The reticulocyte count

K. G. BADAMI

INTRODUCTION

The reticulocyte count is a simple, useful, inexpensive and often undervalued test in the investigation of anaemias. When correctly performed and assessed, it serves as a reliable index of effective erythropoiesis. This article discusses the causes of a high, low and inadequate increase in the reticulocyte counts.

The reticulocyte is a young red cell. It is produced in the bone marrow where it normally has a life of 2.8 days before entering the circulation where it spends a further day before becoming fully mature.

The reticulocyte count is an index of effective erythropoiesis, that is, it reflects the amount of red cells being produced in the marrow and entering the peripheral blood. In normal healthy persons, the reticulocyte count is low (0.5% to 1.5% in adults and children and 2% to 6% in full term infants at birth) because there is only a small steady rate of destruction of aged abnormal red cells and only a low level of marrow activity is required to maintain normal haemoglobin levels.

As a result of haemorrhage, haemolysis or an abnormal pooling of red cells, the marrow in the healthy individual steps up its production of red cells up to 4 to 8 times its basal levels. Indeed, the marrow may be so successful in restoring normal haemoglobin levels that a raised reticulocyte count may be the only indication that there is something amiss. This situation is known as a compensated haemolytic state. It is not clear still, how the marrow maintains this increased rate of erythropoiesis in the absence of a hypoxic stimulus. However, not every individual is able to generate such a response, and this is discussed later.

It is important to remember that a bone marrow response leading to a rise in the reticulocyte count is the reaction that ought to occur in the face of anaemia. A 'normal' reticulocyte count in this situation implies a suboptimal response whose cause should be sought. Although there is no easy way to predict the reticulocyte count for a given degree of anaemia, the highest counts are generally found in the most anaemic patients.

Reticulocytes are easy to assess. They contain residual RNA which imparts a diffuse grey-blue colour with a Romanowsky stain, and take on the characteristic reticular appearance after supravital staining with a dye such as methylene blue. The amount of reticular substance present depends on the age of the reticulocyte with the most mature forms showing only a few granules. Because of the errors involved in manual counting, particularly with lower counts, automated methods are gradually being adopted.

AN INDEX OF EFFECTIVE ERYTHROPOIESIS?

The reticulocyte count is expressed as the percentage of red cells that are reticulocytes. A falsely high impression of effective erythropoiesis may be conveyed by the observed reticulocyte count for two main reasons.

1. The observed reticulocyte count (expressed as the percentage of red cells that are reticulocytes) does not take into consideration the fact that the total number of red cells is reduced in anaemia. For example, three patients, each with an observed reticulocyte count of 10% and red cell counts of 2, 3 and 5 million/μl respectively, apparently have a similar erythroid response, but the actual number of reticulocytes per μl is 200,000 in the first, 300,000 in the second and 500,000 in the third.

2. Raised erythropoietin levels due to anaemia induced hypoxia may, besides other effects, cause premature release of reticulocytes into the circulation. Thus, a reticulocyte may actually spend as much as 2 to 2.5 days in the peripheral blood instead of having a normal maturation time of 1 day. It is not entirely clear why this happens.

A possible explanation is that erythropoietin may promote the accelerated acquisition of a critical amount of haemoglobin so reducing the average number of cell divisions, the expulsion of the nucleus and the premature release of the reticulocyte into the circulation. These cells are large (2 to 3 times the volume of normal red cells) and are called macroreticulocytes or stress reticulocytes. They may be produced in response to intense erythropoietin stimulation. With lower levels of erythropoietin, such as those under normal basal conditions, the reticulocytes produced are smaller, show much less basophilia and only a few granules after staining with methylene blue. They may be quite difficult to recognize on a Romanowsky stained peripheral smear.

There might thus be a link between intracellular haemoglobin content and the ability of the reticulocyte to enter the circulation. It is clear therefore that reticulocytes which have been 'pushed' into the peripheral blood before their time do not reflect erythropoietic activity.
THE CORRECTED RETICULOCYTE COUNT

A valid assessment of the rate of erythropoiesis therefore requires correction for both anaemia and premature delivery of red cells.9

Correction for anaemia

The observed reticulocyte percentage can be manipulated in two ways to compensate for the discrepancy introduced by the variation in the number of circulating red cells.

