Original article

Role of immature reticulocyte fraction in evaluation of aplastic anemia in cases of pancytopenia

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Abstract:
The immature reticulocyte fraction (IRF) which is a new diagnostic indicator measured in automated cell counters are based on flow-cytometric determination of erythrocyte RNA content. It gives a basic idea about the marrow erythropoietic activity and its response to drugs and therapy at an early stage thereby useful for monitoring therapy by the physicians without seeing the marrow. Also IRF helps in segregating aplastic anemia from common nutritional deficiency anemia's that presents as pancytopenia, which is not possible by a reticulocyte count since it is more or less equal in most of the cases.

In our study 172 cases of pancytopenia were studied. Complete blood count (CBC) and bone marrow were done in 172 patients out of which 58 showed IRF 0% among which 54 were diagnosed as aplastic anemia and 4 as aplastic crisis in hemolytic anemia. Hence, IRF has replaced all other reticulocyte indices and can be routinely incorporated in evaluation of pancytopenia cases.

Keywords: Pancytopenia, Immature Reticulocyte Fraction, Aplastic anemia

Introduction:
Pancytopenia is an important clinico-haematological entity worldwide but with varying patterns in clinical presentations. Bone marrow aspiration is considered of primary importance in evaluating and diagnosing the cause of pancytopenia. But before doing a bone marrow aspiration or biopsy, a note on the newer reticulocyte indices given by automated cell counters other than reticulocyte percent and absolute reticulocyte count helps us to get a picture about the marrow erythropoietic activity. These newer reticulocyte indices are the Immature reticulocyte fraction (IRF). Most of the physicians use only reticulocyte percentage and absolute reticulocyte count in evaluating anemia’s. We attempted to emphasize the importance of Immature Reticulocyte Fraction (IRF) over other reticulocyte indices in diagnosis of aplastic anemia and in assessment of marrow response to therapy.

Material and methods:
A prospective study was done in our department from November 2013 to August 2015. Patients, who satisfied the criteria for pancytopenia with hemoglobin <9g/dl, WBC <4000/µl, Platelets <1,00,000/µl were included in our study. Detailed clinical history was collected.

All the patients were clinically examined for pallor, icterus, lymphadenopathy, hepatosplenomegaly, splenomegaly and sternal tenderness. Two ml of EDTA blood sample were collected in vacutainers. Complete haemogram with reticulocyte indices were done with automated hematology analyzer sysmexxt- 2000i. A reticulocyte percentage of (0.2-2%) and IRF of <5% is taken as the normal reference range. Also peripheral blood
smear and simultaneous bone marrow aspiration and biopsy were performed in all the cases. All necessary and relevant data were processed. Data were evaluated by standard statistical methods.

**Result:**
Totally 172 cases of pancytopenia were studied. Patients presented with different age groups with most common affected age group of 30-40 years. Males were more affected than females. Complete blood count with reticulocyte indices were performed in all the cases. Bone marrow aspiration was performed in all the 172 cases to diagnose the cause of pancytopenia. The most common cause of pancytopenia was found to be aplastic anemia 58 (33.7%) followed by megaloblastic anemia (32.55%). Other less common causes were myelodysplastic syndrome, myeloproliferative neoplasms, aleukemic leukemia, storage disorders, hypersplenism and acute blood loss. The reticulocyte count and IRF were compared in all the cases and the results were correlated and tabulated (Table-1). Based on the result we categorise the pancytopenia cases into four major categories (Table 2).

Among 58 (33.7%) cases under category I, 54 (31.38%) were diagnosed as aplastic anemia and 4 (2.32%) as aplastic crisis in hemolytic anemia (sickle cell anemia). Here, in all the cases IRF was found to be zero but reticulocyte count was variably low.

Category 2 includes 22 (12.7%) cases of megaloblastic anemia, 10 (19.7%) cases of MDS, in all 32 cases both reticulocyte count and IRF was found to be variable. We found no correlation between reticulocyte count and IRF in these cases.

