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Investigates the genetic types of acute lymphoblastic leukemia in full remission Indonesian patients: a cross-sectional analysis

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Abstract

Acute lymphoblastic leukemia (ALL) is most common in children, especially those aged 1–4 years old, and the second most common acute leukemia in adults. The updated International Consensus Classification (ICC) of B-acute lymphoblastic leukemia (B-ALL) and T-acute lymphoblastic leukemia (T-ALL) includes recent clinical, cytogenetic, and molecular data. Transcriptome sequencing (RNA-seq) was performed on 200 bone marrow specimens using TruSeq library preparation and HiSeq 2000/2500 or NovaSeq 6000 sequencers (Illumina). The purpose of this study was to define the frequency of chromosomal abnormalities of ALL patients in adults and children in Indonesian patients after full remission for international collaboration has improved and advanced the diagnosis and treatment of ALL in Indonesia. Our resulting data showed that the most common structural abnormality was t(9;22) in 15% of the patients. The frequency of genetic abnormalities was 69 % and 60% for numerical and/or structural in the B-ALL and T-ALL patients, respectively. The adults had a higher incidence of t(9;22) and a lower incidence of hyperdiploid than children. In conclusion: The results of this study molecular subtypes differ strikingly in their responses to treatment that needs more assessment.

Keywords: Cytogenetic; Transcriptome sequencing; Acute lymphoblastic leukemia (ALL)

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Introduction

Acute lymphoblastic leukemia (ALL) is an aggressive neoplasm that comprises B- and T- acute lymphoblastic leukemia (B-ALL; T-ALL) [1]. The updated International Consensus Classification (ICC) includes revisions to ALL entities previously described in the 2016 WHO classification, as well as several newly described ALL subtypes [2]. Most entities from the 2016 WHO are retained, but several modifications incorporate findings from recent genomic studies [3]. Many of the newly described entities have characteristic clinical features, and the use of the updated classification will assist in risk stratification and treatment selection for these patients [4].

Non-communicable diseases (NCDs), such as heart diseases, diabetes mellitus, and cancers, are increasingly more widespread than communicable diseases globally, including in Indonesia [5]. From

2012 to 2030, Indonesia will have spent $4.47 trillion on NCDs, with breast cancer alone accounting for 15.7 percent of the total cost. As a result, the expense of all cancers will be significantly higher than the estimated 15.7 percent [6]. Considering this, it will be indispensable to know the incidence rates, prevalence proportions, and mortality rates of childhood ALL to plan healthcare strategies for the future. Yet, the exact incidence rate of childhood ALL in Indonesia is still unknown.

A frequently mentioned incidence of childhood ALL is 2.5 to 4.0 new cases per 100,000 children in Indonesia [7]. However, upon tracing the literature, the source states that the incidence mentioned is only an estimation and that "there was no international or national publication on the incidence of childhood cancer and childhood leukemia in Indonesia" [8]. The next best estimate is achieved from publications that calculated ALL incidence in a single institution, which developed the first institution-based cancer registry in 2000 [9, 10]. Indonesia is the fourth most populous country, with more than 13,000 islands [11]. Therefore, such data on incidence from a single institution might not be enough to represent the incidence of childhood ALL in all of Indonesia [12].

B-ALL with t(9;22)(q34.1;q11.2)/BCR::ABL1: lymphoid only and multilineage involvement The new ICC divides the entity “BCR::ABL1-positive B-ALL” into two subtypes: “B-ALL with t(9;22)(q34.1;q11.2)/BCR::ABL1 with lymphoid only involvement” (BCR::ABL1+ ALL-L), and “B-ALL with t(9;22)(q34.1;q11.2)/BCR::ABL1with multilineage involvement” (BCR::ABL1+ ALL-M) [13]. These cases cannot be distinguished by immunophenotyping or differences in the fusion protein (p190 versus p210) [14]. The underlying difference between these subtypes reflects the target cell for the transformation event, with a multipotent progenitor serving as the target for BCR::ABL1+ ALL-M, and a later progenitor targeted in BCR::ABL1+ ALL-L [15].

The former thus appears akin to chronic myeloid leukemia presenting in the lymphoid blast phase (CML-LBP) [16] and the latter to de novo B-ALL [12]. Although optimal therapy for each has not yet been established, prognosis and treatment may differ, particularly in pediatric patients [17].

The purpose of this study was to define the frequency of chromosomal abnormalities of ALL patients in adults and children in Indonesian patients after full remission for international collaboration has improved and advanced the diagnosis and treatment of ALL in Indonesia.

