Absolute Mature Reticulocyte Count As An Indicator Of Red Blood Cell Production

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Abstract: The reticulocyte production index (RPI), a standard metric used in the diagnosis of anemia, is calculated using two formulas. The first formula adjusts for anemia using the patient's hemoglobin or hematocrit level to generate an expected hemoglobin/hematocrit ratio. The second formula adjusts for prematurely released reticulocytes based on the reticulocyte maturation time, as determined from the patient's hematocrit level. However, modern automated hematology analyzers provide a wide array of data in addition to reticulocyte count (calculated as a percentage), including both the absolute reticulocyte count and the immature reticulocyte fraction. Using these parameters, we developed a new indicator, the absolute mature reticulocyte count (AMRC) which we evaluated relative to RPI values. A total of 1,349 samples were tested in terms of complete blood count (CBC) and reticulocyte count using a fully automated hematology analyzer (XE-2100; Sysmex Corporation, Kobe, Japan). The AMRC, calculated as AMRC = reticulocyte count × LFR (low fluorescence ratio), was compared with the RPI, calculated based on the "continuous maturation time" (RPI-C) and "stepwise maturation time" (RPI-S). The AMRC and RPI-C, and the AMRC and RPI-S, showed strong positive correlations (r2 = 0.87 and 0.76, respectively). Although the RPI is widely used for estimating red blood cell (RBC) production in the clinical setting, AMRC was shown to correlate well with RPI. Moreover, because AMRC does not require hematocrit correction, it can be readily automated and practically applied.

Index Terms: Reticulocyte production index, AMRC, anemia, LFR, XE-2100

1 INTRODUCTION

RED cell parameters and reticulocyte indices play an essential role in the differential diagnosis and treatment of anemia [1-3]. Reticulocytes are produced in the bone marrow by the nuclear exclusion of orthochromatic normoblasts, and are released into the peripheral blood after maturation where they further differentiate into mature red blood cells (RBCs) [4]. Although the reticulocyte count in the peripheral blood reflects erythropoietic activity, this count is significantly affected by anemia status. Two measurements, i.e., the absolute reticulocyte count (ARC) and reticulocyte production index (RPI), are currently used to assess reticulocyte production and differentiate between anemia caused by bone marrow production failure and peripheral consumption [1]. The RPI is calculated using two formulas: the first adjusts for anemia based on the patient's hemoglobin or hematocrit level to generate an expected hemoglobin/hematocrit ratio, while the second adjusts for prematurely released reticulocytes based on the reticulocyte maturation time, as determined from the patient's hematocrit level. Although the RPI is important for classifying anemia, its use is limited by the difficulty of the calculation. However, modern automated hematology analyzers provide additional data to the reticulocyte count, including both the ARC and the immature reticulocyte fraction (IRF). Using these parameters, we developed a new indicator of reticulocyte production, the absolute mature reticulocyte count (AMRC). To obtain this score, correction for anemia was performed using the ARC, and correction for maturation time was done by multiplying the ARC by the low fluorescence ratio (LFR). which is used to quantify the mature reticulocyte fraction.

2 MATERIAL AND METHODS

A total of 1,349 samples were analyzed; the complete blood count (CBC) and reticulocyte count were obtained using a fully automated hematology analyzer (XE-2100; Sysmex Corporation, Kobe, Japan). The XE-2100 uses electric and optical methods to obtain the CBC. In the electric impedance method, cells are classified by size based on direct-current resistance and alternating-current capacitance. The optical method quantifies samples using a combination of forward- and side-scattered light. These methods may be combined with chemical methods [5] and the AMRC. The RPI ("stepwise maturation time", RPI-S; "continuous maturation time" RPI-C) were calculated as follows:

AMRC = Absolute reticulocyte count × LFR
RPI-S = corrected reticulocyte count × stepwise maturation time
RPI-C = corrected reticulocyte count × continuous maturation time
Stepwise maturation time = hematocrit 40~45:1.0; 35~39: 1.5; 25~34: 2.0; 15~24: 2.5; <15: 3.0
Continuous maturation time = 3.25 – (5 × hematocrit level)

Statistical analysis was conducted using R software (R Development Core Team, Vienna, Austria)

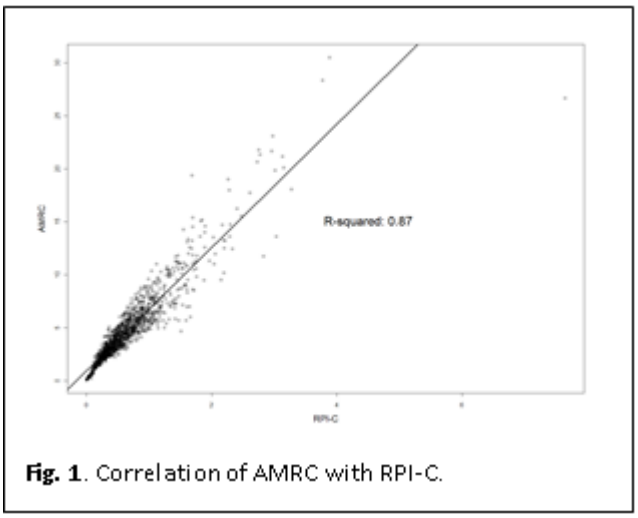

Fig. 1. Correlation of AMRC with RPI-C.

