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Original Paper

Modern Diagnostic Approach of Acute Lymphoblastic Leukemia in Children and Adolescents – Experience of a Single Pediatric Hematology-Oncology Center

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REZUMAT

Abordul diagnostic actual al leucemiei acute limfoblastice la copil și adolescent – experiența unui singur Centru de Hematologie-Oncologie pediatrică

Autorii analizează un lot de 33 de copii și adolescenți (sub 18 ani) consecutiv internați în Clinica de Pediatrie Fundeni, diagnosticați cu Leucemie Acută Limfoblastică (LAL) și tratați în perioada 01.09.2008 – 31.12.2011. Sunt luate în discuție metodele moderne de diagnostic și stratificare a riscului (imunofenotipare, citogenetică, biologie moleculară) și sunt prezentate rezultatele analizei, în scopul stabilirii unor corelații utile pentru abordul clinic al cazurilor. Introducerea noilor metode diagnostice permite o mai bună abordare terapeutică, ameliorând rata supraviețuirilor îndelungate fără semne de boală.

Cuvinte cheie: copil și adolescent, leucemie acută limfoblastică, diagnostic, stratificarea riscului

ABSTRACT

The authors analyzed a cohort of 33 children and adolescents (<18 years of age) consecutively admitted in the Pediatric Clinic of Fundeni Clinical Institute, diagnosed with ALL (acute lymphoblastic leukemia) and treated in the period of time 09.01.2008-12.31.2011. They discuss the role of modern techniques for diagnosis and risk stratification (immunophenotyping, cytogenetics, molecular biology) and present the results of their analysis, aiming to establish useful correlations for clinical management of cases. Introduction of new diagnostic methods allows a better therapeutic approach of cases, leading to superior event-free survival (EFS).

Key words: children and adolescents, acute lymphoblastic leukemia, diagnosis, risk stratification

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most frequent malignancy in children, representing 25% of all the neoplastic diseases in people younger than 15 years [1]. The peak of incidence is between ages 2 and 3 years [1]. In

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adolescents (15 to 19 years), ALL comprises only 7% of all cancers [2]. In the United States, it is estimated that 2500 – 3500 children are diagnosed with ALL every year [1]. The incidence appears to be increasing. In Europe it was reported a 1.4% increase in incidence from 1970 to 1999 [3].

The pathogeny of ALL is complex, but it was shown that genetic factors play an important role. Many congenital / familial genetic disorders confer a high risk to ALL (**Table 1**), but they are quite rare.The majority of children with ALL have instead acquired genetic abnormalities detected in leukemia clones (**Table 2**).

ALL is a biologically heterogeneous disease [9] arising at any stage of lymphoid differentiation and characterized by an arrest in maturation and uncontrolled proliferation of lymphoid cells (blasts).

Clinical manifestations depends on extent of bone marrow infiltration with blast cells, as well on extramedullary infiltration of leukemic cells. Hematologic manifestations reflect the extension of bone marrow with blast cells, that cause anemia, neutropenia; the signs and symptoms arising from these manifestations are pallor, fatigue, petechiae, bruising, bleeding and fever, the last one due to infections. Extramedullary infiltration by leukemic cells causelymphadenopathy, hepatomegaly and splenomegaly, which are usually asymptomatic. Bone pain due to periosteal involvement is present in about 25% of children with ALL. Children with central nervous system (CNS) involvement may present symptoms like headache, vomiting, and lethargy. Testicular infiltration (in approximately 2% of males) and rare involvement of other organs than CNS, liver, spleen, and lymph nodes can also occur (eg, skin, eyes, pleural space, ovaries) [1, 10, 11].

