

The Role of Kappa and Lambda in Subclassification of B Cell Lymphoblastic Leukemia in Sudanese Patients Using Flow Cytometry

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How to cite this paper: Abdalla, H.A.E., Humeida, A.A.K., Abbass, E., Altayeb, O.A. and Marghani, G.M. (2016) The Role of Kappa and Lambda in Subclassification of B Cell Lymphoblastic Leukemia in Sudanese Patients Using Flow Cytometry. *Open Journal of Blood Diseases*, 6, 44-52.
<http://dx.doi.org/10.4236/ojbd.2016.63007>

Received: May 24, 2016

Accepted: September 10, 2016

Published: September 13, 2016

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Abstract

Background: B-cell Acute lymphoblastic leukemia (B-ALL) is a neoplasm of lymphoblasts which are of B-cell lineage typically composed of small to medium sized blast cells, moderately condensed to dispersed chromatin with scanty cytoplasm and inconspicuous nucleoli, involving the bone marrow and/or blood. **Methods and materials:** This is a prospective cross-sectional study in which 50 blood and/or bone marrow samples of newly diagnosed patients (B-ALL) were tested for immunophenotyping. All samples were prepared for surface and cytoplasmic markers including kappa and lambda human antibody for 10 minutes in dark place and then run by the Flow Cytometer. **Results:** 64% of the study populations were males and 36% were females. Cases were classified according to immunophenotype and the age into different subtypes and showed the following frequencies: Pro B-ALL (8%), early pre B-ALL (56%), common B-ALL (16%), Pre-B-ALL (14%) and Mature B-ALL (only 6%). Surface immunoglobulin was positive in 10% and negative in 90% of all patients, showing 100% positivity in mature B-ALL and totally negative in other subtypes. While cytoplasmic immunoglobulin was positive in 16% and negative in 84% of all patients and was positive in 100% of Pre-B-ALL and in 50% of mature B-ALL. Surface kappa was more expressed in mature B-ALL than lambda giving a ratio of 2:1, while cytoplasmic kappa:lambda was 6:1 in Pre-B-ALL. **Conclusion:** Kappa and lambda have important role in B-ALL classification which necessitates their presence in immunophenotyping of B-ALL.

Keywords

B Cell Acute Lymphoblastic Leukemia, Immunoglobulin, Kappa, Lambda, Flow Cytometer

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common form of leukemia in children; constituting 75% of acute leukemia under six years of age, and 80% - 85% of ALL cases in children are of precursor B-lineage, with good prognosis in children and cure rate of approximately 80% [1] [2].

1.1. B-Cell Acute Lymphoblastic Leukemia (B-ALL)

B-cell Acute lymphoblastic leukemia (B-ALL) is a neoplasm of lymphoblast which are of B-cell lineage typically composed of small to medium sized blast cells, moderately condensed to dispersed chromatin with scanty cytoplasm and inconspicuous nucleoli, involving the bone marrow and/or blood.

Classification/Subtypes Are Classified According to French-American-British (FAB) and World Health Organization (WHO)

1) FAB classification for ALL:

Subtyping of the various TYPES of ALL is done according to the French-American-British (FAB) classification, which was used for all acute leukemia's.

- ALL-L1: small uniform cells, with inconspicuous nucleoli.
- ALL-L2: heterogeneous population of blasts.
- ALL-L3: large cells with cytoplasmic vacuoles and basophilic cytoplasm.

2) WHO 2008 classification for ALL:

The recent WHO (2008) International panel on ALL recommends that the FAB classification be abandoned, since it depends on morphology with no clinical or prognostic relevance. And the WHO advocates the use of the genetics and molecular genetics with immunophenotypic classification mentioned below.

- 1. Acute lymphoblastic leukemia/lymphoma. Synonyms: Former FAB L1/L2.
- i. Precursor B acute lymphoblastic leukemia/lymphoma.
- ALL with recurrent cytogenetic abnormality:
 - t (12; 21) (p12, q22) TEL/AML-1.
 - t (1; 19) (q23; p13) PBX/E2A.
 - t (9; 22) (q34; q11) ABL/BCR.
 - T (V, 11) (V; q23) V/MLL.
- B-ALL with hyperdiploidy.
- B-ALL with hypodiploidy.
- B-ALL not otherwise specified [1].
- Immunological classification of B-ALL.

