ORIGINAL RESEARCH PAPER

Volume-7 | Issue-10 | October-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EMERGING ROLE OF NEWER HEMATOLOGICAL PARAMETERS FOR DIAGNOSIS AND RECUPERATION IN POST HEMATOPOIETIC STEM CELL TRANSPLANTATION

Pathology

Gahlot Gaurav PS MD, Assistant Professor, Dept of Lab & Mol Med, Army Hospital (Research &Referral) Delhi, India

Pandey Pankaj MBBS, Junior Resident, Dept of Lab Sciences & Mol Med, Army Hospital (Research &Referral) Delhi, India

Badwal Sonia MD, Professor & Head of Department Dept of Lab & Mol Med, Army Hospital (Research &Referral) Delhi, India

Ahuja Ankur* MD, DM, Assistant Professor, Dept of Lab & Mol Med, Army Hospital (Research &Referral) Delhi, India *Correspnding Author

Beenu Singh DLO, Pursuing Secondary DNB at Medanta-The Medicity Gurgaon Haryana

ABSTRACT

BACKGROUND: The present study will emphasize the critical role of new hematological parameters generated by Automated Cell Counters (ACCs) along with traditional parameters of the complete blood cell counts required for diagnosis and follow up cases especially in the setting of Pre & Post Hematopoietic Stem Cell Transplant (HSCT)

AIMS: To analyze the clinical applications of newer hematological parameters provided by ACCs for expanding their diagnostic potential beyond conventional blood cell counts

STUDY DESIGN: This is a cross sectional observational study of results obtained from EDTA blood samples of 40 patients of Pre & Post HSCT over a period of 18 months

SUBJECTS & METHODS: Blood samples were collected in EDTA vacutainer and processed through ACCs i.e. ADVIAR 2120i and SYSMEX XT- 4000i. The peripheral blood film examination in each case was performed on Leishman-Geimsa stained smear.

STATISTICAL ANALYSIS USED: The hematological parameters obtained from ACCs/microscopic were assessed by Student's t-test, chi-square test using the SPSS 21.0 software with considering p value < 0.05 as significant

RESULTS: Newer reticulocyte indices like Immature reticulocyte fraction and Mean reticulocyte volume found to be significantly raised in pre & post HSCT cases with p value <0.05.

KEYWORDS

Automated Cell Counters (ACCs), Hematopoietic Stem Cell Transplant (HSCT), Reticulocyte indices, Immature Reticulocyte fraction (IRF), Mean Reticulocyte Volume (MCVr),

INTRODUCTION

Over the years with the advancement in technology, the components of the Complete Blood Counts (CBCs) have expanded beyond the estimation of Red Blood Cells (RBCs), Hemoglobin (Hb) concentration, Mean Corpuscular Volume (MCV), White Blood Cells (WBCs) and Platelet count etc. The newer ACCs like Advia®2120i and Sysmex XT-4000i additionally provide parameters like Nucleated Red Blood Cells (NRBCs), Immature Reticulocyte Fraction (IRF), Mean Reticulocyte Volume (MCVr) and Mean Reticulocyte Hemoglobin Content (CHr) etc. These recent parameters have an important role in early diagnosis of disease and monitoring response to treatment especially in anemia, hematopoietic stem cell transplant (HSCT) & hematolymphoid malignancies. The newer instruments also provide numerous flagging systems for any abnormal cells, in built quality control programs and automated maintenance etc.

In 1896, method of blood cells counting in a test tube filled with diluted blood was evolved by George Oliver that can be considered as the forerunner of automated blood count.[1] In 1940s, Wallace Coulter observed an increase in electrical impedance by the particles and developed a simplified blood cell analysis tool for rapid screening of large number of blood samples.[2] In 1973 George & Groner evolved light scattering technique of flow cytometer.[3] Therefore this progressive improvement in these instruments has allowed enumeration and evaluation of blood cells with great accuracy, precision and speed at low cost.

