A profile of hematological malignancies diagnosed in a tertiary care center of Assam: Highlighting the experience from a resource constraint perspective

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ABSTRACT

Background: Hematological disorders require a systematic approach, from clinical examination to a complete blood count, followed by a bone marrow study for diagnosis. To effectively plan, treat, and prognosticate, immunophenotyping and molecular studies are essential to incorporate. **Aims and Objectives:** The aim of our study is to understand the pattern of neoplastic hematological conditions encountered in Assam and share our experience during the diagnosis. **Materials and Methods:** This was a single-centered, observational study carried out for 18 months (January 2022–June 2023). A thorough hematological workout was done, including cytochemical staining and conventional polymerase chain reaction for the BCR-ABL transcript, after satisfying inclusion and exclusion criteria. The data were analyzed and correlated before arriving at the final diagnosis. **Results:** The total of 38 neoplastic cases confirmed by bone marrow study. Out of 38 cases, 14 were chronic myeloid leukemia, 10 were chronic, 4 were in the blastic phase, and BCR-ABL was positive in 13 cases. Two cases were pediatric, of which 1 was in the blastic phase. All 8 cases of plasma cell dyscrasia presented with anemia, bone lesions, and hypercalcemia. The acute leukemia category consisted of 4 acute myeloid leukemia cases, of which 1 was acute promyelocytic leukemia. A total of 10 acute lymphoblastic leukemia (ALL) cases, where 1 case was T-ALL and the rest was B-ALL. Two cases showed a subleukemic presentation in peripheral blood. **Conclusion:** The experience of pathologists and their meticulous approach can greatly aid in accurate diagnosis, but the immunophenotyping facility and molecular hematology setup can provide most of the relevant information for the best decision-making.

Key words: Bone marrow; Multiple myeloma; Acute leukemia; Chronic leukemia; BCR-ABL transcript

INTRODUCTION

Health systems are challenged by a rapidly aging population, which is causing an increase in the burden of hematologic malignancies.[1,2] Evidence regarding the disease incidence, disease prevalence, and disease burden associated with hematologic malignancies worldwide is limited. Only few local studies have reported the burden of individual diseases.[3,4]

Hematological disorders can affect any age and gender and usually present with anemia. The spectrum of hematological disorders is relatively different in the developing world than in developed countries.[5] Hematologic neoplasia is comparatively common, accounting for around 9% of all cancers and being the fourth most frequently diagnosed cancer in both men (after prostate, lung, and colorectum) and women (after breast, lung, and colorectum) in economically developed regions of the world.[6]

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The geographical distribution of various neoplastic hematological disorders has been provided by various authors, but no such data has been published regarding the northeastern states. For the diagnosis of hematological neoplastic disorder, a systematic approach starting from the clinical examination to a complete blood count and bone marrow study is required. The bone marrow profile includes both bone marrow aspiration (BMA) and trephine biopsy, which are the two most important diagnostic tools for neoplastic hematological disorders. BMA provides reliable information regarding the morphology and stage of maturation of blood cells.[7] Trephine biopsy provides additional information regarding the marrow architecture, cellularity, and infiltration.

The aim of this study was to understand the pattern of neoplastic hematological disorders encountered in our set up by thorough clinico-hematological workout.

Aims and objectives

1. To understand the pattern of neoplastic hematological conditions encountered in a tertiary care center
2. To share the experience during the diagnosis in a resource-limited setup.

MATERIALS AND METHODS

- This was a single-centered, observational study of ambispective direction, where enrolment of cases were both retrospective and prospective, done in the Department of Pathology (Hematology Section) with the Department of Medicine, the Cytogenetics Laboratory of the Anatomy Department, and the Medical Research Unit of the institute. The study was carried out over a period of 18 months (January 2022–June 2023).
- The study was approved by the Institutional Ethics Committee for Human
- We followed standard operating procedures (SOP) for the evaluation of hematological disorders. The information collected were clinical details of the patients, like age, gender, chief complaints, and lab investigations such as complete blood count, coagulation profile, reticulocyte count, biochemical parameters, and radiological investigations with clinical diagnosis. Peripheral blood smear (PBS) and BMA and trephine biopsy slides were retrieved, re-examined, and correlated with the records in the case of retrospective enrollment.
- For prospective enrollment, the same SOP was followed in addition to a physical interview for clinical history. Patients' complete blood count, coagulation profile, reticulocyte count, and peripheral blood films were done first, followed by BMA, and a biopsy were done simultaneously under aseptic and antiseptic

