

Immature reticulocyte fraction is an early predictor of bone marrow recovery post chemotherapy in patients with acute leukemia

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ABSTRACT

الأهداف: لتحديد فوائد الخلية الشبكية غير الناضجة (IRF) باستخدام عداد خلايا الدم الإلكتروني (ANC) في التنبؤ باستعادة نخاع العظم بعد العلاج الكيميائي.

الطريقة: أجريت دراسة ملاحظة استطلاعية في أقسام علم الأمراض، الطب وطب الأطفال، جامعة كبانغسان، ماليزيا، في المركز الطبي (UKMMC) خلال فترة 19 شهراً من أبريل 2009م إلى ديسمبر 2010م لتقييم مدى شفاء نخاع العظام في المرضى الذين يعانون من سرطان الدم الحاد. في هذه الدراسة تم تسجيل 22 مريضاً كانوا في مراحل خمود. جمعت عينات الدم من اليوم الأول بعد العلاج الكيميائي، وكل ثلاثة أيام حتى يتعافى المرضى الدم. تقاس جميع عينات الدم لتعداد الخلايا المتعادلة الكلي والخلية الشبكية الغير ناضجة باستخدام عداد خلايا الدم الإلكتروني (Beckman-Coulter LH750).

النتائج: تبين نسبة المرضى المتعافين من الخلية الشبكية غير ناضجة IRF أسرع من المتعافين ANC وكانت 63.3% (14 من أصل 22). كان هناك اختلاف واضح في متوسط عدد أيام التعافي الخلية الشبكية غير ناضجة IRF بالمقارنة مع تعافي ANC (14.05 و 17.18 أيام) على التوالي، $p=0.005$.

الخلاصة: أثبتت الدراسة أن الخلية الشبكية غير ناضجة IRF كانت أكثر فائدة في توقع تعافي نخاع العظم من المرضى المصابين باللوكميا الحادة بعد العلاج الطبيعي مقارنة بعدد خلايا الدم الإلكتروني ANC. لا تتأثر الخلية الشبكية الغير ناضجة عن طريق العدوى ولكنها تقاس بسهولة وغير مكلفة فهي إحدى المؤشرات الموثوقة لتقييم إعادة تشكيل نخاع العظم.

Objectives: To establish the benefits of immature reticulocyte fraction (IRF) measurement using an automated hematology cells analyzer over absolute neutrophil count (ANC) in predicting bone marrow recovery post induction chemotherapy.

Methods: A prospective observational study was carried out in the Departments of Pathology, Medicine, and Pediatrics, Universiti Kebangsaan Malaysia, Medical Center (UKMMC), Kuala Lumpur, Malaysia during a period of 19 months from April 2009 to December 2010 to assess the bone marrow recovery in patients with acute leukemia. A total of 22 patients in remission induction phases were enrolled in this study. The blood specimens were collected from day zero after chemotherapy, and every 3 days until patients recovered hematologically. All blood samples were measured for ANC and IRF using an automated hematology analyzer (Beckman-Coulter LH750).

Results: The percentage of patients showing IRF recovery earlier than ANC recovery was 63.6% (14 out of 22 patients). There was a significant difference in the mean number of days for IRF recovery as compared with ANC recovery (14.05 and 17.18 days), $p=0.005$.

Conclusion: This study proved that IRF was more useful in predicting bone marrow recovery in a patient with acute leukemia post induction chemotherapy compared with ANC. The IRF is not affected by infection, is easily measured, and inexpensive; thus, it is a reliable parameter to evaluate bone marrow reconstitution.

Saudi Med J 2014; Vol. 35 (4): 346-349

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Received 25th September 2013. Accepted 18th February 2014.