- Immune phenotype of B-ALL:
- The phenotype of blasts has prognostic impact in B-ALL. Common B-ALL has the best prognosis.
- B-ALL marker.
- CD19/cCD79a/TdT/cCD22.
- Divided into pro-B-ALL (early pre-B-ALL), /CD10-).
- Common ALL (CD10+).
- Pre B-ALL (CD10+/-/cytoplasmic IgM).
- Mature B-ALL (CD10+/rarely, CD20+, CD22+, surface IgM+)
- Other marker used to diagnosis of B-ALL (CD34, CD45) [1]-[6].

1.2. Childhood Acute Lymphoblastic Leukemia

Childhood acute lymphoblastic leukemia comprises different biological subtypes of acute leukemia which is defined by cell morphology, immunophenotype, and genetic abnormalities, affecting the prognosis, and the treatment protocols.

Immunophenotypic Classification of Childhood All

1) Early pre-ALL

The leukemia cells always express CD19 and almost all cases have cytoplasmic CD22, CD79a. CD10 and TdT are expressed in 90% of cases and CD34 in more than 75% of cases. Surface CD22 expression is seen in most cases. And blasts lack expression of surface and cytoplasmic immunoglobulin, and light chains.

2) Pre-B-ALL

The pre B immunophenotype is found in approximately 20% - 25% of cases and is defined by presence of cytoplasmic immunoglobulin μ heavy chains with absence of surface immunoglobulin.

3) B-Cell ALL (Mature B-ALL)

Mature B-ALL constitutes 2% - 3% of childhood cases and blasts express surface immunoglobulin μ heavy chains plus either κ or λ light chains [3].

1.3. Immunoglobulins

Immunoglobulins are glycoprotein molecules which function as antibodies that are produced by plasma cells in response to an immunoglobulin. All immunoglobulins have a four chain structure as their basic unit. They are composed of two identical light chains (23 kD) and two identical heavy chains (50 - 70 kD).

1.3.1. Immunoglobulin Classes

Based on differences in the amino acid sequences in the constant region of the heavy chains, immunoglobulins can be divided into five different classes. All immunoglobulins within a given class will have very similar heavy chain constant regions. The monoclonality of the immunoglobulin is used as evidence of clonality in lymphoid malignancies. These different types of immunoglobulins are detected by sequence studies or more commonly by the use of antibodies directed to these differences.

1. IgG—Gamma heavy chains.
2. IgM—Mu heavy chains.
3. IgA—Alpha heavy chains.
4. IgD—Delta heavy chains.
5. IgE—Epsilon heavy chains.

1.3.2. Immunoglobulin Light Chains

Immunoglobulins have two types of light chains which are kappa and lambda. In each immunoglobulin only one type of light chain is found. Light chain types are based on differences in the amino acid sequence in the constant region of the light chain. These differences are detected by serological means:

1. Lambda light chains.
2. Kappa light chains.

Light chain restriction is another way to confirm clonality [4].

1.4. Definition of Flow Cytometry

Flow cytometry (FC) is a powerful technique for the analysis of multiple parameters of individual cells within heterogeneous populations what will be studied simultaneously. Flow cytometers are used in a range of applications in research and diagnostic laboratories from immunophenotyping, to ploidy analysis, to cell counting and glycoprotein expression analysis. The flow cytometer performs this analysis by passing thousands of cells (which are suspended in sheath fluid) per second through a laser beam and detecting the light that emits from each cell as it passes through by detectors. The emitted light is then converted into signals by the electronics to report cellular characteristics such as size, complexity, and display them [5].

The monoclonal antibodies used in FC are conjugated with fluoro chromes which are stimulated by laser beam in the Flowcytometer [6].

These antibodies are against antigens found on the surface, cytoplasm or nuclei of the studies cells to identify their type.