Category 3 includes 05 (2.9%) cases of malaria with hypersplenism and 7 (4.06%) cases of acute blood loss. Here, IRF was found to be very high in all the cases but reticulocyte count was normal/elevated. Hence, IRF increases much earlier than reticulocyte count indicating earliest indicator of bone marrow erythroid hyperplasia.

Category 4 includes, cases on treatment for megaloblastic anemia (19.7%), and cases recovering from drug induced bone marrow suppression 20 (11.62%). These cases showed low to normal reticulocyte count but IRF was found to be increased in all the cases which reflects the marrow erythropoietic activity is accelerated on withdrawal of drug or the patient’s marrow is responding to treatment.

Hence, IRF clearly differentiates category 1 and 2 cases from category 4 since IRF was zero to low (<5%) in first two categories but normal to high (<5%) in category 4 whereas the reticulocyte count was variable in both the categories. It is also specific in diagnosis of aplastic anemia cases since IRF is absolute zero where reticulocyte count is variable low. Category 3 causes had very high IRF with high reticulocyte count indicating the increased marrow erythropoiesis than normal thus indirectly indicating a peripheral cause of pancytopenia while bone marrow is normal. IRF raises in cases of increased marrow erythropoiesis before the reticulocyte count would rise. Hence, IRF was found to be the earliest indicator of marrow erythropoietic activity. Also we found the difference in reticulocyte scatterogram between cases of aplastic anemia and other causes of pancytopenia.

The scatterogram from sysmexxt2000i shows the amount of forward scatter and side scatter indicating the amount of immature reticulocytes in case of megaloblastic anemia (figure 1) which is absent in cases of aplastic anemia (figure 2).
<table>
<thead>
<tr>
<th>Causes of pancytopenia</th>
<th>No. of cases</th>
<th>Reticulocyte count (%)</th>
<th>IRF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>95% range</td>
<td>Median</td>
</tr>
<tr>
<td>Megaloblastic anemia</td>
<td>22 (12.7%)</td>
<td>1.1</td>
<td>0.4-1.8</td>
</tr>
<tr>
<td>Megaloblastic anemia</td>
<td>34 (19.7%)</td>
<td>4</td>
<td>2-6</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>54 (31.38%)</td>
<td>0.36</td>
<td>0.01-0.71</td>
</tr>
<tr>
<td>Aplastic crisis in hemolytic anemia</td>
<td>04 (2.32%)</td>
<td>0.21</td>
<td>0.02-0.4</td>
</tr>
<tr>
<td>Early marrow recovery</td>
<td>20 (11.62%)</td>
<td>0.635</td>
<td>0.07-1.2</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>10 (5.81%)</td>
<td>0.98</td>
<td>0.48-1.48</td>
</tr>
<tr>
<td>Aleukemic leukemia</td>
<td>12 (6.97%)</td>
<td>1.34</td>
<td>0.58-2.1</td>
</tr>
<tr>
<td>Storage disorder</td>
<td>04 (2.32%)</td>
<td>1.295</td>
<td>0.59-2.0</td>
</tr>
<tr>
<td>Hypersplenism</td>
<td>05 (2.9%)</td>
<td>5.65</td>
<td>1.3-10</td>
</tr>
<tr>
<td>Acute blood loss</td>
<td>07 (4.06%)</td>
<td>5.35</td>
<td>2.2-8.5</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>172</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1:** Causes of pancytopenia with correlation of reticulocyte percent and IRF. (Normal reference ranges are mentioned in the text)
<table>
<thead>
<tr>
<th>Category</th>
<th>Criteria</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>Zero IRF with low reticulocyte count</td>
<td>Aplastic anemia, aplastic crisis in hemolytic anemia.</td>
</tr>
<tr>
<td>Category 2</td>
<td>Normal/high IRF with low/normal reticulocyte count</td>
<td>Megaloblastic anemia, Myelodysplastic syndrome, acute leukemia.</td>
</tr>
<tr>
<td>Category 3</td>
<td>High IRF with normal to high reticulocyte count</td>
<td>Hypersplenism, Acute blood loss, post malarial hemolytic state.</td>
</tr>
<tr>
<td>Category 4</td>
<td>High IRF with normal to low reticulocyte count</td>
<td>Early marrow recovery from chemotherapy or infections, megaloblastic anemia responding to treatment.</td>
</tr>
</tbody>
</table>

