Variation in histomorphometric estimates across different sites of the iliac crest

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SUMMARY Bone specimens were obtained from four different sites in the ilium of necropsy cases using a Jamshidi 8G trephine needle. Undecalcified histological sections were prepared and two structural histomorphometric parameters were estimated. Estimates of the percentage of trabecular bone volume (%TBV) deviated systematically, with variation of the biopsy site around the usual location, increasing in magnitude from the anterior to the posterior ilium. Such differences highlight the importance of understanding the degree of variation which exists in histomorphometric data. The histomorphometry of a vertical biopsy specimen of the iliac crest did not differ significantly from that of a transiliac biopsy specimen of similar diameter taken from an adjacent site.

When taken at the standard site, and when providing a long core of cancellous bone, with minimal discomfort to the patient, the vertical biopsy specimen compares well with the transiliac biopsy specimen for use for investigating metabolic bone disorders.

There is little doubt about the value of the iliac crest biopsy specimen for the investigation of metabolic bone disorders. The results of studies on various methods of biopsy have been well reported, but there is still some confusion about the importance of the histomorphometric variation seen in these results.

A general feature of bone which may impede the interpretation of such data is the structural heterogeneity occurring at different anatomical locations. This “site variation” is particularly prominent in the ilium and complicates the comparison of different biopsy specimens. It makes such a difference that one region of the ilium (2 cm below the iliac crest and 2 cm behind the anterior aspect) has become the preferred site for biopsy (subsequently referred to as the usual biopsy site).

Because it is unrealistic to expect that all patients will always be biopsied from the same location, it is essential to know the amount of variation that exists around the iliac crest biopsy site. In this way comparisons of morphometric data from one patient’s biopsy specimen can be compared with either an established range of reference values or with the data from a previous biopsy specimen. Equally important is an understanding of morphometric differences between these conventional transiliial (horizontal) biopsy specimens and the vertical biopsy specimens which are preferred by some clinicians.

Material and methods

Bone samples were obtained from 35 necropsy subjects with no evidence of malignancy or other condition likely to affect the bone. In 14 cases two cores were taken in the transiliial plane, slightly anterior to the usual biopsy site. Two further cores were taken an equal distance posterior to this site. They were separated by no more than 3 mm and were labelled 1, 2, 3 and 4 from anterior to posterior. In the remaining subjects cores of bone were taken horizontally from the usual biopsy site, and vertically from the anterior superior iliac spine, immediately adjacent to the horizontal sample.

All samples were taken by one operator (RM) using a Jamshidi 8G trephine instrument (American Pharmaseal Laboratories, Glendale, USA). They were fixed in 10% neutral buffered formalin for up to 24 hours and processed into Araldite resin (Ciba-Geigy, Australia), without decalcification.

Three sections, 7 μm thick, were cut using a Jung K motorised sledge microtome (Reichert Jung, Heidelberg, West Germany), and stained with a combined von Kossa/van Gieson stain after removal of the resin. Estimates of two histomorphometric parameters (per-
Percentage of trabecular bone volume (%TBV) and surface density of bone (MS/UV) were obtained by the Quantimet 720 Image Analysing Computer (Cambridge Instruments, Cambridge, UK), linked to a Hewlett Packard HP9000 Series 200 microcomputer (Hewlett Packard, Fort Collins, USA).

**DEFINITION OF VARIABLES**
The following parameters were defined: %TBV—that proportion of the total bone specimen volume (including marrow space) which is occupied by trabecular bone (mineralised or osteoid); MS/UV (mm²/mm³), the surface area of trabeculae (mm²) per unit volume of whole bone tissue (mm³), including bone and marrow.

Specimen lengths were compared using Student’s paired t test, with significance set at the 5% level. All other comparisons were made using a mixed model (Type 111) two way analysis of variance for a two factor experiment with significance set at the 5% level.

**Results**
There were 21 subjects in the “horizontal v vertical” study (14 men and seven women), with a mean age of 59.0 (range 27–81) years. The vertical cores were almost twice as long as the horizontal cores (19.9 (SD 7.2) mm v 10.1 (3.5) mm, p < 0.05). In the other part of the study which compared bone samples from four sites along the iliac crest, there were 14 subjects (eight men and six women), with a mean age of 53.1 (range 19–88) years. The cores from these cases were not significantly different in length.