conditions after giving 2% xylocaine as local anesthesia. For a complete blood count, an automated hematology analyzer (Sysmex {XN 550}) with six parts was used, followed by microscopic examination of PBS.
- Cytochemical stains such as myeloperoxidase (MPO) and PAS were used whenever necessary.
- MPO method:
  Blood smears from both tests and the normal sample as a control were dried and covered with a wash burn stain containing benzidine base, basic fuschin, and sodium nitroprusside for 3 min. Then an equal quantity of freshly prepared H_2O_2 is used to flood the slide for 2 min. Then it is washed thoroughly with tap water for 1 min, dipped in 95% alcohol for 1 min, and then again washed in tap water for 1 min. Then the slide is flooded with Wright's stain for 5 min, and twice the volume of buffer solution is used for 30 min. Finally, the slide is washed in water, dried, and examined.[8] More than 3% of blasts positive for MPO were interpreted as positive.
- Bone marrow study:
  BMA was performed using Salah's needle, and 0.25–0.5 mL of aspirate was withdrawn from the posterior superior iliac spine, and smears were prepared immediately.[9] BMA was followed by a bone marrow biopsy (BMB) (Figure 1 shows a single core of BMB), which was done through the same incision by using a Jamshidi needle, taking the specimen 0.5–1 cm away from the aspirated site. Guidelines from Naresh et al., 2006 are followed while processing for BMB.[10]
- Molecular method (polymerase chain reaction [PCR]):
  For the molecular study of chronic myeloid leukemia cases, RNA was extracted from peripheral blood and complementary DNA was prepared with the aid of the reverse transcriptase PCR method. To identify the *BCR-ABL* transcript type of the patients, a qualitative PCR was carried out with one specific primer.

BCR forward primer (5′-GGA GCT GCA GAT GCT GAC-3′) and ABL reverse primer (5′-TCA GAC CCT GAG GCT CAA-3′). Along with this, a housekeeping primer (β-globulin), (5′AGT GCT GCA AGA AGA ACA ACT ACC3′) and (5′CTC TGC ATC ATG GGC AGT GAG CTC3′), was used to avoid the false negative results.
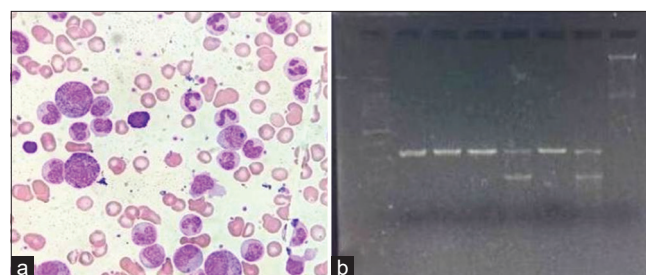

Figure 1: Chronic myeloid leukemia (a) PBS study. (b) BCR-ABL transcript positivity

In difficult cases, the consensus of three pathologists was taken as final:

Inclusion criteria

All cases of hematological were malignancies confirmed by bone marrow studies, and complete relevant clinical and laboratory workup.

Exclusion criteria

Cases with inconclusive evidence, incomplete reports in records, incomplete workups, or inadequate bone marrow samples.

Statistical analysis

This was an observational study; the data obtained were recorded on an Excel sheet. The descriptive statistics were expressed in terms of numbers and percentages. Tables and graphs were used to present the final data.

RESULTS

This study was done in a tertiary care hospital, where a total of 38 cases of hematological malignancies were included during 18 months.

Table 1 shows hematological malignancy and its distribution. Chronic myeloid leukemia (CML): 14 cases (36.84%); acute leukemia: 14 cases (36.84%); plasma cell dyscrasia: 08 cases (21.05%); and chronic lymphocytic: leukemia 02 cases (5.27%).