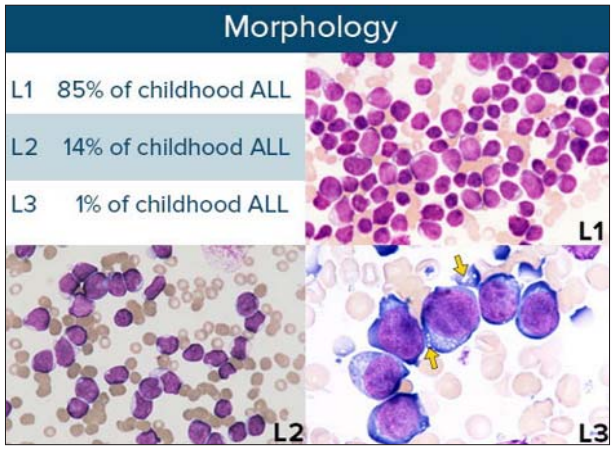

Figure 1. Common morphological variants of ALL (FAB classification)[4, 2]
Photo credit / source: reference.medscape.com [13]
● L1 (85% of childhood ALL): small blasts, scant cytoplasm, indistinct nucleoli; ● L2 (14% of childhood ALL): large blasts, abundant cytoplasm, prominent nucleoli; ● L3 (1% of childhood ALL): large blasts, abundant basophilic cytoplasm, prominent vacuoles (arrows), prominent nucleoli

The gold standard of diagnosis is bone marrow aspirate/biopsy, a mandatory investigation used to confirm diagnosis, to evaluate the number of blasts present, and to establish the leukemic phenotype. The morphology of blasts using the French-American-British (FAB) criteria is no longer in favor, because the subgroups (**Fig. 1**) do not correlate with the lineage or risk category [9]. Immuno-

Table 1. Congenital genetic syndromes conferring high risk for acute lymphoblastic leukemia (ALL) in children [1, 4]

• Down's syndrome (increases risk by 10- to 20-fold)
• Bloom's syndrome
• Neurofibromatosis
• Fanconi anemia
• Shwachman-Diamond syndrome
• Congenital immunodeficiency syndroms with increased risk for ALL (Wiskott-Aldrich syndrome, Ataxia-telangiectasia, etc)

Table 2. Common recurrent chromosomal abnormalities in pediatric ALL [5–7]

Genetic abnormalities	Genes involved	Frequency	Prognostic significance
t(12;21)(p12;q22)	TEL(ETV6) / AML1(RUNX1)	20-25%	Favorable
Hyperdiploidy	> 50 chromosomes	25%	Favorable
t(9;22)(q34;q11)	ABL / BCR	3-5%	Unfavorable
t(X;11)(X;q23)	MLL rearrangements	5-6% but 60% in infant ALL	Unfavorable
t(1;19)(q23;p13)	PBX1 / E2A	5-6%	Favorable overall outcome but increased risk of CNS relaps [8]
Hypodiploidy	< 44 chromosomes	1%	Unfavorable

MLL = Myeloid/lymphoid or mixed-lineage leukemia; ABL/BCR = Breakpoint cluster region-Abelson Kinase

phenotyping of blast cells is now considered a superior method of diagnosis, because the expression of cell surface markers depends on their lineage (B or T) and stage of maturation. B-cell precursor ALL (CD 10+, CD 19+, CD 20+) represents 80-85% of childhooh ALL [4]. T-cell ALL (15-18%) is diagnosed by positivity for CDs 2,3,5,7 and 8 [4, 10, 14]. Early T-cell precursor phenotype (CD 8-, CD 5dim) is considered as having poor prognosis [5]. Mature B-cell leukemia (Burkitt; 2%-3%) is characterized by specific surface and cytoplasmic immunoglobulins and negativity for TdT(terminal deoxynucleotidyl transferase) marker [4]. Lumbar puncture (LP) is performed in ALL-patients to assess for CNS involvement. Cerebrospinal fluid (CSF) cytospin preparations are used to assess for the presence of lymphoblasts and to categorize the patient's CNS status at leukemia diagnosis, as follows [9]:

- CNS1:absence of blasts and WBC in the CSF <5/μl;
- CNS2: presence of blasts and WBC count less than 5/μl;
- CNS3: presence of blasts and WBC count greater than 5/μl,if the LP was nontraumatic.