The reticulocyte count (immature non-nucleated RBC) is a vital indicator of effective erythropoiesis.[4] In healthy individuals, reticulocytes circulate in the peripheral blood for 1-2 days before they lose sufficient RNA to become mature red blood cells. In case of increased erythropoietic demand, reticulocyte life span in peripheral blood increases to >3 days, due to premature release of immature reticulocytes from the bone marrow. On the basis of fluorescence intensity signals, reticulocytes are classified into three maturation stages: low fluorescence reticulocytes (LFRs), medium-fluorescence reticulocytes (MFRs), and high-fluorescence reticulocytes (HFRs). IRF is the combination of HFRs & MFRs and its value of >5% is a reliable marker for hemopoietic recovery, but is limited to bone marrow transplantation (BMT) procedures.[5,6] The recent development of automated (rather than manual) reticulocyte counts has permitted more precise, quantitative counting of IRF (ratio of immature reticulocytes to the total number of reticulocytes). Serial determination of IRF after BMT can be used to demonstrate successful engraftment. In most cases; a rise in the IRF even before absolute neutrophil count (ANC) has been observed.[7,8] An increase in IRF of >20% from the post BMT value suggests successful erythroid engraftment. Thus IRF constitutes as a very early, sensitive index of marrow erythropoietic activity and can be used with the reticulocyte count to follow up the response of HSCT. Reticulocyte Production Index (RPI) gives a snapshot of the functional iron available for incorporation into hemoglobin within RBCs over the previous 3-4 days. An increased RPI of >3 can be seen hemolytic anemias, recent hemorrhage and marrow response to therapy.[9] Other reticulocyte cellular indices such as MCVr (mean reticulocyte volume), CHr (reticulocyte hemoglobin content), CHCMr (reticulocyte hemoglobin concentration) generated by Advia®2120i further allows the assessment of functional state of the erythropoiesis in the diagnosis and monitoring of post HSCT.

MATERIAL AND METHOD

This cross sectional observational prospective study was conducted at a "Tertiary Care Super-Speciality Hospital" of North India in the Department of Pathology from Oct 2015 to Mar 2017. Forty pre & post-HSCT samples of such patients were analyzed to access the role of various traditional and recent hematological parameters in diagnosis and follow up of HSCT. The relevant clinical data was accrued from the details available in the blood collection centre/wards/concerned departments. Blood samples of the selected patient were collected under aseptic precautions in EDTA vacutainer and processed within two hrs of collection through any of the two new generation hematology analyzers i.e. ADVIA®2120i or SYSMEX XT-4000i with commercial quality control specimens. The peripheral blood film examination in each case was performed on Leishman-Geimsa stained smear by two experienced technologists and the results

were confirmed by the hematopathologist. The association between hematological, morphological and clinical features were tested using Student's t-test for continuous variables and the chi-square test for qualitative variables. All statistical analyses was performed using the SPSS 21.0 software considering p value <0.05 as significant.

RESULTS

In addition to conventional hematological parameters; newer ones like immature granulocytes (myelocytes, metamyelocytes & bands) and reticulocyte indices as IRF, CHr, MCVr and CHCMr were also analyzed by Advia^{R} 2120i at pre HSCT and 28th day of Post HSCT in 40 such cases. Among these parameters, statistically significant increased values of IRF (1.818 to 12.423) and MCVr (89.890fL to 98.534fL) at pre HSCT and after 28 days of HSCT were observed (p value<.05). Reference range of MCVr 95.1fL to109.6fL and cut off value of IRF as <4.1% were considered. [Figure 1]

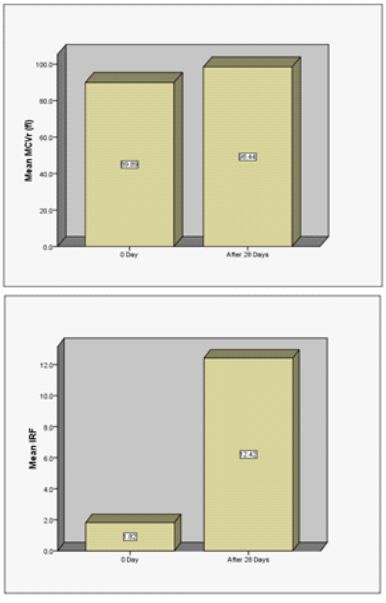

Figure 1 MCVr and IRF are increased in post transplant cases after 28 days. (Advia^{R} 2120i)

