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Original Research Article

Detection and Classification of Leukemia Based on Advia 2120i Scattergram/ PANDA Algorithm and Comparison with Flow Cytometric Diagnosis

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Abstract

Purpose: To study the ability of ADVIA 2120i, five part cell counter scattergrams by PANDA approach to accurately detect and classify leukemia and its comparison with final Flow Cytometric results.

Methods: This is a retrospective analysis of 48 consecutive patients over a 6 months period from October 2018 to March 2019. The CBC was performed on ADVIA 2120i and the cell counter scatter diagrams were evaluated based on PANDA algorithm. The scatter diagram results were compared with final flow cytometric results.

Results: The scatter diagram analysis showed PPV of 95% with accuracy of 79.16% (38). Out of total 48 leukemia patients, highest accuracy was observed that in CML (100%) and APML (100%) while it was 91.67% for ALL. The accuracy of the scatter diagram was more in patients with total leucocyte count of >50000 cells/cmm (86.36%) as compared to the ones with <50000 cells/cmm (76.92%).

Conclusion: The timely identification and classification of leukemia is crucial for patient survival, and is essential to prompt further investigations to confirm the diagnosis and to initiate treatment as soon as possible.

Keywords: ADVIA 2120i scattergrams; PANDA algorithm; Scattergram analysis; Scattergram analysis comparison with flow cytometric results; Use of automated cell counters.

Introduction

Leukemia is characterized by the uncontrolled proliferation of hematopoietic cells and displacement of normal precursors in the bone marrow. Our understanding of leukemia diagnosis has advanced a lot, since the time Rudolf Virchow discovered leukemia in year 1845. Despite advances in therapy, mortality associated with leukemia remains high due to many factors, one of which is the diagnostic delay.

The Complete Blood Count (CBC), differential count and peripheral blood smear usually are the first line investigations that alert the clinician to a possible underlying leukemia, but further investigations are essential to confirm the diagnosis and classify the leukemia before appropriate therapy can be commenced.[1,2] Since the advent of automated cell counter in the year 1956, this technology has also evolved to such an extent that nowadays some cell counters like ADVIA 2120i from Siemens are using myeloperoxidase method[3] for accurate differential count.

This analyzer has two chambers for counting WBCs, peroxidase and basophil chambers. In the peroxidase method, the RBCs are lysed,

and peroxidase reagents are used to distinguish peroxidase-positive cells (neutrophils, eosinophils, and monocytes) from peroxidase-negative cells (lymphocytes, basophils, and "large unstained cells"–LUC). In the basophil channel, surfactant and phthalic acid are used to lyse RBCs and platelets and to strip away the cytoplasmic membrane from all leukocytes except basophils. Cells are then counted and classified according to size, lobularity, and nuclear density. This channel also provides valuable information about the degree of maturity of each WBC's nucleus by measuring its lobularity and density[3]. Based on the perox and baso distribution of cells the cytograms can be given a particular zone.

Peroxscattergrams can be analyzed and different zones defined based on maximum cells falling into them from P0 to P6. Similarly basoscattergrams are divided as D0 and D1. This is called as PANDA algorithm[1, 2, 4, 5] and based on it a PANDA classification grid can be made[6] (Fig. 1). This can then be utilized for detection and classification of leukemia.

This helps in early detection and classification of leukemia, which allows the clinician to promptly initiate the most appropriate treatment plan, as the prompt initiation of chemotherapy has been shown to confer a better prognosis.[2]

The aim of this study was to check the reliability of white blood cell (WBC) cytogram assessment from an automated hematology analyzer, Siemens Advia 2120i using PANDA algorithm, in the classification of leukemia when compared to flow cytometric analysis.

Materials and Methods

Present study was carried out in the Hematology section of the Central pathology laboratory, Department of Pathology, MGM Medical College, Aurangabad. This is a retrospective analysis of forty-eight patients of leukemia, attending the hematology OPD over a six month period from October 2018 to March 2019. The CBC of these patients was done on Automated Hematology Analyzer from Siemens, Advia 2120i. The scatter diagram analysis[4] was carried out using PANDA algorithm[1,2,4,5] as described by Siemens and other researchers namely, Rachna Agarwal, Manish Singhal[1] and Vincenzo Roccoo.[2] The scatter diagram diagnosis thus obtained was compared with flow cytometric results, immunophenotyping and cytogenetic analysis, which was done at the outsourced laboratory in Hyderabad.

