Post Chemotherapy Bone Marrow Recovery in Acute Leukaemias- Comparison of Immature Reticulocyte Fraction with Absolute Neutrophil Count

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Abstract
Background: To compare Immature Reticulocyte Fraction (IRF) with Absolute Neutrophil Count (ANC) in post chemotherapy acute leukaemia patients to assess marrow recovery.

Methods: In this comparative study, 45 diagnosed cases of AML and ALL on chemotherapy for remission induction/consolidation, between age group of 2-60 years were included. Complete blood count (CBC) was done. Immature reticulocyte fraction was done by using automated hematology analyzer Sysmex XT 2000i. Absolute neutrophil count was calculated from total leucocyte count and differential count of white blood cells. True positive and true negative patients, showing recovery on the basis of IRF and ANC were calculated. Ch- square test was used. P-value <0.05 was considered statistically significant.

Results: Age ranged from 3-39 years while the mean age was 15 ± 11 years. Nineteen (42%) patients were male and 26 (58%) patients were female. The mean recovery day for IRF was 11.7 ± 7.1, while that for ANC was 17.5 ± 9.32. Out of 45 patients, IRF predicted recovery in 41 patients and was unable to show recovery in 4 patients. Similarly ANC predicted recovery in 36 patients, but was unable to predict recovery in 9 patients. In 34 patients, both ANC and IRF showed recovery. So the True positive patients were 34 (75%). In these 34 patients, IRF was early predictor in 24 (70.5%) cases and ANC in 10 (29.5%) cases. Similarly patients who had not shown recovery both by IRF and ANC were 4. So the True negative patients were 4 (8.8%). Patients who were negative on IRF were 4. Similarly total patients positive on ANC were 36 and patients who were negative on ANC were 9. Patients showing recovery both by IRF and ANC were 34 out of 45.

Conclusion: IRF can predict bone marrow recovery earlier than ANC count. IRF can serve as an additional parameter in patients who are in need of evaluation of bone marrow recovery after chemotherapy.

Key Words: Acute leukemia, Immature Reticulocyte Fraction, IRF, Absolute Neutrophil Count, ANC, Bone marrow recovery.

Introduction
Acute leukemia is a malignant disorder in which neoplastic white cells arising from haemopoietic stem cell infiltrate blood and bone marrow.1 It has been shown that in 100,000 newly diagnosed cases of acute myeloid leukaemia (AML) is about 2.5% and acute lymphoblastic leukaemia (ALL) is about 1.3%.2 A study has shown that among the malignant haematological disorders AML was common (27.69%) followed by ALL (9.04%).3 Intensive chemotherapy is the treatment option which is available, that can be combined with stem cell transplantation. This treatment however is followed by a period of severe bone marrow failure in which patients have low blood counts.4 This is the most critical period because of risk of infection and bleeding in these patients.

Reticulocytes are immature red cell. Reticulocyte count can be used to assess erythropoiesis or haematopoietic recovery after chemotherapy. Microscopic quantification of reticulocytes has now been replaced by automated flow cytometric counters.5 These counters can also measure immature reticulocyte fraction (IRF) which indicates the less mature reticulocytes population.6 Absolute neutrophil count (ANC) is used for monitoring chemotherapy and it has been shown that this parameter has sensitivity >96%. Present practice is to assess bone marrow recovery after chemotherapy through monitoring of absolute neutrophil count, but it has been shown that IRF is more reliable and can provide this information earlier. According to a study performed to assess bone marrow recovery after chemotherapy IRF did show early bone marrow recovery in 78% cases as compared to ANC. Another study has shown that IRF has a sensitivity of 97%.9 Haematology analyzers are now equipped to perform a reticulocyte count which has IRF as an important adjunct. IRF is now considered as an important parameter of clinical utility because its
increase occurs before an increase in total reticulocyte count and absolute neutrophil count, so it can be used to monitor bone marrow recovery after transplant or chemotherapy.10

Patients and Methods
This comparative study was performed at Haematology department of Fauji Foundation Hospital, Rawalpindi, from February 2013 to August 2013. According to WHO sample size calculator, Calculations Confidence level was taken 95%, anticipated population proportion was taken 97% and absolute precision required was 8%. Sample size (n) was approximately 40 patients. Diagnosed cases of AML and ALL on chemotherapy for remission induction/consolidation, between age group of 2 - 60 years were included.

Complete blood count (CBC) was done by using automated haematology analyzer Sysmex XT 2000i. Immature reticulocyte fraction was done by using automated haematology analyzer Sysmex XT 2000i. Determination of IRF by sysmex is a flow cytometry based technique. Sysmex XE 2100 uses patented polymethine fluorescent dye to stain RNA content of reticulocytes. Reticulocyte differential count which is done on this analyser is based on RNA content of the cell. Three fractions high fluorescence ratio(HFR), middle fluorescence ratio(MFR) and low fluorescence ratio(LFR) are determined. IRF is sum of HFR AND MFR. Absolute neutrophil count was calculated from total leukocyte count and differential count of white blood cells. Measurement technique bias was controlled by applying standardized laboratory test. Selection bias was controlled by applying inclusion and exclusion criteria. For quantitative variables like age, ANC, IRF mean ±SD was calculated. True positive and true negative patients, showing recovery on the basis of IRF and ANC were calculated. Chi-square test was used. P-value <0.05 was considered statistically significant. For qualitative variables like gender and accuracy, frequency and percentage were calculated.

