Original article:

Bone Marrow Evaluation of cases of Leukemia in Pathology Department, BJGMC, Pune

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Abstract:

Introduction: Bone marrow evaluation is an important and effective way of diagnosing and evaluating primary hematologic and metastatic neoplasms as well as non-hematological disorders.

Materials and methods: The present study is an observational study carried out in our institute from June 2012 to June 2014. We studied 63 cases of Bone Marrow Aspiration and Bone Marrow Biopsy received in department of pathology. Case selection was based on clinical features and supported by laboratory evidences. General particulars like age, sex and detailed clinical history was recorded for every patient. Detailed clinical history and physical examination was done as per the proforma annexed. A clinical diagnosis was made based on history and findings of physical examination.

Results : On FAB classification, the most frequent type was AML-M2 found in 6 cases , followed by AML-M0, AML-M1, AML-M3 and AML-M4 found in 3 cases , 3 cases , 2 cases and 2 cases respectively out of the total 63 cases of Leukemia. There were no cases of M5, M6 and M7. In the present study, there were 2 cases of subleukemic leukemia, all were diagnosed as AML-M2 on bone marrow studies.

Conclusion: In the present study, the sex wise distribution of cases showed a male dominance, found in 65.07% with the overall male : female ratio being 1.8:1. The median blast cell percentage in our study was highest for AML (60%)

Keywords: FAB classification

Introduction:

Bone marrow evaluation is an important and effective way of diagnosing and evaluating primary hematologic and metastatic neoplasms as well as non-hematological disorders. Bone marrow evaluation is important in patients with an unexplained excess or deficiency of any peripheral blood cell type , splenomegaly or fever of unknown origin. A through bone marrow study involves examination of peripheral blood smears, direct particle and buffy coat bone marrow smears, trephine biopsy imprints, particle and trephine biopsy sections and marrow volumetric data. The information obtained from the study of these various specimens is usually complimentary. Bone marrow aspiration and biopsy are complimentary in diagnosing cases of acute and chronic leukemia.

Biopsy is of particular value in patients with inadequate aspirate specimens or dry taps with packed or empty marrows in acute leukemia. The present study comprises of evaluation of bone marrow aspiration and biopsy in 63 cases of leukemia carried out in Department of Pathology, BJGMC, Pune.

Aims and objectives:

1) To study the age-wise distribution of leukemia.

2) To study the bone marrow findings in leukemia.

3) To study pattern of leukemia in our institution.

Materials and methods:

The present study is an observational study carried out in our institute from June 2012 to June 2014. We studied 63 cases of Bone Marrow Aspiration and Bone Marrow Biopsy received in department of pathology. Case selection was based on clinical

features and supported by laboratory evidences. General particulars like age, sex and detailed clinical history was recorded for every patient. Detailed clinical history and physical examination was done as per the proforma annexed. A clinical diagnosis was made based on history and findings of physical examination.

Inclusion criteria: Patients of all ages and sexes who had pathological diagnosis suggestive of leukemia on Bone Marrow aspiration and biopsy study from June 2012 to June 2014.

Exclusion criteria: Dry tap on Bone Marrow aspiration and clotted Bone Marrow aspiration samples were excluded from the study.

All the patients were evaluated with the following detailed parameters:

Blood in EDTA bulb received from concerned departments was processed and complete haemogram was performed on Electronic cell counter i.e. Cellenium and Erma.

Peripheral Blood Smear: PBS was obtained and was stained by Leishman stain. Peripheral smear examination - was done systematically under low power, high power and oil immersion.

Bone Marrow Study: Bone Marrow Aspiration with Jamshidi needle either from iliac spine or sternum in adults and from tibia in infants was done followed by its staining and studying its morphology and cytochemical analysis.Bone Marrow biopsy from iliac spine was done for studying the cellularity and architecture of the marrow and its structure.

Materials- Bone marrow aspiration needle, sterile disposable syringes with needles, xylocaine, spirit, clean and dry glass slides, spreader slides, gloves, drapes, formalin, gauze, antiseptic solution scalpel, Bouin's fluid, Haematoxylin and Eosin stains, special stains MPO, PAS and Reticulin stains, wherever required.

Cytochemistry: Studies with special stains like Myeloperoxidase (MPO), Periodic acid-Schiff (PAS) and Non-specific Esterase (NSE) were done whenever required.Morphological subtyping was done according to the French–American–British (FAB) classification.

Other investigations:

X-ray, USG, CT, MRI, Lymph node biopsy was carried out in some patients.

