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Genetic and immunophenotypic diversity of acute leukemias in children

Review

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Abstract

Acute leukemias are the most commonly diagnosed malignancies in children. Acute leukemias constitute a heterogeneous group of cancers resulting from clonal outgrowth and accumulation of immature precursor cells of different hematologic lineages. Cancerous transformation begins with disruption of cell maturation mechanisms triggered by particular environmental or endogenic factors, including innate and acquired immunodeficiencies as well as autoimmune diseases.
Research in the field of acute leukemias has revealed many possible genetic abnormalities in leukemic cells, including both structural and numerical aberrations. The former can produce some particular fusion genes, yielding fusion protein products which can have an oncogenic potential in hematopoietic cells. Some of them, including translocations resulting in fusion product formation *BCR-ABL1* and different fusion products involving the *KMT2A* gene, are markers of adverse prognosis, whereas numerical aberrations with high hyperdiploidy and chromosome number exceeding 51 are markers of favorable prognosis. Detection of these aberrations already has a well-grounded clinical significance in acute lymphoblastic leukemia and plays an important role in patient risk stratification. The appearance of particular genetic changes often correlates with the expression of certain markers on the surface of leukemic cells. Determination of expression or lack of specific antigens, that is, immunophenotyping, is possible with the use of the flow cytometry technique. Flow cytometry is currently considered as a fast and broadly available technique which can provide clinically useful information in a relatively short time after biological specimen collection. Flow cytometry also enables appropriate classification of acute leukemias.

Keywords

acute leukemia • children • DNA index • aneuploidy • prognostic factor • immunophenotype

1. Epidemiological data

Acute leukemia (AL) is the most frequently diagnosed childhood cancer in Poland; these account for about a third of all cancers in this age group. Acute lymphoblastic leukemia (ALL) accounts for approximately 80% of all leukemia cases in the population under 18 years of age, while the remaining 15–20% include acute myeloid leukemia (AML) [1]. The standardized incidence rate of leukemia is 35.4/1 million people in Poland. The incidence of each type of leukemia depends on age. Most ALL cases occur between the ages of two and five.

For AML in children, the peak incidence rate occurs in the first year of life and then gradually declines until age four, and then remains relatively constant throughout childhood [2]. Literature data indicate that children respond well to treatment. With the improvement of diagnosis and treatment criteria, the cure rate for ALL is close to 90%, and for AML it is about 60–70% [3].

2. Etiopathogenesis

Acute leukemias are a heterogeneous group of hematopoietic malignancies that occur as a result of clonal growth and accumulation of immature cells of the lymphoid or myeloid lineage in the bone marrow. Despite great interest from researchers, the pathogenesis of most childhood acute leukemias remains unknown. It is known, however, that as a result of disturbance of the mechanisms controlling the maturation process of normal cells, neoplastic transformation begins. This process is multistage, it results in the displacement of normal cell lines, and it is initiated as a result of various external factors. Established and suspected risk factors for leukemia can be classified as familial, genetic, environmental (benzene exposure, high doses of ionizing radiation, chemotherapy, electromagnetic fields), and lifestyle (smoking, obesity) [4]. Moreover, endogenous factors have an impact, including primary and secondary immune deficien-

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cies and chronic stimulation of the immune system with its own antigens in the course of autoimmune diseases [5, 6].

Growing evidence from epidemiological studies strongly suggests that the increased incidence of leukemia is possibly associated with an abnormal immune response to infections in the early period of life. Research shows that the development of leukemia requires two factors: genetic and environmental. The first stage involves a genetic mutation that occurs before a fetus is born and which predisposes babies to leukemia. The second factor may be a childhood illness, as a result of exposure to one or more common infections [7, 8]. Recent experiments supporting the hypothesis of "delayed infection" as the cause of childhood B-cell lymphoblastic leukemia have shown that transcription factors determine the proper course of lymphocyte differentiation, while deregulation of factors such as ETV6, PAX5, IKZF1 significantly influences the susceptibility to ALL [8, 9].

The complex process of neoplastic transformation in acute leukemias requires at least two events resulting in damage to the cell's DNA. The abnormalities may concern both point mutations and double-stranded DNA breaks and result in chromosomal translocations, deletions, duplication, and inversion. Chromosomal translocations result in a fusion gene, most often acting as a transcription factor or signaling molecule. The fusion (chimeric) gene expression product is a fusion protein with oncogenic potential for hematopoietic cells. In the process of leukemogenesis, the altered cell loses the ability to differentiate and becomes insensitive to regulatory signals, including those that initiate the processes of apoptosis; however, the constant division and proliferation favor the formation of further genetic aberrations. The direct cause of leukemia manifestation are secondary mutations, including activating mutations of transcription factors or kinases, inactivating mutations, and deletions of genes encoding factors that inhibit the neoplasm growth [10].