Chemistry panels, liver and renal function studies, coagulation studies, other tests, procedures or imaging studies are based on the patient's clinical and current laboratory findings [1], [4], [10]. Chest radiograph is done to assess for the presence of mediastinal mass, most common in older children and in those with T-cell ALL. The presence of a mediastinal mass may signal a possible medical emergency (the risk of imminent respiratory arrest caused by compression of the trachea or a risk of superior vena cava syndrome). Other investigations are ultrasonography of testis, magnetic resonance imaging (MRI) of the brain in the presence of CNS signs/symptoms, a basis echocardiogram for assessing the risk of anthracyclines toxicity, etc [15].

Modern ALL treatment protocols (ALL IC-BFM 2002, Interfant-06) [16, 17] imply risk-based therapy, in order to reduce toxicity in patients with low-risk ALL and to indicate aggressive therapy for those with a high-risk of relapse (high-risk ALL)[18]. The clinical and laboratory features used for risk stratification are summarized in **Table 3**.

Original study

Background

The aim of this study was to identify the cytogenetic and molecular abnormalities in pediatric ALL, in correlation with evolution, treatment results and prognosis. The study was a descriptive and observational one.

Objectives

- Evaluation of clinical and biological features at diagnosis;
- Analysis of the immunophenotyping, cytogenetics and molecular data at diagnosis and correlation with clinical and biological profile of cases;
- Inclusion in specific risk groups standard risk (SR), intermediate risk (IR), and high risk (HR);
- Treatment outcomes according to risk groups;
- Evaluation of complications, relapses and causes of death;
- A comparison of our results with international studies;

MATERIALS AND METHODS

Patients were eligible for inclusion if their age at inclusion was under 18 years and if they were diagnosed and treated in Pediatric Clinic - Fundeni Clinical Institute in the period of time 09.01.2008-12.31.2011.

Exclusion criteria were:

- Presence of physiologic statuses which contra-indicate the treatment (pregnancy, breast feeding);
- Refused Protocol/Refused Consent to the Treatment - by family or adolescent patients after they have read the Informed Consent;
- Initiation of cytostatic therapy in another medical institution and following the treatment for longer

Table 3. Risk stratification [1] of new diagnosed pediatric ALL

Risk Factor	Favorable	Unfavorable
NCI [19] Risk Category		
Age at diagnosis	1 – 10 years	<1 y and > 10 y
WBC at diagnosis	<50 x 10^3/Ml	>50 x 10^3/μL
Phenotype	B – lineage ALL	T – lineage ALL
CNS status	CNS 1	CNS 2, CNS 3
Testicular disease	No	Yes
Genetics	Hyperdiploidy	Hypodiploidy
	Trisomy 4, Trisomy 10	Trisomy 21
	t(12;21) / ETV6-RUNX1	t(9;22) / BCR-ABL, MLL rearrangements, iAMP21
Induction failure	No	Yes
End of induction MRD	Negative	Positive

NCI = National Cancer Institute (US); WBC = white blood cell; CNS = Central Nervous System; iAMP21 = intrachromosomal amplification of chromosome 21; MRD = minimal residual disease

than 1 month;
- Treatment in our institution for a shorter period than 30 days;
- Secondary ALL;
- Congenital ALL and other forms with rapid fatal outcome (less than 30 days from diagnosis);
- Lack of complete diagnosis or Protocol completion.

Study oversight

The study was conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonisation (ICH) [20], it was approved by the local Fundeni Clinical Institute Committee for Bio-Ethicsand all families and adolescents > 16 years old have signed Informed Consent.The study has no sponsors, and the investigators personally collected data and analyzed them. The first author wrote the draft of the manuscript, and all the authors reviewed and made the revisions to submit it for publication.

Patients

The study analyzed 33 consecutive patients, 17 males (51,51%) and 16 girls (48,48%);ages at diagnosis were comprised between 3,5 month and 17,8 years (median of ages: 4,33years).