DISCUSSION

The recent development of ACC like AdviaR 2120i led to more precise counting of reticulocytes variables which are very early index of marrow erythropoietic activity. This study was performed to establish the role of newer hematological parameters in diagnosis and follow up of 40 HSCT cases. Our results of IRF increase and its use as indicator of engraftment after stem cell transplantation were in concordance with studies of Kabata et al.[5] and George et al.[7,8,10,11] In addition, role of increased MCVr as to measure hematopoietic recovery in HSCT was supported by Torres et al[12]

CONCLUSION

IRF constitutes as an inexpensive, non-invasive, objective, first sign, early and sensitive index of marrow erythropoietic activity and can be used with the reticulocyte count to monitor the response of HSCT.[13] Our study concluded that IRF and MCVr showed ten times and approximately 10% increase respectively at pre and 28th day post HSCT. Despite the essential role of automation, microscopic correlation of pathologic samples remains essential.

Informed consent: A written consent in the language the patients understands was taken from all the subjects being enrolled after explaining the objectives and benefits of the study to them.

Ethical clearance: The study was then undertaken after due approval of the hospital ethics committee.

Funding resources: No funding was obtained from any external source.

REFERENCES

1. Oliver G. A contribution to the study of the blood and the circulation. Lancet. 1896;147:1541-47.
2. Coulter WH. Means for counting particles suspended in a fluid. 1953;10:508-13.
3. George W, Groner W. Method and apparatus for analysis of leukocytes using light scattered by each leukocyte at absorbing and non-absorbing wavelength. 1973;2:336-46.
4. Advani SH, Giri NK. Acute lymphoblastic leukemia in childhood : treatment, results and prognostic factors. Indian journal cancer. 1989;26:180-8.
5. Kabata J, Tichelli A, Gratwohl A, Speck B. Flow cytometric pat¬tern of leucocyte recovery after therapy induced aplasia. Acta Hematol Pol. 1994;25:329-42.
6. Kuse R. The appearance of reticulocytes with medium or high RNA content is a sensitive indicator of beginning granulocyte recovery after aplasiogenic cytostatic drug therapy in patients with AML. Ann Hematol. 1993;66:213-14.
7. Kuse R, Foures C, Jou JM, d'Onofrio G, Paterakis G. Automatedreticulocyte counting for monitoring patients on chemotherapy for acuteleukaemias and malignant lymphomas. Clin Lab Haematol. 1996;18:39-43.
8. Fisher CP, V Spanish. Flow cytometric reticulocyte quantification in the evaluation of hemato¬logic recovery. Eur J Haematol. 1994;53:293-7.
9. Brugnara C, Schiller B, Moran J. Reticulocyte hemoglobin equivalent (Ret He) and assessment of iron-deficient states. Clin Lab Haematol. 2006;5:303-8.
10. George P, Wyre RM, Bruty SJ, Sweetenham JW, Duncombe AS. Automated immature reticulocyte counts are early markers of engraft-ment. J Hematother Stemm Cell Res.2000;9:219-23.
11. 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;18:39-43.
12. Torres A, Sanchez J, Lakomsky D, Serrano J, Alvarez MA,Martin C, Valls C, Nevado L, Rodriguez A, Casano J, Martinez F, GomezP. Assessment of hematologic progenitor engraftment by complete reticulocyte maturation parameters after autologous and allogeneic hematopoietic stem cell transplantation. Haematologica. 2001;86:24-98.
13. Łuczyński W, Ratomski K, Wysocka J , Krawczuk-Rybak M , Jankiewicz J. Immature reticulocyte fraction (IRF) – an universal markerof hemopoiesis in children with cancer? Eur J Haematol. 1994; 53: 293-7.