Correlation of Cytomorphology with Flowcytometric Immunophenotyping of Acute Myeloid Leukemia in Tertiary Care Hospital

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ABSTRACT

Introduction: WHO classification utilizes morphology, genetic information, immunophenotyping, biologic and clinical features to define specific disease entity. Although it gives an accurate detailed diagnosis, immunophenotyping by flow cytometry gives an immediate prompt diagnosis. Morphological diagnosis for leukemias may sometimes be not correlating with flow cytometry diagnosis. Study objectives were to correlate morphological and flowcytometric results of patients diagnosed with acute myeloid leukemias.

Material and methods: Study was conducted in department of pathology. Cases were classified as Acute leukemia based on CBC, peripheral smear, bone marrow morphology, special stains cytochemistry and Flow cytometry Immunophenotyping. Categorization was done based FAB system.

Results: Total 92 cases of AML were diagnosed on cytomorphology, cytochemistry and Flow cytometry were studied. Out of which M0 were 6.5%, M1-13%, M2-27.2%, M3-17.4%, M4-15.2% and M5 were 20.6%. There was 88% correlation between cytomorphology and flowcytometry.

Conclusion: Interpretation of immunophenotyping by flowcytometry, done in close conjunction with morphology, is mandatory for appropriate diagnosis of acute myeloid leukemia. However morphology combined with cytochemistry is also very helpful in the diagnosis of AML if facility of flowcytometry is not available.

Keywords: Acute Myeloid leukemia, Morphology, Cytochemistry, Flowcytometry

INTRODUCTION

The WHO classification of acute myeloid neoplasms relies on the morphological, cytochemical, and immunophenotypic features of the neoplastic cells to establish their lineage and degree of maturation. The classification is based on criteria applied strictly to initial specimens obtained prior to any therapy. Blast percentages in the peripheral blood and bone marrow, is of practical importance for categorizing myeloid neoplasms and determining their progression. A peripheral blood smear and Bone marrow aspiration should be examined and correlated with the results of a complete blood count. Cytochemical studies are useful in determining the lineage of blasts, although in some laboratories they have been supplanted by immunological studies using flow cytometry. Cytogenetic and molecular genetic studies are required at the time of diagnosis not only for recognition of specific genetically defined entities, but also for establishing a baseline against which follow-up studies can be interpreted to assess disease progression.[1] Multiparameter flow cytometry is the preferred method of immunophenotypic analysis in AML due to the ability to analyze large numbers of cells in a relatively short period of time with simultaneous recording of information about several antigens for each individual cell.[2]

Cytology, possibly supplemented by cytochemistry, is the starting point for the diagnosis and classification of the acute leukemias. The 2016 revision of the 2008 WHO classification of acute leukemias is part of a broader classification of tumors of haemopoietic and lymphoid tissues. It builds on the work of the French–American–British (FAB) group and on earlier WHO classifications published in 1999, 2001 and 2008. The WHO criteria for regarding a patient as having acute myeloid leukemia (AML) differ from the FAB criteria in that cases with at least 20% bone marrow or peripheral blood blasts are categorized as AML.[3]

Study aimed to correlate cytomorphology with Flowcytometric Immunophenotyping of Acute Myeloid Leukemia and to classify AML as per FAB classification

MATERIAL AND METHODS

Retrospective study was conducted in the department of Pathology of a tertiary care hospital and referral centre using case record of patients diagnosed with acute leukemia from Jun 2016 to Jun 2018. File records and computer data of patients previously diagnosed to have acute leukemia on the basis of clinical findings, complete blood counts, results of peripheral blood films, bone marrow morphology and cytochemistry (Periodic Acid Schiff stain – PAS and Myeloperoxidase stain - MPO) and flow cytometry findings were recorded. Those cases diagnosed as AML on morphology, cytochemistry and flow cytometry were

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How to cite this article: Shailendra Jambhulkar, Nitin Y. Shende, Purnima Kodate, Jayshree Tijare, D. T. Kumbhalkar. Correlation of cytomorphology with flowcytometric immunophenotyping of acute myeloid leukemia in tertiary care hospital. International Journal of Contemporary Medical Research 2019;6(2):B19-B22.

