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Lorenz Bastian

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Diploma thesis

Academic year:
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1. INTRODUCTION

1.1 Childhood leukemias

Leukemias are malignant diseases of the blood. Due to genetic abnormalities in a hematopoietic cell, there is an unregulated (higher) clonal proliferation of these cells. Malignant transformation of these cells leads to accelerated growth compared with normal cells and decreased apoptosis. This results in an accumulation of so-called blasts and leads to disruption of normal bone marrow function and finally bone marrow failure. There are different types of leukemia with different clinical features, laboratory findings and different response to therapy. Leukemias are the most common cancers in childhood (1).

1.2. Epidemiology

Childhood hematopoietic malignant diseases are the most common cancers in children. Leukemias together with lymphomas (Hodgkin and non Hodgkin Lymphoma) account for about 40% of pediatric malignant diseases (2). Leukemias are more common than lymphomas and account for approximately 31% of cancers diagnosed in children younger than 15 years in the US (1). Most common types of pediatric leukemia are acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (1). The rest like chronic lymphocytic leukemia and juvenile myelomonocytic leukemia (JMML), which is neither acute nor chronic, are very rare in children (1,3). ALL is by far the most common and accounts for approximately 77%, followed by AML with approximately 11% and CML which accounts for only about 2 to 3% in pediatric population of the United States (1). A different geographical distribution has been noticed for different subtypes of childhood leukemias around the world. As already said ALL by far the most common one has an annual incidence of up to 40 cases per million children among industrialized western European countries whereas it seems to be less common in less developed countries (4). ALL is most commonly diagnosed at 2t o3 years of age. It occurs more often in boys than girls at all ages (1). It is also more common in children with genetic abnormalities, for example, Down syndrome (1,5). AML, the second most common pediatric leukemia, has a stable worldwide incidence of 5–9 cases per million per year (4). AML is more commonly diagnosed if the child gets older and increases especially in adolescence with about 36% of cases of leukemia in 15 to 19 years old patients in the US (1). It is also more common in a population with chromosomal abnormalities, especially Down syndrome (6).

The incidence of CML, the rarest childhood leukemia, is increasing with increasing age of the child. It is more common in adolescence, like AML. It causes approximately 2% of

all leukemias in children younger than 15 years and 9% of all leukemias in adolescents between 15 and 19 years (7). It is strongly connected to a chromosomal abnormality (1).

1.3. Classifications and Pathogenesis

1.3.1. ALL

ALL is a lymphoproliferative disease with abnormal proliferation of lymphoblasts, a precursor cell of the lymphocytes. There is no minimal limit of blasts for the diagnose of ALL but usually, there has to be more than 20 to 25 % of blasts in the bone marrow (8). ALL can be classified by morphology, immunophenotyping, cytogenetic and molecular abnormalities of leukemic cells. The current classification system of the WHO distinguishes ALL by his phenotypical surface markers, depending on whether it is from the B or T cell lineage. The most common subtype of ALL is B lymphoblastic leukemia accounting for approximately 85%. This includes the previously called precursor B-ALL or pre–B-ALL. T-lymphoblastic leukemia accounting for approximately 15% (1). T-ALL affects boys more often than girls and occurs at an older age than B-cell ALL (9). Burkitt leukemia is a rare B cell leukemia of mature B lymphocytes and accounts for about 1% of ALL. This WHO classification, based on immunophenotype, it is important for choosing the right treatment (1). In the 2008 WHO classification based on genetics, there are seven categories of B-ALL with recurrent genetic abnormalities, the rest of B-ALL without those genetic abnormalities are classified as B-lymphoblastic leukemias which are not otherwise specified (NOS). There is no subclassification of T-ALL based on genetics(8).