1. If accurate red cell counts are possible (with an electronic counter, for example), the absolute reticulocyte count expressed as reticulocytes per μl of blood may be determined.
2. If this is not possible, the observed reticulocyte count is related to a haematocrit (Hct) of 45% as follows:

   \[ \text{Observed reticulocyte count} \times \frac{\text{Patient's Hct}}{45} \]

Correction for the premature release of reticulocytes from the marrow

The absolute reticulocyte count, or percentage obtained in the first step, needs to be further corrected to remove the discrepancy caused by the premature release of reticulocytes into the circulation. These ‘shift’ cells can easily be recognized on the peripheral smear by their polychromasia and their presence calls for the second correction.

As the time spent by the reticulocyte in the circulation is proportional to the degree of anaemia, an approximate correction is to divide the absolute reticulocyte count or percentage by a factor of 2, assuming an increase in the maturation time to be 2 days, but ideally, 1.0, 1.5, 2.0 and 2.5 days should be considered as average maturation times with haematocrits of 45%, 35%, 25% and 15% respectively. Therefore, it is important when interpreting times with haematocrits of 45%, 35%, 25% and 15% 2.5 days should be considered as average maturation times with haematocrits of 45%, 35%, 25% and 15% respectively.

The double corrected absolute reticulocyte count and percentage (which is also known as the reticulocyte production index) provide a good idea of the rate of effective erythropoiesis. The normal absolute reticulocyte count varies from 25 000 to 125 000/μl and a value less than 40 000/μl is considered to be evidence of depressed erythropoiesis. The basal reticulocyte production index is taken as 1. Values less than 1 suggest impaired red cell production while a reticulocyte production index of 3 (i.e., 3 times the basal level) or more, suggests haemolysis.

It must be remembered that it takes time for the marrow to deliver freshly produced red cells to the circulation. The first response to an acute demand for red cells is ‘shift reticulocytosis’ which manifests as polychromasia on the peripheral smear after 6 hours. There is no change in the erythroid/myeloid ratio till the second or third day and the reticulocyte production index increases (i.e., the delivery of freshly made red cells to the circulation begins) around the fifth day. Around the tenth day, all three variables, viz. polychromasia on the peripheral smear, the erythroid/myeloid ratio in the marrow and the reticulocyte production index are in concordance. Polychromasia without erythroid hyperplasia or an increased reticulocyte production index suggests ‘shift reticulocytosis’. On the other hand, erythroid hyperplasia in the marrow without an increase in the reticulocyte production index, whether or not accompanied by polychromasia, suggests intramedullary destruction of red cell precursors or ineffective erythropoiesis.

INTERPRETATION OF THE RETICULOCYTE COUNT

The interpretation of the reticulocyte count in various settings is discussed briefly below, and summarized in Table I.

1. Normal haemoglobin with a normal reticulocyte count
   (a) It occurs in normal healthy persons.
   (b) Immediately after acute blood loss (within 6 hours).
2. Normal haemoglobin with a high reticulocyte count
   (a) Compensated haemolytic disorders
   (b) After splenectomy. The normal spleen selectively traps reticulocytes so a modest increase in the reticulocyte count may favour splenectomy.
   (c) Extramedullary haematopoeisis—lack of normal controls may permit the reticulocyte to enter the circulation, usually accompanied by other immature cells.
   (d) Damage to the bone marrow micro-architecture by fibrosis, infiltrative malignancies etc.
3. Low haemoglobin with a high reticulocyte count
   (a) Classically found with acute haemolysis, haemorrhage or red cell pooling. With normal marrow and adequate iron, the reticulocyte response will reflect the degree of anaemia.
   (b) In any anaemia responding to therapy, for example in a case of iron deficiency responding to iron.
4. Low haemoglobin with a low reticulocyte count or with an inadequate reticulocyte response
   (a) Due to defects in the marrow itself as in hypoplastic anaemias red cell aplasia, megaloblastic anaemia and marrow infiltration by leukaemia, lymphoma, myeloma, metastatic cancer, storage cells or granulomas. With marrow infiltration a leucoerythroblastic picture with marked red cell changes but without reticulocytosis is characteristic.
   (b) Due to defects in the red cell precursors or in the mature red cells. Defects in the red cell precursors (dyserythropoietic states) lead to intramedullary destruction of these cells and hence ineffective erythropoiesis as in the thalassaemias, megaloblastic anaemia, megalodysplastic syndromes, congenital dyserythropoietic anaemia and sideroblastic anaemia.

The mature red cells in some conditions may show a decreased affinity to oxygen leading to increased oxygen delivery to the tissues relative to the anaemia and consequently a low erythropoietin drive. A less than expected...
rise in the reticulocyte count is found in these conditions such as in haemolytic anaemias due to sickle haemoglobin or some haemoglobin variants and in enzyme defects like pyruvate kinase (PK) deficiency. In PK deficiency there is also increased reticulocyte destruction in the spleen. After splenectomy the reticulocyte count shows a marked increase.