Table 2 shows 10 cases of CML in the chronic phase and 4 cases in the blastic phase, along with age and hematologic characteristics.

Table 3 shows 5 cases of acute myeloid leukemia (AML), 10 cases of acute lymphoblastic leukemia (ALL), and other hematological characteristics.

Table 4 shows the AML profile. Age ranges from 36 to 68 years, with a male-to-female ratio of 1.5:1.

DISCUSSION

Hematological disorders exhibit a wide spectrum, which frequently requires bone marrow examination to attain a final diagnosis. In our study, we obtained 38 cases of hematological malignancy that satisfied the adequacy criteria (Table 1). We excluded incomplete workups, incomplete clinical information, and incomplete records in retrospective cases. There was one case of metastatic malignancy. As metastatic

Table 1: Distribution of malignant hematological disorders

S. No.	Disorder	Total number of cases	M:F	Percentage
1.	Acute leukaemia	14	1.2:1	36.84
2.	Plasma cell dyscrasia	08	3:1	21.05
3.	Chronic myeloid leukaemia	14	2:1	36.84
4.	Chronic lymphocytic leukaemia	02	1:1	5.27

Table 2: Pattern of distribution of CML

Phase	No. of patient %	Age	Hb	Total count	Blast ()	Basophil (%)	Platelet count
Chronic	10 (71.4)	7–69 years	5.0–8.2 g/dL	2–3 lacs	2–7	3–5	4–5 lacs
Blastic	4 (28.6)	8–85 years	2.4–4.8 g/dL	1.5–2 lacs	30–80	10–12	40,000–50,000
Total	14 (100)						

CML: Chronic myeloid leukemia

Table 3: Pattern of distribution of acute leukaemia

Category	No. of patients	Age	Hb	Total count	Blast (%)	Platelet count	Cytochemistry MPO/PAS
AML	4	36–71	4.5–7.0 g%	36,000–1,30,000	38–90	20,000–70,000	MPO positive (4 cases)
ALL	10	2–25	3.2–7.2 g%	45,000–1,32,000	29–64	30,000–80,000	PAS positive (9 cases)

AML: Acute myeloid leukemia, ALL: Acute lymphoblastic leukemia

Table 4: Acute myeloid leukaemia profile

S. No.	Age (years)	Sex	Hb (g/dL)	Total count	Platelet count	Blasts (%)
1.	36	M	6.5	36,000	41,000	70
2.	47	F	5.2	1,04,000	70,000	46
3.	68	M	4.5	1,30,000	20,000	90
4.	53	M	7.0	55,000	62,000	38

malignancy is not a primary hematological disorder, it was not included in the hematological malignancy category.

The CML was the largest hematological neoplasm and constituted 36.84% of all neoplasms. The age group ranges from 7 to 85 years old. We had 3 cases of pediatric CML (7 years, 8 years, and 13 years, and the others were adults). The M: F ratio was 2:1, in contrast to 1.6:1.0 in the Indian study and 1.09:10 in the western study.[11] The majority of the cases of CML were provisionally diagnosed from the PBS study (Figure 1a shows leucocytosis with the presence of immature myeloid cells like myelocytes, metamyelocytes, and bands along with basophils). Most of the CML cases presented with chronic phase: 10 cases (71.4%) and only 4 cases with blastic phase (28.6%) (Table 2). One of the striking features of our study group was that the youngest was 7 years female, followed by another 8 years female. The median age of CML was 33 years in our study, which is almost similar to Indian studies published.[12] In all 14 cases of CML, PCR was done for detection of the BCR-ABL transcript, and 13 cases were confirmed as positive. The commonest transcript was p210 (Figure 1b). Imatinib drug was the first signal transduction inhibitor (STI) used in a clinical setting. It prevents a BCR-ABL protein from exerting its role in the oncogenic pathway in CML.[13] Imatinib is freely available for patients, being the public hospital in our setup.