Regardless of the etiology, the first step in the process of neoplastic transformation is the occurrence of chromosomal instability (CIN), resulting in a disturbance of the regulation of oncogene expression and/or loss of neoplastic function of suppressor genes. CIN—that is, the accumulation of numerical and structural aberrations of chromosomes—is one of the characteristic features of neoplastic cells. Numerical and structural CINs arise during errors in chromosome segregation during mitosis. Abnormal duplication or segregation of chromosomes can promote whole chromosome gain or loss and/or changes in chromosome structure, including but not limited to translocations and deletions. Moreover, both numerical and structural changes predispose to the accumulation of successive changes in chromosomes, thus increasing CIN and changing the properties of the cell in an unpredictable manner, for example by increasing the rate of cell proliferation, increasing neoplasm malignancy or greater drug resistance. CIN is a source of genetic variation that promotes the adaptation of neoplasms to stressful environments or to cytotoxic antineoplastic drugs [11]. The phenomenon of CIN promotes carcinogenesis as it increases the probability of oncogene activation and inactivation of suppressor genes, and consequently allows the escaping out of control of regulatory mechanisms. Unfortunately, the mechanisms underlying CIN are not yet fully recognized. The abnormal course of the cell cycle leads to disturbances in the functioning of the cell life cycle checkpoints, errors in the attachment of the kinetochore to microtubules, abnormal sister chromatid cohesion, abnormal centrosome replication, abrasion of telomeres, and abnormalities in the checkpoint of the karyokinetic spindle syndrome (SAC) [12, 13]. In rapidly dividing cells, the number of chromosomes may be multiplied (polyploidy) or an incorrect number may be created by adding one or more chromosomes (aneuploidy) [14]. The appearance of aneuploidy may both promote and inhibit the formation of neoplasms [12].

3. Classification of acute leukemias

In immunological, genetic, and molecular terms, acute leukemias are a heterogeneous group of neoplasms characterized by the clonal proliferation of immature cells of various hematopoietic lines. The proliferation of immature cells of the lymphoid lineage leads to ALL, which can be derived from B-lymphocyte precursor cells (B-cell precursor acute lymphoblastic leukemia: BCP-ALL) or T-lymphocytes (acute lymphoblastic leukemia:

T-ALL). In contrast, clonal transformation of cells of the myeloid, monoid, erythroid, or megakaryocytic lineage leads to acute myeloid leukemia (AML) [10].

The most popular classification from 1976 was the FAB (French-American-British) classification, which includes the division of ALL into three subtypes – L1, L2, and L3 – and AML into eight subtypes – M0–M7 – distinguished on the basis of morphological cell features and cytochemical tests [15]. In clinical practice, however, the FAB classification turned out to be insufficient in the case of low-differentiated acute myeloid leukemias and acute lymphoblastic leukemias, where it is particularly important to determine the degree of maturity and the lymphocyte lineage from which cell proliferation occurs. The FAB classification is supplemented by the determination of the immunophenotype of the tested cells: that is, determination of the expression of specific antigens of cluster differentiation (CD) occurring both on the cell surface and in the cytoplasm and in the cell nucleus. In 1995, the classification of acute lymphoblastic leukemias was introduced to the European Group for Immunological Classification of Leukemia (EGIL), whose criteria are based entirely on the blast cell immunophenotype [16]. The immunological classification of ALL is presented in Tables 1 and 2.

