



Multilineage dysplasia as assessed by immunophenotype in acute myeloid leukaemia, a prognostic tool in the genetically undefined category

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ABSTRACT

Acute myeloid leukaemia (AML) is a type of cancer in which abundance of abnormal blood cells is produced by bone marrow. In India, 1-2 persons per 100,000 people are affected by AML each year. The present research has been carried out on individuals who were suffering from AML and diagnosed with MLD using morphological, immunophenotypic, and molecular criteria. Samples were gathered from the Government Cancer hospitals' Oncology departments in Bangalore, India. For the trial, a total of 300 participants were enrolled. The observed association with morphology confirmed the capacity of MFC-based approach to draw attention to dysplasia. Insight into the prognosis was limited when looking at MLD data evaluated by an immune-phenotypic score (IPS) either globally or within the narrowly defined genetic groupings. It's interesting to note that IPS-related dysplasia gave critical predictive information while focusing on the genetically undefinable patients who tested triple-negative for FLT3, NPM1, and CEBPA (TN-AML-MLD). While there is no significant correlation between IPS scores and age (p 0.09) or gender (male p 0.67 & female p 0.77), the dysplasia lineage was significantly higher in the absence of dysplastic characteristics (IPS₀), WBC (p 0.01), neutrophile (p 0.01), & platelet (p 0.04). In the ever-changing AML-MLD treatment environment, the influence of the IPS category preserved its validity and gave insight into the prediction of responses.

Keywords: *Acute myeloid leukaemia; Multilineage Dysplasia; Immune-phenotypic score; India*

INTRODUCTION

An excessive number of abnormal blood cells are produced by the bone marrow in acute myeloid leukaemia (AML), a particular type of malignancy. In the classification of myeloid neoplasms by the World Health Organisation (WHO), AML is listed as a distinct entity. Patients with any of the following illnesses and at least 20% blasts in their bone marrow or peripheral blood now fall into this category:

(a) AML with multilineage dysplasia (b) Analysis of the immunophenotype in AML (Weinberg et al.,2019).

AML with multilineage dysplasia is defined as the presence of at least 20% blasts and at least 50% of cells exhibiting one of three types of dysplastic characteristics: dysgranulopoiesis, dyserythropoiesis, or dysmegakaryopoiesis.

In AML, one or more cytopenias & dysplastic alterations in two or more myeloid lineages (erythroid, granulocytic, and megakaryocytic) define myeloid related changes (MRC) with relation to multilineage dysplasia (Weinberg et al.,2019). So, AML along with myelodysplasia-related changes (AML-MRC) is a separate biological subgroup of AML that makes up 25–34% of the AML cases compared to non-MRC AML (Rozman et al.,2014). Detection of myeloid dysplasia in two or more myeloid lineages (erythroid, granulocytic, and megakaryocytic) using immunophenotyping with multiparameter flow cytometry (MFC) technique in which multiple individual cell properties such as size & internal complexity, as well as antigen expression, are correlated within the cell population (Falinie et al.,2010). This MFC determines whether or not abnormal cells are present as part of the diagnosis, as well as whether or not the cells are cancerous or non-malignant. Immunophenotypic score (IPS), a non-invasive method for determining immunophenotype MFC, might calculate the severity of dysplasia based on the deviation from the expected profile.

In India, 1-2 individuals out of every 100,000 are affected with MLD in AML each year. Less than 1 per 100,000 adults under the age of 30 experience the incidence, which rises to 17 per 100,000 people over the age of 75. In India, AML-MLD is more prevalent in older people since prognosis is greatly influenced by age. The risk rises after the age of 50 and is highest for those between the ages of 70 and 89. AML accounts for 80-90% of cases of acute leukaemia in adults. Males are more likely to be affected than females. AML with MLD makes up roughly 3% of all malignancies, with a median age of 32 years, although it can also affect youngsters. AML with MLD makes up roughly 3% of all malignancies, with a median age of 32 years, although it can also affect youngsters. According to the Delhi Population-Based Cancer Registry, less than 10% of all leukaemia occurs in children under the age of 10 and between 25% and 30% in those between 10 and 15 years (ICMR, 2019).