There were gross splenomegaly vague symptoms like tiredness and weakness in the chronic phase. 4 cases of CML were presented in the blasts crisis phase, where there was severe anemia (2.4–4.8 g%), thrombocytopenia, high basophilia, marked leukocytosis (total count 1.5–2 lacs), and a blast counts up to 80%. All the blastic cases presented with deteriorating clinical conditions. One CML case with blast crisis is a pediatric age of 8 years deteriorated rapidly, similar to the case published by Dey and Dutta (2023),[14] and its behavior is much more aggressive than that of adult CML in blast crisis. This child died within 1 month of diagnosis during Imatinib therapy.[15]

Multiple myeloma (MM) is an incurable disease with a relapsing-remitting nature. It is characterized by the neoplastic proliferation of clonal plasma cells producing excess monoclonal immunoglobulin, light chains, or both, often resulting in a multitude of target organ damage.[16] Among these ones, one of the most fascinating but, at times, most difficult is that of a morphological diagnosis. There happen to be a wide variety of morphological forms of plasma cells, which may create difficulties in the diagnosis.[17] In our study, we diagnosed a total of 8 cases of plasma cell dyscrasia MM in BMA (Figure 2a shows the presence of Mott cells and biopsy; Figure 2b shows the presence of plasma cells), and on imprint, the age group ranged from 41 to 70 years, and M: F was 3:5. The mean age of presentation in this small group was 53.25 years, in contrast to a large study, which showed a mean age of 65 years.[16] All 8 cases (100%) were presented with multiple bone lesions, hypercalcemia, and anemia; 7 cases (87.5%) showed the presence of M protein (Figure 2c); and 3 cases showed kidney impairment (creatinine > 2 mg/cubic ml of blood). We diagnosed this category as having more than 10% plasma cells in bone marrow and the presence of one or more CRAB features (hyercalcemia, renal involvement, anemia, bone involvement) as per the criteria laid down by the American Society of Clinical Oncologists.[18] A case of plasma cell dyscrasia is labeled as multiple yeloma when there are 10% or more clonal plasma cells on bone marrow examination or a biopsy-proven plasmacytoma plus the presence of one or more myeloma-defining events. Myeloma-defining events include the presence of one or more CRAB features or one or more biomarkers of malignancy.[16]

The next common malignancy was acute leukemia, constituting 36.84% of hematologic malignancy, of which 4 cases were AML and 10 cases were ALL (Table 3).

All the cases of acute leukemia were diagnosed with the help of an initial PBS study, followed by BMA and BMB. The cases were initially suspected in PBS based on findings of more than 20% blast with other usual findings of severe anemia, leucocytosis, and thrombocytopenia (Figure 3a shows the presence of myeloblasts, a prominent Auer rod in the cytoplasm, and prominent nucleoli). However, two cases were atypical with sub-leukemic presentations and were diagnosed as acute leukemia on a bone marrow study. Acute leukemia cases were further classified on the basis of cytochemistry like MPO (Figure 3b shows MPO positivity) and PAS stain. However, two cases remain inconclusive enough to be put into any of these
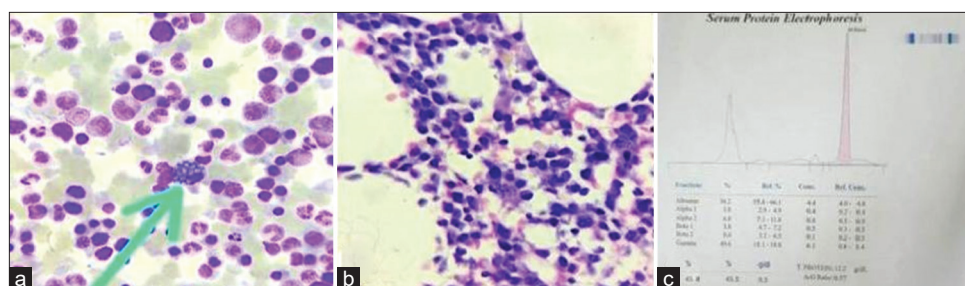