The data collected at diagnosis were :
- Complete personal and familial history;
- Weight, height, corporal surface and BMI (Body Mass Index);
- Complete physical examination, focused on adenopathy, fever and symptoms/signs of infection, bleeding, symptoms and signs of CNS involvement, and symptoms and signs of other extramedullary infiltration (liver, spleen, testes, cutis,etc).

Laboratory investigations at diagnosis are presented in **Table 4**.

The morphologic diagnosis was established in presence of >25% blasts in bone marrow. Peripheral blood and bone marrow aspirate smears were submitted to May-Grónwald-Giemsa (MGG) as well to Periodic acid–Schiff (PAS), α-Naphthyl Acetate Esterase and Myeloperoxidase (MPO) colorations.

Immunophenotyping was conducted on peripheral blood or bone marrow collections. The blasts from peripheral blood or bone marrow harvested on EDTA (Ethylene diamine tetraacetic acid) were isolated by density gradient centrifugation using Ficoll-Hypaque. Then they were subject to flow cytometry using a Becton Dickinson FACS scan (FACS - Fluorescence-activated cell sorting), the panels of monoclonal antibodies being presented in **Table 5**. To establish the cellular lines, we used the EGIL Score (EGIL 2002 – European Group for the ImmunologicalCharacterization of Leukemias) [21]. Coexpression of myeloid antigens was defined as simultaneous expression of ≥1 myeloid antigens (CD 13, CD 33, CD 65) on more than 20% of lymphoblasts.

Cytogenetics - The chromosomes were prepared in 24 hours culture on usual media (RPMI 1640, MEM Eagle, TC-199, IC 65) supplemented with bovine fetal serum (FBS), L-glutamine, N-16 solution, antibiotics and phytohemagglutinin (PHA). The preparats were then examined by standard cytogenetic technique and G-band analysis (karyotyping); chromosomal anomalies were assessed conforming to ISHCN (International System for Human Cytogenetic Nomenclature).

Classification

We classified our patients in 3 risk groups (SR, IR, HR) conforming to ALL IC-BFM 2002 Protocol Criteria (**Table 6**). In infants, the allocation to SR and HR group was made conforming to Interfant 2006 Protocol criteria (**Table 7**).

Table 4. Laboratory and imagistic investigations at diagnosis

• CBC differential
• BMP – aspirate , trephine biopsy for morphology , cytochemistry, immunophenotyping, cytogenetics and molecular biology
• ESR, CRP
• Liver screening tests = ALT, AST, Bilirubin, GGT
• Renal function screening tests: urine, serum creatinine, urea, uric acid
• Glucose, triglycerides, cholesterol
• Serum and urinary electrolytes
• Viral infection markers: EBV, HSV, CMV, HAV, HBV, HCV, HIV 1/2
• Coagulation (PT, AP, INR, aPTT, fibrinogen, FDP, d-dimers)
• Blood group
• Bacteriology (as clinical indicated): blood cultures, nasal and pharyngeal, swabs, urine culture, coproculture, skin and other foci cultures
• Thorax Rx (PA, LAT)
• Abdominal ultrasound
• ECG and echocardiography
• Lumbar puncture (cytology)

Table 5. Panel of monoclonal antibodies for immunophenotyping

• Marker of progenitor cells: CD 34
• Pan-hematopoietic marker: CD 45
• B-cell markers: CD 10, CD 19, CD 20, CD 22, CD 24,Cμ,sμ, s IgM,κ and λhight chains
• T/NK cell marker: CD 1a, CD 2, CD 3, CD 4, CD 5, CD 7, CD 8, CD 16, CD 56, TCR $\alpha\beta/\gamma\delta$
• Myelo-monocytic markers: CD 11b, CD 13, CD 14, CD 15, CD 33, CD 64, CD 119
• Red cell line marker: Glycophorin A
• Platelets marker: CD 61

