sites sampled. Such fractures could, in theory, lead to a change in bone density as a result of progressive accumulation of microcallus foci with increasing age. In the present study no attempt was made to examine the trabeculae for microfractures and microcallus. However, the topographical difference in bone density was found to an equal degree in younger and in older subjects, and

ageing had no significant effect on the values obtained (Table 6). Moreover, the quantitative difference between the two sites was often accompanied by a qualitative difference in subarticular bone texture, and this difference in architecture was already apparent in young adults (Fig. 3). For these reasons, and those given in the preceding paragraph, it is concluded that there is most probably an inherent difference in subarticular calcified tissue density at the two sampling sites (though specimens from children would be needed to establish this with certainty). This conclusion does not discount the possibility that an accumulation of microfractures during ageing or in osteoarthritis might also affect the subarticular density.

Macroscopically apparent degenerative lesions of the uncalcified articular cartilage, with or without bone exposure, were noted with similar frequency in a surface region (L) topographically related to the 'lateral' subarticular site when compared to a surface region (M) related to the less dense 'medial' subarticular site (Fig. 4). Thus the difference in bone density did not appear to be an important factor in the initiation of macroscopically apparent cartilage degeneration. However, cartilage lesions on the 'lateral' region had, once present, a significantly greater ($P = 0{\cdot}034$) potential to progress to bone exposure. At least two hypotheses can be suggested to account for this difference, namely:

(1) Degenerate cartilage on the 'lateral' region will have a more dense, and hence less resilient, calcified base, and this might make it more susceptible to the progression of wear from mechanical damage during compressive loading;

(2) The higher density of the subarticular base, and the higher susceptibility to progressive cartilage wear on the 'lateral' region, might both be attributable, independently, to features in the mechanical environment of that part of the patella (e.g. consequences of angulation of the femur on the tibia, different in men and women) and not directly related one to the other as cause and effect.

The present observation that cartilage lesions on the 'lateral' and 'medial' regions (Fig. 4) differ in their susceptibility to the progression of wear may seem in conflict with a previous observation (Meachim *et al.* 1977) that, during patellar wear, articular cartilage thinning on the 'lateral' region correlates with thinning on the 'central plus medial' segment. The explanation is that degeneration on the 'central plus medial' segment recorded in the previous study affects the 'central' much more than the 'medial' region of the present study.

Bone exposure from progressive thinning of patellar articular cartilage is more common in women than in men (Meachim *et al.* 1977). The present findings suggest that this difference cannot be explained in terms of a sex difference in the subarticular calcified tissue density (Tables 1, 2, 3).

SUMMARY

Topographical variation in subarticular calcified tissue density has been studied by point counting photomicrographs from transverse slabs from a random series of 25 human left patellae from necropsies. A 'lateral' site and a 'medial' site were selected for counting, each corresponding to an area 4 mm across and 0·8 mm in depth in the vertically cut histological sections. The density of subarticular bone plus calcified cartilage was similar in men and women, but for both sexes was significantly greater at the 'lateral' compared to the 'medial' site. A topographical difference was still apparent when the calcified cartilage was excluded from the analysis. It was found to be an inherent difference between the two sites, and not attributable to the

effects on subarticular bone or calcified cartilage which may occur from (*a*) degeneration of the overlying uncalcified cartilage, (*b*) trabecular microfractures, (*c*) pathological osteosclerosis in osteoarthritis. Patellae with osteoarthritic bone exposure were excluded from this part of the study.

The frequency of degenerative change in the uncalcified articular cartilage was then compared on a 'lateral' and a 'medial' region of the patella; these regions each represented a larger patellar *en face* surface than did the subarticular sampling sites. For this part of the study, 85 left patellae (the original 25 plus a further 60) were used, and specimens with osteoarthritic bone exposure were included. Macroscopically apparent degeneration of the articular cartilage, with or without bone exposure, had a similar frequency in the two regions, but degeneration affecting the 'lateral' region had a significantly greater potential to progress to bone exposure. This topographical variation is discussed in relation to that found in subarticular calcified tissue density.

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