Results

The total 48 leukemia patients, highest number was observed that of AML (28) followed by ALL (12) (Table 1). There were 05 cases of CML, 03 of APML. Our study group did not have any CLL patient. The number of males (33) outnumbered females (15) by 2.2 times (Table 1).

The accuracy of scattergram analysis by PANDA algorithm was maximum in cases of APML (100%) and CML (100%) with least accuracy in AML (67.85%) (Table 2). Using the PANDA algorithm, 79.16% of cases *i.e.* 38 out of 48 cases were accurately classified. The positive predictive value (PPV) was 95% (Table 3). False positives in our study were only 08 cases while false negatives were seen only in 02 cases. The accuracy was seen to be increased in patients who presented with initial WBC count > 50,000/cmm than those presenting with <50,000/cmm WBC counts (Table 4).

Table 1: Spectrum of hemato-lymphoid malignancies in study group.

Category	No. of Males	No. of Females
AML	18	10
APML	2	1
ALL	9	3
CML	4	1
CLL	0	0
Total	33	15

Table 2: Comparison of Siemens Advia 2120i scattergram and final diagnosis by flow Cytometry.

Category	Siemens Advia 2120i	Final by Flow Cytometry
AML	19 (67.85%)	28
APML	3 (100%)	3
ALL	11 (91.67%)	12
CML	5 (100%)	5
CLL	0 (0%)	0
Total	38 (63.34%)	60

Table 3: Summary of final diagnosis obtained from PANDA analysis of scatter grams on Advia 2120i.

Investigations used for Analysis	True Positives N = 60	False Positives N = 60	False Negatives N = 60	Accuracy %	PPV %
PANDA Algorithm	38	08	02	79.16	95

True positives: PANDA algorithm correlates with the final diagnosis.

False Positives: PANDA algorithm does not correlate with the final diagnosis.

False Negatives: No diagnosis obtained from PANDA algorithm

PPV: Positive Predictive Value.

Table 4: Accuracy of scatter diagrams based on total leucocyte counts (in cells/cmm).

Total Leucocyte Count (in cells/ cmm)	Diagnosis by Siemens Advia 2120i	Diagnosis by Flow Cytometry
>50000	19 (86.36%)	22
<50000	20 (76.92%)	26

Discussion

In our study, the accuracy of Advia 2120i scattergram to classify leukemia was 79.16%, which is more than the study done in 2018 by Rachna Agarwal and Manish K. Singhal et al[1] in Jaipur (61.4%). Another study done by Dashini Pillay et al showed 48% accuracy with PPV of 88%.[6] The increase in accuracy in our study can be explained due to less number of study cases leading to low number of false negatives i.e. only 02 cases out of total 48. The PPV of all cases using the PANDA algorithm in our study was 95%. The low accuracy in study by Dashini Pillay et al can be attributed to high number of false negatives (64 out of 150 cases).

A study done by Shelat, S.G., Canfield and W. Shibutani et al,[7] demonstrated 100% sensitivity in detecting blasts while our study showed sensitivity of 95% with false negatives in 02 cases out of 48. The accuracy was 77.8% in a study done by Gibbs et al,[5] which is comparable with accuracy of our study (79.16%). Onofrio et al did a similar study and found the accuracy to be 91.1%[8] which was much higher than that of accuracy in our study (79.16%).

In our study all the cases of APML, a correct diagnosis was made based on scattergram alone. The advantage of this finding is the impact it has on the immediate management of the patient in terms of initiation of all trans retinoic acid.[2,7] Similarly all the cases of CML were accurately diagnosed which can be helpful in initiating treatment in asymptomatic patients in the chronic phase itself. There was 91.67% accuracy in cases of ALL and 67.85% in that of AML.

In conclusion the analysis of WBC scattergrams by PANDA algorithm improves the overall assessment of leukemic cases by providing a provisional classification to the clinicians. Furthermore scattergram analysis alone has the potential to differentiate myeloid from lymphoid malignancies and to identify specific malignancies such as CML and APML.

The timely identification and classification of leukemia proves crucial for patient management, and helps in further investigations to confirm the diagnosis and initiation of treatment as soon as possible.[9] Adequate training of both technologists and pathologists in the interpretation of cytograms in conjunction with morphology can lead to improved utility of advanced cell counters like ADVIA 2120i.

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