Role of Flow Cytometric Immunophenotyping in the Diagnosis and Classification of ALL

Flow cytometric immunophenotyping is important for the distinction between acute and chronic leukemias and between ALL and AML, identification of lymphoblast lineage (B-ALL or T-ALL), and moreover the subtype of ALL (immunological classification) and assessing response to treatment, by the detection of minimal residual disease. Prognosis of ALL depends of the phenotype having significant cytogenetic and molecular abnormalities. For example, B-cell ALL with CD9+, CD10+, CD19+, CD20– or only partial, CD34– phenotype is a sensitive marker for t (1; 19) (q23; p13). B-cell ALL with a CD10–, CD15+, CD24– or partial phenotype is associated with MLL abnormality. However, there is little correlation between immunophenotyping and cytogenetic and molecular characteristics of the blasts [7] [8].

2. Materials and Methods

A descriptive cross-sectional study was conducted in Flow Cytometry laboratory for Leukemia & Lymphoma Diagnosis, Khartoum, Sudan in the period from September 2015 to March 2016. The study included 50 patients with B cell ALL. The samples were fresh venous blood or bone marrow aspiration samples containing not less than 20% blast cells. 2 ml of blood or bone marrow (BM) aspirate samples collected in EDTA containers and were received in the hematology unit after collection and then mixed gently.

2.1. Surface Preparation

All tubes were labeled then 100 ul were pipette into each tube from venous blood or bone marrow then washed using 1000 ul PBS in centrifuge for 5 min at 500 rpm, and the supernatant was discharged, 10 ul of Kappa-FITC/Lambda-PE (Immunostep, SL, Spain) were added and mixed gently, incubated in dark for 10 min at RT then After incubation 1000 ul of lysing solution were added, mixed gently, Incubated for 10 min in dark and finally samples were analyzed using the Flow Cytometer (4 COLOR EPICS XL-MCL BECKMAN COULTER, Miami, USA).

2.2. Cytoplasmic Preparation

100 ul of whole blood or BM were added to test tube, then washed using 1000 ul phosphate buffer saline (PBS) in centrifuge for 5 min at 500 rpm, the supernatant was discharged, 100 ul of fixative solution were added, then incubated for 10 min in RT, after that 1000 ul of PBS were added and centrifuged for 5 min in 500 rpm and then supernatants were discharged s, 100 ul of permeabilization solution were incubated for 5 min at RT, 10 ul of kappa-FITC/Lambda-PE or Lambda PE were incubated for 10 min in dark, then it have been washed by PBS and re-suspended in 1000 ul PBS finally analyze were run by flow cytometer [9]-[13].

2.3. Quality Control

Positivity was considered when 20% or more of the population expressed the marker as a cut off points between negative and positive scale for every marker. The percentages, mean fluorescence intensity and the parentheses were also recorded for most of the markers.

3. Results

In this study we analyzed 50 patients newly diagnosed as B-ALL, 32 patients (64%) were males and 18 (36%) were females and according to patients' ages they gave the following frequencies: (6%) patients in group (0 - 12 months), (38%) in group (1 - 5) years, (28%) in (6 - 16) years, (24%) in group (17 - 40) years and (4%) in group (41 - 60) years (**Figure 1**).

Regarding the mean of TWBCs count, it was 29×10^3 c/cumm in all cases, and showed different counts in the B-ALL subtypes (PRO: 64×10^3 c/cumm, Early PRE: 21×10^3 c/cumm, Common: 47×10^3 c/cumm, Pre B-ALL: 29×10^3 c/cumm and Mature:

11×10^3 c/cumm).

In our study B-ALL subtypes were stated in the study population giving the following frequencies: 4 (8%) patients PRO B-ALL, 28 (56%) patients early pre B-ALL and 8 (16%) patients common B-ALL, 7 (14%) patients Pre-B-ALL and 3 (6%) patients Mature B-ALL (**Figure 2**).

Surface and cytoplasmic immunoglobulins for kappa and lambda were tested in the study population, while surface immunoglobulin was positive in 10% and negative in 90% of all patients, and was positive in 100% of mature B-ALL and was totally negative in other subtypes.

Cytoplasmic immunoglobulins were positive in 16% and negative in 84% of all patients. The study population showed 100% positivity in Pre-B-ALL and 50% in mature B-ALL. Surface Kappa was positive in 8% of all subtypes and surface lambda was positive in 4% giving a ratio of 2:1 (kappa:lambda). The expression of cytoplasmic kappa was positive in 12% and cytoplasmic lambda was positive in 2% cytoplasmic kappa:lambda ratio of 6:1 (**Table 1**).

