# Incidence and Primary Immunophenotypic Characterization of Acute Lymphoblastic Leukemia (ALL) by Flow Cytometry at the Children's Clinical University Hospital from 2022 to 2024

**Alma Strupule[1,2], Iveta Gorela[1] , Dagne Grāvele[1]**

[1] Children's Clinical University Hospital, Riga, Latvia; [2] Riga Stradiņš University, Riga, Latvia

## INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy diagnosed in pediatric patients, accounting for more than 25% of all pediatric cancer cases [1].

Although the etiology of ALL can be attributed to inherited genetic syndromes such as Down syndrome, Klinefelter syndrome, and Fanconi anemia, as well as congenital immunodeficiency disorders including Wiskott-Aldrich syndrome, X-linked agammaglobulinemia, and GATA2 haploinsufficiency in some patients, the underlying cause remains largely unidentified in the majority of cases [1,2,3].

Over the past three years, the incidence of diagnosed ALL cases at the Children's Clinical University Hospital has increased, highlighting the need for research not only into the potential causes and pathophysiological processes of ALL but also into diagnostic possibilities and the characteristic features of their primary immunophenotypes using the NAVIOS EX 10-color flow cytometer.

## OBJECTIVES

To investigate the incidence and primary immunophenotypic characterization of pediatric ALL cases using flow cytometry at the Children's Clinical University Hospital from August 24, 2022, to January 31, 2024, while adhering to principles of data security, ethics, and confidentiality.

## METHODS

This retrospective cohort study included 26 pediatric oncohematological patients diagnosed with C91.0 (Acute Lymphoblastic Leukemia (ALL) according to the ICD-10 [4]).

The study evaluated patient demographics including age, gender, and region of residence in Latvia.

Patients underwent an acute leukemia orientation tube (ALOT) protocol examination, as per the standardized NOPHO protocol, at the Clinical Laboratory of the Children's Clinical University Hospital. Additionally, further B- or T-lymphoblast leukemia phenotyping was conducted to ascertain the primary immunophenotype of the blasts.

The patient samples used were either bone marrow (BM) aspirate specimens or peripheral blood (PB) samples.

Using the NAVIOS EX 10-color flow cytometer, the results from the acute leukemia orientation tube (ALOT) protocol examination and subsequent B- or T-lymphoblast leukemia phenotyping of the patients reflected the total blast count, the percentage (%) expression of the antibody markers within the blast population, the expression intensity within this population, and the identification of the corresponding ALL subtype (B-ALL or T-ALL) based on the obtained data.

## RESULTS

The incidence of ALL determined by flow cytometry at the Children's Clinical University Hospital from August 24, 2022, to January 31, 2024, revealed a total of 26 patient cases.

Of these, 23 cases (~88%) were diagnosed with B-cell ALL (B-ALL), and 3 cases (~12%) with T-cell ALL (T-ALL).

Among the 26 patients, 16 were female (~62%) and 10 were male (~38%). At the time of ALL diagnosis, the most common age was 2-3 years old (8 out of 26 cases, corresponding to 31%).

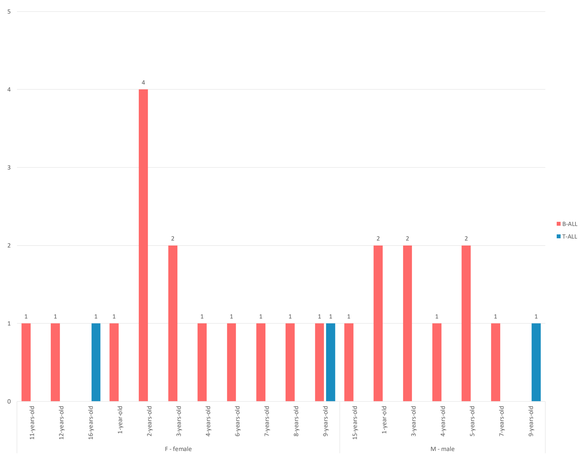


**Figure 1.** *Distribution of Pediatric ALL Cases by Age Group and Gender, with ALL Subtype Classification (B-ALL vs. T-ALL).*

12 out of 26 patients (~46%) resided in Riga.

4 patients (~15%) lived in districts adjacent to Riga (Mārupes, Ķekavas, Ropažu, and Ādažu districts).

1 patient each resided in Liepāja, Ventspils, Talsi, Jelgava, Sigulda, Ogre, and Limbaži districts, while 2 patients (~8%) resided in Madona district.

**Figure 2.** *Incidence of 23 Pediatric ALL Cases Treated at the Children's Clinical University Hospital by Districts Where the Patients Resided on the Latvian Map[5] (August 24, 2022 – January 31, 2024), with Patient Count Highlighted in Red.*

In flow cytometry, among the 26 patients, BM aspirate samples were analyzed for 23 patients, while PB samples were analyzed for 3 patients.

The percentage (%) of blast population in ALL patients varied on the first day of diagnosis, as illustrated in Figure 3, however, in general, the most common percentage of blast population fell within the range of 70% to 100%.

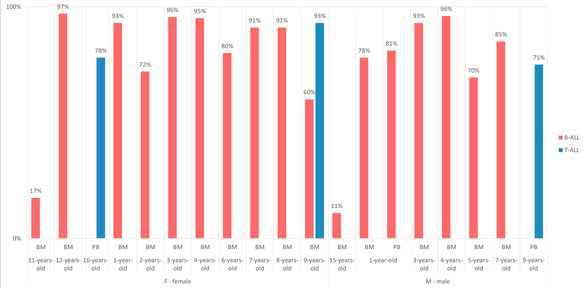


**Figure 3.** *Distribution of Total Blast Population Percentage (%) in ALL Patients by Age, Gender, and Sample Type (BM or PB) with Subtype Classification (B-ALL vs. T-ALL).*

The antibody markers used for analyzing the SSC/CD45 blast population in primary immunophenotyping of B-ALL were CD2, smCD3 (surface membrane CD3), cyCD3 (cytoplasmic CD3), CD4, CD8, CD5, CD9, CD10, CD11c, CD13, CD14, CD15, CD19, CD20, smCD22, cyCD22, CD24, CD33, CD34, CD38, CD58, CD66c, CD73, cyCD79a, CD81, CD99, CD117, CD123, CD133, CD304, HLA-DR, cyMPO, cyIgM, NG2, nTdT, kappa and lambda.