Table 2: Categorisation of causes of pancytopenia depending on reticulocyte count and IRF.

FIGURE 1: Reticulocyte scatterogram of megaloblastic anemia seen in automated cell analyzer (IRF=52.4%).
Discussion:
Heilmeyer\[7\] was one of the first to propose a classification of reticulocytes based on maturation as judged by the quantity of reticulo filamentous particles as seen under microscope after staining with brilliant cresyl blue. Later, it was demonstrated that the reticulum is composed of protein and ribosomal RNA.\[8\] The introduction of cytometric methods that use dyes that selectively bind RNA and, therefore, are able to generate reproducible signals proportional to the nucleic acid content has reposed the reticulocyte maturation index.

The term immature reticulocyte fraction was introduced to indicate the less mature reticulocyte fraction.\[9\] There are, however, various expressions according to the analyzer used. Some divide the reticulocytes into 3 distinct populations and others into only 2 based on RNA content; thus, the reference intervals are different and the comparison of samples analyzed with different techniques can be problematic.\[10\] The Immature Reticulocyte Fraction (IRF) represents the proportion of young reticulocytes with the highest RNA content.\[11\] Reticulocytes in automated cell counter Sysmex xt-2000i are counted using forward scatter and side fluorescence using the dye auramine O in Sysmex xt-2000i.\[12\]

The difference in staining makes possible the identification of the youngest highly fluorescent reticulocytes from the more mature low fluorescent reticulocytes based on which they are divided into Low fluorescence reticulocyte (LFR), Medium fluorescence reticulocyte (MFR) and High fluorescence reticulocyte (HFR).\[6\] The sum of Medium fluorescence ratio and High fluorescence ratio is given as the Immature reticulocyte fraction (IRF). Immature reticulocytes normally constitute less than five percent (5%) of the total number of reticulocytes.\[13\]

IRF>5% were taken as bone marrow recovery.\[14\]

The Manual reticulocyte counts enumerate all RNA stained cells, and simply lump immature and mature reticulocytes together. So that differentiating mature and immature reticulocyte manually becomes difficult which can be done by automated cell counters. Also manual reticulocyte counting is laborious and time consuming. IRF also replaces other Reticulocyte indices like absolute reticulocyte count and reticulocyte production index (RPI) which is calculated from the reticulocyte percent to see the
degree of erythropoietic activity. Also we found that IRF of 0% is highly specific in the diagnosis of aplastic anemia. Thus IRF helps in differentiating aplastic anemia from other causes of pancytopenia.

**Conclusion:**

Thus in our study we found that correlating IRF and reticulocyte count we are able to get an idea about the marrow erythropoietic activity and early marrow response to treatment before the reticulocyte count would change. It also aids in diagnosis of aplastic anemia. It can be used in follow up studies during the course of treatment as bone marrow aspiration and biopsy are invasive procedures which cannot be repeated very often.

It also helps in differentiating aplastic anemia from megaloblastic anemia which are the two most common cause of pancytopenia in India, so that treatment can be decided, where the former requires frequent red cell transfusions whereas the later do not.

The only disadvantage is the reference range of IRF differ from counter to counter depending upon the dye and method used for counting reticulocyte, which put the clinicians and pathologists into trouble in making decisions. Hence, it is important to standardize these reference ranges and more studies have to be done on these indices to make it useful at the most.

**References:**