1.3.2. AML

In AML there is an abnormal proliferation of myeloid cells. In comparison to ALL, there has to be a blast count of more than 20% in the bone marrow to diagnose AML (8). The classification of AML has changed significantly. The 2008 classification of the WHO is now mainly based on chromosome abnormalities and specific gene mutations, since it is now known that these two factors are much more important for the prognosis than morphology. Morphology was the main indicator in the previously used FAB classification (1,8).

1.3.3. CML

CML is a chronic disease, which differentiates it from ALL and AML. The disease has two or three phases, the initial chronic phase, followed by an accelerated and/or blast phase. The first chronic phase usually lasts about 3 to 4 years. There are malignant blasts produced but not in a high number and normal cells are still predominating (1). Blood or bone marrow contains less than 10% leukemic blasts (10). Switching to accelerated phase the number of normal cells is decreasing and the number of blasts is increasing. This usually lasts about 3 to 9 months (11). Blasts are increasing from 10 to 19% (10). Finally, the blast phase, also called blast crisis is characterized by a high number of malignant clones and is similar to acute leukemia (1). Usually, there are more than 20% blasts in blood and bone marrow. The blast crisis can be either of myeloid (most common), lymphoid, mixed, erythrocytic, megakaryocytic (both are rare in pure forms) or of mast cell (extremely rare) form (10).

1.4. Risk factors

In most of the cases, the exact etiology of childhood leukemia is not known. But there are some known genetic and environmental risk factors (1).

1.4.1. Environmental risk factors

Ionizing radiation is an environmental risk factor which has been significantly linked to an increased risk of developing ALL and AML (12). Alkylating drugs used in chemotherapy of prior cancers are known to increase especially the risk of AML later on (10). AML is also connected to benzene exposure (10). Exposure of the mother during pregnancy to unspecified residential pesticides also have been associated with an increased risk of childhood leukemia (13).

1.4.2. Genetic risk factors

Genetics are known to be a risk factor for the development of leukemia. There is the two-step model proposing that development occurs after both a first mutation which usually occurs in utero (chromosomal translocation) and a second mutation occurring after birth (14). If there are twins and one is diagnosed with leukemia the lifetime risk for the second one is estimated to be 20%. The incidence of siblings of a patient with leukemia is four times greater than in the normal population (15). Some genetic conditions are predisposing factors for children to develop leukemia (1).

1.4.2.1. Down syndrome

It is well known that children with Down syndrome (DS) have an increased risk to develop ALL as well as AML (1,5). Acute leukemia is 10 to 20 times more common in children with trisomy 21 than in population without DS. The ratio of AML to ALL is the same, but from the first to the third year of life AML is more common in DS patients compared to non-DS patients (1). ALL affects 1 in 300 children with DS, and the prognosis in children with DS is worse than in children with non-DS (5). Children with DS-AML have much better outcomes than children without Down syndrome (1).

1.4.2.2. Other genetic abnormalities

Li Fraumeni syndrome, a family syndrome predisposing to cancers due to the p53 mutation, leukemia can be one of the multiple cancers. Also, neurofibromatosis is predisposing to the development of leukemia (15). Genetic abnormalities of the immune system like Ataxia-telangiectasia, Bloom syndrome, Schwachman-Diamond syndrome are also known to carry an increased risk of developing leukemia (1).

1.5. Clinical manifestations

Children with leukemia often present with signs and symptoms that reflect bone marrow infiltration and/or extramedullary disease. When leukemic blasts replace the bone marrow, patients have signs of bone marrow failure. Extramedullary disease can, for example, affect the spleen, liver or testis (1).

1.5.1. ALL

ALL commonly presents at the beginning unspecific with anorexia, fatigue, malaise, and asthenia. Later on, the patient often develops clinical manifestations like pallor, listlessness, and bleeding (purpuric and petechial skin lesions, or mucous membrane hemorrhage). Other common clinical manifestations include bone pain, fever, and fatigue. In addition, organ infiltration can cause lymphadenopathy, hepatosplenomegaly, testicular enlargement or central nervous system (CNS) involvement (1,16). The white blood cell count in B-ALL varies. Most patients have a mild increase of the WBC. The median count at presentation is 33000 (1). But the WBC count can also be low or normal. The hemoglobin of patients is in most cases moderately to markedly reduced. Thrombocytopenia is seen in over 90% of patients (15). Symptoms of CNS involvement, such as headache, vomiting, lethargy, and nuchal rigidity are rarely noted at initial diagnosis but are more common in T-ALL and mature B cell ALL (17).