In 2016, the World Health Organization (WHO) introduced a new, currently binding classification that is the basis for the classification of hematopoietic neoplasms. This classification complements the FAB classification, and in addition to the morpho-

Table 1. Immunological classification of acute lymphoblastic leukemia based on B-lymphocyte precursor cells according to EGIL

	cytCD79, CD19, CD22	CD10	cytIgM	kappa/ lambda
Pro-B-ALL (B-I)	+	-	-	-
Common-B-ALL (B-II)	+	+	-	-
Pre-B-ALL (B-III)	+	+/-	+	-
Mature-B-ALL (B-IV)	+	+/-	+/-	+

cyt – cytoplasmatic expression

Table 2. Immunological classification of acute lymphoblastic leukemia based on T-lymphocyte precursor cells according to EGIL

	cytCD3, CD7	CD2, CD5, CD4 and/or CD8	CD1a	CD3
Pro-T-ALL (T-I)	+	-	-	-
Pre-T-ALL (T-II)	+	+	-	-
Cortical-T-ALL (T-III)	+	+/-	+	-
Mature-T-ALL (T-IV)	+	+/-	-	+

cyt – cytoplasmatic expresion

logical and cytochemical features of blast cells, it also includes immunophenotypic and cytogenetic and molecular characteristics, on the basis of which it is possible to initially determine the prognosis. The WHO classification has identified new AL subtypes or modified some of the existing ones. For example, among ALLs, the T-ALL subtype from early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) has been distinguished, characterized by CD7 expression, lack of CD1a antigen, CD3 antigen surface expression, and lack of or low CD5 expression, with possible expression of myeloid antigens and frequent occurrence of specific mutations [17, 18]. In turn, among AML types, the previous FAB M3 AML subtype is now referred to as acute promyelocytic leukemia (APL) based on the presence of t(15; 17)(q22; q12) rearrangement or the PML/RARA translocation product detected by appropriate methods.

The classification of AML and ALL according to WHO is presented in Tables 3 and 4, respectively. Currently, acute leukemia is diagnosed when the percentage of blasts in the bone marrow is above 20% [18]. Genetic and molecular tests are becoming necessary in selecting the appropriate treatment regimen, including targeted therapy. Therefore, the WHO classification is updated on an ongoing basis based on new research results [19].

4. Prognosis and prognostic factors in acute leukemias

Acute leukemia is a heterogeneous group of diseases, varied in terms of a course, response to a treatment, and the occurrence of cytogenetic and molecular changes. In recent years, attempts

Table 3. WHO immunological classification of acute myeloid leukemia [20]

Acute myeloid leukemias with repeated genetic changes
AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T
AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFB-MYH11
APL with t(15;17)(q24.1;q21.2); PML-RARA
AML with t(9;11)(p21.3;q23.3); KMT2A-MLLT3
AML with t(6;9)(p23;q34.1); DEK-NUP214
AML with inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM
AML with t(1;22)(p13.3;q13.1); RBM15-MKL1
AML with t(9;22)(q34.1;q11.2); BCR-ABL1
AML with NPM1 mutation
AML with CEBPA mutation
AML with RUNX1 mutation
Acute myeloid leukemias with myelodysplasia
Treatment-induced acute myeloid leukemias and myelodysplastic syndromes
Acute myeloid leukemias, not classified anywhere
Acute myeloid leukemia minimally differentiated
Acute myeloid leukemia without maturation
Acute myeloid leukemia with maturation
Acute myelomonocytic leukemia
Acute monoblastic and monocytic leukemia
Acute erythroblastic leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with fibrosis
Myelosarcoma
AML associated with Down syndrome

have been made to identify the prognostic factors that predict the course of the disease at the time of diagnosis of AL. In new treatment protocols used in Poland—that is, AIEOP-BFM 2017 in ALL and AML-BFM 2019 in AML—stratification of patients into risk groups (high, intermediate, standard) is based on the response to treatment with a specific level of minimal residual disease (MRD) and the presence or absence of specific genetic disorders [21]. In designated risk groups, the intensity of therapy is varied, therefore the early identification of risk factors is important. Thanks to advances in chemotherapy and hematopoietic cell transplantation (HSCT), the long-term survival of ALL patients in children now exceeds 90% [22]. However, approxi-

Table 4. WHO immunological classification of acute lymphoblastic leukemia [20]

Leukemia / B-cell lymphoblastic lymphoma, unspecified
B-cell leukemia / lymphoblastic lymphoma with reproducible genetic changes
Acute leukemia / B-lymphoma with t(9;22)(q34.1;q11.2); BCR-ABL1
Acute leukemia / B lymphoblastic lymphoma with t(v;11q23.3); with KMT2A (MLL) rearrangements
Acute leukemia / B-lymphoma with t(12;21)(p13.2;q22.1); ETV6-RUNX1
Acute leukemia / B lymphoblastic lymphoma with hyperdiploidy
Acute leukemia / B-lymphoblastic lymphoma with hypodiploidy
Acute leukemia / B-lymphoma with t(5;14)(q31.1; q32.3); IL3-IGH
Acute leukemia / B-lymphoma with t(1;19) (q23;1332); TCF3-PBX1
Acute leukemia / B lymphoblastic lymphoma, similar to BCR-ABL1 +
Acute leukemia / B-lymphoblastic lymphoma with intra-chromosomal disease chromosome 21 amplification
Acute leukemia / T-lymphoblastic lymphoma
Acute leukemia / lymphoblastic lymphoma from early T-cell precursors
Acute leukemia / NK lineage lymphoblastic lymphoma