Methods

From December 2019 to December 2022, we reviewed all cases with a final diagnosis of ALL including 200 cases of ALL. Definite diagnosis in all the cases was established based on morphology, cytochemistry, immunohistochemistry, and flow cytometric analysis in our center. All the cases were referred from affiliated hospitals in Indonesia. Pretreatment bone marrow aspirations or peripheral blood samples were cultured. Briefly, the samples were cultured in RPMI 1640 basal medium, containing 10% fetal calf serum (Gibco-Invitrogen-USA), for 72 hours at 37°C, and then treated with 0.1 microgram/ml of colcemid (Gibco-Invitrogen-USA) to stop the cells in the metaphase of mitosis. After harvesting with hypotonic solution (0.068 mol/L KCL) and fixation with acetic acid /methanol, (3/1)the chromosomes were spread and stained using the standard G-banding technique. For each case, a minimum of 20 metaphases were analyzed by using the CytoVision® chromosomal

karyotyping automatic system (Genetix CompanyUSA). Karyotype was written according to the International Chromosome Nomenclature (ISCN 2009). A successful cytogenetic analysis required the detection of at least 2 or more cells with the same structural change or chromosomal gain, 3 or more cells with the same chromosomal loss, in at least 20 metaphases. The patients' karyotypes were thereafter subdivided into groups based on the WHO classification (2008).

Statistical analysis

In this cross-sectional, descriptive study, the mean age and incidence of cytogenetic abnormalities, using the SPSS software package (version 18). Moreover, we performed comparisons in terms of cytogenetic subclasses and age groups using the Pearson chi-square test with MED CALC software.

Results

We conducted a cytogenetic analysis of 200 ALL patients, comprising 170 B-ALL and 30 T-ALL cases. The 170 B-ALL patients were comprised of 50 females at a mean age of 22 ± 12.09 years and 120 males at a mean age of 21.11±12.23 years (mean age=13.78±15.2 years, range=1 month to 79 years). Children accounted for 108 (70.1%) cases at a mean age of 5.79±3.73 years (lower than 15 years), and adults comprised 46 (29.9%) cases at a mean age of 35.36±14.82 years. The 30 T-ALL patients were composed of 10 children and 7 adults, and all of them, except one, were male (94.9%). Karyotyping was unsuccessful in 20 patients; 12 specimens were cultured but did not have metaphases and 10 samples had too few metaphases to be adequate or had too poor quality to be interpreted. There were 130 cases of successful cytogenetic analysis of B-ALL patients, with 50 (40.3%) cases, 19 (12.5%) adults and 40 (25.7%) children, showing normal karyotypes. Normal karyotypes were found in 8 out of the 17 (46.1%) T-ALL patients. The frequency of cytogenetic abnormalities, including numerical and/or structural changes, was 69.9% and 55.9% in the B-ALL and T-ALL patients, respectively.

Table 1.

Distribution of the genetic abnormalities in the T-cell acute lymphoblastic leukemia patients

Karyotype	Frequency %	Male/Female
Normal	7(49)	7/0
Dupl 21	1(8.87)	1/0
t(11;14)	1(8.87)	1/0
Near tetraploidy	1(8.87)	1/0
Hypodiploidy, del 1	1(8.87)	1/0
Del6(q21)	1(8.87)	1/0

There were 13 T-ALL patients with successful karyotyping: 7(49) patients had normal karyotype and the main abnormalities were Dup21, del 6q21, der 13, dup 1, t(11;14), near tetraploidy, and del 1. Table 1, and Table 2, depict the distribution of the cytogenetic abnormalities in the T-ALL patients.

Table 2.

Distribution of the other cytogenetic abnormalities in pseudodiploid B-precursor acute lymphoblastic leukemia pediatric patients

Abnormalities	Number of cases	Percentages of cases (in total 200)
t(10;12), inv12	1	1.13
t(4;9)	1	1.13
t(1,4)	1	1.13
t(7;14)	1	1.13
t(6;12)	2	2.27
del X	1	1.13
dup 1	1	1.13
del 12p	2	2.27
Total	10	11.4

The main cytogenetic abnormality was hyperdiploidy (47 to >65 chromosomes) in 47 (37.8%) B-ALL patients. In the children group, the most common abnormality was hyperdiploidy in 32 (39.9%) patients in comparison with the adults, in whom hyperdiploidy was found in 9(20%) patients. Hyperdiploidy with 51-65 chromosomes, as the sole abnormality, was significantly more frequent in the children (24/27.3%) than in the adults (1/2.5%).