Observation and result:

The present study was carried out in our department from June 2012 to June 2014. The data collected was studied and analysed. A total of 63 cases of leukemia were studied. Out of the 63 cases, 40 cases were studied both on both Bone Marrow Aspiration and Bone Marrow Biopsy and 23 were studied only on Bone Marrow aspiration. Out of the total 63 cases diagnosed as leukemia, 27 cases (42.9%) were of ALL, 16 cases (25.4) were of AML, 16 cases (25.4) were of CML and 4 cases (6.3) were of CLL.

Table 1. Distribution of cases of leukemia.

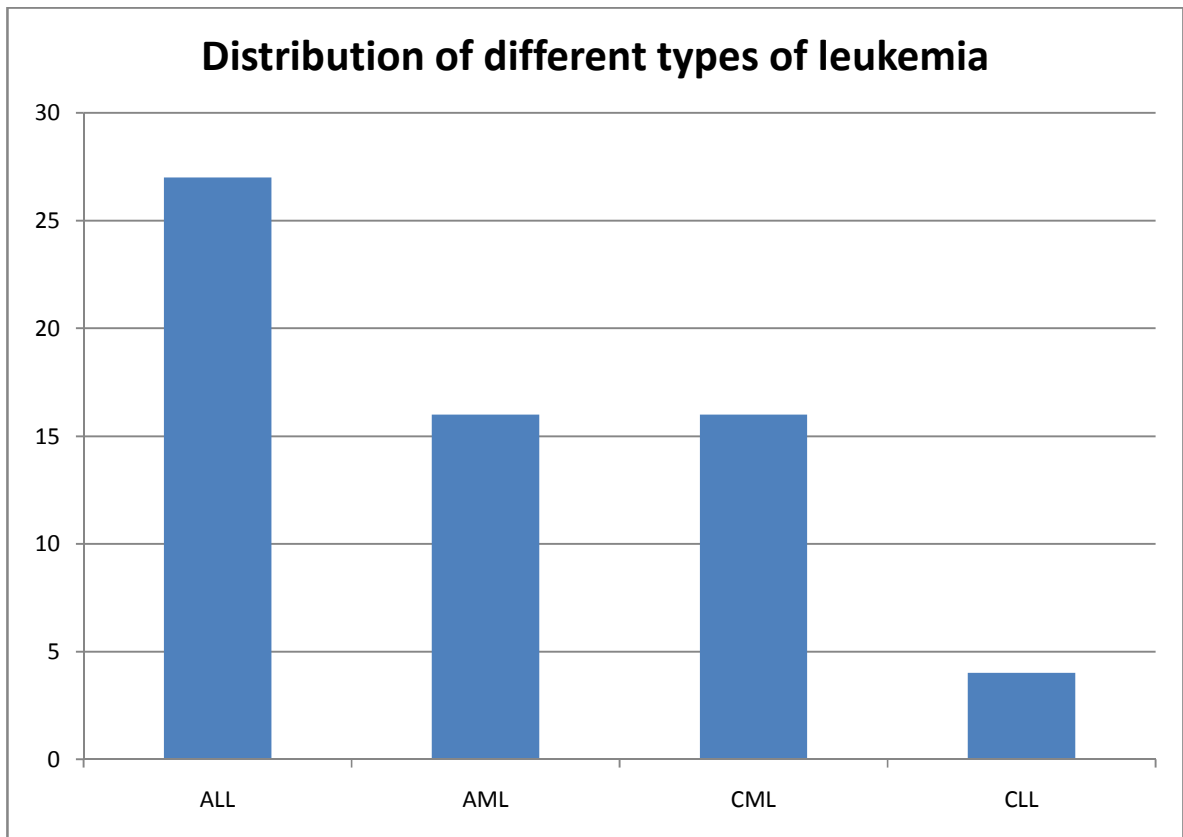| Type of Leukemia	Frequency	Percentage
Acute	43	68.25
Chronic	20	31.75

In our study, Acute Leukemia was more common compared to Chronic Leukemia. Acute Leukemia was found in 43 cases (68.25%) and Chronic Leukemia in 20 cases (31.75%).

Table 2. Distribution of different types of leukemia.

Type of Leukemia	Frequency	Percentage
ALL	27	42.9
AML	16	25.4
CML	16	25.4
CLL	4	6.3
Total	63	100.0

In our study, Acute Lymphoblastic Leukemia was the commonest finding and was seen in   27 cases (42.9%). followed by Acute Myeloid Leukemia and Chronic Myeloid Leukemia found in 16 cases (25.4%) each and Chronic Lymphocytic Leukemia in 4 cases (6.3%).

