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Quantitative reticulocyte counting: Clinical applications

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Abstract: Erythropoietic activity within the marrow is a dynamic process. Red cells emerge into the circulation containing remnants of the erythroblast RNA that was responsible for the synthesis of haemoglobin in the developing cell. Latterly, it has been possible to identify newly emerged cells by their RNA content. The earliest applications related to monitoring the erythropoietic response to recombinant human erythropoietin. Quantitative reticulocyte counters also allow other features of the reticulocyte population to be characterized. Quantitative reticulocyte counting has only lately become available. All the received haematological wisdom on this topic must be re-evaluated in the light of such real data.

Marrow erythropoietic activity is a dynamic process. It results in the production of 2-3 million new red cells per second in a normal human subject. The red cells that emerge into the circulation contain remnants of the erythroblast RNA that was responsible for the synthesis of haemoglobin in the developing cell. Originally, these cells were recognized by the appearance of a reticulum when they were exposed to supravital stains such as new methylene blue. Hence, the eponym, reticulocytes. This reticulum was related to the polysomal remnants within the newly emerged cells. As these cells became adult erythrocytes over the course of 1-2 days the reticulum disappeared. The process by which this takes place is thought to involve removal of these cytoplasmic remnants by the reticuloendothelial cells of the spleen. This also appeared to result in the loss of some cell volume and other cytoplasmic contents. Latterly, it has been possible to identify newly emerged cells by their RNA content. This involves using specific RNA/DNA stains to identify the cells *(1)*.

Originally reticulocytes were identified and estimated by visual inspection of a stained blood smear. Now it is possible to identify cells containing RNA and to count them in large numbers by flow cytometry. In practice the visual estimate of the proportion of reticulocytes in the smear is at best semiquantitative. A number of studies have pointed to the high coefficient and variation of the estimate which may range up to 100% *(2)*. The advent of quantitative flow cytometric techniques has brought the reticulocyte count in line with other blood cell counts with coefficient variation closer to 5%.

The ability to reliably quantitate the number of reticulocytes in circulation has allowed a direct estimate of current erythropoietic activity. The best estimates of the reticulocyte life span in vivo suggest that this is of the order of 0.5 - 2.5 days. Changes in the reticulocyte count therefore reflect current changes in erythropoietic activity. Anecdotal studies in normal subjects have demonstrated that the mechanisms controlling erythropoiesis respond rapidly to changes in the subject's circumstances. Modest changes of altitude and decreased oxygen tension will result in an increase in the erythropoietic activity and reticulocyte count in a matter of days. Conversely, mild infections may have a pronounced affect on suppressing erythropoietic activity. In the absence of such changes erythropoietic activity remains constant and there is no evidence of any diurnal variation.

The clinical application of quantitative reticulocyte counting is widespread. One of the earliest applications has related to monitoring the erythropoietic response to recombinant human

erythropoietin. This has demonstrated the brisk increase in erythropoiesis that occurs in the first two weeks after the commencement of therapy *(3)*. During this time the reticulocyte count will increase by 1.5 - 2 times its starting level. In those patients who are going to maintain their response to erythropoietin the reticulocyte count shows a steady increase towards a peak level later in therapy. This then falls as a normal balance of production and destruction is re-established. However, in those patients who are not going to prove to be good responders the initial rise in the reticulocyte count is not maintained. By the fourth week after the commencement of therapy the reticulocyte count will have fallen back towards its base line level. This enable patients who will not respond to erythropoietin to be identified at a relatively early stage.

At the other extreme it has been possible to confirm the presence of increased erythropoietic activity in polycythaemic patients. The absence of such an increase in patients with increased haemoglobin concentration is a clear indication of haemo-concentration. When the erythropoietin concentration is not increased to match the increased reticulocyte count this is clear evidence of a primary polycythaemia which is erythropoietin independent.

Increased erythropoietic activity is reflected by an increased reticulocyte count but when there is increased mature red cell destruction the proportion of reticulocytes to erythrocytes will be increased. In patients with a compensated haemolysis there will be both an increased reticulocyte count due to stimulated erythropoiesis and an increased reticulocyte percentage due to red cell destruction. The result is a gross elevation in the reticulocyte percentage. Even a visual reticulocyte estimate can often detect this. It has however, proved impossible using such visual techniques to detect relatively minor but significant increases in haemolysis. The precision of the quantitative flow cytometric reticulocyte estimate allows such marginal changes to be observed. It is thus possible to identify small but persistent increases in red cell destruction in patients with heart valve protheses.

Outside these relatively clear cut conditions there are numerous other applications of the reticulocyte count as a dynamic measure of the balance of red cell production and destruction. For example, in patients treated with vitamin B12 who were previously deficient in this haematinic it is possible to identify and confirm an erythropoietic response well in advance of any change in haemoglobin concentration.

Quantitative reticulocyte counters also allow other features of the reticulocyte population to be characterized. In particular the amount of RNA within the different cells can give an indication of the age distribution of the population. In patients receiving bone marrow transplant after previous marrow and patient therapy an increase in the proportion of very young RNA rich cells is the earliest indication of engraftment *(4)*. In addition, one family of counters will allow the reticulocyte indices, that is their haemoglobin content, concentration and volume, to be measured independently of the mean red cell indices *(5)*. Early studies using such instruments confirm that there is a significant loss in cell volume from the reticulocyte to the erythrocyte. This loss appears to be greatest amongst those cells which will become mature microcytes *(6)*. Early indications are that the reticulocytes preceding such cells are not smaller than their normal counterparts. In contrast there is no significant loss of haemoglobin content as the reticulocyte matures towards the erythrocyte. Thus, in a stable and balanced setting red cell haemoglobin content will approximately equal that of the reticulocyte. However, when erythropoiesis is stimulated or modified there will be a significant change in reticulocyte haemoglobin content. An increase in this parameter is the earliest mark of response to iron therapy, it seems possible that this might form the basis for a therapeutic test of functional iron deficiency.

Quantitative reticulocyte counting has only lately become available. All the received haematological wisdom on this topic must be re-evaluated in the light of real data.

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