**HISTOMORPHOMETRY**
The histomorphometric estimates of %TBV and MS/UV from the bone samples are shown in the table. The results of a two way analysis of variance performed on the data comparing horizontal and vertical biopsy specimens, show that for both parameters, there was significant variation due to “cases” and also due to “interaction mechanisms” (p < 0.05). The method of sampling did not contribute significantly to the variance in estimates of either parameter.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(n =)</th>
<th>% TBV Mean* (SD)</th>
<th>MS/UV Mean* (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal</td>
<td>21</td>
<td>17.5 (5.7)</td>
<td>2.09 (0.77)</td>
</tr>
<tr>
<td>Vertical</td>
<td>21</td>
<td>17.4 (7.1)</td>
<td>2.50 (1.13)</td>
</tr>
<tr>
<td>Site 1</td>
<td>14</td>
<td>21.6 (9.0)</td>
<td>2.22 (0.69)</td>
</tr>
<tr>
<td>Site 2</td>
<td>14</td>
<td>19.4 (7.2)</td>
<td>2.47 (1.35)</td>
</tr>
<tr>
<td>Site 3</td>
<td>14</td>
<td>14.1 (5.7)</td>
<td>1.84 (0.67)</td>
</tr>
<tr>
<td>Site 4</td>
<td>14</td>
<td>13.5 (4.6)</td>
<td>1.85 (0.54)</td>
</tr>
</tbody>
</table>

*The mean case value is the mean of the estimates from replicate slides in each subject.

The results of a two way analysis of variance comparing the data from different sites around the iliac crest show that there was significant variation due to “site” and “interaction mechanisms” for the estimation of %TBV (p < 0.05), while the estimation of MS/UV was not significantly affected. There was also significant biological variation between cases in the estimation of both variables (p < 0.05).

**Discussion**
Quantitative histomorphometry of bone biopsy specimens is increasingly being used for the detection and management of metabolic bone disorders. The objective nature of the methods and the potential for standardisation of many parameters make this type of investigation reasonably acceptable, but there still remains some uncertainty with respect to some aspects of the procedure. One of these aspects is biopsy size, and we have dealt with this in a previous publication.

The site variation which is observed within even a small confined region of the iliac crest is of clinical and pathological importance. Because it is unreasonable to expect that all patients will be sampled from one specific location each time they present for investigation, it is essential to know the variance in histomorphometric parameters around the “ideal” biopsy site. Only then is it possible to interpret confidently any histoquantitative data obtained from a given biopsy specimen.

Estimates of %TBV were observed to decrease significantly and systematically towards the postero-superior region of the ilium. The estimation of MS/UV, however, was unaffected by the same variation in sampling site. A similar decrease in %TBV in the posterior aspect of the ilium has previously been reported, though another report indicated that neither estimates of %TBV nor MS/UV fluctuated significantly with variation in anterior-posterior site. It is thought that bone remodels constantly in an area measuring about 5 cm diameter around the usual biopsy site, suggesting that if the biopsy specimens are taken relatively close to each other, morphological differences will probably not be significant. Indeed, the closest of the bone samples in this study, separated by no more than 3 mm, showed almost identical morphological estimates, while those separated by a greater distance showed strikingly different results when compared pairwise. It is clear that the precise aim of the needle which was obtained in this necropsy study is not achievable in the clinical situation, and this alone confirms the importance of the findings.

There is little doubt that the vertical method of sampling has the potential to produce longer cores, and indeed this was observed in our study. This is because the transilial sample is limited in length by the
width of the ilium, while the length of the vertical core is limited only by the length of the sampling needle. In some cases the ilium below the iliac crest is extremely thin, and it is almost impossible to take either a vertical core or a horizontal sample if the necropsy sample is taken too low. It was found in this study, however, that cores produced by both methods were equally suitable for histomorphometric analysis, with the only apparent difference being that the vertical cores were twice as long as the horizontal cores.

A prominent feature of the vertically oriented core is the heterogeneous arrangement of the trabeculae in the ilium. Immediately inferior to the cortical bone at the iliac crest is a transitional region of bone showing widely varying architecture which is neither purely cortical nor cancellous in appearance. Beneath this is the truly cancellous zone. It has been shown that these different regions vary with respect to their cancellous bone density, and there are problems associated with comparing horizontal biopsy specimens from these levels of the ilium. Similarly, significant spatial variability has been observed in the trabeculae immediately below the iliac crest.

Estimates of %TBV and MS/UV in this study were not significantly different when horizontal and vertical specimens from the same ilia were compared, suggesting that the direction in which the samples are taken has no significant effect on these parameters. This is similar to findings reported elsewhere, but in contrast to one investigation in which estimates of %TBV from the vertical biopsy specimen were found to be about 2.5% higher than the horizontal biopsy specimen. This may have been due to the inclusion of some paracortical trabecular structures in the analysis of the vertical biopsy specimens. It is thought that the inconsistently variable pattern of bone structure in this zone which has been included may account for the marginally higher standard deviation seen in the estimates of the vertical cores. Uncertainty in determining precisely the point at which cortical and cancellous bone are distinguished may lead to significant discrepancies in histomorphometric results, particularly with respect to vertically orientated biopsy specimens.

Further study of histomorphometric variation between different vertical sites along the iliac crest, based on the translivial study in this investigation with a comparison of paracortical and deeper trabecular architecture, is a possibility for the future.

The potential for errors resulting from inconsistent sampling methods, serves as a reminder that biopsy methods and quantitative procedures must be universally standardised to make valid comparisons of morphometric data and there is already a growing trend towards this goal.

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References


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