Graph 1. Distribution of different types of Leukemia.

The 63 cases were further typed with the help of FAB classification for Leukemia.

Table 3. Distribution of different types of ALL by FAB classification:

Type of Leukemia	Frequency	Percentage
ALL-L1	11	40.74
ALL-L2	13	48.15
ALL-L3	3	11.11
Total	27	100

On FAB classification, the most frequent type was ALL-L2 found in 13 cases (48.15%) followed by ALL-L1 and ALL-L3 found in 11 cases (40.74%) and 3 cases (11.11%) respectively.

Table 4. Distribution of different types of AML by FAB classification:

Type of Leukemia	Frequency	Percentage
AML-M0	3	18.75
AML-M1	3	18.75
AML-M2	6	37.50
AML-M3	2	12.50
AML-M4	2	12.50
Total	16	100

On FAB classification, the most frequent type was AML-M2 found in 6 cases , followed by AML-M0, AML-M1, AML-M3 and AML-M4 found in 3 cases , 3 cases , 2 cases and 2 cases respectively out of the total 63 cases of Leukemia. There were no cases of M5, M6 and M7. In the present study, there were 2 cases of subleukemic leukemia, all were diagnosed as AML-M2 on bone marrow studies.

We also had 3 cases of CML in Blast crisis out of 16 cases of CML. They were distinguished for AML on the basis of massive splenomegaly on systemic examination, increased Platelet count and presence of basophilia on Peripheral smear examination and presence increased megakaryocytes on Bone marrow examination. Also, all the cases had increased fibrosis on Reticulin stain performed on Bone marrow Biopsy.

In our study, Chronic Leukemia was found in the higher age group compared to acute leukemia. The median age group for chronic leukemia (50 years) is higher compared to acute leukemia (21 years).

Table 5. Median age affected in different types of leukemia.

Type	Median Age (in years)
ALL	15.5
AML	45.5
CML	40.5
CLL	61

In our study, the median age affected in ALL was the lowest (15.5 years) and in CLL was the highest (61 years).

Table 6. Distribution of cases according to sex:

Sex	Acute	Chronic	Total	Percentage
Female	14	8	22	34.92
Male	29	12	41	65.08
Total	43	20	63	100.0

In our study, the sex wise distribution of cases in acute and chronic cases of leukemia showed a male dominance. We found male distribution in 41 cases (65.07%) and female in 22 cases (34.93%) out of 63 cases

Graph 2. Distribution of cases according to sex.

Table 7. Distribution of cases of different types of leukemia according to sex:

	Frequcncy(%)	
Type	Male	Female
ALL	19 (70.37)	8 (29.63)
AML	10 (62.5)	6 (37.5)
CML	10 (62.5)	6 (37.5)
CLL	2 (50)	2 (50)

In our study, we found male dominance in ALL, AML and CML whereas in CLL, an equal distribution of male and female cases was noted. Overall Male:Female ratio was 1.8:1.

Table 8. Findings of Median Blast % found in different types of leukemia.

Type of Leukemia	Median Blast cell %
ALL	40
AML	60
CML	8
CLL	35

The median blast cell percentage in our study was highest for AML (60%) and the lowest for CML (8%).

Table 9. Findings of MPO stain on Acute Leukemia cases

MPO	Lymphoid	Myeloid	Frequency
Absent	27	0	27
Present	0	16	16
Total	27	16	43

In our present study, MPO stain was positive in 16 cases (100%) of AML and was negative in all the 27 cases (100%) of ALL.

Table 10. Findings of PAS stain on Acute Leukemia cases

PAS	Lymphoid	Myeloid	Frequency
Absent	9	15	24
Present	18	1	29
Total	27	16	43

In the present study, PAS stain was positive in 18 cases (66.67%) of ALL and was also positive in 1 case (6.25%) of AML. PAS stain was negative in 9 cases (33.33%) cases of ALL and 15 cases (93.75%) of AML.

In the present study, NSE stain was positive in 2 cases (100%) of AML M4.

In the present study, Reticulin stain was performed on the Bone Marrow biopsies. All the cases were graded as 1-2 on Reticulin Stain except 3 cases of CML(Blast crisis) which showed Grade 3 on Reticulin stain.

Table 11. Findings of Iron stores by Perl's Stain on Bone marrow aspiration and Bone Marrow Biopsy.

Type	Normal iron stores (2+ to 3+)	Reduced iron stores (0+ to 1+)
ALL	22	5
AML	12	4
CML	14	2
CLL	4	0
Total	52 (82.53%)	11 (17.47%)

In our present study, the iron stores on Perl's stain were normal in 52 cases (82.53%) out of the total 63 cases of leukemia.