1.5.2. AML

AML is, of course, presenting in a similar way. Pallor, fever, swollen lymph nodes, hepato- or splenomegaly are common clinical manifestations (1). Due to bone marrow infiltration, we usually see anemia. The median WBC count at diagnoses is 20 000. Thrombocytopenia with platelets below 100000 is seen in more than 75% of the patients (18). In comparison to ALL signs and symptoms like subcutaneous nodules, “blueberry muffin” lesions, infiltration of the gingiva and laboratory findings of disseminated intravascular coagulation (DIC) are more specific for the diagnose of AML and less commonly seen in ALL (1).

1.5.3. CML

The presenting symptoms of CML can be the usual clinical manifestations like fever, weight loss, pallor, and organomegaly. In the Lab, we usually see anemia, leukocytosis, and thrombocytosis (1,10).

1.6. Prognosis

Nowadays the overall prognosis for pediatric patients with leukemia is quite good. For ALL the 5-year survival rate is about 90%. For AML the survival rate is about 60 to 70%. The prognosis differs for different risk groups of patients depending on age, count of white blood cells and different genetic abnormalities (1). More details on different prognosis due to genetic abnormalities will be mentioned in the next section.

1.7. Cytogenetics

Every cell has a chromosome set and the basis of our human life is gene expression of the DNA. The biological basis of cancer is the progressive accumulation of genetic and epigenetic alterations leading to disruption of normal cell functioning (19). There are proto-oncogenes, carrying important information for normal function of the cell, and oncogenes in which protooncogenes can develop and can lead to the formation of malignant cells There are five main mechanisms by which oncogenes work: they can code for growth factors, growth factor receptors (both leading to uncontrolled proliferation), changing signal transducers or transcription factors in the cell and interfere with controlled cell death (apoptosis). On the genetic basis, there are three main mechanisms of activating a protooncogene: amplification, point mutations, and translocation (6).

- Amplification leads to an increased number of copies of a gene (20)(Figure 1)
- Point mutations are mutations where a single nucleotide base is changed, inserted or deleted (20)(Figure 1)
- Translocation is the transfer of chromosomal material between chromosomes. There is an abnormal recombination after breakage of two chromosomes (21). Translocation is an important mechanism in a few leukemias. It can lead to so-called fusion genes. The transcription of a fusion gene can lead to the production of novel proteins which can be oncogenically active (6)(Figure 1)