Results
Age ranged from 3-39 years while the mean age was 15 ± 11 years. Nineteen (42%) patients were male and 26 (58%) patients were female. The mean recovery day for IRF was 11.7± 7.1, while that for ANC was 17.5 ± 9.3 (Table 1). Out of 45 patients, IRF predicted recovery in 41 patients and was unable to show recovery in 4 patients. ANC predicted recovery in 36 patients, but was unable to predict recovery in 9 patients. In 34 patients, both ANC and IRF showed recovery. So the True positive patients were 34 (75%). In these 34 patients, IRF was early predictor in 24 (70.5%) cases and ANC in 10 (29.5%) cases. Similarly patients who had not shown recovery both by IRF and ANC were 4. The True negative patients were 4 (8.8%). Patients who were negative on IRF were 4. Total patients positive on ANC were 36 and patients who were negative on ANC were 9. Patients showing recovery both by IRF and ANC were 34 out of 45.

Table 1.: Recovery day for IRF and ANC (n=45)

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<thead>
<tr>
<th>Recovery day for IRF</th>
<th>Recovery day for ANC</th>
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<tr>
<td>Mean = 11.7</td>
<td>Mean = 17.5</td>
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<td>S.D = 7.1</td>
<td>S.D = 9.3</td>
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<td>p-Value &lt; 0.002*</td>
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Table 1.: Recovery day for IRF and ANC (n=45)

* p = Significant

Discussion
Patients of ALL are more responsive to chemotherapy as compared to AML. After chemotherapy there is suppression of bone marrow activity and patients are prone to infection which can further augment marrow suppression. The bone marrow recovery after chemotherapy can be assessed with the help of parameters like ANC, reticulocyte percentage, absolute reticulocyte count (ARC), and Immature reticulocyte fraction (IRF). It has been seen that IRF normally constitute about less than 5% of total number of reticulocytes. The normal reference range for IRF is from 3.0% - 15.9% in males and 2.3% - 13.4% in females. During periods of intense erythropoietic stimulation they are released into peripheral circulation. Different studies have shown that an increase in IRF is an early predictor of recovery as compared to other parameters after bone marrow ablation.8
In a study by Briggs et al it has been shown that in dyserythropoietic conditions such as acute leukaemias, MDS, etc. IRF may be higher at diagnosis, (more than the reference range). It drops after chemotherapy. As the bone marrow recovers IRF starts rising again. Rising IRF is considered a sign of haemopoietic recovery. Our study showed similar trend i.e IRF was higher at diagnosis and then gradually dropped to a low level after chemotherapy, recovery was assessed with the help of rising IRF. Studies revealed IRF as a strong indicator of post-chemotherapy aplasia and also the first sign of recovering bone marrow.

In a study done by Yesmin et al the mean recovery day was 16.6 ±4.6 for IRF and 23.2±6 for ANC. In the study by Spanish multicentric study group, haemopoietic recovery was studied in three groups, an autologous bone marrow transplant group, an allogenic bone marrow transplant group and a group of patients receiving chemotherapy for acute leukaemia. It was seen that in all the three groups IRF recovery was earlier than ANC and a rising IRF was the first sign of recovery. In our study only one group of patients was taken and they were being treated with chemotherapy. In our study 91% patients had showed recovery by IRF and the mean day of bone marrow recovery by IRF was 11.7 and that for ANC 17.5. So our results are comparable with the results of this study.

In a study by Kuses et al it was observed that IRF fractions like mid-florescence-ratio(MFR) and high-florescence-ratio(HFR) can act as indicators of bone marrow recovery. In this study two groups, an acute leukaemia and a malignant lymphoma group were taken. However in this study IRF fractions were compared with absolute reticulocyte count in addition to ANC. It was observed that in acute leukaemia group MFR showed earlier recovery in comparison with ANC. The study showed that mid-fluorescence-ratio. and High-Florescence- -ratio can act as sensitive parameters to predict bone marrow recovery. Torres et al also showed that IRF is an earlier predictor than absolute neutrophil count in monitoring bone marrow recovery. They studied patients who were undergoing allogenic and autologous transplant. In this study it was observed that there was earlier increase in reticulocyte parameters than rise in absolute neutrophil count.

Conclusion

Immature Reticulocyte Fraction can predict bone marrow recovery. It shows recovery earlier than ANC. It can be used as an additional parameter in patients who need evaluation of bone marrow recovery after chemotherapy.

References

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