mately 15–20% of children with ALL experience a recurrence (relapse). The relapse rate in patients with the first ALL recurrence is 71–93%, depending on the time and location of the relapse. Early relapse, up to 36 months after initial diagnosis, has a worse prognosis than isolated extramedullary or late relapse occurring more than 36 months after initial diagnosis. Although clinical remission can be achieved in the majority of ALL recurrences, long-term survival rates range from 40% to 50% [23, 24]. With regard to AML, the overall survival rate is approximately 70% [1, 25]. Recurrent AML is found in approximately 30% of patients, and the longterm survival rate in this group is approximately 40% [25].

In recent years, the progress in research on acute leukemia in children has contributed to the discovery of numerous anomalies related to, among other things, mutations of genes involved in the maturation of lymphocytes and in the regulation of the cell cycle. Some of these chromosomal aberrations, such as aneuploidy or chromosomal translocations, are already of broadly recognized diagnostic and prognostic importance, are included in modern therapeutic protocols, and contribute to the stratification of patients into specific risk groups.

In ALL, structural rearrangements are important, mostly including translocations. In BCP-ALL, the most common aberration is the t (12; 21) (p13; q22) translocation resulting in the ETV6-RUNX1 (TEL-AML1) fusion gene, which is a favorable prognostic factor. The t (12; 21) (p13; q22) translocation occurs in nearly 25% of children and its detection enables the stratification of patients into a group with a favorable prognosis. The disease-free survival rate is above 90%. On the other hand, patients with t (9; 22) (q34; q11) translocation with the formation of the BCR-ABL1 fusion gene and the so-called Philadelphia chromosome and patients with rearrangements involving the KMT2A (MLL) gene are classified to more intensive chemotherapy. KMT2A (MLL) rearrangements occur most frequently in infant ALL, however the occurrence of KMT2A-AFF1 t (4; 11) (q21; q23) translocation is associated with an unfavorable prognosis in all age groups. Sometimes, as in the case of the CDKN2A deletion, no influence of the aberration on the clinical course of the disease is observed [10, 26]. A common phenomenon in BCP-ALL is hyperdiploidy. High hyperdiploidy with a chromosome number above 51 is a favorable prognostic factor if the MRD level is not taken into account [27, 28]. On the contrary, hypodiploidy with the number of chromosomes below 44 is an unfavorable prognostic factor [29, 30, 31]. The most common genetic abnormalities are presented in Table 5.

In T-ALL, the most common chromosomal structural rearrangements include loci of genes encoding T-cell receptor (TCR) subunits and causing activation of oncogenes (TLX1, TLX3, TAL1, LMO1, LMO2, HOXA). Depending on the oncogene, the incidence of these genetic aberrations is 40–50%. The prognosis is favorable for t(10;14) (q24;q11) with TLX1 overexpression, and unfavorable for t(5;14)(q35;q32) with TLX3 overexpression. Activating mutations in the NOTCH1 gene, observed in various subtypes of pediatric T-ALL, have a direct impact on the increase in the self-renewal capacity of hematopoietic cells. The mutation has no unequivocally confirmed prognostic value, but a favorable prognosis is observed in some therapeutic protocols. Frequent rearrangements and fusion gene formation also take place within the 11q23 region of the KMT2A (MLL) gene, which has a negative impact on the expression of HOX genes, which are involved in normal hematopoiesis [10, 26]. The most common genetic abnormalities in childhood T-ALL are summarized in Table 6.

Detecting genetic disorders is also a basic element of AML diagnostics. The detection of disorders such as the t(8;21), t(15;17) translocation, chromosome 16 inversion, or the finding of chromosome 11 abnormalities involving the KMT2A gene are the bases for the recognition of specific AML subtypes. Moreover, according to the latest WHO classification from 2016, for the diagnosis of leukemia, it is more important and sufficient to determine the presence of t(8;21), t(15;17) or inv(16) inversion regardless of the percentage of bone marrow blasts [7]. The most common genetic abnormalities found in pediatric AML are presented in Table 7.