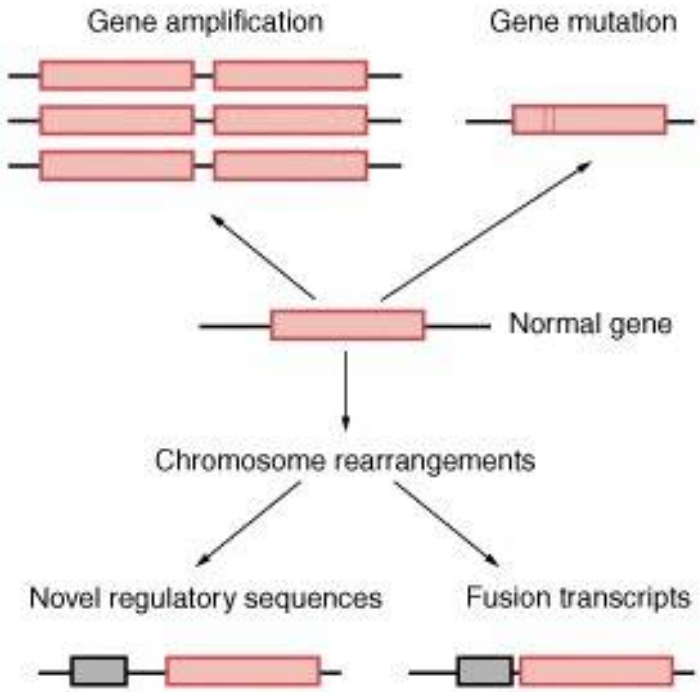


Figure 1. Schematic representation of the main mechanisms of oncogene activation, Taken from: Pierotti MA, Sozzi G, Croce CM. Mechanisms of oncogene activation. Holland-Frei Cancer Medicine. 6th edition. 2003

Pediatric leukemia is, in general, a biologically and genetically heterogeneous disease. Cytogenetic analysis of genes involved in the disease-specific translocations interrupt oncogenes which play a causative role in leukemic transformation (22). Chromosomal deletions, which result in the loss of tumor suppressor genes, are much less common in pediatric leukemias (23).

1.7.1. ALL

The nowadays used classification of ALL is mainly based on cytogenetics and immunophenotype. Especially for B-ALL, it is very important and there are seven types of B-ALL in the 2008 WHO classification with recurrent genetic abnormalities. These genetic abnormalities plus information about the patient's age and the WBC count can give us more information about the prognosis and treatment response. B cell leukemias not fitting in that subclassifications have been classified as B-ALL not otherwise specified (8). Identification of specific recurrent genetic abnormalities is critical for disease evaluation, optimal risk stratification, and treatment planning. Subtypes of B-cell ALL with recurrent genetic abnormalities include the following: hyperdiploidy (51 to 65 chromosomes); hypodiploidy (< 44 chromosomes); t(9;22)(q34;q11.2), *BCR-ABL1*; t(v;11q23), *MLL* rearrangement; t(12;21)(p13;q22), *ETV6-RUNX1*; t(1;19)(q23;p13.3), *TCF3-PBX1*;and t(5;14)(q31;q32), *IL3-IGH* (24).

1.7.1.1. B-ALL with t(12;21) (p13;q22), *TEL-AML1* (*ETV6-RUNX1*)

This type of B-ALL is the most common ALL with recurrent genetic abnormalities in children. It accounts for 20 to 30%. Usually, it appears between 2 to 10 years of age and it is rare in infants (8,25). A fusion gene is produced between *ETV6* gene (*TEL*) on chromosome 12 and the *RUNX1* (*AML1*) on chromosome 21. This translocation lacks unfavorable markers and can be treated with a low-intensity regiment of chemotherapy. It has a favorable outcome and children remain usually relapse-free (8,22).

1.7.1.2. B-ALL with Hyperdiploidy

Hyperdiploidy is a chromosome set of more than 50 chromosomes in the blasts. It is after t(12;21) translocation the most common recurrent genetic aberration seen in childhood leukemia. It accounts for about 25% and is also most often seen between 2 to 10 years of age (8). Usually, it causes a B-ALL but can be very rarely also seen in T-ALL. It has a favorable outcome but it has been discovered that there is a slightly inferior outcome for patients having a lower hyperdiploidy (chromosome number of 51 to 55) than patients having a higher hyperdiploidy (chromosome number of 56 to 67). As well it has an influence on prognosis which chromosome is gained (22). Chromosome 4, 10 and 17 are the most commonly gained and are connected to a favorable outcome (8). Whereas the addition of chromosome 5 or isochromosome 7 has an inferior outcome (22).

1.7.1.3. B-ALL with Hypodiploidy

A chromosome number less than 46 is defined as hypodiploidy and accounts for about 7% of ALL leukemias in children. In general, a lower number of chromosomes leads to a worse outcome. The most of these leukemias have a chromosome set of 45 chromosomes and have a better prognosis than leukemias with a chromosome set of less than 45 (22). A chromosome set of fewer than 45 chromosomes is very rare and accounts for only about 1 % of pediatric B-ALL. These rare types can occur at any age (8).

