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SIGNIFICANCE OF HEMATOLOGICAL PARAMETERS IN HEMATOLYMPHOID MALIGNANCIES AND INFECTIONS

Pathology

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KEYWORDS

INTRODUCTION:

The technology of Automated Cell Counters (ACCs) has advanced and expanded to count neutrophils, eosinophils, basophils, lymphocytes, monocytes, large unstained cells (atypical lymphocytes; lacks peroxidase activity) and large immature cells like blasts & immature granulocytes. ACCs i.e. Advia^{R} 2120i and Sysmex XT-4000i further generate newer parameters like Immature Granulocytes (IGs), Nucleated Red Blood Cells (NRBCs), Immature Reticulocyte Fraction (IRF), Mean Reticulocyte Volume (MCVr), Mean Reticulocyte Hemoglobin Content (CHr) etc. Numerous flagging systems, in built quality control programs and automated maintenance are other inherent advantages of modern hematological instruments.[1] This study was conducted to access the vital role of newer parameters in early diagnosis and monitoring the response of the treatment especially in anemia, sepsis and hematolymphoid malignancies.

Immature granulocytes are maturing granulocytic myeloid cells; comprise of myelocytes, metamyelocyte and band forms.[2-4] Increased IGs with neutrophilia are observed in bacterial infections, myeloproliferative diseases, acute inflammatory diseases and metastatic BM cancers etc:[2]

Nucleated Red Blood Cells are precursors of reticulocytes and mature erythrocytes. Presence of NRBCs in an adult's peripheral blood smear (PBS) is pathogenic as raised demand activates the bone marrow to produce & release immature RBCs into the circulation. The reference range of mean NRBCs by manual PBS is 0/100WBC and by Advia^{R} 2120i range is 0-0.2x10^{3} cells/cumm respectively.

The reticulocyte count is an important indicator of effective erythropoiesis.[5] IRF is quantitative proportion of all younger reticulocytes; derived as a ratio of immature reticulocytes to total number of reticulocytes. IRF is a very early and sensitive index of marrow erythropoietic activity and its fraction in excess of 5% is a reliable marker for hemopoietic recovery.[6]

IRF	Absolute reticulocyte count	Clinical conditions
Decrease	Decrease	Aplastic anemia, chronic renal failure
Decrease	Decrease	Early erythropoietic response after anemia
Increase	Decrease	Repopulating bone marrow
Increase	Increase	Response to erythropoietin treatment or early acute hemorrhage or hemolytic anemia

The monitoring of BM regeneration after chemotherapy/radiotherapy is further vital as high risk of infection is associated by prolonged time of aplasia.[7]

Determination of percentage of blasts in PBS and BM is essential for diagnosis and classification into Acute lymphoblastic leukemia (ALL), Acute Myeloblastic Leukemia (AML), Myelodysplastic Syndromes (MDS) and lymphoproliferative disorders like Chronic lymphobcytic leukemia (CLL), Chronic myelocytic leukemia (CML). Post-therapy PBS blast percentage is an important prognostic index that reflects the outcome in ALL.[8] Chemotherapy is the main stay of treatment in acute leukemia, thus should be monitored by IRF & presence of blasts in bone marrow (Minimal Residual Disease).[9]

MATERIAL AND METHODS:

This cross sectional observational prospective study was conducted in the Department of Laboratory Sciences & and Molecular Medicine at a "Tertiary Care Super-Speciality & Research Hospital" of North India for a duration off 18 months. The study population comprises of 40 cases of hematolymphoid malignancies and 10 clinical cases of infections with the aim to analyze multiple traditional and recent hematological parameters in pre and post treatment stages. PBS with >10% of blasts were selected to assess their patterns using ACCs. Twenty out of forty cases had blast counts of >10% on routine PBS manual differential counts and reported as acute leukemia whereas remaining twenty cases were reported as chronic leukemia. Blood samples were collected in EDTA vacutainers; under aseptic precautions and processed within 2 hours of collection through two new generation hematology analyzers i.e. ADVIA^{R} 2120i and SYSMEX XT- 4000i. For the validation of the ACCs results, 200-cells manual differential counts were performed by two experienced technologists from Leishman- Giemsa stained PBS and these results were confirmed and compared by a hematopathologist. The association between various 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:

Immature granulocytes (IGs) results were analyzed in forty hematolymphoid malignancy patients (20 cases each of myeloid leukemia & lymphoid leukemia) and ten patients having infections. IG% was increased both types of patients in sepsis & myeloproliferative malignancies and the results were validated with PBS finding. Mean IGs % in sepsis (12.41%) and control (0.36%) cases was calculated by Advia^{R} 2120i with reference range of 0.21% to 0.49%. These cases on follow up were found to have decreased IG% number on remission. Pre-chemotherapy mean IGs % was maximally raised in CML (26.8%) followed by AML (10.8%), ALL (1.0%), CLL (0.8%) with control value of 0.4% as analyzed by Advia^{R}2120i.

Mean value of White Blood Cells in Perox channels (WBCP) increased to 19.56x10^{3} cells/uL in sepsis as compared to control value

of 0.0x10^3 cells/uL. Mean value of WBCP among hematolymphoid malignancies was maximally raised in CLL 62.9 x10^3 cells/uL followed by ALL 56.2 x10^3 cells/uL, AML 26.9 x10^3 cells/uL and CML 26.0 with control value of 0.0 x10^3 cells/uL.