According to the currently used AIEOP-BFM 2017 protocol, independent factors with confirmed prognostic significance in ALL are: response to therapy expressed as MRD level on day 15 of remission-inducing treatment assessed by flow cytometry and on day 33 and before the start of the M protocol assessed by PCR.

Table 5. Selected genetic abnormalities most frequently found in pediatric BCP-ALL [10, 26]

Genetic aberration	Occurrence frequency	Effect	Prognostic significance
t(9;22)(q34;q11.1)-BCR-ABL1	2–3%	Mitogenic signalling activation, apoptosis suppression, altered cell adhesion	Unfavorable
t(12;21)(p13;q22)-ETV6-RUNX1	20–25%	HOX genes impaired expression in lymphopoiesis	Favorable
t(1;19)(q23;p13.3)-TCF3-PBX1	5–6%	Differentiation disorder	Favorable in intensified treatment; not relevant in AIEOP-BFM 2017
KMT2A (MLL) rearangements	5–8% (infants appr 70%, children > 1 year old, appr 2%)	Effect on HOX gene expression	Unfavorable in infants; unfavorable in the presence of t(4;11)(q21;q23) regardless of age
Chromosome hyperdiploidy > 51	20–30%	Mechanism poorly recognized	Favorable (especially in the presence of trisomy 4, 10 i 17); not relevant in AIEOP-BFM 2017
Chromosome hyperdiploidy ≤ 44	5–6%	Mechanism poorly recognized	Unfavorable
CDKN2A deletion	2–30%	Proliferation control loss	Not relevant

Table 6. Selected genetic abnormalities most often found in pediatric T-ALL [10, 26]

Genetic aberration	Occurrence frequency	Effect	Prognostic significance
Translocations of oncogenes responsible for TCR (TLX1, TLX3, TAL1, LMO1, LMO2, HOXA) expression	40–50%	Protein overexpression, impaired differentiation	t(10;14) with TLX1 overexpression associated with favorable prognosis; TLX3 overexpression with unfavorable prognosis
del(1)(p32) with SIL–TAL1 fusion	20–30%	TAL1 overexpression, impaired differentiation	Not relevant
CDKN2A deletion	60–70%	Loss of proliferation control	Not relevant
KMT2A (MLL) rearangement	5–10%	Effect on HOX gene expression	Not relevant
NOTCH1 mutation	40–50%	Self-renewal capacity increase	Favorable in some therapeutic protocols

Table 7. Selected genetic abnormalities most often found in pediatric AML [10]

Genetic aberration	Occurrence frequency	Effect	Prognostic significance
t(8;21)(q22;q22) RUNX1–RUNX1T1	10–12%	Impaired differentiation, effect on cell proliferation and survival	Favourable
inv(16) or t(16;16)(p13;q22) CBFB–MYH11	5–6%	Impaired differentiation, effect on cell proliferation	Favorable
KMT2A (MLL) rearrangements	10–15%	Altered HOX gene expression pattern, abnormal differentiation	The presence of t(9;11) (p21; q23) contributes to favorable prognosis; in other cases unfavorable
t(15;17) (q22;q21) PML–RARA	5–9%	Differentiation impaired	Favourable
Monosomy 7	5%	Suppressor gene function loss	Unfavorable
Intra-tandem FLT3 duplication	10–15%	Increased proliferation	Unfavorable

MRD levels on day 15. ≥ 10% and absence of complete remission by day 33 are unfavorable prognostic factors for both BCP-ALL and T-ALL, as is a poor response to prednisone on day 8 of treatment. In addition to the response to treatment, equally important risk factors are repeated genetic aberrations, including the presence of KMT2A, TCF3-HLF rearrangements, hypodiploidy < 45 chromosomes [17]. The immunophenotype of leukemic blasts and the distinction between BCP-ALL and T-ALL are of less importance, although within BCP-ALL the pro-B-ALL CD10- phenotype is strongly correlated with KMT2A rearrangement. Moreover, a better prognosis is associated with the DNA index (DI) of leukemic cells higher than 1.16 [32, 33].