The expression of CD34 was positive in 50% of all cases and the expression was as follow among the different subtypes: Pro-B-ALL (50%), Early pre B-ALL (34%), Common B-ALL (6%), Pre B-ALL (6%), and showed no expression in mature B-ALL (**Figure 3**).

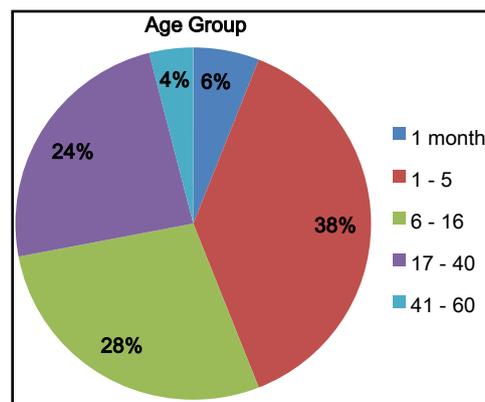


Figure 1. This figure showed the age groups of the study population.

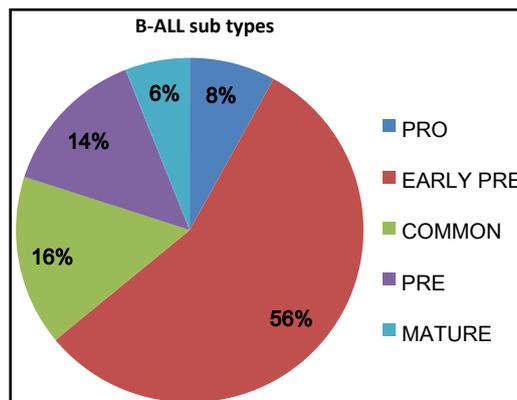


Figure 2. This figure showed the frequencies of B-ALL subtypes among the study population.

CD10 was also tested and was positive in 80% of the study population, and showed no expression in Pro-B-ALL while was 100% positive in common B-ALL, it was also positive in 50%, 10% and 4% of early pre-B-ALL, pre-B-ALL and mature B-ALL cases respectively (Figure 4).

Table 1. Frequency between CD10, CD34, cyto (kappa, lambda), surface (kappa, lambda) positive among diagnosis.

Remark	PRO	EARLY PRE	COMMON	PRE	MATURE
CD10	0%	89%	100%	71%	66%
CD34	50%	60%	37%	42%	0%
c (kappa/lambda)	0%	0%	0%	100%	33%
s (kappa/lambda)	0%	0%	0%	40%	100%

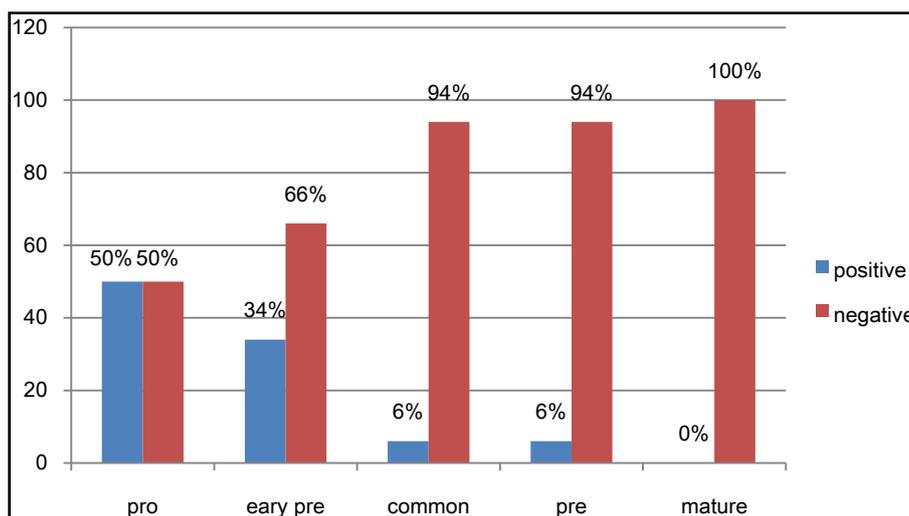


Figure 3. This figure showed the expression of CD34 remark in B-ALL and subtype.

