Hypochromic macrocytes: are they reticulocytes?

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Abstract

Automated full blood counters measuring the size and haemoglobin concentration of individual red cells by high and low angle light scatter can provide estimates of the percentage of cells which are abnormal in either or both of these variables. The hypothesis that an increase in the number of large cells with a reduced haemoglobin content ("hypochromic macrocytes") was indicative of a reticulocytosis was investigated. A correlation was shown between the percentage of hypochromic macrocytes and the reticulocyte count. This correlation was too weak to allow the actual reticulocyte percentage to be predicted from the percentage of hypochromic macrocytes. An increased percentage of hypochromic macrocytes, however, often indicates an increased reticulocyte count and thus serves to chart the haematologist to this possibility.

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An increased reticulocyte count is important in the differential diagnosis of anaemia because it indicates increased marrow output of red cells and, in the absence of recent administration of haematinic agents, suggests the possibility of haemorrhage or haemolysis. A visual reticulocyte count, however, is not only imprecise but also time consuming and therefore expensive, while an automated reticulocyte count requires major capital

expenditure. The prediction of an increased reticulocyte count, by using the variables of an automated full blood counter could, if possible, be clinically useful.

The H.1 and H.2 automated full blood counters (Technicon Division, Bayer Diagnostics, Basingstoke, England) estimate the volume and haemoglobin concentration of individual red cells by measuring high and low angle light scatter.¹ Such measurements are represented graphically in a red cell cytogram (fig 1A) in which volume (V) is plotted against haemoglobin concentration (HC). Cells of more than 120 fl are classified as macrocytes with the presence of more than 4% of such cells giving rise to a "macro" flag. Cells with a haemoglobin concentration of less than 280 g/l are considered to be "hypochromic", with the presence of more than 4% of such cells giving rise to a "hypo" flag. During routine use of this instrument, we noted that a number of patients with autoimmune haemolytic anaemia had an increase in the percentage of hypochromic macrocytes (fig 1B). Furthermore, patients receiving haematinic agents often showed an increase in the percentage of this cell population which paralleled the increase in the reticulocyte percentage. Reticulocytes are generally considered to be larger and less dense than mature red blood cells. We investigated the hypothesis that hypochromic macrocytes, as detected by the H.1 automated counter, might represent reticulocytes by making a systematic comparison of the percentage of these two cell types: we sought to determine if this new variable would serve as a flag for an increased reticulocyte count.

Figure Red cell volume (V) plotted against haemoglobin concentration (HC) for (A) a blood sample from a healthy volunteer and (B) a blood sample from a patient with a severe autoimmune haemolytic anaemia with a reticulocyte count of 35%. (B) shows an increase of hyperchromic cells, corresponding to spherocytes, and an increase in hypochromic macrocytes.

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Methods

Reticulocyte counts were carried out for clinical purposes. They were estimated on a Sysmex R-1000 analyser (Toa Electronics Company, Milton Keynes), using auramine-O as the nucleic acid fluorochrome. Patients had a variety of diagnoses, including renal failure, autoimmune haemolytic anaemia, sickle cell anaemia, β thalassaemia major, drug induced haemolysis, and vitamin B₁₂ or iron deficiency anaemia responding to treatment. Reticulocyte counts, which ranged from 0.5–43%, were compared with the percentage of hypochromic macrocytes (cells with V >120 fl, HC <280 g/l), as determined by an H.1 automated full blood counter. Automated blood counts were performed within 6 hours of venepuncture.

Results

The percentage of hypochromic macrocytes showed a weak but significant correlation with the reticulocyte count ($r = 0.35$; $p < 0.001$; intercept of regression line, $a = 0.56$, slope of regression line, $b = 0.41$).

Twelve of the 150 patients had more than 4% hypochromic macrocytes, and in eight of these cases the reticulocyte count was above 2%; but only five of the 13 patients with more than 5% reticulocytes had hypochromic macrocytes above 4%.

Discussion

The percentage of hypochromic macrocytes determined by the H.1 automated full blood counter showed a significant correlation with the percentage of reticulocytes. This observation supports the hypothesis that, on average, reticulocytes are larger than mature red cells and have a lower haemoglobin concentration. The correlation between the two variables was weak, however, with only 12% of the variation in the percentage of hypochromic macrocytes being explained by the variation in the reticulocyte percentage.

It is clear from our findings that not all reticulocytes are hypochromic macrocytes, and we have in fact observed that as reticulocytosis develops there is often also an increase in hypochromic normocytic cells. It is like-

wise evident that, although most of the patients with an increased percentage of hypochromic macrocytes had an increased reticulocyte count, not all hypochromic macrocytes were reticulocytes. We have found that a strong drive to erythropoiesis can lead to iron deficient erythropoiesis²; if hypochromia is due to an inadequate supply of iron to the developing erythroblast the resultant red cell would be expected to remain hypochromic throughout its lifespan; a cell produced when the iron supply was adequate would be expected to increase its haemoglobin concentration to normal as redundant membrane was removed and it matured from a reticulocyte to a red cell. Furthermore, in conditions of haemopoietic stress when erythropoietin concentrations are high there is production of 'shift erythrocytes' which are larger than normal erythrocytes; such cells are likely to remain larger than normal red cells throughout their lifespan. The category of hypochromic macrocytes is thus likely to include cells which have lost their RNA and are therefore no longer reticulocytes but are nevertheless still hypochromic and macrocytic. We also observed an increase in hypochromic macrocytes in patients with a low reticulocyte count but with dyserythropoiesis—for example, in megaloblastic anaemia, in the myelodysplastic syndromes, and in thalassaemia major.

Our observations support the hypothesis that an increase in the percentage of hypochromic macrocytes is often due to an increase in the percentage of reticulocytes and that reticulocytes are often hypochromic macrocytes. The correlation between these two variables is too weak for the percentage of hypochromic macrocytes to be used to predict the reticulocyte percentage. An increase in the percentage of hypochromic macrocytes, however, serves to indicate the possibility of reticulocytosis or dyserythropoiesis.

- 1 Mohandas N, Kim YR, Tycko DH, Orlik J, Wyatt J, Groner W. Accurate and independent measurement of volume and hemoglobin concentration of individual red cells by laser light scattering. *Blood* 1986;68:506–13.
- 2 Macdougall IC, Cavill I, Hulme B, *et al.* Detection of functional iron deficiency during erythropoietin treatment: a new approach. *BMJ* 1992;304:225–6.