1.7.1.4. B-ALL with t(9;22) (q34;q11.2), *BCR/ABL1*

This translocation creates the well-known fusion gene "Philadelphia" chromosome. It causes about 4% of childhood B-ALL (8). It translocates sequences of the tyrosine kinase *ABL* proto-oncogene from chromosome 9 to the *BCR* gene on chromosome 22. Usually, this translocation is seen in CML and adult leukemia (22). At the genetic level, the translocation is the same as in CML. But it has been shown that the breakpoint of the *BCR* gene on chromosome 22 (of a patient with B-ALL Ph +) is more centromeric than in CML. This leads to a shorter *BCR-ABL* fusion gene, forming a smaller hybrid protein (185 kDa vs 210 kDa) (25). Children with B-ALL Ph+ most often have an early B-cell phenotype. They are older than ten years and often have unfavorable markers (high WBC count) leading to an unfavorable outcome. There is frequently an involvement of the CNS (22). These children usually need aggressive chemotherapeutic protocols (8).

1.7.1.5. B-ALL with t(v;11q23), *MLL* Rearranged

This genetic aberration accounts for about 5% of childhood ALL and it is most commonly found in infants (children younger than one year) (8). *MLL* gene is located on chromosome 11 and needed for normal hematopoiesis. Most often a translocation between chromosome 4 and 11 leads to a rearrangement of the *MLL* gene (*MLL-AF4*) and to an abnormal hematopoiesis. This results in an immunophenotype of pro-B/mixed B-ALL leukemia. It is very resistant to therapy and often associated with CNS infiltration, leading to an unfavorable outcome (1,26).

1.7.1.6. B-ALL with t(5;14)(q31; q32), *IL3-IGH*

Very rare type with an incidence of less than 1% of childhood ALL not much known about (8).

1.7.1.7. ALL with t(1;19), *E2A/PBX1* (*TCF3/PBX1*)

Has an incidence of 5% of childhood ALL. A fusion gene between *TFC3* gene located on chromosome 19 with the *PBX1* gene on chromosome 1 is produced, coding for a transcription factor. It is most common around 5 years of age (8). The influence on the prognosis of this translocation is not known (1).

1.7.1.8. T-ALL

The WHO classification of 2008 does not subclassify T-ALL by cytogenetics. Even though 50 to 70% have a genetic abnormality in their blasts but to less recurrent groups have been identified so far for a subclassification (8). A translocation between chromosome 10 and 14 has an incidence of about 7% of childhood T-ALL cases. The outcome of this translocation is favorable (1).

1.7.2. AML

Cytogenetics is now known to be very important for the prognosis of children with AML. In the 2008 WHO classification there are nine sub-classifications of AML with recurrent genetic aberrations included (8). Furthermore, the cytogenetic abnormalities t(8;21)(q22;q22), t(15;17)(q22;q12), inv(16)(p13.1q22) or t(16;16)(p13.1;q22) included in the subgroups of AML with recurrent genetic abnormalities, are sufficient for the diagnosis of AML regardless of the blast percentage in the peripheral blood or bone marrow (27).

1.7.2.1. AML with t(8;21)(q22;q22), (*RUNX1-RUNX1T1*)

It is a very common translocation in children with AML, with an incidence of 8 to 13% (8). *RUNX1* gene on chromosome 21 (also called *AML1* and affected in the t(12;21) of B-ALL) fuses with the *ETO* (*MTG8*) gene on chromosome 8 (28). This results in a protein disrupting the CBF transcription factor which is important for normal hematopoiesis (8). It is usually connected with a good outcome (18).

