Assessment of cellularity in bone marrow fragments

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SUMMARY Bone marrow cellularity was measured using the Quantimet 720 Image Analyser in 50 biopsy samples. In haematologically normal subjects the percentage cellularity of the marrow was between 40 and 63%. Subjective assessment of cellularity was an unreliable indicator of the quantitative measure.

There are a number of methods for evaluating bone marrow production activity. A functional measurement of erythroid production can be obtained by using ferrokinetic techniques and an extension of this allows the total erythroblast population in the body and the total cell production to be calculated. A simpler and more traditional method of assessing erythropoiesis has been based on either the reticulocyte count in the peripheral blood or the myeloid:erythroid ratio in marrow smears. The inherent inaccuracy and variability of these techniques limit the usefulness of this approach to the detection of marked changes. In practice it is usual for haematologists simply to give a subjective evaluation of the cellularity of marrow section preparations.

In this study we have measured the reproducibility and observer variation of this subjective assessment and compared it with a quantitative measure of the cellularity of aspirated bone marrow using the Quantimet 720 Image Analyser.

Subjects and methods

Subjects
Bone marrow cellularity was evaluated in 50 biopsy samples from 12 haematologically normal subjects and 38 patients. The normal subjects were all undergoing minor elective surgical procedures (removal of cyst, repair of hernia, simple gynaecology) and gave their fully informed consent to additional investigations. Their mean (±SD) haemoglobin concentration was 13.7 ± 1.2 g/dl; mean WBC (±SD) was 7.3 ± 1.9 x 10⁹/l; mean (±SD) MCV 91.0 ± 5.7 fl. They all had normal bone marrow morphology. Marrow aspirates were taken, while the subjects were anaesthetised, from either the anterior iliac crest (nine subjects) or from the posterior iliac crest (three subjects). Of the 38 patients whose marrows were being examined as part of a haematological investigation, 10 had been reported as having normal marrow morphology. Four patients had a myeloproliferative disorder (three polycythaemia rubra vera and one essential thrombocythaemia) with haemoglobin concentrations between 17.5 and 19.5 g/dl. Four patients also had a mild autoimmune haemolytic anaemia (haemoglobin concentration 10.7–12.0 g/dl). Twelve patients with megaloblastic marrows were also studied. Three had a serum vitamin B₁₂ concentration of less than 120 ng/l, 5 had a serum folate level of less than 2.1 μg/l, one had chronic renal failure and three subsequently had a peripheral blood response to a pharmacological dose of vitamin B₁₂. Three had sideroblastic anaemia, while two patients had anaemia associated with chronic renal failure, two had iron deficiency anaemia and one had a refractory anaemia.

Methods
Bone marrow aspirates were immediately diluted in 20 ml of a formalin, acetic acid, distilled water (2:1:7) fixative. The samples were left for six hours at room temperature to complete fixation and then transferred to 70% alcohol. The granules were washed free of contaminating red cells, dehydrated and embedded in paraffin. Between 30 and 50 sections (5 μm thick) were cut from each block. In our hands this represented the minimum thickness that could be reliably cut from a paraffin block.

The sections were cut at 20–50 μm intervals depending upon the size of the embedded marrow to minimise cellular overlap. A total of 10 slides were examined from each block and sections were stained with haematoxylin using a standard staining procedure, except that the slide was left in haematoxylin for five rather than eight minutes.
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The characteristics of this assessed microscopic view into each frame of view. The apparatus was set to detect randomly that the apparatus have been described in detail. In essence it converts the microscopic view into a digitalised screen display which it then analyses quantitatively. It can be used either to count the number of cells, the area of those cells or the area of a contrasting portion of the cell image within each frame of view. In this study the apparatus was set to detect the number of nuclei (N), the total area of the nuclei (A) and the total cell area (C) in each field of view. The threshold was set so that stained regions were fully detected. The identification of each parameter was checked on the screen and where necessary this was edited using a light pen. All measurements were made using a \( \times 40 \) objective so that each picture point on the screen was equal of 0.093 \( \mu \text{m}^2 \). Ten fields selected randomly from each slide were counted within a 9294 \( \mu \text{m}^2 \) frame which was always contained within the granule tissue. The percentage cellularity was calculated simply from the proportion of the frame occupied by cells. A total of 100 fields were examined from each block. In this way a minimum of 4500 and up to 16 000 cells were examined and counted. In addition, the mean nuclear area was calculated from the total nuclear area (A) divided by the total cell count (N), the mean cellular area per nucleus from the total cell area (C) divided by the total cell count (N) and the nucleus:cell area ratio from the total nuclear area (A) divided by the total cell area (C).