CD=Cluster of differentiation; TCR=T cell receptor

Table 6. ALL risk stratification according to IC-BFM 2002 Protocol

Standard Risk Group (SR)	Intermediate Risk Group (IR)	High Risk Group (HR)
• Age 1-6 years *and*	• Blasts number D8 <1000/μL(PGR) *and*	• Blasts number D8 >1000/μL(PPR) *or*
• Leucocyte count at diagnosis <20 000/μL *and*	• Age > 1 - >6 years *or*	• BM – M2, M3, D33 *or*
• Blasts number D8 <1000/μL(PGR) *and*	• Leucocyte count at diagnose >20 000/μL *and*	• Presence of t(4;11) / MLL-AF4 *or*
• BM – M1, M2, D15 *and*	• BM – M1, M2, D15 *and*	• Presence of t(9;22) / BCR-ABL *or*
• BM – M1, D33 (complete remission – CR)	• BM – M1, D33 *or*	• Patient in the IR group and BM – M3, D15 (induction failure)
	• SR criteria	
	• but BM – M3, D15	
	• and BM – M1, D33	

PGR=Prednisone good responders; PPR=Prednisone poor responders; BM - M1/2/3=BM status according to morphology; D8,15,33=Day 8,15,33.

Table 7. Risk group definition recording to Interfant 2006 Protocol

Standard Risk (SR)	Medium Risk (MR)	High Risk (HR)
No MLL rearrangement	MLL status unknown *or*	MLL rearrangement *and*
	MLL rearrangement *and* age > 6 months (183 days)	Age < 6 months (183 days) *and*
	or MLL rearrangement *and* Leukocyte count at diagnosis <300 000/μL	Leukocyte count at diagnosis ≥ 300000/μL

Treatment

We used for chemotherapy the ALL IC-BFM 2002 Protocol in patients aged 1-18 years, and Interfant-06 Protocol in infants [16, 17].

Statistical analysis

The data obtained from analysis of clinical sheets were submitted to methods of descriptive statistics. Using the IBM SPSS statistics 20, Epi Info and Microsoft Excel 2010, we computed relative frequencies and realized the graphic representations. In order to test statistical signification of differences we used the χ^2, log-rank tests and Cox proportional regression model [22].

RESULTS

Hereditary factors

We find in the families of our patients 4 cases having malignant proliferation in their histories: 3 grade 2 relatives (grandparents) with Hodgkin disease, non-Hodgkin malignant lymphoma and lung cancer respectively. Also, 1 grade 3 relative (grand-grandfather) had a non-specified malignancy.

Clinical picture

The main clinical signs and symptoms in our cohort of patients are shown in the **Fig. 2**.

Laboratory

CBC (complete blood count) at diagnosis revealed an association between aregenerative anemia, leukocytosis, thrombocytopenia and presence of blasts in peripheral blood (**Table 8**).

We have to mention that only statistical correlation was the strong tendency to have significant bleeding at a platelet count less than 50 000/μL (RR=2,4561 and OR = 4,0749).