According to the guidelines of the AML-BFM 2019 protocol, factors with a good prognostic value in AML in children include: the presence of t(16;16), t(8;21), t(1;11), inversion of chromosome 16 (inv (16)) and presence of NPM1 gene mutation or CEBPA biallelic mutation, with normal karyotype. In turn, the unfavorable prognostic factors include abnormalities within the 12p chromosome, the presence of t(2;12), t(4;11), t(5;11), t(6;11), t(10;11) translocations, t(6;9), t(7;12), t(9;22), t(16;21), chromosome 5 monosomy (or 5q arm), inversion of chromosome 3 (inv (3)), gene mutations WT1, intra-tandem FLT3 gene duplication (FLT3-ITD), or a complex karyotype with three or more aberrations, including at least one structural aberration [34].

5. Diagnostic importance of numerical aberrations in acute leukemias

5.1. Numerical aberrations in ALL

The most common numerical abnormality in ALL is hyperdiploidy, that is, the presence of more than 50 chromosomes in a blast cell. The classification based on the modal number of chromosomes (MN) – that is, the number of chromosomes most often found in the total population of cancer cells – allows for the distinction of the following ALL subtypes: hyperdiploidy near tetraploidy, hyperdiploidy near triploidy, high hyperdiploidy and low hyperdiploidy. Leukemias with a diploid number of chromosomes occur with a frequency of 40%, while pseudodiploid leukemias with a chromosome number of 46 and structural changes occur with a frequency of about 10–15% [35]. The characteristics of the specific ALL subtypes are presented in Table 8.

Hyperdiploidy near tetraploidy (NT), which is unfavorably related to the presence of 82–92 chromosomes, is found sporadically (approx. 1% of children), mainly in T-ALL [37, 38]. According to the literature, NT is associated with the L2 subtype and T-ALL. It is more common in old age, and in as many as 40% of NT cases, it is associated with the expression of at least one of the myeloid markers, such as CD13, CD15, or CD33. Most patients achieve remission in a relatively short time [37].

Hyperdiploidy near triploidy (MN: 66 to 73 chromosomes) is a rare subtype of childhood leukemia. Studies have confirmed the relationship of this subtype of hyperdiploidy with the pre-B-ALL immunophenotype [37].

In children with ALL, high hyperdiploidy (HH) is observed in about 25–30% of all cases (with the maximum occurrence in the 1–4 year age range) [27, 38]. HH is characterized by the presence of 51–65 chromosomes and is associated with a good prognosis. In children, hyperdiploidy over 50 chromosomes corresponds to DI > 1.16 [39]. The most frequently observed extra chromosomes are 4, 6, 8, 10, 14, 17, 18, 21, or X [27, 28, 40, 41]. HH is also associated with other factors associated with a good prognosis, such as low leukocytosis and age. Hyperdiploidy is a particularly favorable prognostic factor if chromosome 4, 10, or 17 trisomy is present in the karyotype with the number of chromosomes above 50 [42]. Single nucleotide polymorphism (SNP) analysis showed that in almost 80% of BCP-ALL with high hyperdiploidy, there are additional genetic disorders [43]. The rare allele of PRDM9, encoding histone H3 methyltransferase, characteristic for the meiosis, which controls DNA replication, may be associated with the development of high hyperdiploidy. The activity of PRDM9 in the early stages of meiosis is believed to lead to genetic instability, confirming its potential role in leukemogenesis [44]. Studies have shown that hyperdiploidy in BCP-ALL promotes a good response to both steroid therapy and chemotherapy, and patients with DI > 1.16 are sensitive to antimetabolites such as mercaptopurine, thioguanine and cytarabine, and to L-asparaginase [44]. The

Table 8. Hyperdiploidy characteristics in ALL [36]

	MN	Characteristics	Prognosis
Tetraploidy near hyperdiploidy (NT)	82–94	L2; T-ALL immunophenotype; older age on the day of diagnosis; CD13, CD15, and CD33 expression	Unfavorable
Tetraploidy near hyperdiploidy	66–73	pre-B-ALL immunophenotypy	Favorable
High hyperdiploidy (HH)	51–68	L1 or L2; presence of additional chromosome u X, 4, 6, 10, 14, 17, 18, or 21; low WBC and LDH; the age between 5 and 10; CD123, CD10, CD66c overexpression, no CD45 expression	Favorable
Low hyperdiploidy	47–50	Presence of additional chromosome 21, 8, 10, or X	Intermediate

mechanisms of greater sensitivity of hyperdiploid ALL to treatment have not been fully understood, but it has been observed that cells with hyperdiploidy above 50 chromosomes undergo apoptosis more easily, which may be the result of overexpression of neoplasm suppressor genes located on chromosome 21, which is frequently trisomized [45].

