Immature reticulocyte fraction (IRF) – an universal marker of hemopoiesis in children with cancer?

Łuczyński W[1], Ratomski K[2], Wysocka J[2], Krawczuk-Rybak M[1], Jankiewicz J[3]*

[1] Department of Pediatric Oncology, Medical University of Białystok, Poland
[2] Department of Laboratory Pediatric Diagnostics, Medical University of Białystok, Poland
[3] Students Scientific Section at Department of Pediatric Oncology, Medical University of Białystok, Poland

Abstract

Purpose: Anemia is one of the most frequent side effects of anticancer treatment, it is also caused by disease itself. Reliable laboratory tests indicating hematological recovery after chemo- and radiotherapy are needed. Effective erythropoiesis can be monitored by quantitative measurement of reticulocytes. The amount of RNA in these cells can be assessed with flow cytometry and divided into low- (LFR), middle- (MFR) and high-fluorescence reticulocytes (HFR). This distribution is correlated with their maturation.

Material and methods: The aim of our study was to find the most sensitive indicator of anaemia among reticulocyte subpopulations assesed by flow cytometry in children with cancer. 46 children with different neoplasmatic diseases were enrolled into the study.

Results: 1) we did not find any differences between control and examined group at the time of diagnosis except for IRF, which was higher in examined group ($p=0.001$); 2) IRF was lower already 2-4 days after end of chemotherapy ($p=0.03$), and rised before next regimen ($p=0.0006$).

Conclusions: In conclusion we showed that IRF is not only the first sign of hematologic recovery but also very strong indicator of postchemotherapy aplasia in children with cancer and may serve as a additional parameter of bone marrow function in clinical studies.

Key words: anaemia, reticulocytes, IRF, flow cytometry, cancer, children.

* CORRESPONDING AUTHOR:
Department of Pediatric Oncology
Medical University of Białystok
ul. Waszyngtona 17, 15-274 Białystok, Poland
Tel/fax: +48 85 7450846
e-mail: w.luczynski@wp.pl (Włodzimierz Łuczyński)

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Introduction

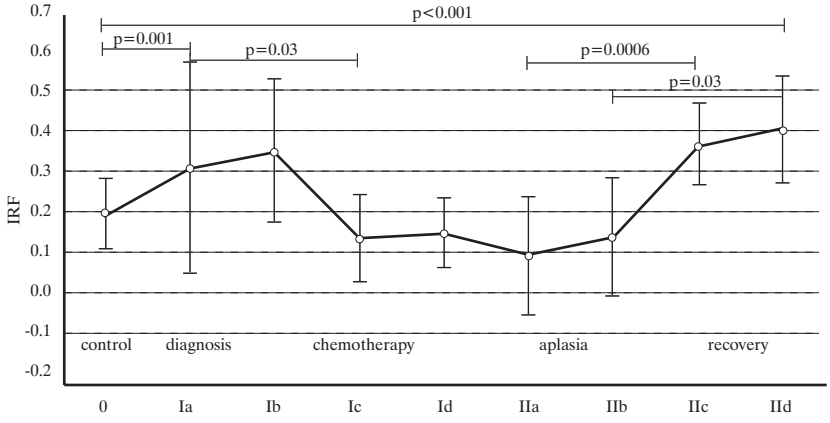
Anemia is one of the most frequent side effects of anticancer treatment, it is also caused by disease itself. Reliable laboratory tests indicating hematological recovery after chemo- and radiotherapy are needed. Effective erythropoiesis can be monitored by quantitative measurement of reticulocytes [1,2]. The amount of RNA in these cells can be assessed with flow cytometry and divided into low- (LFR), middle- (MFR) and high-fluorescence reticulocytes (HFR). This distribution is correlated with their maturation. HFR fraction represents the most immature reticulocytes. The immature reticulocyte fraction (IRF) is the sum of HFR and MFR fractions. The usefulness of flow cytometric analysis of reticulocytes, as a predictor of engraftment in autologous bone marrow transplantation and for granulocyte recovery after polychemotherapy in leukaemic patients was reported in many studies. Earlier studies suggested HFR as a strongest predictor of hematological recovery, others – IRF. The aim of our study was to find the most sensitive indicator of anaemia among reticulocyte subpopulations assesed by flow cytometry in children with cancer.