(c) Due to deficiencies in substances necessary for erythropoiesis like iron, vitamin B₁₂, folic acid, pyridoxine, riboflavin and protein. Erythropoietin deficiency occurs in renal disorders. In addition there may be a decreased marrow response to erythropoietin due to some, as yet unknown, toxin. Hypothyroidism, hypopituitarism and hypogonadism cause deficiencies of hormones necessary for erythropoiesis. In the anaemia of chronic disorders, iron is not available to erythroid precursors because it is trapped in the macrophages.

(d) Immediately after acute haemorrhage, haemolysis or sequestration of red cells occurs. This is because it takes time for the marrow to increase production of red cells. This is normally 2 to 3 days and it may be 5 days before newly formed reticulocytes are delivered to the circulation.

(e) When immune factors act against the mature red cells as well as against red cell precursors and erythropoietin, as may happen rarely in cases of autoimmune haemolytic anaemia.

5. High haemoglobin with a high reticulocyte count. This occurs in conditions called the secondary polycythaemias caused by high erythropoietin levels, either physiological (i.e. secondary to hypoxia) as in residents at high altitude, smokers, and in various pulmonary and cardiac diseases, or due to the inappropriate erythropoietin secretion which occurs in some neoplastic and non-neoplastic conditions.

6. High haemoglobin with a normal or low reticulocyte count. This may occur because of suppression of erythropoietin production after overtransfusion. In polycythaemia vera many factors can affect the reticulocyte count. In the initial stages there is increased red cell production but in spite of finding polychromasia on the peripheral smear the reticulocyte, iron and folate deficiency and splenic sequestration may bring the reticulocyte count down.

THE RETICULOCYTE COUNT IN CLINICAL PRACTICE

The reticulocyte count is a particularly useful investigation in the situations listed below.

1. As an indicator of a haemolytic process. It is important to realize that while haemolytic anaemias are usually associated with raised reticulocyte counts, sometimes this may not occur as in:
   (a) Folate deficiency in a chronic haemolytic disorder.
   (b) Iron deficiency limiting the marrow response. Frequently overlooked causes include haemosiderinuria in paroxysmal nocturnal haemoglobinuria (PNH) and associated chronic disease.
   (c) Conditions such as thalassaemia which are associated with ineffective erythropoiesis.
   (d) Transient erythroid hypoplasia (aplastic crisis), a feature of some haemolytic anaemias like sickle cell anaemia, hereditary spherocytosis and also other haemolytic anaemias such as autoimmune haemolytic anaemia and paroxysmal nocturnal haemoglobinuria. In some cases this aplastic crisis is caused by a parvovirus infection.
   (e) Haemolytic anaemias with low oxygen affinity.
   (f) Autoimmune, haemolytic anaemia secondary to a lymphoma infiltrating the marrow. Similarly a drug used for the treatment of a condition associated with haemolytic anaemia may cause hypoplasia of the marrow and reduce the reticulocyte count.
   (g) Immune haemolytic anaemias when the antibodies also act against the red cell precursors.

2. To diagnose a fully compensated haemolytic state.

3. In the differential diagnosis of pancytopenia. A low reticulocyte count points to hypoplastic anaemia, pure red cell aplasia, PNH, infiltrative disorders such as acute leukaemia, myelodysplastic syndromes or to megaloblastic anaemia, whereas a high count points to either hypersplenism or immunological destruction of all three cell lines.

The reticulocyte count is important in the follow up of patients with anaemia on therapy. A rising reticulocyte count (retic response) means that the anaemia is responding to therapy as in a patient with megaloblastic anaemia receiving vitamin B₁₂ or one with iron deficiency on iron therapy. Similarly, a fall in the reticulocyte count with a rise in haemoglobin levels indicates a favourable response in a patient with autoimmune haemolytic anaemia on corticosteroids.

So important is the reticulocyte response to therapy that it forms the basis of tests for anaemias caused by iron, vitamin B₁₂ and folic acid deficiency by therapeutic trials.
CONCLUSION
It is important that the reticulocyte count be done in the investigation of any case with anaemia. Unfortunately there is always a tendency to do painful tests such as a bone marrow aspiration or expensive ones such as serum ferritin assays in the initial stages. The simple combination of a haemoglobin estimation, reticulocyte count and the examination of a peripheral blood film will often provide a diagnosis or indicate which further tests will be most helpful.

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REFERENCES