Low hyperdiploidy with 47–50 chromosomes occurs in about 10–15% of ALL cases and is often associated with the presence of an extra 21, 8, 10 or X chromosome. The survival rate is lower than in HH [38, 46, 47].

According to the literature data, some immunophenotypic profiles of leukemia may be associated with certain genetic aberrations, which is why they are indirectly considered to be of prognostic nature. For example, in the majority of ALL patients with HH on leukemic blasts, the CD10 antigen, characteristic of the immune subtype of common-B-ALL, is detectable [48]. Moreover, high expression of CD123, CD66c, and weak expression of CD45 on the surface of blasts are observed in hyperdiploidy [49]. Overexpression of the CD123 antigen in the majority of cases coexists with hyperdiploidy, so that the CD123 antigen may be an additional prognostic marker in BCP-ALL. Moreover, this marker is useful in MRD monitoring [50, 51]. Interestingly, unlike BCP-ALL, in T-ALL, hyperdiploidy is not associated with CD123 expression [51].

The numerical aberrations which are a bad prognostic factor and qualify the patient to a high-risk group include hypodiploidy, characterized by a chromosome count of less than 46, detected in less than 7% of childhood ALL. There are three types of hypodiploidy: near-haploidy with 23–29 chromosomes, low hypodiploidy with 33–39 chromosomes, and HH with 42–45 chromosomes. Structural disturbances within these three types most often occur with low hypodiploidy, usually observed in patients over 15 years of age. On the other hand, hypodiploidy similar to haploidy most often occurs in the age group under 15 [43, 52, 53]. BCP-ALL patients with high hypodiploidy show better survival rates compared to patients with a lower chromosome number, whose 3-year event-free survival (EFS) is only 30% [52, 54]. Both in children and adults, the number of chromosomes lower than 44 is associated with a worse prognosis, regardless of the therapy used [35, 54]. There is also a situation in which hypodiploid cells are reduplicated, as a result of which a hyperdiploid karyotype is observed (a phenomenon called "masking"), which in turn makes correct genetic classification difficult and contributes to the selection of inappropriate treatment for these patients [44, 55, 56]. In recent years, a number of recurring genetic disorders have been identified that distinguish patients with haploidy near aneuploidy from low hypodiploidy. Haploidy near ALL cases show disturbances of the receptor tyrosine kinase (RTK) signaling pathway (RTK) and the Ras protein dependent pathway (NF1), as well as an increased frequency of mutations within the IKAROS gene family, particularly IKZF3 (Aiolos), encoding the zinc finger transcription factor AIOLOS. In turn, in ALL with low hypodiploidy, mutations in the TP53, RB1, and IKZF2 genes (Helios) are more often observed. Both low hypodiploidy and haploidy near ALL show activation of signaling pathway dependent on the Ras protein and phosphatidylinositol-3-kinase (PI3K), which are sensitive to PI3K inhibitors, such as rapamycin, which at the same time suggests their use in therapeutic treatment [44, 57]. Poor prognosis related to the occurrence of hypodiploidy may not be related to resistance to cytostatics, but be related instead to the presence of only single copies of the suppressor genes [45]. Characteristics of specific subtypes of hypodiploidy in ALL are shown in Table 9.

5.2. Numerical aberrations in AML

The occurrence of chromosome number abnormalities in AML is much rarer than in ALL, and little is known about its impact on prognosis. Some subtypes have already been well defined, such as AML in patients with Down syndrome, which is associated with chromosome 21 trisomy and the FAB M7 phenotype and occurs in about 10% of patients [58]. There are few studies on the cytogenetic profile and treatment outcomes of patients with a hyperdiploid karyotype involving 49–65 chromosomes. Studies by Lazarevic et al. [59] showed that the presence of high hyperdiploidy in AML is associated with poor prognosis. Studies of another group of researchers conducted on a group of 1,563 patients showed that 14% (n = 221) have chromosomal abnormalities, including an additional chromosome 8, 13, or 21. This study did not show a relationship between the occurrence of hyperdiploidy and gender or WBC, but did show more frequent

Table 9. Hypodiploidy characteristics in ALL [36]

