IMMUNOPHENOTYPING OF ACUTE LEUKAEMIAS : A CRITICAL ANALYSIS OF 35 CASES

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ABSTRACT

Accurate classification of acute leukaemias is essential for proper case management. The utility of monoclonal antibodies in the diagnosis and classification of acute leukaemias is well established. This diagnostic utility relates primarily to two points: firstly the distinction between acute myeloid leukaemia and acute lymphoblastic leukaemia and secondly to subtypes of acute lymphoblastic leukaemia. Leukaemic cells were immunophenotyped using the alkaline phosphatase antialkaline phosphatase techniques. The monoclonal antibodies were very useful in distinguishing cases of acute myeloid leukaemia from acute lymphoblastic leukaemia. Cases of acute lymphoblastic leukaemia with CD10 positivity showed a better prognosis. T-cell acute lymphoblastic leukaemia was uncommon and was associated with unfavourable prognosis. The alkaline phosphatase antialkaline phosphatase technique served as a reliable and convenient method for immunophenotyping of leukaemias.

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Introduction

Leukaemias, till recently, were identified and classified by morphologic and cytochemical criteria [1,2]. Recent advances in immunology have led to important insights into the stages of human lymphocyte and granulocyte differentiation [3]. These stages can be precisely defined using monoclonal antibodies [mo abs] enabling a more accurate classification of leukaemias [3,4].

The established protocols of immunophenotyping acute leukaemias, especially the acute lymphoblastic leukaemias (ALL), are resource intensive and beyond the reach of most of the developing countries. In this study, a modified protocol was evaluated for immunophenotyping acute leukaemias. The mo abs were used with two primary aims:

- a. To evaluate their role in distinguishing acute myeloid leukaemia (AML) from ALL.

- b. To determine the cells of lineage in cases of ALL and classify them either as B-cell lineage (precursor B-cells) or T-cell lineage.

Material and Methods

Thirty five cases of acute leukaemia diagnosed on peripheral blood smear and bone marrow examination were included in this study. There were 20 cases of ALL, 14 cases of AML and one case of acute undifferentiated leukaemia. The classification of leukaemia was based on the criteria laid by the French-American-British (FAB) cooperative study group [1,5]. Cytochemical stains such as myeloperoxidase (MPO), Sudan Black B (SBB) were always utilised. Immunophenotyping was carried out on routinely prepared blood and bone marrow smears using the alkaline phosphatase antialkaline phosphatase (APAAP) technique [6,7]. The protocol followed was :

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1. Prepare blood and bone marrow smears.
 2. Air dry for 2-18 hours.
 3. Fix in acetone : methanol for 90 seconds.
 4. Transfer directly to Tris buffer saline (TBS).
 5. Leave in TBS for 1-5 minutes.
 6. Add suitably diluted primary mouse mo ab and incubate in a moist chamber at room temperature for 30 minutes.
 7. Tap off antibody and place slide in TBS for 5 minutes.
 8. Add anti-mouse-immunoglobulins. Incubate for 30 minutes.
 9. Tap off antibody and place slide in TBS for 5 minutes.
 10. Add APAAP complex and incubate for 30 minutes.
 11. Tap off APAAP complex. Place slide in TBS for 5 minutes.
 12. Add alkaline phosphatase substrate and incubate for 30 minutes.
 13. Wash in TBS and then with tap water. Counterstain with haematoxylin and mount in an aqueous mounting medium.
- All incubation was done in room temperature in a moist chamber.

Positive and negative controls (e.g. normal peripheral blood smear, known case of leukaemia) were used for comparison.

The following mo abs were used : CD 2, CD 7, CD 10, CD 13, CD 14, CD 19, CD 20, and CD 33. The B-cell antigen expression was defined as CD 19 positivity. CD 20 was used as an additional B-cell marker. The T-cell antigen expression was CD 2 and CD 7 positivity. The cells of B and T lineages were by definition negative for myeloid cytochemical markers and mo abs CD 13 and CD 33. CD 14 was used as a monocytic marker. The common ALL antigen (CALLA) was defined as CD 10 positivity. A positive reaction was seen as cell having red staining of an intensity greater than that seen in the background. The criterion for surface marker positivity was its expression in at least 20% of the leukaemic blast cell population [8].