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Sepsis and anemia are the most frequent side effects of chemotherapy in patients with acute leukemia. During intensive chemotherapy, more than 10% of patients die because of treatment complications rather than progression of their underlying leukemia. An analysis of causes of death during intensive chemotherapy carried out by Creutzig et al,¹ has shown that high incidences of fatal infections were due to chemotherapy-induced severe neutropenia. Absolute neutrophil count (ANC) has been used to guide decision making on cessation of antibiotic therapy, discharge from the hospital, and resumption of chemotherapy.^{2,3} However, even though it is an easy and practical method for evaluating bone marrow recovery, the count may drop during periods of clinical or subclinical infection.³ This may provide inaccurate information of the actual bone marrow status. As reticulocytes are not affected by underlying infection, they are a better parameter for use as a marker of bone marrow recovery in neutropenic patients. It has been known from many years that the number of reticulocytes in the peripheral blood corresponds to bone marrow activity.⁴ A previous study showed an increase in immature reticulocytes to be the first sign of hematopoietic recovery.⁵ Other researchers also used the same parameters to assess recovery in a group of autologous transplanted patients.⁶ They observed that reticulocyte parameters rose earlier than neutrophil count. Immature reticulocytes are released into the peripheral blood during periods of intense erythropoietic stimulation, such as hemorrhage, certain anemias, or in response to therapy designed to stimulate bone marrow production.⁶ The immature reticulocyte fraction (IRF) may be termed the red cell equivalent of the "left shift" typically associated with neutrophilic white cells, providing additional red cell information that may shorten the time from diagnosis to therapy or shorten therapy itself. The literature suggests that the IRF, in combination with the reticulocyte count, might be useful in improving the classification of anemias, monitoring bone marrow recovery, and monitoring anemia therapies. A few studies^{5,7-9} utilizing flow cytometry in the measurement of reticulocyte parameters showed that most of the cases had earlier IRF recovery compared with ANC. These studies showed that IRF could be a better parameter of bone marrow regeneration than ANC since the parameters were not influenced by infection, and can therefore be used to

assess bone marrow recovery status especially in patients with persistent neutropenia. The aim of this study was to evaluate the usefulness of IRF compared with ANC in predicting bone marrow recovery in patients with acute leukemia after received chemotherapy.

Methods. This prospective observational study was carried out in the Universiti Kebangsaan Malaysia (UKMMC), Kuala Lumpur, Malaysia between April 2009 and December 2010. Twenty-two patients with newly diagnosed acute leukemia and who were receiving treatment at this center were recruited into the study. Out of these patients, 12 (54.5%) were acute lymphoblastic leukemia (ALL) cases, and another 10 (45.5%) were acute myeloid leukemia (AML) patients. All subjects signed informed consent forms. This study was approved by the Institutional Human Research Ethical Committee and was carried out in accordance with Helsinki Declaration.

Acute leukemia patients received the standard chemotherapy protocol for ALL or AML that has been used in UKMMC. Peripheral blood samples (3 ml) were collected in EDTA tubes starting on day zero of chemotherapy and every 3 days until the patients recovered hematologically. All blood samples were measured for full blood count and IRF using an automated hematology analyzer (Beckman-Coulter LH750, City, State, USA) in the Hematology Unit, Department of Laboratory Diagnostic Services, UKMMC. The measurements were carried out within 2 hours of collection.

Automated reticulocyte analysis is based on flow cytometry combined with hydrodynamic focusing. Cells were identified by simultaneous 3-dimensional analysis using Coulter VCS technology (volume, conductivity, and light scatter). The Coulter procedure uses new methylene blue stain to precipitate residual RNA within erythrocytes. Erythrocytes containing residual RNA scatter more light than the mature red cells and young reticulocytes contain more stain, and scatter more light, than older reticulocytes. The Coulter Maturity Index (MI) parameter expresses the number of early reticulocytes as a proportion of the total reticulocyte count, which was equivalent with IRF. The ANC was calculated from total leucocyte count and differential count of white blood cells (WBC). Serial hemogram with IRF and ANC was carried out every 3 days until patients recovered hematologically. Day zero was taken on the day before administration of chemotherapy. The mean values for IRF and ANC were calculated for each category according to the day of blood sampling. The day of recovery was selected based on the day with a sudden significant increase in the ANC and IRF value.⁹

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company.

Percentages of early, late, or equal recovery using both measurements were also calculated.