MLD may be the outcome of the pathologic maturation or differentiation of the leukemic clone, a physiologically pre-existing clonal hemopoiesis, or both. The importance of MLD has diminished in some well-defined genetic entities, like AML-bearing CEBPA (bi-allelic) or

NPM1 modifications and therapeutic attempts shouldn't rely on it in these circumstances. The aim of the study is to evaluate MLD in AML using an approach different from morphology, namely the Immunophenotyping Score (IPS), which is steadily being recognized as a good methodology to evaluate dysplasia.

METHODOLOGY

The study was conducted on patients who were identified with AML and were later diagnosed with MLD. Morphological, immunophenotypic, & molecular criteria were used to diagnose MLD. The samples were gathered from the Government Cancer Hospitals' Oncology Department in Bangalore, India. A total of 300 patients were enrolled in the study after providing written consent. Two and a half mL of venous blood samples were collected in an EDTA tube under aseptic conditions for examination of the peripheral blood film and haematology.

Following are the procedures and assessment of the AML patients:

- The May-Grunwald-Giemsa method-stained bone marrow smears from AML cases underwent morphological revision. With the use of WHO guidelines, the degree of dysplasia was assessed. The observation of dysplasia in at least two cell lineages in bone marrow smears (>50% of dysplastic cells per lineage) formed the basis for the classification of multi-lineage dysplasia (MLD). A granularity disorder known as pseudo Pelger-Huet anomaly is defined as the presence of agranular, hypogranular, or hyposegmented neutrophils in 50% or more of polymorphonuclear neutrophils. At least >25 erythroid precursors were having at least 50% of the following dysplastic characteristics: megaloblastoid features, when three megakaryocytes or >50% of six cells exhibited dysplastic characteristics like micromegakaryocytes, many separated nuclei, or extremely big single nuclei, dysmegakaryopoiesis was determined to be the cause.
- In accordance with standard practises, written informed consent was obtained prior to any bone marrow aspiration. Blood-stained smears from bone marrow & peripheral blood aspirates were examined

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- under a microscope to determine the shape & number of blasts.
- Genetic testing, such as looking for an abnormal cytoplasmic NPM1 expression or NPM1 gene alterations, were used to identify the NPM1 mutation, FLT3 internal tandem duplications (ITD), & CEBPA mutations were also looked into. In AML-MLD patients, these mutant genes are investigated utilising the flow cytometry method.
- AML-MLD by immunophenotype score (IPS) was calculated for granulocytes, erythrocytes, and thrombocytes compartments, as well as other characteristics such as age, gender, and bone marrow blast percentage. IPS parameters were expressed in form of median range within a cell compartment. Multilineage dysplasia was evaluated for triple-negative disease (TN-AML) using an immunophenotypic score (IPS). AML was diagnosed using the immunophenotypic scoring

method, which also serves to distinguish AML from other B cell cancers. In contrast to other B-cell malignancies, where scores are typically >3, AML typically has scores between 0 and 2.

Statistical Aspects

The Kruskal-Wallis test or the Mann-Whitney test for continuous variables were used to evaluate all the collected data. The significance was set at p-value less than 0.05. Utilising SPSS version 26 and R version 3.5.0, statistical analyses were carried out.

RESULTS

In the study, all AML 300 patients having maturing cell compartments (i.e., less than 0.01% of all cells) were assessed for dysplasia, were included. Their morphology characteristics are shown in Table 1.