Discussion:

This study was carried out on 63 cases admitted in our institute from June 2012 to June 2014 for the evaluation of leukemia.

Table 12. Comparison of Studies of Distribution of cases of leukemia.

Study	Year	Acute	Chronic	Total
D'Costa G G, et a[1]	1984	140	102	242
Kulshrestha R, et al[2]	2002	121	75	196
Laishram R, et al[3]	2011	88	15	103
Present Study	2014	43	20	63

D'Costa, et al (1984),[1] Kulshrestha R, et al (2002),[2] Laishram R, et al (2011),[3] in their studies found out 140 cases (57.85%), 121 cases (61.73%) and 88 cases (85.43%) respectively and observed a larger number of cases of acute leukemia over chronic leukemia.

In present study, out of 63 cases of leukemia, 43 cases (68.25%) were of acute leukemia and 20

cases (31.75%) were of chronic leukemia. There was a higher frequency of Acute leukemia in our institution. The present study is comparable to studies by D'Costa et al (1984)[1] , Kulshrestha R et al (2002)[2], Laishram R, et al (2011)[3] who also had a similar finding.

Table 13. Comparison of studies of frequency of different types of leukemia

Study	Year	ALL	AML	CML	CLL
Kulshrestha R, et al[2]	2002	39	56	69	6
Laishram R, et al[3]	2011	34	54	13	2
Present	2014	27	16	16	4

Kulshretha R, et al(2002)[2] in their study found out largest number of cases diagnosed as CML and lowest number of cases diagnosed as CLL.

Laishram R, et al (2011)[3] in their study found out largest number of cases diagnosed as AML and lowest number of cases diagnosed as CLL.

In the present study we found out largest number of cases diagnosed as ALL and lowest number of

cases diagnosed as CLL. The finding of highest number of cases as ALL was because of the highest number of samples obtained from the age group of 0-20 years. The finding of the lowest number of cases as CLL is comparable to studies by Kulshretha R, et al(2002)[2] and Laishram R, et al (2011)[3].

Table 14. Comparison of Studies of distribution of cases of ALL on FAB classification.

Study	Year	L1	L2	L3	Total
Kulshrestha R, et al[2]	2002	15	23	1	39
Preethi CR[4]	2008	0	13	0	13
Laishram R, et al[3]	2011	4	28	2	34
Present	2014	11	13	3	27

Kulshrestha R,et al (2002)[2] in their study found out ALL-L2 to be the commonest subtype of ALL present in 23 cases (58.97%) out of 39 cases of ALL. Preethi CR,et al (2008)[4] in their study found out ALL-L2 to be the commonest subtype of ALL present in 13 cases (100%) out of 13 cases of ALL.

Laishram R, et al (2011)[3] in their study found out ALL-L2 to be the commonest subtype of ALL present in 28 cases (82.4%) out of 34 cases of ALL.

In the present study we found out ALL-L2 to be commonest subtype of AML present in 13 cases (40.74%) out of 27 cases of ALL. The finding in our study is comparable to the studies by Kulshrestha R,et al (2002)[2], Preethi CR,et al (2008)[4] and Laishram R, et al (2011).[3]

Table 15. Comparison of Studies of distribution of cases of AML on FAB classification.

Study	Year	M0	M1	M2	M3	M4	M5	M6	M7	Total
Kulshrestha R, et al[2]	2002	0	5	30	13	3	4	1	0	56
Preethi CR[5]	2008	0	10	22	3	11	4	0	0	50
Laishram R, et al[3]	2011	4	5	15	21	5	1	3	0	54
Present	2014	3	3	6	2	2	0	0	0	16

Kulshrestha R,et al (2002)[2] in their study found out AML-M2 to be the commonest subtype of AML present in 30 cases (53.57%) out of 56 cases of AML.

Preethi CR,et al (2008)[5] in their study found out AML-M2 to be the commonest subtype of AML present in 22 cases (44%) out of 50 cases of AML.

Laishram R, et al (2011)[3] in their study found out AML-M3 to be the commonest subtype of AML present in 21 cases (38.9%) out of 54 cases of AML.

In the present study we found out AML-M2 to be commonest subtype of AML present in 16 cases (37.50%) out of 63 cases of AML and is comparable to the study by Kulshrestha R,et al (2002).[2]

Table 16. Comparison of Studies of median age found in different types of leukemia.