Figure 2: Plasma cell dyscrasia (a) Mott cell in bone marrow aspiration. (b) Bone marrow biopsy. (c) Presence of M band in serum electrophoresis
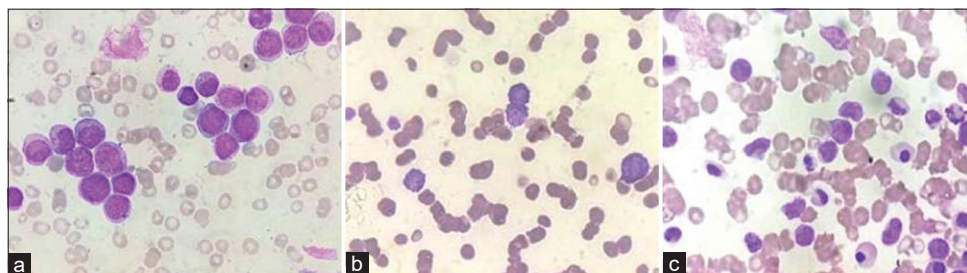
Figure 3: Acute leukemia (a) acute myeloid leukemia – Auer rods in peripheral blood smear (PBS). (b) Myeloperoxidase positivity. (c) ALL in PBS

categories. We followed up on inconclusive cases where one case was diagnosed as T-cell ALL and the other was AML (M0). As in the FAB classification, AML (M0) is undifferentiated. MPO remains negative (<3%). In that particular case, blast% was 90% with no auer rods in PBS. Another case was promyelocytic leukemia, which clinically presented with hemorrhage and features of disseminated intravascular coagulation.

ALL constituted 10 cases, whose ages ranged from 2 to 25 years, with a median age of 5 years. Only two adult cases were noted. PBS findings in a few cases were difficult to recognize as blasts, so some provisional reports were given as atypical lymphocytes (Figure 3c shows leucocytosis with the presence of blasts), but bone marrow examination revealed sheets of atypical lymphocytes, followed by a negative for MPO staining confirmed as ALL. All cases presented with thrombocytopenia and moderate to severe anemia at the time of diagnosis. Immunophenotyping revealed eight cases to be B-cell ALL (common ALL) and one T-cell ALL on follow-up. The B-cell ALL showed immuophenotype positivity for CD10, CD19, CD 20, CD13, HLA-DR, and CD79a, with aberrant expression of the myeloid lineage marker CD13 in two cases similar to a previous study.[19] The T-cell ALL showed innunophenotyping positive for CD3, CD5, CD7, HLA-DR, and TdT.

There were 2 cases of chronic lymphocytic leukemia; both were males over 55 years old (55 and 60 years old) and presented with vague symptoms of weakness. The hematological profile revealed absolute lymphocytosis >5000 cells/cu ml of blood and a differential lymphocyte count of 55–85% in PBS. PBS showed cells smudging and occasional prolymphocytes, larger in size with a moderate amount of cytoplasm, coarse chromatin, and inconspicuous nucleoli. There was mild anemia and leukocytosis in both cases. Further BMB showed more than >30 lymphocytes present in interstitial spaces.

Limitations of the study
As we have done the study within a resource constraint set up, we had to strictly follow up on the cases that participated in our study for immunophenotype results done outside our facility for further confirmation and subclassification in cases of acute leukemia.

The morphologic accuracy was 84.6% in acute leukemia diagnosis, and a small subset of cases were difficult to classify between ALL and AML. However, for all practical purposes and treatment decisions, including hematopoetic stem cell treatment, a molecular signature, according to the World Health Organization (WHO), is essential. The WHO classification of tumors of various organ systems, also known as the WHO Blue Books, has provided a unified tumor classification system, enabling people across the world to share their knowledge and research results. Newer editions with updates have been made every 5–10 years to reflect our better understanding of these diseases through the ongoing research work conducted by many researchers and physicians.[20,21] This tertiary center is one of the largest in this region and provides specialty care in a patient bed with over 1500 beds. Currently, the treatment protocol for hematologic malignancy depends on WHO criteria-defined diagnosis. In CML and CLL cases, the accuracy level was 100%, which was possible due to strict morphological criteria followed during applying the conventional methods, the experience of the pathologists, consensus building amongst pathologists, and incorporating clinical and radiologic findings.

CONCLUSION

Experience of pathologists and meticulous approach can greatly aid in accurate diagnosis, but immunophenotyping facilities and molecular hematology setups can provide most of the relevant information for best decision-making. Whereever treatment is available and affordable for the public, priority for adequately resourced laboratory service is the ultimate need for the best outcome.

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