Bone marrow cellularity assessed by point-counting

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SUMMARY A simple method for rapid point-counting of bone marrow biopsies is described; the method gave an accurate assessment of cellularity in 100 aspiration biopsy specimens. Measurements of marrow cellularity by the visual point-counting technique correlated well with those obtained with a Quantimet 720 image-analysing computer.

The assessment of bone marrow cellularity is essential in the diagnosis of marrow hypoplasia or aplasia (de Gruchy, 1970) and also in the differentiation of polycythaemia vera from secondary erythrocytosis (Kurnick *et al.*, 1972).

Evaluation of the cellularity of biopsies is usually based on the microscopist's experience rather than on objective criteria. Morley and Blake (1975) compared direct visual assessment of marrow cellularity in the mouse with the nucleated cell count in suspensions of whole marrow and found that the former method failed to distinguish between many normal and abnormal marrows. Nearly all cases of mild or moderate hypocellularity were missed, and, in addition, the degree of reproducibility of visual microscopy was low.

Point-counting has been shown to be a very accurate method of assessing the relative proportions of different tissue components (Dunnill, 1968). The application of point-counting to aspiration marrow biopsies presents particular problems. The specimen is fragmented, and the cellularity of the fragments is not randomly distributed because of their different rates of sedimentation during fixation and processing. The primary purpose of this paper is to describe a simple point-counting procedure that permits accurate assessment of bone marrow cellularity. The results are correlated with those obtained with a Quantimet 720 image-analysing computer.

Material and methods

One hundred unselected bone marrow biopsies were retrieved from the files of the Department of Pathology, Ninewells Hospital and Medical School.

These specimens had been fixed and processed to paraffin in a standard way and embedded in Peel-A-Way embedding moulds (Peel-A-Way Scientific, California, USA; size S22). These result in paraffin blocks that, on sectioning, have a cut surface area of approximately 20 × 20 mm. From each block sections were cut at a microtome setting of 7 µm, and in three cases sections 200 µm apart were obtained from the entire block. The sections were stained with Mayer's haemalum and eosin and mounted in synthetic resin.

An area of graph paper 40 × 40 mm scaled in 10, 5, and 1 mm was photographed and reduced to produce a negative 20 × 20 mm scaled in 5, 2.5, and 0.5 mm: this was then contact-printed onto Kodak fine grain positive film to produce photographic grids that could be superimposed on the marrow preparations.

A graticule (10 × 10 mm) with 121 intersection points was fitted in a ×6.3 focussing eyepiece; the four corner intersections were ignored and the remaining 117 were used for point-counting. With a ×40 objective, the image in the eyepiece graticule was most of the content of the smallest (0.5 mm) square in the photographic grid.

POINT-COUNTING METHOD

The photographic grid was placed over the marrow preparation and positioned so that the quadrants labelled A, B, C, D (Fig. 1) each contained approximately the same amount of marrow; the grid was then secured to the slide with narrow strips of adhesive tape.

Point counts were made in each quadrant A, B, C, and D. Within each quadrant the method of selecting fields for point-counting depended on the total area of the marrow sample. If the sample was large, fields nearest the centre of the 5 mm squares labelled I, II, III, IV were chosen; in a small sample,

Fig. 1 Photographic grid, 20 × 20 mm, scaled in 5, 2.5, and 0.5 mm. Square I in quadrants B, C, and D is shown by the dotted lines.

where the marrow was confined to the innermost 5 mm square labelled I in each quadrant, fields nearest to the centre of the 2.5 mm squares labelled 1, 2, 3, 4 were chosen.

A minimum total of 600 points were counted. The assessment was based on an equal number of fields from each quadrant or all of the marrow specimen. The number of fields within which counts were made varied, depending on the degree of fragmentation of the marrow sample, but ordinarily did not exceed four in each quadrant A, B, C, D. For each field, the number of points lying on cell nuclei, cytoplasm, erythrocytes, and fat spaces were separately recorded using multiple tally counters. Erythrocytes at the periphery of marrow fragments or in large clefts within them were excluded because it was considered that they were derived from peripheral blood. The counts on marrow tissue for each field were summed, and the totals for cell nuclei, cytoplasm, erythrocytes, and fat were expressed as percentages.

MEASUREMENTS WITH THE QUANTIMET 720

The Quantimet 720 image analysing computer system used in this study comprised a Leitz Orthoplan microscope with Auto Focus; a Plumbicon Image Scanner with Auto Shade Corrector; a Variable Frame; a 1D Auto Detector and two 2D Amenders; an MS3 Standard Computer; a Classifier-Collector; a Calculator Field-Feature Interface; and a Hewlett-Packard 9810A Calculator. All measure-

ments were made using a × 40 objective and a × 10 eyepiece.