TABLE 1: Demographic, WHO classification and genetically undefined AML –MLDs variables of study population

S ₂ No.	Variables	N=300
1	Age, median (range)	57 (18–75)
2	Gender n (n%) Male Female	188 (62%) (38%)
3	WHO Classification,	n (n%)
3.1	Recurrent genetic abnormalities	139 (46.3)
3.2	Therapy-related	10 (2.3)
3.3	Myelodysplasia-related changes (MRC)	73 (23.8)
3.4	Not otherwise classified	(26.6)
4	Prognosis of genetically undefined AML –MLD (Triple negative disease NPM1, FLT3 and CEBPA)	
4.1	Favourable	83 (29.6)
4.2	Intermediate-1	134 (45.7)
4.3	Intermediate-2	23 (8.3)
4.4	Adverse	60 (19.2)

Table 1 shows the demographic data like age & gender of the patients described. The patients age range lie between 18-75 and median 57 (Figure 1) years, whereas study population contain male

frequency (62%) and female (38%) (Figure 2). Patients with AML show prognosis related to cytogenetic testing more in stage of Intermediate 1 in the study population (Table 1).

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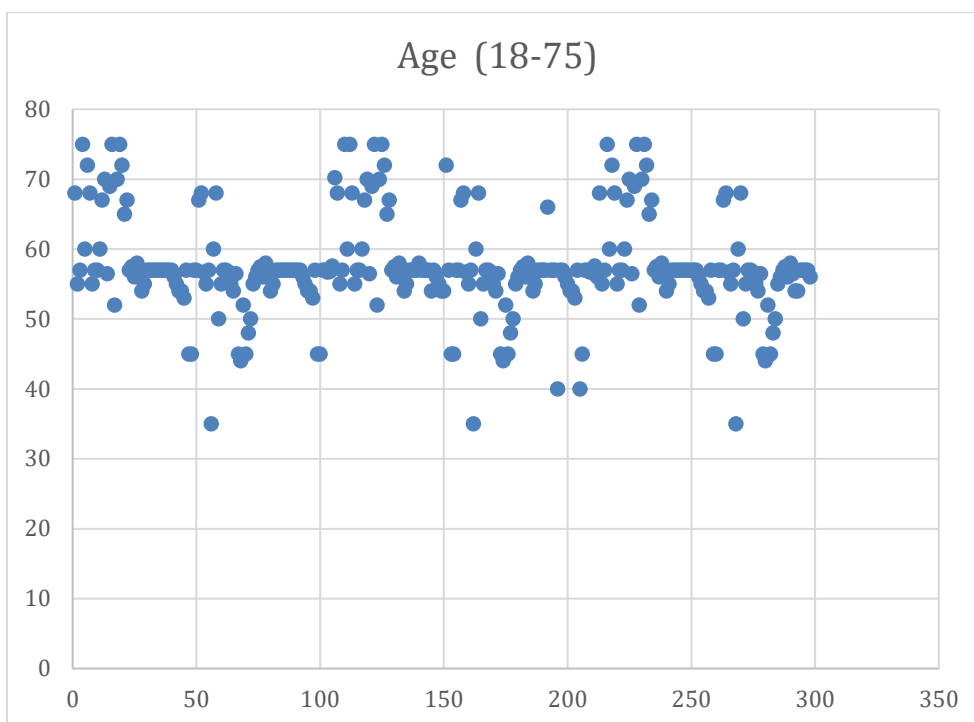