Electron microscopy was done for detection of MPO in the case diagnosed as acute undifferentiated leukaemia using the method of Frahm and Karnovsky [9].

The cases were treated using standard chemotherapeutic regimens and their outcome was correlated with the type of leukaemia.

Results

There were 20 cases of ALL. Amongst the ALL, there were 9 of FAB L1 and 11 of FAB L2 subtypes. After immunophenotyping, the cases of ALL were placed in three groups (Table 1).

TABLE 1
Immunophenotypes of ALL

Cell type		Total	Adult	Children
Precursor B cell ALL	CD10 + CD19+ CD20+/-	12	2	10
T-ALL	CD2+ CD7+	3	2	1
Precursor B cell ALL	CD10- CD19+ CD20+/-	5	3	2

CD 10 positivity was seen in 12 out of 20 cases of ALL. In childhood ALL, 10 out of 12 cases reacted with CD 10 and were mostly FAB L1 subtype (Table 2). The adult group showed mostly FAB L2 subtype and a lesser CD 10 positivity (Table 3). CD 19 expression was seen in all cases of precursor B-cell ALL. CD 20 was seen in 6 out of the 20 cases.

The CALLA positive (i.e. CD 10 positive) group, on an average, achieved remission in 30-45 days after chemotherapy. All the cases had a disease-free period for at least 1 year after remission except one infant 3 months old who died during the induction phase of chemotherapy, surviving for only 25 days.

The second largest group was CD 10 negative precursor B-cell ALL. The cases were mostly adults (Table 3). There was a low rate of remission in these cases and the relapse

rate was high. There were 3 cases of T-ALL (15%), and were mostly associated with an unfavourable prognosis (Tables 2 and 3). All cases of ALL were negative for MPO SBB, CD 13 and CD 33. No correlation was observed between the FAB classification and the ALL immunophenotypes.

TABLE 2

FAB and immunophenotypes of childhood ALL and their response to therapy

Cell type	L1	L2	Response to chemotherapy
CALLA + precursor B cell ALL	6	4	Remission by 30-45 days. One male infant died on 25th day of chemotherapy. Other disease-free for at least 1 year.
T-ALL	—	1	Remission after 113 days, relapse after 8 months.
CALLA - precursor B cell ALL	1	1	One case remission in 36 days, relapsed after 8 months. Other case remission in 111 days, relapse after 10 months.

TABLE 3

FAB and immunophenotypes of adult cases of ALL and their response to therapy

Cell type	L1	L2	Response to therapy
CALLA + precursor B cell ALL	—	2	a. Remission by 30 days b. Remission in 90 days, disease free for 1 year.
T-ALL	1	1	No remission
CALLA - precursor B cell ALL	1	2	No remission in two cases, third case in remission in 4 weeks, relapse in 3 months.

There were 14 cases of AML and 1 case of acute undifferentiated leukaemia. The blast cells of this case were positive for myeloid markers CD 13 and CD 33. Ultrastructural analysis also showed peroxidase positivity. The FAB subtypes of AML and their reactivity is depicted in Table 4.

TABLE 4

Cytochemistry and reactivity of cases of AML with monoclonal antibodies

FAB subtypes	MPO/SBB	CD 13	CD 33	CD 14	CD 7
AML M0	—	1	1	—	—
AML M1	5	5	3	—	—
AML M2	1	1	1	1	—
AML M3	—	—	—	—	—
AML M4	2	2	2	2	1
AML M5	6	5	4	4	—
AML M6	—	—	—	—	—

Fig. 1 : APAAP staining of leukaemic cells with mo abs. A positive reaction is seen as red staining around the cell. (CD 13 was used).

Fig. 2 : Electronmicrograph of myeloblast showing peroxidase positive granules (x 19,000).

CD 13 was positive in 14 and CD 33 in 11 out of the final total of 15 cases of AML. CD 14 positivity was seen in 6 out of 8 cases of leukaemias of monocytic lineage (AML M4/M5). An odd CD 7 positivity was observed in an otherwise confirmed case of AML M4. All cases of AML were positive for atleast one myeloid marker CD 13 or CD 33.