Analysis was carried out using the Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA) version 18.¹⁰ Differences in the mean number of days for IRF recovery were compared to ANC recovery using paired Student t-test. Statistical significance was taken the level of $p < 0.05$.

Results. During the study period, 27 patients with newly diagnosed acute leukemia were recruited. However, 5 patients succumbed due to their malignancy. The data were analyzed based on the remaining 22 living patients who were available for follow up. The mean age was 20.1 years, ranging from 3 months to 73 years. There were 12 female (54.5%) patients, and 10 male (45.5%) patients. According to ethnicity, they were 14 Malays (63.6%), 5 Chinese (22.7%), and 3 Indians (13.6%). There were 12 patients (54.5%) diagnosed as ALL, and another 10 (45.5%) were AML. Serial measurements of ANC were carried out on these 22 patients blood samples starting from day zero (before starting chemotherapy) and then every 3 days. These measurements showed a mean ANC of $18.5 \times 10^3/\mu\text{L}$ (± 24.5) at day zero, and then a severe drop started on day 3 with a mean of $0.95 \times 10^3/\mu\text{L}$ (± 0.92). The ANC reached its lowest measurement on day 18 with a mean of $0.05 \times 10^3/\mu\text{L}$ (± 0.07), and then started to increase on day 21 with a mean of $0.1 \times 10^3/\mu\text{L}$ (± 0.0) (Figure 1). Concurrently, reticulocyte parameters were measured, which included reticulocyte percentage, absolute reticulocyte count, mean reticulocyte volume,

and IRF. At day zero the mean was 0.30 ± 0.01 with a slight increase on day 3 0.33 ± 0.1 , and started to drop reaching the lowest measurement at day 12 with a mean of 0.10 ± 0.10 , and then started to increase at day 15 with a mean of 0.15 ± 0.00 (Figure 2). These result showed that the recovery of IRF is earlier than ANC. Out of these 22 cases, 14 patients (63.6%) showed earlier recovery of IRF compared with ANC, 5 (22.7%) patients showed late recovery of IRF, and 3 (13.6%) showed that they both recovered at the same time. There was a significant difference in the mean number of days for IRF recovery as compared with ANC recovery (14.05 and 17.18 days, mean difference 4.09, SD 6.108, $p = 0.005$). These indicated that measurement of IRF is better than ANC in predicting of bone marrow recovery.

Discussion. Absolute neutrophil count is the usual target criterion in decisions regarding cessation of antibiotic therapy, discharge from hospital, and resumption of chemotherapy because it saves time and money.² Though it is an easy and practical method for evaluating recovering bone marrow function, it may drop during periods of clinical or subclinical infection,³ and this may give a false impression of the actual bone marrow status, thus, ANC might not be useful in predicting bone marrow recovery.

Reticulocytes have come a long way from being a morphologic and staining curiosity to an exceedingly important and widely used parameter for assessing the adequacy of the erythropoietic response.⁶ Flow cytometric reticulocyte analysis is more precise and more sensitive than manual reticulocyte counting.¹¹

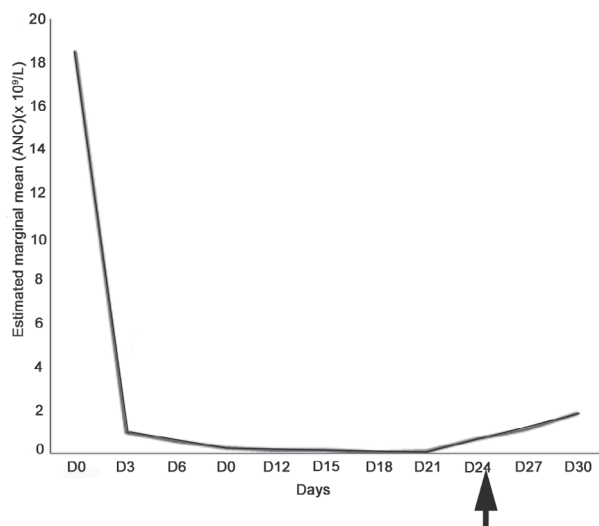


Figure 1 - Estimated marginal means for patients after serial measurement of absolute neutrophil count (ANC). Arrow indicated the recovery day (n=22).