FIGURE 1: Depicting age range of AML-MLD patients

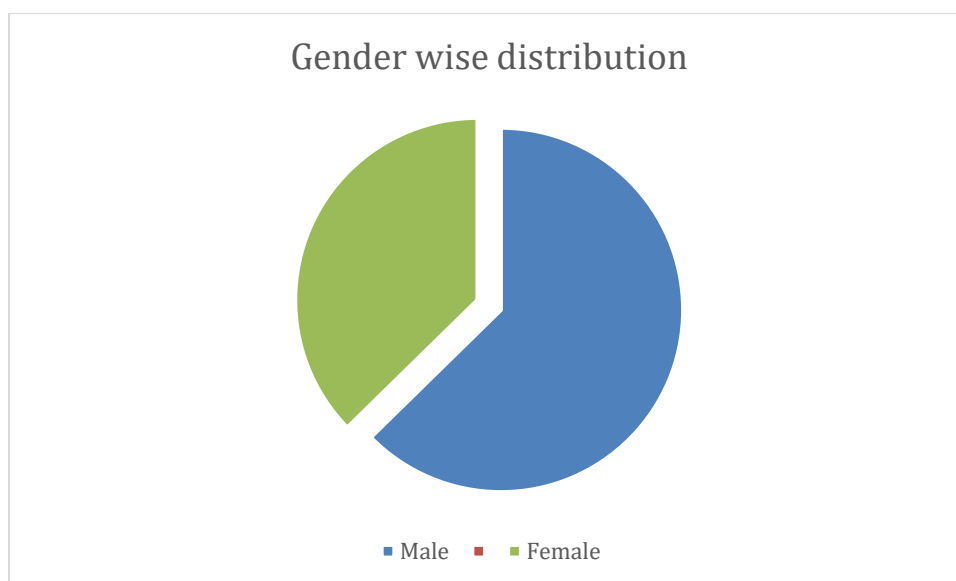


FIGURE 2: Gender distribution among the study population with AML-MLD

TABLE 2: Triple negative disease (TN- AML-MLD) subset characteristics of AML-MLD patients based on IPS.

S.No			Triple negative disease subset Intermediate 1 (n=134)		p-value
			IPS_0 N = 80	IPS_1-2 N = 54	
1	Age	Median value (range)	56.5 (35–72)	57 (22–73)	0.958

2	Gender: Male	Percentage (%) %	55%	34%	0.67
3	Female	%	29%	21%	0.77
4	White blood cells (WBC) unit 10⁹/Ltr,	Median value (range)	3.2(0.5–36.9)	6.7 (0.5–36.9)	0.018
5	Neutrophils unit 10⁹/Ltr,	median (range)	2.0 (0.0-10.5)	5.5 (0.0-10.5)	0.015
6	Hemoglobin (Hb) unit g/dl	Median value (range)	9.5(4.6–13.3)	9.0 (4.6–14.9)	0.350
7	Platelets (Plt* 10⁹/ Ltr)	Median value (range)	73.6 (16–281)	53.2 (16–281)	0.040
8	bone marrow blasts (%)	, Median value (range)	86 % (20–100%)	79.8% (20–100%)	0.910

Table 2 illustrates the relevance of the genetically ambiguous individuals with intermediate risk & triple-negative outcomes for FLT3-ITD, NPM1 & CEBPA (TN-AML-MLD). Thus, the correlation between IPS and prognosis dysplasia lineages white blood cells (p=0.01), neutrophils (p=0.01) & platelets (p=0.04) shows significant difference between immune-phenotyping score system, whereas gender, age, haemoglobin & bone marrow blasts percentage were non-significant between the groups. In subsequent analyses, individuals were combined IPS_1 and IPS_2 as both have unexpectedly identical prognosis as compared to the IPS_0 subgroup. When compared to IPS_1-2 with IPS_0 characteristics revealed a significantly lower WBC, neutrophil, and platelet count. Both IPS_0 & IPS_1-2 shows no significance with age and gender. Thus, IPS_0 shows a significant prognostic impact on genetic undefined abnormalities.

DISCUSSION

There is considerable debate over the prognostic significance of the MLD in AML. Here, the main aim of our research is to investigate dysplasia using an immunophenotype predictive tool in AML patients. For example, immunophenotypic score (IPS) was used to estimate MLD in AML. An established role for immunophenotyping based on IPS is in the diagnosis & categorization of acute leukaemia. Intriguingly, when focusing on individuals with genetically unexplained

conditions who were triple-negative for NPM1, FLT3, & CEBPA, IPS-related dysplasia provided important predictive information (Falini, et al.,2010).