1.7.2.2. AML with t(9;11)(p22;q23), *MLLT3-MLL*

This translocation leads to rearrangement of the *MLL* gene on chromosome 11. As already mentioned for B-ALL with t(v;11q23), *MLL* Rearranged, this gene a has an important role in normal hematopoiesis. Changes in *MLL* genes are seen in 9 to 22% of pediatric patients with AML. The t(9;11) translocation causes about 7% of childhood AML. It can present with bleeding as a clinical sign, due to extramedullary disease and DIC. In general, *MLL* rearrangements lead to a worse prognosis. But if it is caused by t(9;11) it has a favorable outcome (8).

1.7.2.3. AML/AP (Promyelocytic) with t(15;17)(q22; q12),*PML/RARA*

Promyelocytic AML is seen in 4 to 10% of pediatric patients with AML. The translocation between chromosome 15 and 17 is the most common genetic abnormality causing it. It is also associated with bleeding due to DIC and has a favorable outcome (8,18).

1.7.2.4. AML with inv(16)(p13.1q22) or t(16;16)(p13.1; q22), *CBFβ/MYH11*

Has an incidence of 5 to 10% of AML diagnosed in pediatric population. Ever the involution or the translation on chromosome 16 cause the *MYH11* protein disrupting the *CBF* transcription factor which important for normal hematopoiesis. These cytogenetic abnormalities are connected with a favorable outcome (8).

1.7.3. CML

CML is very strongly connected with a genetic aberration. In children, 99% are connected to the "Philadelphia chromosome" which can also lead to ALL (1). The translocation between chromosome 9 and 22 leads to the *BCR/ABL1* hybrid gene. The difference is that in CML the fusion gene is longer than in ALL Ph+ and this results in the M-bcr ("major") fusion gene which codes for a bigger protein than m-bcr ("minor") fusion gene seen in ALL (22,25). The BCR-ABL is an oncoprotein with a molecular weight of 210 kDa (Figure 2). It has several functional domains leading to malignant transformations. These domains, for example, stimulate the proliferation, activate signal transduction proteins or phosphorylate signal and adaptor proteins leading to leukemogenesis of the cells (10).