The measurement of marrow cellularity by the Quantimet 720 was compared with that of a manual point counting procedure in 10 biopsies. Ten sections were selected at equal intervals through the block and from each of these three fields were taken at random. The field was viewed on a projecting microscope with a \( \times 63 \) objective giving a 116 \( \mu \text{m} \times 116 \mu \text{m} \) field. This was overlayed by a grid of 100 equally spaced points. The percentage of the points falling on each of the three components, fat, cell nuclei and cytoplasm, was scored and taken as equal to the proportional area of each. The total number of nuclei was counted in each field. The percentage cellularity was calculated as the sum of the points falling on the cytoplasm and nuclei.

To evaluate the subjective assessment of bone marrow cellularity in sections by light microscopy three haematologists examined 37 specimens which had been assessed by the Quantimet. Two slides from each specimen were collected and numbered randomly so that each specimen was examined independently on two occasions by each observer. The sections were classified into three categories of cellularity: hypocellular, normocellular, and hypercellular.

Results

Bone marrow cellularity as measured by the Quantimet in the 12 haematologically normal subjects ranged between 40% and 60% (mean ± SD, 52.6 ± 5.31%). The mean (±SD) nuclear area was 17.4 ± 1.9 \( \mu \text{m}^2 \). This gave a mean (±SD) nucleus:cell area ratio of 0.44 (± 0.07). In the ten patients who were reported to have a normal marrow the measured cellularity of their marrows was generally less than that seen in the haematologically normal subjects. The mean (37.2%) was significantly less than that for the normal group (U = 4, p = 0.002). The mean nuclear area (15.4 \( \mu \text{m}^2 \)) mean cell area (43.8 \( \mu \text{m}^2 \)) and mean nucleus:cell ratio (0.38) were all less, but not significantly less, than in the normal subjects.

The measurements of bone marrow percentage cellularity by the Quantimet were closely correlated with the results obtained by the point counting method (\( r = 0.96 \)). Comparison of the nuclear count per unit area estimated by the Quantimet and by direct visual counting on the projecting microscope showed that the results for the two methods were also highly correlated (\( r = 0.87 \)). The percentage of the cells which was estimated to be non-nuclear cytoplasm was similar for each of the two methods although much less closely correlated (\( r = 0.62 \)).

Percentage cellularity in the patients fell largely within the broad limits found in the normal subjects. In one patient with a megaloblastic marrow cellularity reached 80% while in one with sideroblastic anaemia and one with haemolytic anaemia the cellularity was between 70 and 80%. Only three patients, one with rheumatoid arthritis one with refractory anaemia and one with polycythaemia, had a percentage cellularity less than 30%. Similarly both the nucleus and cell sizes in the patients were generally within the ranges found in the normal subjects, except in the patients with megaloblastic anaemia where the mean nucleus area was significantly greater (\( U = 36, p < 0.05 \)) than the normal mean.

