Technique for identifying cutting artefacts in sections of undecalcified bone biopsy specimens


Abstract
Areas of fragmentation found in trabecular and cortical bone of iliac crest biopsy specimens have been described as bone quality defects and were thought to be the major factor responsible for femoral neck fractures. These appearances have also been regarded as cutting artefacts and to resolve this difference of opinion consecutive sections cut at right angles to each other in biopsy specimens from 15 patients with femoral neck fractures were compared. Sections were assessed by four independent observers; agreement by at least three was required before an area was accepted as a bone quality defect. In all, 270 were identified. Of the 161 found in sections cut parallel to the cortices, there were only 20 (12%) in coincident areas in consecutive sections. This study shows conclusively that areas of fragmentation previously described as bone quality defects are not artefacts which may be created or excluded depending on the plane of section.

Bone quality defects have been described as areas of fragmentation found in trabecular and cortical bone of iliac crest biopsy specimens from patients with femoral neck fractures (fig 1). It was concluded that they may be the major factor responsible for femoral neck fractures in the population aged over 60 years. These areas of fragmentation are not uncommon, and in our laboratory were always considered to be cutting artefacts. To resolve this difference of opinion, we compared consecutive sections cut at right angles to each other.

Methods
Iliac crest biopsy specimens were taken from 15 elderly patients undergoing surgery for subcapital femoral fracture. Standard 7 mm diameter biopsy specimens, including superficial and deep cortices and intervening trabecular bone, were harvested from a site 5 cm posterior and 5 cm inferior to the anterior superior iliac spine.

A modified block holder was used to hold the biopsy specimen level and was rotated through 90 degrees between sections. This modification of the standard Jung K orientating block holder has four equidistant stabilisers set in the clamp resting on the circular base to ensure that the block face remains orientated in the same plane throughout rotation.

Specimens were fixed in absolute alcohol, dehydrated in Cellosolve, and embedded in the plastic resin Polymaster 1209AC. Undecalcified sections were cut at 6 μm and the modified block holder was rotated between sections. Two sections from each biopsy specimen, cut at right angles to each other, were prepared for analysis. Consecutive sections were also cut without rotating the block, but the microtome blade was moved transversely by 1 mm between sections. Trabecular bone volume, osteoid volume, osteoid surface and osteoid index were determined, but did not differ from previously reported reference values. Black and white photographs (magnification × 100) were used as a tally sheet to record the site of bone quality defects seen on projected colour transparencies. Four independent observers, familiar with previously published work on bone quality defects, mapped out the sites, but agreement by at least three of the observers was required before an area was accepted as a bone quality defect. Photographs of consecutive sections were compared for coincident bone quality defects using a transparent grid overlay.

Of 161 bone quality defects (mean 10.6/sec tion) observed in sections cut parallel to the cortices, only 20 (12.4%) were coincident in sections cut at right angles to the cortices (p < 0.001, Mann Whitney U test). Bone quality defects were frequently seen to disappear completely in consecutive sections in both cortical and trabecular bone (fig 2). A total of 270 were found, 161 in sections cut parallel to the
Identification of cutting artefacts in undecalcified bone

cortices and 109 in those cut perpendicular to the cortices. This difference was not significant and cannot be used as a guide to the optimal direction of section.

The use of this modified block holder has meant that previously described bone quality defects have been identified as cutting artefacts which may be created or excluded depending on the plane of section. These areas of fragmentation are not reliable indicators of the quality of underlying bone.

Figure 2  Bone quality defect in trabecular bone (A) is not seen in the consecutive section (B) cut at right angles to (A).

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**Register of primary immune deficiencies**

In line with other European countries, a Register of all patients in the United Kingdom with primary immune deficiencies is being compiled. This is being organised by Dr J Gooi (Immunology Department, Blood Transfusion Service, Bridge Path, Leeds LS13 7TF).

To gain complete coverage we should be grateful if any physicians or general practitioners, who have not already been contacted and who are currently managing such patients, could send details of their patients to Dr Gooi.

Registration forms are available from Dr J Gooi (0532 645091) or Dr H Chapel (0865 817305, Immunology Department, John Radcliffe Hospital, Oxford OX1 9DU).

**Corrections**

We regret that an error appeared in the Matters Arising "Screening for bacteriuria" 1989:42:557. The microscopy and culture method was described as costing £0.90; it should have read £0.09. Our apologies.

Apologies are extended to Dr G Markey for having changed the gender of the patient reported in his paper 1990;43:282 "Monocyte esterase deficiency in malignant neoplasia". The patient was female.

An error appeared in the last sentence of the paper by Wood et al "Technique for identifying cutting artefacts in sections of undecalcified bone biopsy specimens" 1990;43:516. This study shows conclusively that areas of fragmentation previously described as bone quality defects are not artefacts which may be created or excluded depending on the plane of the section. They, of course, are artefacts.