DOI: http://dx.doi.org/10.21276/ijcmr.2019.6.2.28

Classification of AML	M0	M1	M2	M3	M4	M5	M6	M7
AML cases N= 92	6	12	25	16	14	19	0	0
Percentage	6.5%	13.0%	27.2%	17.4%	15.2%	20.6%	0	0

Table-1: Classification of AML cases based on cytomorphology, cytochemistry and flowcytometry

Classification of AML	M0	M1	M2	M3	M4	M5	M6	M7	Other
Flowcytometry N= 92	6	12	25	16	14	19	0	0	0
Cytomorphology N= 80	3	9	23	15	13	17	0	0	12
Correlation	50%	75%	92%	93%	92%	89%	0	0	

Table-2: Comparison of cases flowcytometry vs cytomorphology and cytochemistry

FAB Category	Degree of Agreement	Kappa Stastatics	' P 'value
AML-M0 and AML –M1	66.67%	0.4545	< 0.0001
AML-M2 TO AML M5	93.15%	0.9097	<0.0001
AML-M0 TO AML-M5	87.91%	0.8545	<0.0001

Table-3: Agreement analysis between cytomorphology and cytochemistry and Flowcytometry using Kappa Statistics

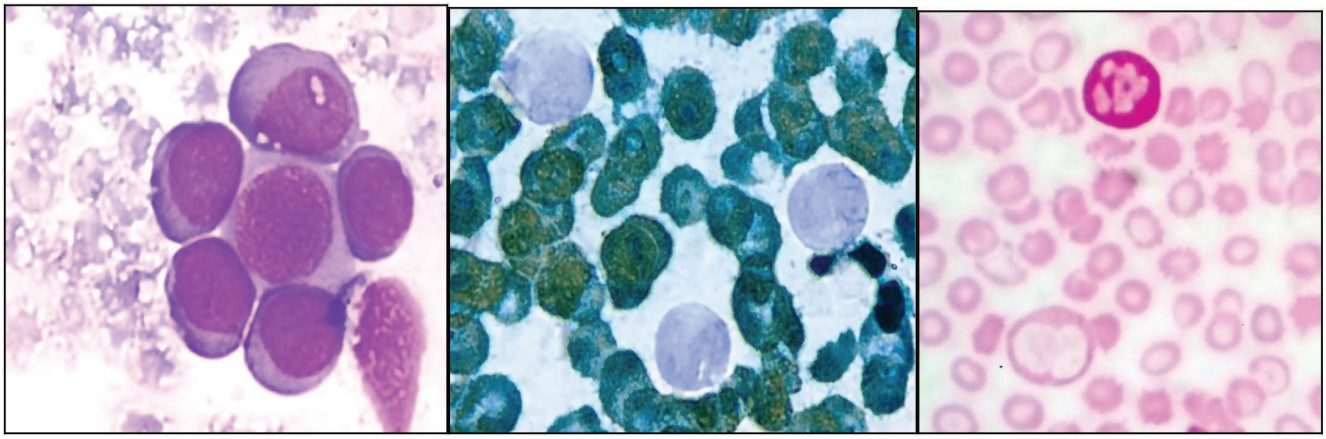

Figure-1: AML M0. Blasts are large with agranular moderate amount of cytoplasm and varying degree of basophilia and round to oval nuclei with fine chromatin. The blasts are MPO and PAS negative