Measurements of bone marrow cellularity were made independently on the same haemalum and eosin preparations used for visual point-counting. In place of the photographic grid, to facilitate the systematic selection of fields, two intersecting lines were drawn on the coverglass with a fine mapping pen and drawing ink so that approximately the same amount of marrow was contained in each quadrant. Within each quadrant three fragments of marrow, each large enough to fill the Quantimet 720 field, were selected for measurement in a random manner. Exceptionally, when the biopsy was particularly fragmented, it was found necessary to use the Variable Frame to reduce the area of the Quantimet 720 field by half. Care was taken to exclude peripheral blood from the measurements.

The method of using the Quantimet 720, in this instance, was a manual one: as each field was located, the controls of the 2D Detector were manipulated by hand, and the area was measured in 'picture-points' at three different detection levels corresponding to the darkly stained cell nuclei, the paler stained cytoplasm, and the unstained fat spaces. Because of the similarity of their staining, erythrocytes could not be distinguished from other cytoplasmic structures. Small artefactual cracks that would otherwise have been included in the measurement of the larger fat spaces were eliminated by using the two 2D Amenders in series. The image was eroded until the cracks disappeared and then dilated by the same number of 'picture-points' so that the fat spaces were restored to their original size. The Hewlett-Packard Calculator was programmed to calculate and print the running and average areas occupied by nuclei, cytoplasm, and fat as percentages of the total. The principles of working of the Quantimet 720 and other automated image analysing systems have been reviewed by Hougardy (1974). According to Fisher (1971), the area of a roughly circular feature of diameter 1/20th of the diagonal of the Quantimet 720 screen is measured to an accuracy better than 2%, and that of a feature of diameter 1/10th of the screen diagonal to within 0.5%. In applying the Quantimet 720 to the measurement of bone marrow cellularity, results reproducible to within 2% were consistently obtained.

Results

With visual point-counting, bone marrow cellularity, expressed as a percentage, was the sum of the percentage figures for cell nuclei, cytoplasm, and erythrocytes. It ranged from 21 to 99% in 100 specimens. In the case of the Quantimet 720 data

Fig. 2 Percentage cellularity as obtained by the visual point counting method and the Quantimet 720 ($r=0.98$, $y = 0.996x + 0.927$).

in which erythrocytes were included in the measurement of cytoplasm, percentage cellularity was obtained by summing the figures for cell nuclei and cytoplasm. The results were closely similar (always within 5%) to those obtained by visual point-counting (see Fig. 2). Similarly, the correlation for cell nuclei, cytoplasm, and fat assessed separately was good.

The reproducibility of the point-counting method was tested by carrying out counts on four marrow preparations with a range in cellularity on six separate occasions. Percentage cellularity on each occasion was within 5% of the original result (mean coefficient of variation = 2.6%).

Variability within marrow samples was tested by carrying out point-counts with the Quantimet 720 on three random biopsies sectioned throughout the entire block at levels 200 μm apart. In all cases percentage cellularity at all levels in each biopsy was within 5% of the result obtained at the first level (mean coefficient of variation = 5.1%).

Discussion

Objective methods of measuring the cellularity of bone marrow biopsies have occasionally been used in the past, the most popular being the volumetric method. This measures the myeloid-erythroid layer (buffy-coat) as a percentage of the total volume of aspirate. Because of dilution of the sample with peripheral blood (which may amount to 40-100% of the total volume (Berlin *et al.*, 1950)), the method is

subject to gross inaccuracy. Berman and Axelrod (1947) and Sturgeon (1951) used a Whipple eyepiece to obtain an assessment of marrow cellularity, but there is considerable observer error associated with this method.

The principles of point-counting have been reviewed by Dunnill (1962). Essentially, the number of points that fall on each component in a tissue section is proportional to the area occupied by that component and, from the theorem of Delesse, to its volume. The accuracy of the method in respect of a particular tissue component is dependent upon the volume density of the component and the number of points counted (Weibel, 1963). Thus, with a cellularity between 25 and 100% and counting 600 points the relative error will be 5%, and 10% if the cellularity is between 7 and 25%.

Point-counting has been used for the assessment of marrow cellularity in the iliac crest (Hartsock *et al.*, 1965) and the second lumbar vertebra (Dunnill *et al.*, 1967). However, in both of these studies blocks of marrow were obtained *post mortem* and the investigators did not have the problem of assessing cellularity in fragmented pieces of marrow with different rates of sedimentation. This problem was solved by point-counting the same number of fragments in each quadrant or in all of the fragments. The visual point-counting method described in this paper has a number of advantages. It requires no expensive apparatus. It is simple and quick, taking approximately 15 minutes for the assessment of one biopsy. The error is about 5% and the results are reproducible, so it should now be possible to assess the clinical significance of moderately hypocellular marrows. In addition, unlike previously described point-counting techniques, the results have been compared with those of the Quantimet 720, thus providing another measure of the accuracy of the results.

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