Blast morphology using FAB classification, was the

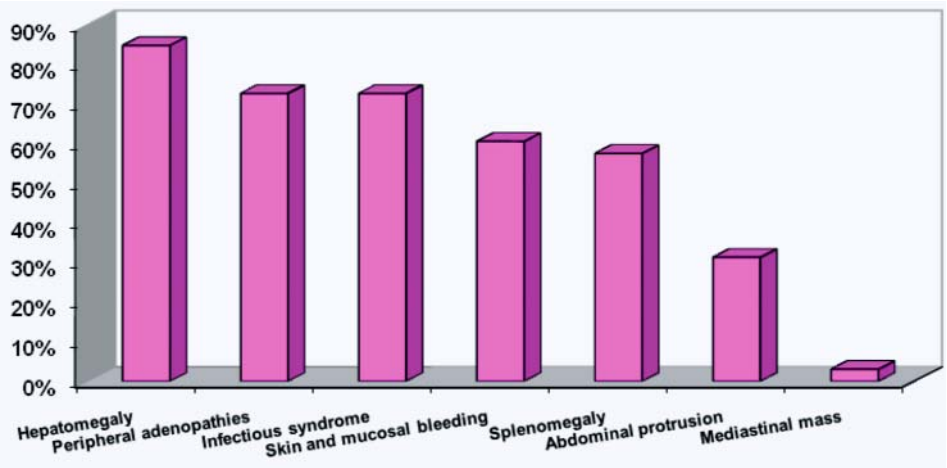

Figure 2. Clinical picture at diagnosis

Table 8. CBC at diagnosis in our patients

Parameter	Minimal value	Maximal value	Average	Median
Leukocyte count (cells/μL)	1 100	440 000	66 837	2 6 000
Hemoglobin (g/dL)	3,5	14	8,64	9,3
Thrombocyte (cells/μL)	10 000	303 000	78 048,48	56 000
Percentage (%) of blasts in PB	1	98	56,9	44

PB=peripheral blood

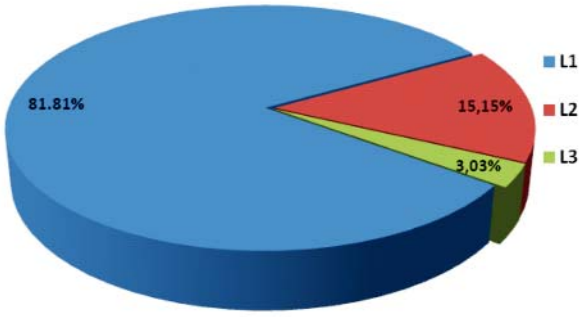

Figure 3. Blast morphology (FAB) in our patients

following: ALL-L1 blasts in 27 patients (81,81%), ALL-L2 blasts in 5 patients (15,15%) and ALL-L3 blasts in one patient (3,03%) (**Fig. 3**).

The immunophenotyping of blasts cells is reveal in **Table 9**.

Distribution of immunophenotypes according to patient"s age is presented in **Table 10**.

Table 9. Results of immunophenotyping

Blast phenotype	Number of patients	Percentage (%)
Pro-B	3	9,09
Pre-B	7	25,21
B-common	20	60,60
B-mature (Burkitt)	1	3,03
T-ALL	2	6,062

We want also to highlight that most of our patients demonstrate aberrant phenotypes ("leukemia" phenotype):

- In 7 from 20 patients with common B ALL (35%) we find co-expression of myeloid markers;
- All the 3 cases of pro-B ALL had aberrant expression of myeloid markers, one of them having also a translocation of MLL gene (his age being less than 1 year);
- One of the 2 cases of T cell-ALL had an aberrant

Table 10. Blast phenotype distribution according to patient's age

Age	B-cell ALL			T-cell ALL		Sum
	Pro-B	Pre-B	B-common	B-mature		
< 1 y	1		1			2
1-6 ys	1	3	12		1	17
6-12 ys	1	2	3	1		7
12-18 ys		2	4		1	7
Sum	3	7	20	1	2	33

Table 11. Cytogenetic abnormalities

	Number of patients	Percentage (%)
Blast ploidy		
Hyperdiploid	4	12,12
46-50 chromosomes	3	9,09
>50 chromosomes	1	3,03
Hypodiploid	2	6,06
40-45 chromosomes	1	3,03
<40 chromosomes	1	3,03
Normodiploid	27	81
Other cytogenetic anomalies		
21 Trisomy (Down)	1	3,03
Inversion of chromosome 1	6	18,18
• with normoploidy	4	12,12
• with hypodiploidy	1	3,03

phenotype (co-expression of B-cell markers and maturational asynchrony).

Cytogenetic abnormalities:

- The ploidy of blasts is depicted in **Table 11**, together with other numeric chromosomal anomalies;
- Molecular biology. In 21 patients (63,63%) we could not demonstrate specific chromosomal translocation; the rest of abnormalities are revealed in **Table 12**.