3 RESULTS

Samples were analyzed based on a combination of forward-scattered light and fluorescence signals captured using the XE-2100 instrument (Sysmex). Analyses were performed to determine the CBC, reticulocyte count, and ARC. As measures of maturity, the low-fluorescence reticulocytes (LFR), medium-fluorescence reticulocytes (MFR), and high-fluorescence reticulocytes (HFR) were also analyzed. The sum of MFR and

HFR denotes the IRF, which indicates the proportion of immature reticulocytes. The AMRC is based on the LFR, i.e., only on mature reticulocytes in peripheral blood. AMRC scores ranged from 0.05 to 30.52, with RPI-C ranging from 0 to 7.64 and RPI-S from 0 to 9.13. Strong positive correlations were seen between AMRC and RPI-C, and between AMRC and RPI-S (r2 = 0.87 and 0.76 respectively; Figs. 1 and 2).

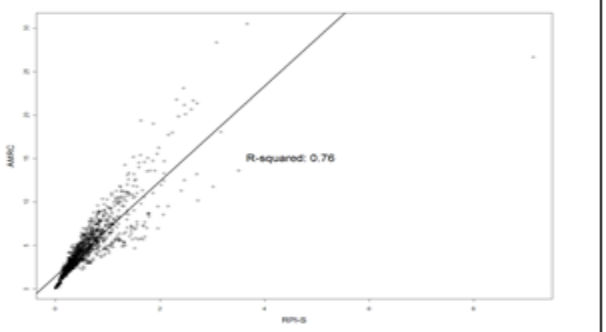

Fig. 2. Correlation of AMRC with RPI-S.

Maturation time is calculated based on hematocrit levels, decreases in which indicate continuous stepwise increases in maturation time. As LFR levels are indicative of the mature reticulocyte fraction, decreases in hematocrit levels are associated with decreases in LFR. Accordingly, it would be expected that as the maturation time increases, LFR would decrease; however, only a weak correlation was evident between LFR and maturation time (r2 = 0.13; Figs. 3 and 4).

4 DISCUSSION

The reticulocyte count, a clinically important measurement for pathophysiological classification of anemia, is used to distinguish between inadequate production of erythrocytes by the bone marrow (which results in a decreased number of reticulocytes) and excessive loss or destruction of erythrocytes (which increases the number of reticulocytes). The reticulocyte count is also used as an early marker of normalization of erythropoiesis by the marrow after therapeutic interventions, such as supplementation with iron, cobalamin, folic acid, or use of erythropoietin-stimulating agents (ESAs). Finally, the reticulocytes count may be obtained after spontaneous or pharmacologically induced aplasia of the marrow, or following bone marrow transplantation [1]. The maturation status of a reticulocyte can be determined by the RNA content. Very young reticulocytes have a dense, coherent mass of RNA and other organelles. The RNA becomes less dense with further maturation and subsequently scatters. These early reticulocytes further develop into mature RBCs after the loss of RNA [7]. The reticulocyte count is usually reported as a percentage of reticulocytes in RBCs. However, the count can be misleading when RBC values are abnormal or erythropoietic stimulation is being applied, such that additional mathematical adjustments are required [8].

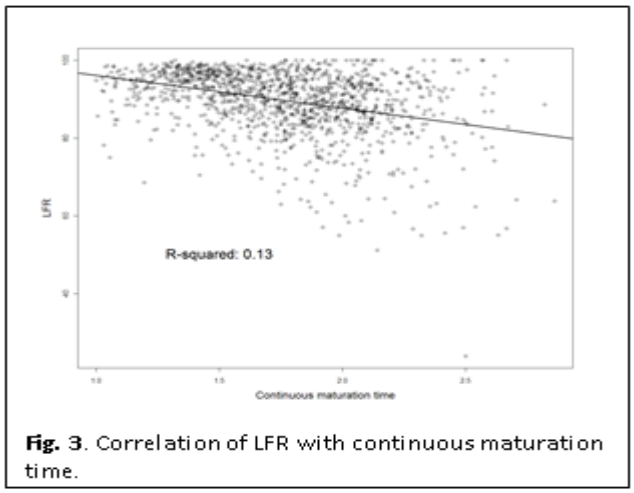

Fig. 3. Correlation of LFR with continuous maturation time.