Study	Year	Median Age(years)			
		ALL	AML	CML	CLL
Idris M, et al[6]	2001	7	26	22	56
Rathee R, et al[7]	2012	22	28	26	58
Hossain MS, et al[8]	2012	27	35	40	60
Present	2014	15.5	45.5	40.5	61

Idris M,et al (2001)[6] in their study, found the median age affected in ALL was the lowest (7 years) and that in CLL was the highest (56 years). Rathee R,et al (2012)[7] and Hossain MS,et al (2012)[8] in their study, also found the median age affected in ALL was the lowest (22 and 27 years respectively) and that in CLL was the highest (58 and 60 years respectively).

In the present study, the median age affected was found to be lowest in ALL (15.5 years) and highest in CLL (61 years). This finding in our study is comparable to studies Idris M,et al (2001)[6], Rathee R,et al (2012)[7] and Hossain MS, et al (2012).[8]

Table 17. Comparison of Studies according to percentage sex wise distribution:

Study	Year	Male	Female
Idris M, et al[6]	2001	56.66	43.34
Babatunde A, et al[9]	2005	56.2	43.8
Hossain MS,et al[8]	2012	69.2	30.8
Present Study	2014	65.08	34.92

Idris M,et al (2001)[6],Babatunde A, et al (2005)[9] and Hossain MS, et al (2012)[8], in their studies found a male dominance in the distribution of cases comprising of 56.66%, 56.2%, 59.83% and 69.2% respectively.

In the present study, a male dominance was found in the distribution of cases (65.08%) and is comparable with the studies of Idris M,et al (2001)[6],Babatunde A, et al (2005)[9] and Hossain MS, et al (2012).[8]

Table 18. Comparison of Studies according to percentage sex wise distribution of Acute and Chronic leukemia.

Study	Year	Sex	Percentage	
			Acute	Chronic
Kulshretha R, et al[2]	2002	Male	58.67	73.33
		Female	41.33	26.67
Rathee R, et al[7]	2012	Male	62.6	71.4
		Female	37.4	28.6
Present	2014	Male	67.44	60
		Female	32.56	40

Kulshretha R, et al (2002)[2] in their study found a male dominance in both acute (58.67%) and chronic leukemia (73.33%). Rathee R, et al (2012)[7] in their study also found a male dominance in both acute (62.6%) and chronic leukemia (71.4%).

In the present study, a male dominance was found in both acute (67.44%) and chronic leukemia (60%) and is comparable to studies by Kulshretha R, et al (2002)[2] and Rathee R, et al (2012).[7]

Table 19. Comparison of Studies according to sex wise distribution of different types of leukemia.

Study	Year		Frequency(%)	
		Type	Male	Female
Babatunde A, et al[9]	2005	ALL	10(55.55)	8(44.45)
		AML	10(55.55)	8(44.45)
		CML	26(61.90)	16(38.10)
		CLL	8(40)	12(60)
Rathee R, et al[7]	2012	Type	Male	Female
		ALL	65(58)	47(42)
		AML	143(65)	77(35)
		CML	176(69.3)	78(30.7)
		CLL	51(79.6)	13(20.4)
Present	2014	ALL	19(70.37)	8(29.63)
		AML	10(62.5)	6(37.5)
		CML	10(62.5)	6(37.5)
		CLL	2(50)	2(50)

Babatunde A, et al (2005)[9] and Rathee R, et al (2012)[7] found a male dominance in all the categories of leukemia.

In the present study, a male dominance was found in ALL, AML and CML and is comparable wit the studies of Babatunde A, et al (2005)[9] and Rathee R, et al (2012).[7] However in CLL, an equal sex wise distribution of cases was noted because of a smaller sample size.

Table 20. Comparison of Studies of Blast cell count percentage

		Median Blast cell %			
Study	Year	ALL	AML	CML	CLL
Rathee R,et al[7]	2012	38	45	42	35
Present Study	2014	40	60	8	35

Rathee R,et al[7] in their study found out the mean blast percentage was highest in cases of AML(45%)

The present study is comparable to the finding of the study by Rathee R,et al[7] Shome et al (1985)[10] and by Preethi CR (2008)[4] in their studies had PAS positivity in 20% and 38.5% cases of leukemia.

Gupta N, et al (2010)[11] in their study found the iron stores to be normal in 23 of their total 26 leukemia cases (88.46%).

In the present study the iron stores were normal on bone marrow and bone marrow biopsy in 56 cases (82.53%). The finding in our study is comparable to the study by Gupta N, et al (2010).

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Conclusion:

In the present study, the sex wise distribution of cases showed a male dominance, found in 65.07% with the overall male : female ratio being 1.8:1.

The median blast cell percentage in our study was highest for AML (60%)

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