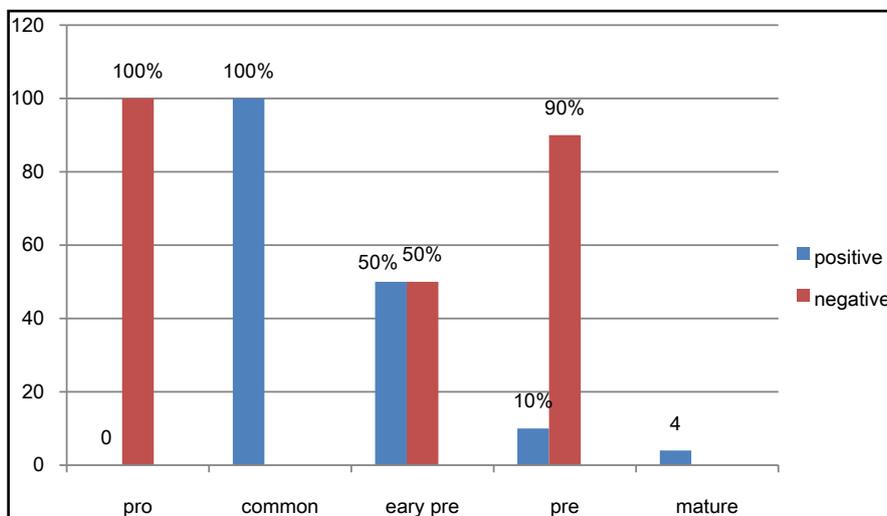


Figure 4. This figure showed expression of CD10 in ALL subtype of B-ALL.

4. Discussion

In this study, we found that most population of B-ALL cases were between 1 - 5 years age group which came in concordances with other study that showed peak between 3 and 5 years and more common in child than adult [14] [15].

The ratio between males and females was found (1:1.7) this findings was also reported by Ward E., De Santis who showed that ALL is slightly more common in males than females with higher frequency in male than female; this may be due to the difference in sample size [15].

Our study confirmed that early pre-B ALL lack cytoplasmic and surface immunoglobulin and Kappa and Lambda while pre-B exhibit cytoplasmic immunoglobulin which agreed with Fatima Bachir *et al.* study that indicated the same result [16].

In this study we found that surface immunoglobulin was positive in mature B-ALL and negative in other subtypes which showed significant result (p-value = 0.000). This result concordant with Fatima Bachir *et al.* that showed 100% of cases with surface Ig (lambda/kappa) and this emphasizes the importance of immunoglobulin and light chain testing in B-ALL for sub classification [16].

Several investigators had similar results concerning the expression of cytoplasmic immunoglobulin in pre B ALL which revealed positive result in all cases (100%) and found a significant finding (p-value = 0.00) [16]. There are some data suggesting that the frequency of surface lambda was much higher than kappa (3 to 1) this result disagree with our finding which showed that the surface immunoglobulin kappa was expressed in 70% of cases with positive light chains while lambda was found in 30%.

CD10 in this study was positive in 80% of all cases and that was considerable higher than that reported in other studies, while that was concordant with other studies [16]-[18] and was found that significant in common B-ALL (p-value 0.000) is showed 92.7% study. The expression of CD34 was negative in mature B-ALL which was significant (p-value 0.000), and agreed with Campana, Dario and Frederick [19].

Fatima Bachir *et al.* (2006) had found correlation between subtypes with the clinical characteristics at presentation and found that the mean TWBCS count was high which also agreed with our study [16].

5. Conclusion

Surface and cytotoplasmic immunoglobulins (Kappa and lambda) have very important role in B-ALL sub-classification which necessitates their presence in immunophenotyping panel of B-ALL.

Acknowledgements

We are indebted to the staff of Hematology Department (Alneelain University). We are grateful to the staff of Flow Cytometry Laboratory for Leukemia and Lymphoma Diagnosis for their collaboration and also the Hematology Department staff in Radiation & n Isotopes Center (RICK), finally special thanks to the patients for being cooperative.

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