Out of 23 cases of B-ALL patients, the most common positive markers identified in primary immunophenotyping for B-ALL were CD10 (positive in ~96% of all cases), CD19 (positive in 100% of all cases), smCD22 (positive in ~52% and heterogeneous in ~22% of all cases), cyCD22 (positive in ~74% and heterogeneous in ~4% of all cases), CD24 (positive in ~96% of all cases), CD34 (positive in ~78% and heterogeneous in ~17% of all cases), CD38 (positive in ~96% of all cases), CD58 (positive in 100% of all cases), CD73 (positive in ~61% and heterogeneous in ~18% of all cases), cyCD79a (positive in 100% of all cases), CD81 (positive in 100% of all cases), and CD123 (positive in ~61% and heterogeneous in ~13% of all cases).

Meanwhile, analyzing the SSC/CD45 blast population, CD2, smCD3, cyCD3, CD5, CD14, CD15, CD117, cyMPO, kappa, and lambda were negative in 100% of all B-ALL cases.

The antibody markers used for analyzing the SSC/CD45 blast population in primary immunophenotyping of T-ALL were CD1a, CD2, smCD3, cyCD3, CD4, CD5, CD7, CD8, CD10, CD13, CD16, CD19, CD20, CD33, CD34, CD48, CD56, cyCD79a, CD99, CD117, CD123, HLA-DR, cyMPO, TCRαβ, TCRγδ, nTdT, kappa and lambda.

Out of 3 cases of T-ALL patients, the most common positive markers identified in primary immunophenotyping for T-ALL were CD2 (positive in ~67% of all cases), smCD3 (positive in ~67% of all cases), cyCD3 (positive in 100% of all cases), CD4 (positive in ~67% of all cases), CD5 (positive in 100% of all cases), CD7 (positive in 100% of all cases), CD99 (positive in 100% of all cases), and nTdT (positive in ~67% of all cases).

Meanwhile, analyzing the SSC/CD45 blast population, CD10, CD13, CD19, CD20, CD33, CD34, CD48, cyCD79a, CD117, CD123, HLA-DR, cyMPO, kappa, and lambda were negative in 100% of all T-ALL cases.

## CONCLUSIONS

This study demonstrated that, out of 26 patient cases, B-ALL was the most frequently detected subtype, occurring in 23 of the 26 ALL cases. This condition predominantly affected females in the 2-3-year-age group, residing in Riga/Districts adjacent to Riga. To gain a more precise understanding of the incidence and primary immunophenotypic characterization of pediatric ALL, this retrospective cohort study should be extended to cover a longer period.

Furthermore, to analyze and more accurately determine the potential causes and pathophysiological mechanisms of ALL in children, it is crucial to perform karyotype analysis. This involves cytogenetic studies designed to identify chromosomal abnormalities, including structural and numerical changes [6]. In addition to karyotyping, molecular analyses are essential as they detect specific genetic mutations and gene rearrangements associated with pediatric ALL [6]. These molecular assays provide detailed insights into the genetic basis of the disease, enabling precise classification, prognosis, and the development of targeted therapies, which is crucial for personalized treatment strategies in children [6].

Additionally, it would be valuable to expand the study to include not only the incidence and primary immunophenotypic characterization of ALL cases but also those of acute myeloid leukemia (AML).

## REFERENCES

[1] Kanwar V.S., Noriko S., Yoon J.M., Willert J.R. Pediatric Acute Lymphoblastic Leukemia. E-medicine Medscape, 2022. Link: *https://emedicine.medscape.com/article/990113-overview?icd=login_success_email_match_norm#a2* (viewed 10.05.2024.)

[2] The American Cancer Society. Risk Factors for Acute Lymphocytic Leukemia (ALL). Last Revised: February 27, 2024. *https://www.cancer.org/cancer/types/acute-lymphocytic-leukemia/causes-risks-prevention/risk-factors.html* (viewed 10.05.2024.)

[3] Raje N, Snyder BL, Hill DA, Streicher JL, Sullivan KE. Severe immunodeficiency associated with acute lymphoblastic leukemia and its treatment. Ann Allergy Asthma Immunol. 2018 May;120(5):537-538. Link: *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5975371/* (viewed 10.05.2024.)

[4] ICD-10 Version:2019 Link: *https://icd.who.int/browse10/2019/en#/C91.0* (viewed 15.05.2024.)

[5] *https://lv.wikipedia.org/wiki/Latvijas_administrat%C4%ABvais_iedal%C4%ABjums#/media/Att%C4%93ls:Latvijas_novadi_2021.png* (Photo link viewed 30.08.2024.)

[6] Mrózek K, Harper DP, Aplan PD. Cytogenetics and molecular genetics of acute lymphoblastic leukemia. Hematol Oncol Clin North Am. 2009 Oct;23(5):991-1010. Link: *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3607311/* (viewed 30.08.2024.)

## ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to my supervisor, Dr. Iveta Gorela, and to Dr. Dagne Grāvele, for their invaluable support and guidance throughout the development of this research.

RESEARCH POSTER PRESENTATION DESIGN © 2019
www.PosterPresentations.com