To compensate for any decrease in mature RBCs, a combination of the reticulocyte index and ARC can be used. The reticulocyte index can be calculated as the reticulocyte count (%) × (hematocrit level / 0.45). The ARC is calculated as the reticulocyte count (%) × RBC count (RBC/l or RBC/mm3), after which a shift correction can be applied. Under intense erythropoietic stimulation, the reticulocyte maturation time in bone marrow is shortened, leading to greater stress on the underlying cells and longer maturation times in the bone marrow. The RPI corrects for this increased maturation time, and is calculated as reticulocyte index / maturation time in peripheral blood [9]. However, the manual microscopic method shows low precision (coefficient of variation [CV] between 68.6% [low concentration] and 16% [high concentration]) [10] when its use is required due to a lack of any alternative method [1].

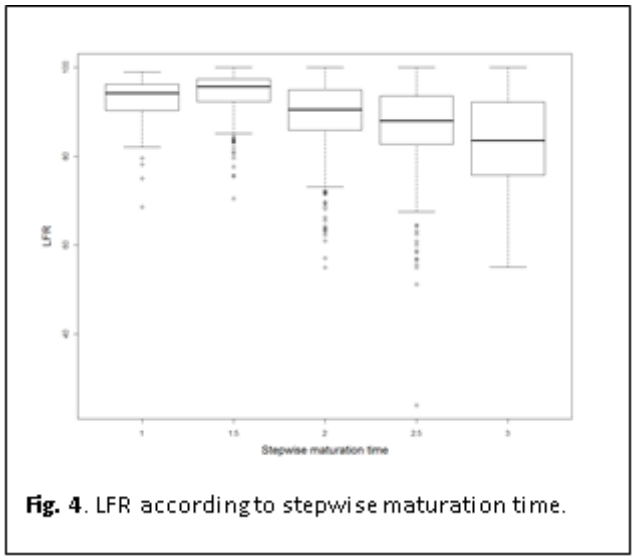

Fig. 4. LFR according to stepwise maturation time.

Starting in the mid-1990s, automated hematology analyzers began replacing traditional microscopic methods [11, 12]. An automated hematology analyzer can provide the reticulocyte count according to the total RBC count, as well as additional parameters including the ARC, IRF, and mRNA content. The maturity of reticulocytes, cell volume, hemoglobin concentration and content, and mean sphered corpuscular volume can also be calculated using automated hematology analyzers. Although more complicated than traditional metrics,

these new reticulocyte indices have proven useful in guiding diagnosis [13, 14]. IRF is among the most important parameters for assessing clinical outcomes, due to its ability to predict the fraction of younger reticulocytes, which is one of the most important markers of erythropoietic activity. For example, an increase in the IRF has been observed during recovery of bone marrow in response to erythropoiesis-stimulating agents [3, 13]. The XE-2100 instrument (Sysmex) reports the LFR, MFR, and HFR values, which are indicative of the maturity of reticulocytes; moreover, the IRF is reported as the sum of the MFR and HFR [5, 15]. The RPI adjusts for the maturation time in peripheral blood, which is itself an adjustment of the ARC. The AMRC uses the ARC to account for a decreased RBC-adjusted reticulocyte count, and the LFR to compensate for increased maturation time. The RPI can be calculated based on peripheral blood maturation time (RPI-C or RPI-S). In this study, the AMRC showed strong correlations with both the RPI-C and RPI-S ($r2 = 0.87$ and 0.76 respectively). Modern hematology analyzers are able to directly report both the reticulocyte count (%) and ARC. To calculate the RPI, hematocrit results should also be obtained. The reticulocyte count, obtained after determining the CBC, can indicate anemia. Obtaining the reticulocyte count alone, i.e., without determining the CBC or other laboratory parameters, is insufficient for calculating the RPI or reticulocyte index. Moreover, whether there is a linear relationship between the hematocrit level and reticulocyte maturation time is not clear. In the absence of CBC test results, additional studies including shift correction are needed to assess reticulocyte production. The AMRC exhibits a strong correlation with the RPI and can be calculated without data on the hematocrit level or maturation time. However, IRF (normal) reference ranges often differ considerably among methods [7], and the data may be further confounded by differences in reference intervals among the analyzers of different manufacturers [1]. Considering these limitations, in addition to the single-center design of this study and the use of only one type of analyzer, a prospective, multicenter study involving multiple analyzers and clinical outcome measures will be necessary to validate our conclusions regarding the usefulness of the AMRC.

5 CONCLUSION

The goal of the present study was to evaluate the AMRC as a newly developed hematologic index of reticulocyte production. AMRC scores are derived using a combination of the ARC and mature reticulocyte fraction, in addition to other metrics such as decreased hematocrit and increased maturation time in peripheral blood, similar to the calculation of the RPI. Our data confirm that the AMRC is strongly correlated with the RPI. Because the AMRC can be calculated without the need for hematocrit results, it can easily be adopted for use with various laboratory information systems.

ACKNOWLEDGMENT

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