Material and methods

46 children with different neoplasmatic diseases were enrolled into the study. Children were suffering from leukaemias, lymphomas and solid tumors. The control group consisted of 40 children subjected to Department of Pediatric Surgery for minor operations with no sign of anaemia.

Peripheral blood was taken according to physicans' orders for blood count. Resting material was used for flow cytometric assessment of reticulocytes. In total over 500 specimens were assessed. Obtained results were divided into 9 groups: control (0), time of diagnosis (Ia), 1st day after chemotherapy (Ib), 2-4 days after chemotherapy (Ic), 5-9 days after chemotherapy (Id), children admitted to the hospital in aplasia: at the beginning of hospitalisation (IIa) – WBC (white blood cells)

Figure 1. **IRF in control and examined group in consecutive stages of therapy**



<1.0 x 10^3/μl, nadir – deep aplasia (IIb) – WBC<0.5 x 10^3/μl, neutrophils <0.2 x 10^3/μl, at the beginning of recovery (IIc) – WBC>1.0 x 10^3/μl, before next chemotherapy (IId).

Reticulocytes and their subpopulations were automatically analysed by flow cytometry as described earlier [3]. Samples of venous blood containing EDTA were analysed within 4 hours using Epics XL (Coulter flow cytometer). Preparation procedure included staining with thiazole orange. Data were processed using Excel' 97 and Statistica 6.0. Results are showed as mean and percentiles. Significance levels were calculated according to the nonparametric Mann-Whitney U-test (difference between the control group – 0 and consecutive stages of therapy Ia-IId) and the Spearman correlation coefficient (correlation between all assessed parameters). Level of $p<0.05$ was regarded as significant.

Results

1) We did not find any differences between control and examined group at the time of diagnosis except for IRF, which was higher in examined group ($p=0.001$).

2) Total percentage of reticulocytes was lower in aplasia (IIa) comparing to the time of diagnosis (Ia) ($p=0.02$), but not directly after chemotherapy (Ib, Ic) and rised before next regimen (IId) ($p=0.03$); very similar tendency was observed in reticulocyte count, HFR count, MFR, LFR count and percentages;

3) We found the differences in IRF in following groups (*Fig. 1*): control group vs time of diagnosis in examined group ($p=0.001$), IRF was lower already 2-4 days after the end of chemotherapy ($p=0.03$), and rised before next regimen ($p=0.0006$);

4) We noted many correlations between all assessed parameters but here we mention only strong ones (positive, $r>0.5$, $p<0.00001$):

- between Hb or E and: number of reticulocytes, number of LFR,
- between WBC and: percentage and number of reticulocytes, MFR, LFR and IRF
- between PLT and: percentage and number of reticulocytes, MFR, LFR, IRF.

Discussion

It is known from many years that number of reticulocytes in the peripheral blood corresponds with bone marrow activity [4]. Our results concerning IRF are similar to those obtained by Spanish Multicentric Study Group for Hematopoietic Recovery: a rising IRF was the first sign of hematopoietic recovery [5]. Kabata et al. also found that HFR + MFR increase was predictive for hematological recovery but limited to bone marrow transplantation procedures [6]. In authors' opinion regeneration of reticulocytes was observed after leucocyte recovery. Torres et al., used flow cytometry to assess same parameters as in our study, but in the group of allo- and autologous transplanted patients. The authors observed earlier increase in reticulocyte parameters than rise in neutrophil count [7]. They conclude that any determined reticulocyte parameter can reliably measure this fraction, but the most useful are mean fluorescence index (MFI) and mean reticulocyte volume (MRV). On the other hand in Kuse's et al. opinion MFR (middle fluorescence fraction) and HFR may serve as indicators of bone marrow recovery after chemotherapy [8]. George et al. found that HFR preceded neutrophil recovery, so in their opinion HFR can be used as an earlier indicator of engraftment after stem cell transplantation [9].