Correlation between clinical and biological characteristics and blasts immunophenotype and cytogenetics/molecular biology

We could establish some correlations between clinical and biological characteristics of our patients and the blast immunophenotype and genetic peculiarities:

- Patients with hyperleukocytosis at diagnose (17; 51,51%) were included in ALL-T group (both cases in our study), as well in ALL-B group;
- B-common ALL were the most frequent, having tendency to have the onset in children aged 1-6 y and 6-9 y (14/20 patients with B-ALL, 70%) and have been associated with favorable prognostic factors (leukocyte count <20 000/μL at diagnose, modest infiltrative syndrome, without CNS involvement, normal or hyperdiploid karyotype, and t(12;21)(ETV6-RUNX1) translocation);
- B-common ALL having t(9;22)(BCR-ABL) translocation – 3 patients in our cohort – had a belated onset (>10 yoa) and were associated with unfavorable risk factors, especially hyperleukocytosis;
- Pro-B and Pre-B ALL had the onset at younger ages (mostly <1 yoa), and have been associated with hyperleukocytose, massive organ infiltration, t(4;11)(MLL-AF4) translocation and hypodiploidy (2/3 cases) – all of these been factors for unfavorable prognosis. Unlike the data from literature, our patients did not have CNS involvement at diagnose.
- 2 patients with T-ALL had the onset in adolescence and mediastinal mass; both of them however had favorable prognostic factors, unlike the data from literature.

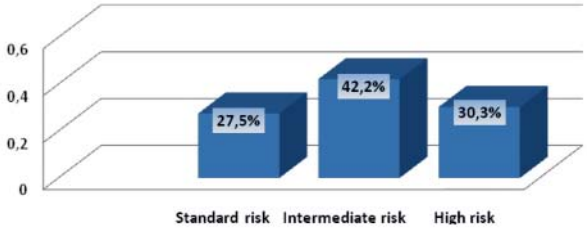

Figure 4. Allocation of patients to risk group

Allocation of patients to risk group is shown in **Fig. 4**.

Correlations between blast immunophenotype and cytogenetics/molecular biology are summarized in **Fig. 5**.

DISCUSSION

Starting from the actual wide-spread belief that ALL in children is a very heterogeneous disease, the aim of this study was to validate the newer methods for diagnose and risk stratification in a single Pediatric Hematology-Oncology Center.

The main deficiency of our study was the limited number of patients, which made difficult finding of valid statistical correlations.

However, we could make some observations presented in the following statements.

Clinical and hematological manifestation in our cohort of patients are similar to data from literature [10], [23], [24], but we have to emphasize the absence of initial CNS involvement in our patients, probably a random situation due to numerical limitations of our study.

Bone marrow (BM) puncture/aspiration and trephine biopsy remains the gold standard for positive diagnosis in pediatric ALL.

It is now more and more clear that FAB classification – based on blast morphology – is no longer able to distinguish inside the biological puzzle of children ALL the peculiarities which could drive the therapy and establish the most adequate prognosis.

Table 12. Molecular biology

Alterations	Absent	MLL-AF4	ETV6-RUNX1	BCR-ABL	SIL-TAL
Number of patients	21	2	3	3	1
Percentage (%)	63,63	6,06	9,09	9,09	3,03

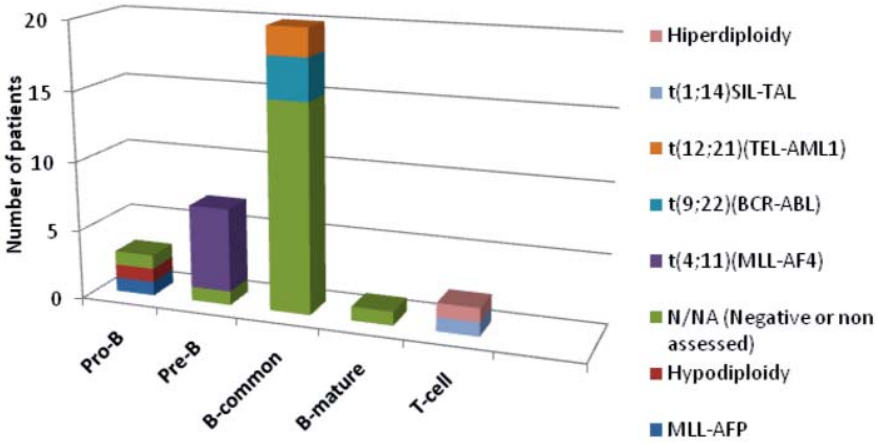

Figure 5. Correlation between blast immunophenotype and cytogenetics /molecular biology