Table 1 demonstrates age and gender of the AML identified patients. The age range was (18-75) with median age 57 years & male frequency (62%) more than female (38%). Patients with AML show prognosis related to cytogenetic testing more in stage of Intermediate 1 in the study population. According to a study, the age-adjusted incidence of AML in India is 1-2 per 100,000 individuals annually, with the seventh decade having the highest incidence (ICMR, 2019). Any age group can develop AML; however, the incidence rises with advancing years (Vardiman et al., 2019). Like as per our study, the average age in the study ranged from 52 to 57 years old. However, in some other research, the mean age ranged from 35 to 47 years, which was lower than in our study (Arber et al., 2016). In another study, no gender difference was observed in AML (2.1 new cases in men and 1.9 in women), there is a dominance for men in AML-MLD (5.1 versus 3.9 new cases). The male to female ratio in another study (Weinberg et al., 2009) was 1.2:1, which indicated a minor male preponderance in multilineage dysplasia AML, in contrast to Rozman et al., 2014, which showed a 2:1 male to female ratio.

Table 2 shows an impact on prognosis for patients with genetically unknown disease or

those with intermediate risk and NPM1, FLT3-ITD, and CEBPA (TN-AML). While gender, age, haemoglobin, & bone marrow blasts were non-significant between the groups, the significant link between IPS and the prognostic dysplasia lineages like the WBC, neutrophils, & platelets were observed. In the subsequent studies, IP_1 and IPS_2 patients were pooled and compared to the IPS_0 sample because they had nearly comparable survival rates. Initial data showed that IPS_0 had a much lower WBC, neutrophil, and platelet count than IPS_1-2. Age and gender have no effect on IPS_0 or IPS_1-2. Thus, IPS_0 shows a significant prognostic impact on genetic undefined abnormalities. In another study (Arber et al., 2016), the efficacy of the MFC-based technique to highlight dysplasia in AML was examined. This study discovered no connection between age or gender and the immunophenotyping score in the selected analysis. Our findings show that when genetically undefinable patients (TN-AML), who made up roughly 44.6% of our group, were the focus, IPS-related dysplasia provided meaningful prognostic information. Patients with MLD in either one (IPS_1) or both (IPS_2) of the assessed multi lineage had a significantly worse prognosis than patients (IPS_0) without dysplastic features. It was demonstrated in a study by (Orathai in 2011) that there was a clinical association between the patients' score and the WBC counts & lymphocyte percentages in the bone marrow & peripheral blood. In comparison to the lymphomas with a less pronounced leukemic phase, patients with scores > 3 (i.e., those who had primarily CLL) had higher WBC counts, marrow lymphocyte percentages, & blood lymphocyte percentages. This was a sign of the major leukemic feature of CLL.

Wandt et al., (2008) study suggest that allogeneic HSCT acknowledged the limitations of a cross sectional study that covers a significant amount of time and resulted in changes to risk assessment and treatment allocation. The dysplastic cases of TN-AML (IPS_1-2) found to be worse prognosis, which may indicate that these patients needed HSCT earlier in the post-CR phase. To summarize the results, factors like age and gender shows that patients mean age was 57 years when diagnosed with MLD, whereas with respect to gender, male frequency dominated the study population. Other than this, with respect to IPS measure, gender, age, haemoglobin & bone

marrow blasts in %, were non-significant between the IPS groups. While significant relation was observed in between IPS and prognosis dysplasia lineages white blood cells ($p=0.01$), neutrophils ($p=0.01$) and platelets ($p=0.04$). Thus, IPS_0 shows significant finding between IPS and dysplasia lineages overall IPS show significant prognostic tool for the TN-AML patients.

CONCLUSION

In conclusion, patients suffering from AML diagnosed with MLD identified between the ages of 18 and 75 were predominantly 57 years old, with men outnumbering women in terms of frequency (68%) and population (32%). Thus, genetically undefinable study participants who tested for NPM1, FLT3, & CEBPA (TN-AML), IPS-related dysplasia provided important predictive information. In this instance, significantly greater dysplasia lineage WBC ($p=0.01$), neutrophil ($p=0.01$), and Plt ($p=0.04$) levels were related to the absence of dysplastic characteristics (IPS_0). IPS_0 & IPS_1-2, on the other hand, did not demonstrate any significance in terms of age ($p=0.09$) or gender (male $p=0.67$ and female $p=0.77$). The influence of the IPS category maintained its validity and gave insight to the response prediction in the ever-changing AML-MLD treatment environment.

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