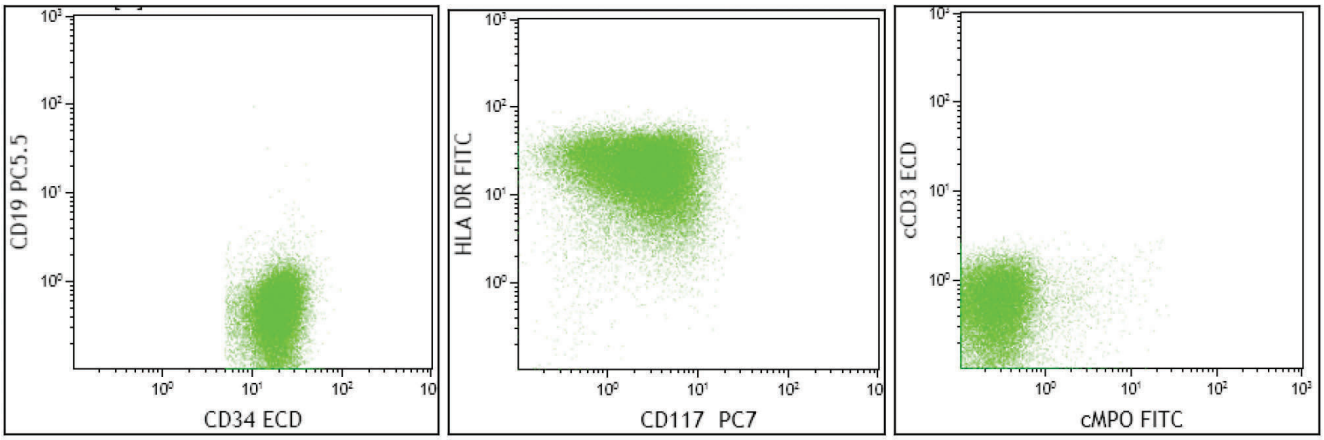

Figure-2: Immunophenotyping of AML M0 by flow cytometer. Blasts are positive for CD34, HLA-DR, CD117 and blasts are negative T- lineage marker cCD3, B-lineage marker CD19 also negative for cMPO

included in our study. Immunophenotyping was done using 3 laser, 10 colour NAVIOS flow cytometer from Beckman and Coulter, using panel of antibodies depending on availability.

RESULT

Total 210 cases of acute leukemia were studied out of which 92 (44%) cases diagnosed as Acute Myeloid Leukemia (AML) and 118 (56%) cases were diagnosed as Acute Lymphoblastic Leukemia (ALL) on clinical history, morphologically, cytochemistry and flow cytometry immunophenotyping (table-1). Cases of ALL diagnosed on morphology and flowcytometry were excluded from the study and we concentrated only of cases of AML. Of 92 cases of AML, 52 patients were male and 40 patients were females with M:F ratio 1.3:1. The age ranged from 2 to 68

years with mean of 42.8 years and SD of ±24.5 years and most patients (72%) belonged to fifth and sixth decade. In the 92 cases of AML, analysis was done on 76 peripheral blood and 16 bone marrow aspirates. Out of 92 cases of AML, 80 cases were diagnosed using morphology and cytochemistry alone. Remaining 12 were reported as acute leukemia only as morphology and cytochemistry was not contributory to differentiate between AML and ALL, and were turned out to be AML on flow cytometry. These were 3 cases each of AML-M0 and AML-M1, 2 cases each of AML-M2 and AML-M5 and 1 case each of AML-M3 and AML-M4. AML cases were sub classified on the basis of morphology and flow cytometry as M0 to M7 based on FAB classification. Out of which M0: 6.5%, M1:13%, M2: 27.2%, M3:17.4%, M4:15.2% and M5 were 20.6%. Morphological correlation with flowcytometry was found in 88% cases of AML. There was average 92.3% correlation in combined cases of AML-M2, AML-M3 and AML-M4 and 50% correlation in cases of AML-M0 which is highly significant with p value < 0.001.

DISCUSSION

The present study of 92 cases of AML diagnosed by cytomorphology and cytochemistry and flowcytometry as per the data analyzed from the files showed the M:F ratio was 1.3:1 and the age of most of the patients were 40 to 60 years. 80 cases out of total 92 cases of AML were diagnosed on cytomorphology and cytochemistry only with 87.91% degree of agreement using kappa statistics (table-3).