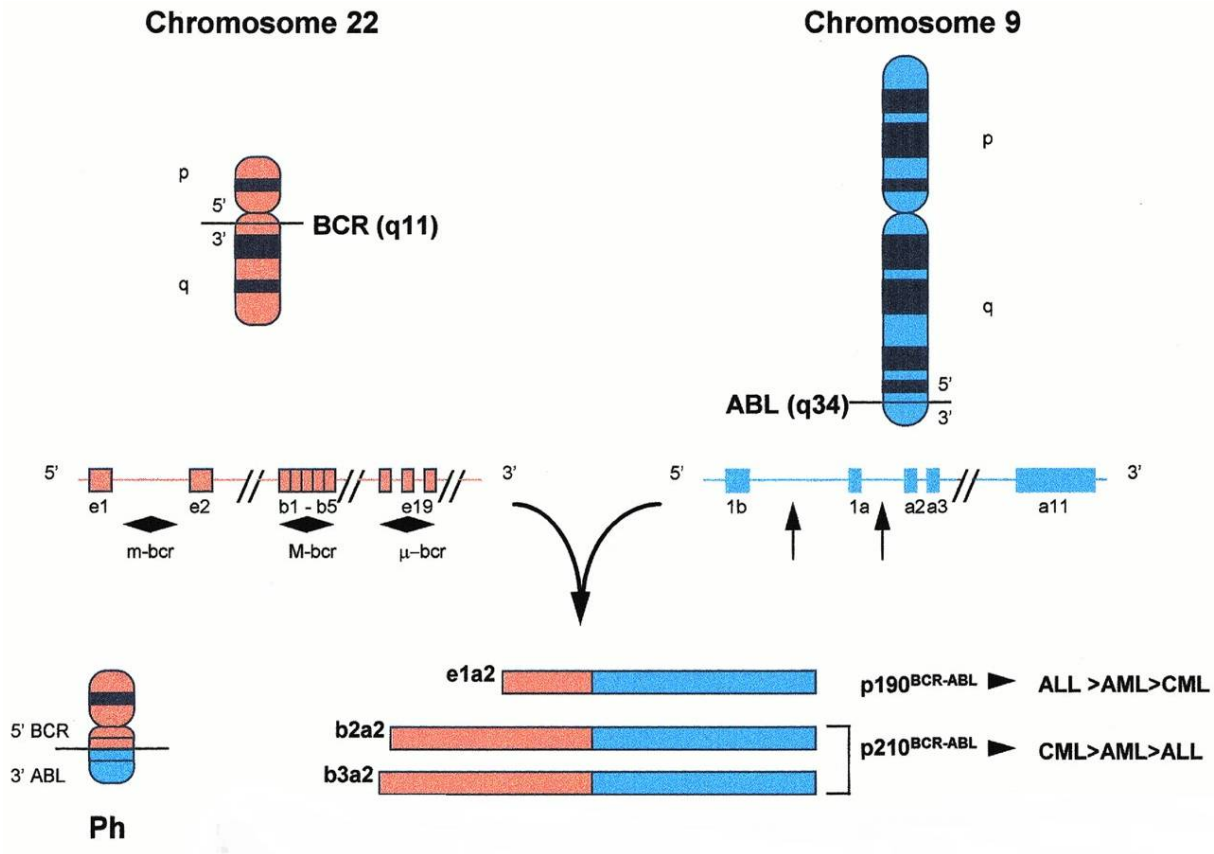


Figure 2. Presentation of "M-bcr" and "m-bcr" fusion proteins. Taken from: Faderl S, Kantarjian H, Talpaz M, Estrov Z. Clinical Significance of Cytogenetic Abnormalities in Adult Acute Lymphoblastic Leukemia. J Am Soc Hematol. 1999;94(5):1495–503.

Recurrent chromosomal abnormalities present in malignant cells often correlate closely with specific clinical and biological characteristics of the disease (23). In AML, unique cytogenetic rearrangements associate with distinct morphological subgroups, such as t(15;17) in acute promyelocytic leukemia (17). In ALL, some changes are specific to particular cell subtypes characterized by immunophenotyping (29). The presence or absence of chromosomal aberrations is a significant prognostic factor for both ALL and AML (22).

The prognostic and diagnostic values of cytogenetic abnormalities of both acute myeloid and lymphoblastic pediatric leukemia have been established worldwide. It has also been demonstrated that the frequency with which chromosomal abnormalities are observed varies among populations (30). Until now, only one study from Croatia has reported the results of cytogenetic analysis in 55 children with acute lymphatic leukemia (31). Despite the fact that cytogenetic analysis is routinely performed at diagnosis in our hospital, data have not been reported until now. The present study was conducted with a view to investigating cytogenetic abnormalities in each type of pediatric leukemia, correlating them with prognosis and clinical presentation at the time of diagnosis.

2. OBJECTIVES

AIMS:

- Investigate the frequency and types of acquired chromosomal aberrations in a group of Croatian children with newly diagnosed pediatric leukemia in a tertiary center

- Investigate the correlation between the most frequent chromosomal aberrations with clinical and biological prognostic risk factors in each pediatric type of leukemia

- Compare results with previously reported results

3. MATERIALS AND METHODS

3.1. Ethical background of data collection

All data which were used for this thesis were gathered at the Department of Pediatrics, University Hospital of Split in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration.

Retrospectively, we reviewed cytogenetic and molecular data of children with newly diagnosed leukemia treated in the Department of Pediatric Hematology/Oncology of the University Hospital Split from January 2000 to December 2017.