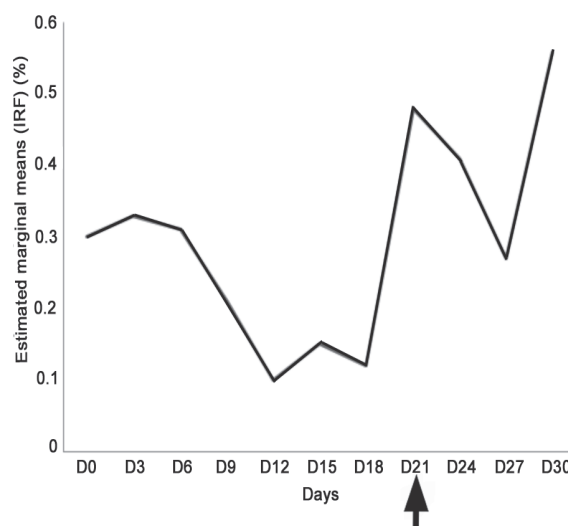


Figure 2 - Estimated marginal means for patients after serial measurement of immature reticulocyte fraction (ITF). Arrow indicated the recovery day (n=22).

The automated flow cytometric analysis of reticulocytes increased the scope of using absolute reticulocyte and the intensity of fluorescence signals to quantitate different fractions to be used in various situations.⁴ The reticulocyte maturity index (RMI) was used as an independent parameter for assessing erythropoiesis.⁵ Reticulocytes are not influenced by infections and therefore would be a better parameter of bone marrow regeneration in patients with persistent neutropenia to guide the modulation of antibiotic strategies in these patients.^{3,7} Das et al,⁷ emphasized that when younger reticulocytes are detected, it is a better indication of recovery. Graziutti et al,⁶ also pointed out that those immature reticulocytes are the most suitable marker to assess erythroid regeneration since other erythroid regeneration markers such as standard reticulocyte percentage and absolute count show fluctuations due to red cells transfusion. The presence of immature reticulocytes means that there is a resumption of erythropoiesis after marrow ablation.

This study aimed to determine the reticulocyte parameters, especially IRF measurement using a Beckman-Coulter LH750 hematology analyzer in newly diagnosed patients with acute leukemia, and compare this with ANC in predicting bone marrow recovery post induction chemotherapy. In this study, we found that IRF showed earlier overall recovery compared with ANC. The paired Student t-test also showed that IRF can be used to predict bone marrow recovery. Our findings were in agreement with past research studies.^{6,8} Graziutti et al,⁶ also used the same parameters to assess a group of autologous transplanted patients, and they found similar results. Their observation showed that there was an earlier increase in reticulocyte parameters compared with neutrophil count. They agreed that any determined reticulocyte parameter can reliably measure this fraction. Das et al,⁷ also used similar methods to look for recovery of pediatric hematology malignancy patients post chemotherapy, and their findings showed that IRF had earlier recovery compared with ANC.

Flow cytometric reticulocyte analysis allows measurement of reticulocytes based on maturity. The RMI or percentage of highly fluorescent reticulocytes (immature reticulocyte fraction) has been shown to be an early predictor of the regenerative activity of the bone marrow, and most of the studies have used a flow cytometer dedicated to automatic reticulocyte analysis, utilizing a fluorescent dye.^{3,5,6,8-10} Buttarello et al⁹ showed that the fluorescence based methods seem to be better than other methods in IRF measurement. Flow cytometry is an expensive and time consuming laboratory method. However, since the differentiation

of reticulocyte maturity does not require monoclonal antibodies, the incubation time of the sample is shorter, and the analysis can be incorporated together with other blood cell parameters using an automated blood cell counter, the cost of IRF analysis can be reduced, and the measurement can be carried out as frequent as needed by the clinician.

In summary, this study has established the superiority of IRF compared with ANC in predicting bone marrow recovery in patients with acute leukemia post induction chemotherapy. Although the sample size was small, this study showed significant findings.

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