Buttarello et al. provided a very interesting comparison in IRF measurement with 5 different automated counters [1]. In their opinion even with slight differences, the fluorescence-based methods seem to be more robust than other methods for IRF measurement.

Flow cytometry is considered as an expensive and sometimes time consuming laboratory method but differentiation of reticulocytes does not require monoclonal antibodies and assessment takes about 1.5 hours (including 1h of incubation). It is also thruth that reticulocyte count is stable after storage for 48 or even 72 hours, but IRF parameters are stable only for 8 hours [10,11].

The monitoring of bone marrow regeneration after chemo- and radiotherapy is also important because of a high risk of infection, which increases with time of aplasia [6]. We have earlier showed a weak correlation between IRF and other parameters of hemopoiesis, same results were obtained in this experiment (no correlation between E or Hb and IRF), so we suggest its independent role as a marker of erythropoietic activity [3]. In conclusion we demonstrate that IRF is not only the first sign of hematologic recovery but also very strong indicator of postchemotherapy aplasia in children with cancer and may serve as a additional parameter of bone marrow function in clinical studies.

References

1. Buttarello M, Bulian P, Farina G, Temporin V, Toffolo L, Trabuio E, Rizzotti P. Flow cytometric reticulocyte counting. Parallel evaluation of five fully automated analyzers: an NCCLS-ICSH approach. Am J Clin Pathol, 2001; 115: 100-11.

2. Choi JW, Pai SH. Reticulocyte subpopulations and reticulocyte maturity index (RMI) rise as body iron status falls. Am J Hematol, 2001; 67: 130-5.

3. Turowski D, Wysocka J, Butkiewicz AM. Peripheral blood reticulocytes and their reference range values for percentage, absolute count, and immature fraction in children, measured with flow cytometry. Folia Histochem Cytobiol, 2000; 38: 31-6.

4. Riley RS, Ben-Ezra JM, Tidwell A, Romagnoli G. Reticulocyte analysis by flow cytometry and other techniques. Hematol Oncol Clin North Am, 2002; 16: 373-420, vii.

5. Spanish Multicentric Study Group for Hematopoietic Recovery. Flow cytometric reticulocyte quantification in the evaluation of hematologic recovery. Eur J Haematol, 1994; 53: 293-7.

6. Kabata J, Tichelli A, Gratwohl A, Speck B. Flow cytometric pattern of leucocyte recovery after therapy induced aplasia. Acta Hematol Pol, 1994; 25: 329-42.

7. Torres A, Sanchez J, Lakomsky D, Serrano J, Alvarez MA, Martin C, Valls C, Nevado L, Rodriguez A, Casano J, Martinez F, Gomez P. Assessment of hematologic progenitor engraftment by complete reticulocyte maturation parameters after autologous and allogeneic hematopoietic stem cell transplantation. Haematologica, 2001; 86: 24-9.

8. Kuse R, Foures C, Jou JM, d'Onofrio G, Paterakis G. Automated reticulocyte counting for monitoring patients on chemotherapy for acute leukaemias and malignant lymphomas. Clin Lab Haematol, 1996; 18 (Suppl. 1): 39-43.

9. George P, Wyre RM, Bruty SJ, Sweetenham JW, Duncombe AS. Automated immature reticulocyte counts are early markers of engraftment. J Hematother Stemm Cell Res, 2000; 9: 219-23.

10. Lacombe F, Lacoste L, Vial JP, Briais A, Reiffers J, Boisseau MR, Bernard P. Automated reticulocyte counting and immature reticulocyte fraction measurement. Comparison of ABX PENTRA 120 Retic, Sysmex R-2000, flow cytometry, and manual counts. Am J Clin Pathol, 1999; 112: 677-86.

11. Peng L, Yang H, Jiang H, Su J, Peng Z. Automated reticulocyte counting using the Sysmex RAM-1. Clin Lab Haematol, 2001; 23: 97-102.