	MN	Characteristics	Prognosis
High hypodiploidy	42–45	complex karyotype containing chromosomes 7, 9, 12; T-ALL or common / pre-B-ALL immunophenotype	Unfavorable
Low hypodiploidy	33–39	monosomy of chromosome 3, 7, 15, 16, 17; common / pre-B-ALL immunophenotype;	Unfavorable
Haploidy near hypodiploidy	23–29	monosomy of chromosome 3, 7, 15, 16, 17; common / pre-B-ALL immunophenotype;	Unfavorable

Table 10. Characteristics of numerical aberrations in AML [36]

	MN	Characteristics	Prognosis
Tetraploidy	81–103	association with additional chromosomes 21, 19, and 8	No data available
Hyperdiploidy	48–65	median age 2 years; women; FAB M7; relationship with the presence of extra chromosomes: 8 (41%), 21 (19%), 19 (18%), and 6 (14%); CD4, TdT, CD19, and CD7 expression	No data available
Hypodiploidy	42–45	older age; men; FAB M2; presence of t(8;21) (q22;q22)	No data available

occurrence in children than in adults (22% vs 13%). The authors suggested that patients with hyperdiploidy should not be automatically assigned an unfavorable prognosis based on the presence of a complex karyotype, but instead chromosomal abnormalities known to have an adverse effect on patient survival should be investigated [60]. In 2014,

the results of a study of 596 pediatric AML patients treated in the years 1993–2012 in the Scandinavian countries were published [44]. For the first time, the clinical characteristics of such a large group of AML patients, taking into account the number of chromosomes in leukemic cells, have been published. Numerical aberrations were observed in 40% of children. A relationship between hyperdiploidy and age (median 2 years), female sex and acute megakaryoblastic leukemia (FAB M7) has been demonstrated. The FAB M2 subtype accounted for 5% of the patient group. The most common were the extra chromosome 8 (41%), 21 (19%), 19 (18%), and 6 (14%). Eight patients had a tetraploid or nearly tetraploid karyotype (MN: 81–103 chromosomes). Tetraploidy most often affected chromosome 21 (16 patients, 3%) or 19 and 8 (2% of patients combined). Structural chromosome aberrations occurred in 390 patients. In turn, hypodiploidy observed in 8% of cases correlated with older age, male gender, FAB M2, and the translocation t(8;21) (q22;q22). The most common loss was the Y chromosome in men (8%, n = 30) or the X chromosome in women (5%, n = 13). Chromosome 7 monosomy occurred in 2% of patients (n = 14) and was a factor of poor prognosis [44].

Leukemia cells in AML showing aneuploidy most often also strongly express CD4 and co-express lymphoid antigens, such as TdT, CD19, and CD7 [61]. Occurrence of numerical aberrations in AML together with the characteristics is presented in Table 10.

6. Summary

This paper presents the current achievements in the field of diagnosis of acute leukemias in children and a review of methods useful in routine laboratory diagnostics. Cytogenetics, immunophenotyping using flow cytometry, and molecular techniques are important, complementary methods used in the diagnosis of acute leukemias, also helpful in identifying risk factors and in drawing conclusions about prognosis. As a result of the enormous progress made in laboratory diagnostics, especially in the field of molecular techniques, in recent years, the number of prognostic markers in acute leukemias has increased significantly. On their basis, patients are currently stratified into risk groups with varying intensity of treatment.

Clinical observations indicate that quantitative cytogenetic disturbances may also affect the effectiveness of ALL treatment, and cytometric analysis of DNA content in leukemic cells is a sensitive and appropriate method for the identification of hyperdiploid clones. Flow cytometry is particularly useful when cell ploidy cannot be determined by cytogenetic methods. Moreover, introduction of methods into diagnostics that allow shortening the waiting time for the result is important from a clinical point of view and results in reducing costs. The identification of biologically distinct leukemia subtypes not only enables patient stratification, but also offers the opportunity to introduce individualized treatment based on the detected molecular target. Examples of already implemented individual therapies are the use of tyrosine kinase inhibitors in ALL with the confirmed presence of the BCR-ABL1 fusion protein. Moreover, immunotherapies with the use of bispecific antibodies and T lymphocytes with chimeric receptors (CAR-T cells) are an effective method of treating leukemia, especially in cases resistant to conventional treatment [22].

Authors' Contribution

M.P.Ś.: contributed to study design, involved in data collection. analyzed the data; **Ł.S.**: contributed to study design, involved in data collection, involved in literature search; **B.M.**: conceived the concept for the study, involved in literature search. **All authors** edited and approved the final version of the manuscript.

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Conflict of Interest

The authors have no potential conflicts of interest to declare.

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