Kheiri et al in 1998 studied 93 cases of acute leukemia. Of 37 cases designated myeloid origin by flow cytometry, 33 (89.2%) were read as myeloid by cytochemistry. The four discordant cases were read as 2 each ALL an 2 as non-diagnostic.[4]

Mhawech et all in 2001, revived 122 cases of acute leukemia, 120 of ALL and 10 of AML and that overall concordance of 81.2% between cytomorphology with cytochemical stains and flowcytometry. In two patients with inconclusive flow cytometry results, cytochemical staining alone provided information sufficient for diagnosis. They recommended that the cytochemical staining should be available for those cases in which flow cytometry results fail to allow a definitive diagnosis.[5]

Gujral et al in 2006, concluded that flowcytometry evaluation is important in those cases of acute myeloid leukemia where blasts do not show Auer rods and are negative for MPO and non specific esterase (NSE) stains like AML-MO and, AML-M7. Classical cases of AML do not require expensive flowcytometry studies.[6]

Belurkar, et al in 2013 studied 50 cases of Acute leukemia with 15 cases of AML, of which 12 were diagnosed on morphology. They found that overall concordance between morphology and flowcytometry was 84% which was in good agreement with the present study.[7]

Murmu et al in 2016 studied 50 cases of acute leukemia with 27 cases of AML and found the concordance of 89% between morphology and Flow cytometry. There were 5 cases of AML – M0 which were difficult to differentiate between AML and ALL, were confirmed by flowcytometry. They concluded that flowcytometry is essential for the diagnosis of AML-M0 and biphenotypic acute leukemia. Their findings are in close agreement with the present study.[8]

Parikh et al in 2017 studied 343 cases of acute leukemia with 136 cases of flowcytometry proven AML. Out of 136 cases of AML, morphology and cytochemistry was able to diagnosed correctly 127 cases of AML. Rest 9 cases, morphologically diagnosed as AML, turned out as ALL on flow cytometry. They concluded that morphology plus cytochemical staining with PAS and SBB was able to correctly diagnose 93.38% cases of flowcytometry proven AML. Their findings are close agreement with the present study.[9]

Das et al in 2018 studied 100 cases of acute leukemia, with 57 cases of ALL, 32 cases of AML, 08 cases of APML and 3 cases of MPAL found the concordance of 88%, 91%, 75% and 0% respectively between cytomorphology and flowcytometry. Their findings are in similar with the present study.[10]

Cytochemical studies are still performed at many institutions and can provide rapid information about the cell type of an acute leukemia (MPO positive versus MPO negative). As more detailed information can now be obtained by flow cytometry in the same time frame, the use of these less specific cytochemical studies has decreased.[11] In the present study we faced major difficulty in diagnosing AML-M0 on morphology and cytochemistry, as we could diagnose only three out of six cases (figure-1,2). In these cases flowcytometry helped to clear the confusion. In other cases from AML-M1 to AML-M5 there was little difficulty in the diagnosis on morphology and cytochemistry and amongst these AML-M3 and AML-M4 were the most easy to diagnose on morphology alone.

In India, Acute leukemias are still diagnosed mainly on the basis of morphology and cytochemistry in many centre, because of its ease and it remains the pillar of diagnosis where flow cytometry is not available. With the concordance of 81% to 93% from various studies between morphology and flowcytometry, morphology and cytochemistry still remains the cornerstone in the primary diagnosis of AML especially where the facilities of flowcytometric immunophenotyping is not available. Only those cases which are not differentiated on morphology between AML or ALL (e.g. minimally differentiated AML or suspected cases of biphenotypic acute leukemia or some ALL) can be referred to flowcytometry analysis. Cytochemical stains are cheap, simple and require no use of special instruments. Cytochemical stains are very much important in developing countries or resource poor regions for the diagnosis of acute leukemia, especially in cases of AML.

CONCLUSION

Many acute myeloid leukemias are correctly diagnosed by morphology and cytochemistry, but there is problem in the diagnosis of minimally differentiated AML. Flowcytometry is very useful tool for the rapid and accurate diagnosis and

subclassification of AML. Flowcytometric analysis, when combined with conventional morphologic and cytochemistry helps to arrive at a narrow differential or even definitive diagnosis of AML. Immunophenotyping multicolor flow cytometry, is now absolutely required to accurately diagnose the lymphoblastic leukemias and AMLs. Rapid interpretation of morphology and flowcytometry can efficiently direct a narrow and specific search for recurrent genetic and molecular lesions in AML. In resource poor region and a setting with lack of facilities for immunophenotyping, morphology and cytochemical analysis best serve the purpose in diagnosis of AML.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 13-01-2019; **Accepted:** 14-02-2019; **Published:** 25-02-2019