Also hyperleukocytosis at diagnose is found in ALL-T group as well in B-ALL group and it was our impression that is not among the very reliable criteria for risk stratification.

Introduction of newer diagnostic methods (i.e. immunophenotyping, cytogenetics, molecular biology) allows better stratification of patients, much closer to biological characteristics of disease [23], [24].

The identification of aberrant ("leukemic") phenotypes is important for minimal residual disease (MRD) assessment and – therefore – for better tailoring the treatment [14, 25-27].

The complex approach of ALL patients enabled us to identify some important correlations between biological features of disease and evolutive tendency, and – due to this ascertainment– to estimate prognosis of ALL [28-31]:

- B common – ALLs, most frequent in our cohort (66,6%) as well as in literature, have tendency to have onset in children aged 1-9 years (70%) and are associated with favorable prognostic factors (initial leukocyte count <20 000/μL, modest infiltrative syndrome, no initial CNS involvement, hyperdiploid or normal karyotype, and favourable translocations, like t(12;21)[ETV6-RUNX1].However, B common – ALLs with t(9;22)BCR/ABL translocations, have a belated onset (> 10 year) and are associated with other unfavorable risk factors, especially initial hyperleukocytosis.
- Pro-B and Pre-B ALLs have the tendency to have onset at younger ages (mostly <1 year) and are associated with hyperleukocytosis, massive organ infiltration, hypodiploidy and unfavorable translocations involving MLL gene – all of these being factors of unfavorable prognosis. We have to mention that –in contrast to data from literature – our patients did not have CNS involvement at the onset.
- T-cell ALLs have the onset in adolescence, the most striking association findings being initial hyperleukocytosis and presence of mediastinal mass (± superior vena cava obstruction syndrome, or respiratory compromise) – all of these known as unfavorable factors. Nevertheless, we should mention that in our cases we did not find CNS involvement at the onset, and that our patients had also favorable prognostic factors, an observation hampered by small number of patients.

Cumulative data from our patients allowed us to stratify them recording to ALL IC-BFM 2002 and Interfant-06 Protocols Criteria which we use in our Clinic. Although the percentages of cases allocated to SR, IR and HR groups differs from those presented in the literature, the post-therapy outcomes are not far from those of other contemporary studies. We intend to present these results in a future paper.

We should also mention that we did not have access to newer diagnostic techniques (i.e. whole genome sequencing, microarray, proteomics, pharmacogenomics) and this situation ischaracteristic for many Centers in low and middle-income countries. The acquisition of these techniques will allow a better understanding of clonal heterogeneity of disease, a better stratification of patients and will permit a better correlation of therapy to biological specificity of ALL patients.

Validation of stratification and of treatment outcomes in correlation to individual risk will be possible only by effective participation in vast international randomized and statistically supervised Protocols. We have now the status of observer in ALL IC-BFM Protocols and we hope to access a higher rank in these studies.

CONCLUSIONS

In our study of 33 children and adolescents consecutively admitted and diagnosed as ALL in a single Pediatric Hematology-Oncology Center we were able to obtain similar results to other contemporary studies regarding the value of new methods (immunophenotyping, cytogenetics and molecular biology) for diagnosis and risk stratification of cases. These techniques allow as to better adapt therapy to the individual specificity of patients. Significant break-

throughs are expected only by introduction of newer diagnostic tools (i.e.newer generation sequencing,whole genome sequencing, microarrays, proteomics, pharmacogenetics), a difficult task nowadays in low and middle-income countries.

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