Discussion

The role of monoclonal antibodies in the classification of leukaemias is well known [3]. The major utility of immunophenotyping lies in the classification of ALL. Early observations go back to the 1970s, when it was observed that children with E-rosette positivity (now T-ALL) had distinct clinical features and a poor prognosis [10]. Following the recognition of T-ALL, a small proportion of cases of ALL were identified as having resemblance to cells of Burkitt's lymphoma. These cells showed surface membrane immunoglobulin of a single light chain, thus indicating a B-cell origin [11]. In 1975, the common ALL antigen (CALLA) was discovered and was found to be present on majority of cases of ALL which did not show B or T cell markers [12]. Cases of ALL which were CALLA positive had a more favourable prognosis [13,14].

Studies have shown that the majority of cases of ALL which have been referred to as "non B, non T" ALL are in fact neoplasm of precursor B cells [14,15]. These precursor B-cells show reactivity to CD 19 and sometimes CD 20. Neame et al document 100% reactivity of non-T ALL with CD 19 [16]. Precursor B-cell ALL is therefore a leukaemia with lymphoblastic morphology with blast cells expressing reactivity to CD 19 and sometimes CD 20 [14]. Precursor B-cell ALL in children expresses CALLA in 90% cases, and is associated with a more favourable prognosis [13,14].

In this study, there were 12 cases of CALLA positive and 5 cases of CALLA negative precursor B-cell ALL. The overall incidence of CD 10 positivity was 60%. Other studies indicate this incidence to be at about 70% [3]. The incidence of CALLA positivity in chil-

dren was 83%. These cases had better rate of remission as compared to other groups (Tables 2 and 3) and a disease-free period of at least one year except an infant aged 3 months who died during induction. Poor prognosis in CALLA positive infantile ALL is recorded [17]. The CALLA negative precursor B ALL had a less favourable prognosis (Tables 2 and 3) as expected [13,14].

CD 2 and CD 7 are considered specific for T-cell lineage in an otherwise confirmed case of ALL [14]. The diagnosis of ALL has to be unequivocal as CD 7 may react with as many as 30% of cases of AML [14,18]. The T-ALL comprise 15-25% of all cases of ALL [10,19]. In this study there were 3 cases of T-ALL giving an overall incidence of 15%.

A major diagnostic utility of mo abs lies in distinguishing AML from ALL. Monoclonal antibodies CD 13 and CD 33 are used as myeloid markers [12,21]. Neame et al reported CD 13 and CD 33 to be positive in 96% of the cases of AML [16]. He reported 87% of cases of AML reacting with CD 13 and 75% with CD 33, while 96% either expressed CD 13 or CD 33 or both. Hansen et al reported 88% of cases of AML reacting with either CD 13 or CD 33 [8]. In our study, CD 13 positivity was seen in 93% and CD 33 in 73% cases and atleast one of these markers was present in all the 15 cases of AML. It needs to be highlighted that around 4% of cases of AML may not react with CD 13 or CD 33 while showing positive reaction for other myeloid markers as myeloperoxidase or Sudan black B [8,16].

The case which was diagnosed as acute undifferentiated leukaemia by light microscopy was positive for myeloid markers CD 13 and CD 33. In addition, peroxidase activity was detected under the electron microscope. This case was subsequently labelled as AML MO. The ultrastructural detection of MPO in blast cells is a valuable tool as a sensitive and specific marker for myeloid cells [21]. The FAB group suggests that demonstration of MPO positive blasts by transmission electron microscopy or reactivity with CD 13 or CD 33 is sufficient for diagnosing AML MO [22].

Whereas a minimum of 3% blast cells must be positive in cases of AML as suggested by the FAB group [1], no such definition exists for ultrastructural myeloperoxidase [20].

CD 14 serves as a monocytic marker and has been reported to be positive in 82-90% of cases of AML M4 and M5 [8,16]. In this study, a positive reaction was seen in 6 out of 8 cases of AML M4 or M5 giving an overall positive reactivity of 75%.

In conclusion, the majority of cases of AML were diagnosed by study of routine morphology and cytochemistry. In these cases, the mo abs did not contribute towards their diagnosis. They, however, proved to be an important tool for identifying blast cells with negative cytochemistry. A case of acute undifferentiated leukaemia could be subsequently classified as AML MO. Cases of ALL could be further subtyped into groups with different prognostic outlook. The CALLA positive precursor B ALL had a more favourable prognosis than other ALL groups. Immunophenotyping cannot be regarded as a "gold standard" for classifying acute leukaemias, however, they provide additional data to improve the current system of patient management [16].

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