Mean value of Large Unstained Cells (LUCs) increased to 0.876x10^3 cells/uL in sepsis as compared to control value of 0.108x10^3 cells/uL with cut off value of >0.31 x10^3 cells/uL. Mean value of LUCs among hematolymphoid malignancies was maximally raised in ALL 28.3 x10^3 cells/uL followed by AML 20.9 x10^3 cells/uL, CML 13.5 x10^3 cells/uL and CLL 2.2 x10^3 cells/uL with control value of 0.1 x10^3 cells/uL.

Forty cases of hematolymphoid malignancy comprised 10 cases each of ALL, AML, CML and CLL. Mean % of blasts suspected in ALL, AML, CML and CLL as measured by Advia^R 2120i were 11.39%, 12.09, 0.34 and 0.35 respectively with control value of 0.4%. Corresponding % of blasts counted by PBS were 64.3%, 70.8%, 0.8% and 0% in ALL, AML, CML and CLL respectively with control value of 0%. There was a huge difference in calculation of blasts% in ALL and AML as counted by Advia^{R}2120i & PBS and their comparison was statistically significant (p value <0.05).

On 28th day post-chemotherapy mean IGs% results were drastically reduced to 1% of each from pretreatment value of 10.8% in AML and 26.8% in CML respectively as measured by Advia^R 2120i. Similarly WBCP & LUCs were also significantly subsided in sepsis and hematolymphoid malignancies. On 28th day post-chemotherapy remission phase, manually there were no blasts seen in acute leukemia, whereas measured mean% blast suspected as measured by Advia^R 2120i for ALL, AML, CML and CLL were 1.41%, 2.33%, 0.8% and 0% respectively with control value of 0%.

DISCUSSION:

This study was performed to access the patterns and role of hematological variables in recovery phase of hematolymphoid malignancies and infections. Immature Granulocytes (IGs) has potential role to predict sepsis in hospitalized patients and may be a sign of inflammatory response to an injury or autoimmune condition or malignancy with bone marrow infiltration, therefore IG% should form part of CBCs. In the index study, 7 & 3 infectious cases were Gram-positive and Gram-negative whereas 6 & 4 cases were with and without toxic granules respectively. Irrespective of the cause; increased IGs% with raised sepsis markers like C-reactive proteins and procalcitonin were found. IG% counts by ACCs will reduce smear reviews, manual differentials and is less time-consuming, less expensive than routine examination of blood smear. ACCs examine thousands of WBCs whereas PBS typically screens 100-200 WBCs. We compared the IGs% results as measured by manual microscopic method and Advia^R 2120i. We found the significant relationship between the two methods of counting IGs (p value< .05) and is in concordance with Mathias Bruegel et al. This validates the replacement of the traditional manual microscopic IG count by the Advia^R 2120i.

An increase in Absolute Neutrophil Count (ANC) of ≥0.5 x10^9/L defines successful myeloid recovery after chemotherapy.[10,11] Some studies have suggested that IRF was the first sign of hematological recovery in 80% of the patients, preceding the rise in ANC on 14th day. The complete reticulocyte picture, total reticulocyte, IRF, RET-H_e, provide less variation than acute phase reactants in patients with inflammation or infection so are direct cellular measurements for a faster indication of patient response.

Manual differential counting is the gold standard procedure for the accurate identification of cells in the peripheral blood.[12] However low cell counts following chemotherapy frequently complicate attempts to obtain a sufficient number of cells to render a meaningful manual differential count.[13,14] In our study, Advia^R 2120i can detect the presence of blasts as % suspected blast, besides determination of the lineage of leukemia unlike in previous studies.[15] Though we have not included that parameter in our study but Advia^R 2120i has peroxidase channel which utilize the presence of peroxidase activity in the myeloid blasts. However from the best of our knowledge no study has done so far to differentiate between the acute leukemia lineages.

Advia^R 2120i calculated most of the lymphoblasts as lymphocytes or lymphocytes mixed with neutrophils or monocytes in ALL cases and counted myeloblasts as monocytes, neutrophils or lymphocytes in AML cases. Our study shows % suspected blast in automated analyzers are not analogized with the blast seen in PBS (p value>0.05). The percentage of blast suspected were decreased in remission phase as decreased in PBS (p value<.05). In chronic leukemia, % blast suspected by Advia^R 2120i and in PBS blast was negligible and corroborated. In this study, we assessed the patterns of blasts counted using Advia^R 2120i and compared the results with manual differential counts. However, we can conclude that the manual differential counts and the judicious slide review criteria remain essential whenever a hematologic disorder is suspected even in cases in which blast flags are not generated. Further studies with more specimens will be necessary to determine whether the type of blast is related to the blast flag sensitivity or simply related to their morphology. Our data showed that differential WBC reports from ACCs should be interpreted with great care, with extra attention paid to suspected blasts and flags because the majority of leukemic blasts may be counted as monocytosis, lymphocytosis, or neutrophilia.

Monitoring of WBCP and LUCs can assist to know recovery in hematolymphoid malignancies and sepsis patients. IRF being non-invasive, inexpensive and objective indicator can also been used with the reticulocyte count to access patient's bone marrow response. Moreover despite the essential role of automation, microscopic examination of blood sample by the pathologist remains Gold standard. LUCs are the aberrant number of larger than normal cells which are unstained on Leishman Giemsa staining. LUCs are larger than normal lymphocytes and may be atypical lymphocytes, myeloperoxidase deficient cells or MPO negative blasts. Advia^R 2120i showed increased LUCs in leukemia patients (except CLL), while in remission phase their numbers was decreased. Thus our study has emphasized the importance of newer parameters generated by ACCs as cost effective, sensitive, specific and faster mode in diagnosis and follow-up cases of hematolymphoid malignancies and infections.

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.

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