Discussion

The mortality of childhood ALL ranges from 0.44 deaths per 100,000 children in one study [14] to 5.3 deaths per 100,000 children in another study [18]. The CFR in this study is 3.58%. While children in developed countries have almost 90% cure rates [19], only 20% of children in low- and middle-income countries (LMICs) survive. Refusal or inability to pay for therapy was the most common reason for treatment failure in Indonesia. Treatment-related mortality was the second most common reason for treatment failure [20].

Twinning an established form of cooperation [620] between Indonesia and the Netherlands produced an Indonesian-specific protocol in 2016 named the Indonesian Acute Lymphoblastic Leukemia (ALL) protocol. The development of this protocol has resulted in an estimated survival rate of 60–70% [21]. However, mortality varies greatly by city. Most of the studies included in this review are based on the

island of Java. With most of the tertiary center hospitals on that island, 40% of children residing outside Java may not have an equal opportunity for early diagnosis and management [22]. Another plausible explanation for the differing mortality and incidence in Indonesia may be an underreporting of cases. Immunophenotyping by either flow cytometry or immunohistochemistry (IHC) can often suggest, and in few cases definitively establish, a diagnosis of a particular subtype of ALL. ETP ALL is defined by flow cytometry, and CRLF2 expression by flow correlates very well with that subset of BCR::ABL1-like ALL with CRLF2 rearrangement [23].

It is important to note, however, that CRLF2 overexpression is not specific for BCR::ABL1-like ALL because the translocation is also found as a secondary event in cases of iAMP21 and hyperdiploid ALL. Also, some cases of Down syndrome with CRLF2-r have a GEX profile that is different from other cases of BCR::ABL1-like ALL. [24] Other phenotypes correlate with many newly defined entities, although few have high specificity. The most promising surrogate is probably co-expression of CD371 and CD2 in DUX4-r B-ALL. CD19+CD27+CD44-/dim is highly but not perfectly predictive of either ETV6::RUNX1 or ETV6::RUNX1-like ALL and can help identify cases of the latter when ETV6::RUNX1 is not identified. Many of the less common B-ALL entities, including KMT2A-r, ZNF384-r (and ZNF384-like), MEF2D-r, and CDX2/UBTF B-ALL, are characterized by dim or negative CD10; additional immunophenotypic features, such as CD15 expression in KMT2A-r or cytoplasmic mu with high levels of CD38 in MEF2D-r, can help narrow down possibilities [25].

Many translocations result in overexpression of proteins that are detectable by IHC, although the relevant data are limited. IHC to detect the N-terminus of DUX4 is sensitive and specific for the DUX4-r subtype of B-ALL [26]. NUT expression, which is also used in the diagnosis of NUT carcinomas, has been reported in cases of NUTM1-r B-ALL [27]. Among T cell cases, BCL11B is expressed in the BCL11B-a subtype of ETP-ALL [7]. Interpretation should be done with caution, however, because other cases of non-ETP T-ALL or T/myeloid MPAL may express BCL11B, so IHC must be used in conjunction with flow cytometry to confirm the diagnosis of ETP. LMO2 IHC can recognize a subset of cases of T-ALL that likely includes the LMO1/2-r subtype [13] although it is not specific because the rare “BHLH, other” subtype has elevated LMO proteins [27].

Other immunophenotypic findings are largely untested, but because there are well-characterized antibodies against PU.1 and TTF1, (the gene products of SPI1 and NKX2.1, respectively) these could potentially be useful to detect cases of these T-ALL subsets [28]. Furthermore, Reverse transcription followed by the polymerase chain reaction (RT-PCR) using primers directed against specific fusion partners is useful for the identification of several rearrangements, including BCR::ABL1, TCF3::PBX1, ETV6::RUNX1, and UBTF::ATXN7L3 (this last rearrangement may also be detected by genomic PCR) [29]. This methodology is particularly useful for the detection of cryptic rearrangements, verification of the expression and structure of novel fusions identified by other assays (e.g. FISH), or if rapid detection is warranted to guide early therapy [30]. RT-PCR may also be used to detect many of the recurrent rearrangements of other subtypes, such as BCR::ABL1-like B-ALL, but is limited by the great diversity in fusion partners for several kinases.

Conclusion

The results of this study's molecular subtypes differ strikingly in their responses to treatment that needs more assessment.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

The authors declared that this study has received no financial support.

Ethics Statement

Not applicable.

Authors' contributions

All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

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