In the subjective assessment of marrow cellularity there was 86–92% agreement between the two independent assessments of the cellularity of each marrow section for the three observers. This high degree of intraobserver consistency was not matched by the same degree of agreement between the observers. On 12 occasions one of the observers disagreed in both assessments with the agreed duplicate assessments of the other two. There were a further three occasions on which two observers clearly differed in their assessment while the third gave inconsistent duplicates. All the disagreements were between normocellularity on the one hand and either hypo- or hypercellularity on the other. Only on 14 occasions was there unanimity in the classification of the marrow cellularity.
Cellularity in the same 37 sections was measured quantitatively using the Quantimet and those with 40–63% cellularity were classified as normocellular. Hypocellularity was then defined as less than 40% cellularity while hypercellularity was taken as greater than 63%. This classification was compared with the subjective assessment of marrow cellularity (Figure). There were only four instances when one of the observers felt that the marrow appeared hypocellular that were not confirmed by direct measurement. This represented a failure rate of 15.4%. The visual definition of normocellularity was however much less reliable. On 34 occasions a hypocellular marrow was reported as normal and on one occasion a marginally hypercellular marrow was classified as normocellular. The overall error rate in the subjective classification of normocellularity was 32.1%. Similarly hypercellularity was thought to be present in 54 instances when the measured cellularity was normal and in four instances when it was less than normal. This represented an error rate of 66%.

Discussion

The assessment of marrow cellularity has always occupied an important place in haematological investigation. While it has long been recognised that increased marrow activity, particularly of the erythroid cells, may involve the spread of bone marrow into new sites it is generally agreed that reduced marrow activity will result in an increased proportion of fat at any one site. The study which we have undertaken relates only to the proportion of fat to cells in sections of granules taken from a single site. It has not proved possible to compare this measure of cellularity at different sites in the same individual but other studies have suggested that the cellularity of the marrow is similar throughout the whole tissue.

Earlier estimates of bone marrow cellularity were based on quantitative visual measurements using either tessellation by a marked Whipple micrometer eye piece or by “point counting". This latter method has been used for the assessment of marrow cellularity from anterior iliac crest and the second lumbar vertebrae of normal subjects. These estimates show a similar but wider range (29–79%) than that found in the present study (40–60%).

The validity of the Quantimet estimate has been established previously and has been confirmed in the present study. The particular advantage of the automated approach is that it enables a greater number of cells to be examined over a representative selection of granule sections. In addition it is also possible to obtain reproducible estimates of the mean nuclear size, mean cell size and the mean nucleus:cell area ratio. Although Ho-Yen and Slidder reported a coefficient of variation (CV) of 2.6% for the point counting estimate of cellularity this was based on only four marrows each counted six times. In our study the CV for this method ranged from 8.2 to 34.7% in 10 marrows each measured 30 times and this seems to reflect the more likely level of reproducibility of the method. By contrast the mean Quantimet CV was 10% or less in all but three of the 50 marrows each of which was measured 10 times.

In the 10 patients whose marrows had been reported as having normal cellularity it was clear that quantitative assessment did not confirm the visual impression. The difficulty of making a visual assessment was confirmed by the more detailed study using three observers. Although there was considerable consistency within each observer there...
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was little agreement between observers. Comparison with the measured cellularity showed that when this was reduced there was only a 36% chance of this being detected by visual inspection although there were only 15% false-positives in those sections identified as hypocellular. Similarly a normocellular marrow (40–62% cellularity) stood a good (42%) chance of being identified as hypercellular but of those sections thought to be hypercellular only 34% were truly so. Perhaps because of this deceptive hypercellularity in normocellular marrows there was no consistent failure to identify a true hypercellularity. It seems, therefore, that visual assessments of cellularity are at best unreliable and may frequently be quite misleading. A similar conclusion had been reached as a result of the measurement of observer error in the assessment of cellularity in mouse marrow sections. Where a true measure of this aspect of the marrow is required this can only be achieved by an objective method.

Taken together the results suggest that in most haematological conditions changes in the degree of cellularity are relatively minor. Even when cellularity is decreased, it does not seem to follow that the ability of the marrow to produce normal, or even increased numbers of red-cells is restricted. Conversely normal percentage cellularity can be associated with a wide range of haemoglobin concentration in the peripheral blood. With the exception of marked hypoplasia the diagnostic usefulness even of an accurate measure of cellularity may be limited.

References


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