3.2. Patients

The study enrolled patients under 18 years of age with newly diagnosed pediatric leukemia that underwent cytogenetic and molecular studies at diagnosis. The diagnosis of pediatric leukemia was based on the French–American–British (FAB) criteria (32). The clinical characteristics at diagnosis, including age, sex, hemogram, central nervous system (CNS) involvement, hepatosplenomegaly, and lymphadenopathy (LAP) were obtained from patient medical records. Relapsed patients were excluded from the study.

3.3. Cytogenetics and molecular techniques

Conventional cytogenetics and molecular cytogenetics (fluorescence in situ hybridization, FISH) complemented by molecular genetics (reverse-transcription polymerase chain reaction, RT-PCR) have been commonly used to detect chromosomal and genetic changes in childhood leukemia in everyday clinical practice. Diagnostic karyotyping of leukemia cells was obtained before initiation of therapy. Conventional cytogenetic analysis of the bone marrow (BM) cells was performed according to standard methods (33). The BM cells were cultured for 24 hours using methotrexate for synchronization of chromosomes. In all cases at least 20 metaphases were analyzed using the conventional trypsin-Giemsa banding technique and karyotyped according to the International System for Human Cytogenetic Nomenclature (34). Abnormal clones were defined as 2 or more metaphase cells with identical structural abnormalities or extra chromosomes, 3 or more metaphase cells with identical missing chromosomes. Normal karyotype required a complete analysis of a minimum of 10 metaphase cells.

FISH analysis is able to detect these abnormalities not only in metaphases but also in interphase nuclei. FISH uses labeled DNA probes directed at selected targets and have a higher resolution than conventional cytogenetics. About 200 cells were analyzed by FISH using LSI specific probes (Vysis; Abbott Molecular, Des Plaines, IL) according to the

manufacturer's instructions. FISH is a very sensitive technique but is limited by the number of FISH-probes.

RT-PCR amplifies specific targets in the genome using specific forward and reverses primers at the RNA-level. This technique requires a reverse transcriptase enzyme that generates copy DNA (cDNA) from RNA. RT-PCR was applied for the detection of specific translocations or fusion genes. This molecular method can detect aberrations that are cytogenetically cryptic and is very sensitive and can be used to monitor minimal residual disease (MRD).

Complementary to conventional cytogenetics FISH or/and RT-PCR analyses with a panel of commercially available probes has been used to screen for the specific translocations/gene fusions: t(1;19) (q23;p13) (*E2A-PBX*), t(9;22)(q34;q11) (*BCR/ABL*),t(12;21)(p13;q22) (*ETV6/RUNX1*), t(8;21)(q22;q22); (*RUNX1-RUNX1T1*) and(15;17)(q22;q12); (*PML-RARA*). FISH analyses were used as a complementary method for the detection of 11q23/*MLL* rearrangements and specific deletions 9p, 7qand inv(16).

3.4. Statistical analysis

Statistical analysis was performed using statistical software Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and MedCalc (MedCalc Software, Ostend, Belgium). Continuous data were presented as mean and standard deviation, whereas categorical variables were presented as whole numbers and percentages.

4. RESULTS

The final sample had a total size of 98 pediatric patients who were diagnosed with leukemia in the University Hospital Split between 2000 and 2017. Most patients were diagnosed with ALL (78%) followed by 20% with AML and only two patients with CML. Most ALL patients were diagnosed with patients B cell lineage ALL (85%). As seen in Table 1, the total distribution of gender was equal. For the gender distribution between T ALL and B-ALL, there has been a significant difference seen with the Chi-squared test (P=0.0126) but it has to be said that the total sample of T ALL were only 13 patients.

The median age of diagnosis of the total sample was 4.8 years with a 95% CI of 3.9 to 6.3 years. The youngest patient was 3 months at the time of diagnoses and the oldest 17.6 years old. The median age of diagnosis for ALL was 4.5 years (95% CI 3.7 to 6.3 years), B-ALL patients where slightly younger with a median age of 4.2 years (95% CI 3.4 to 5.2 years). T-ALL patients were shown to be older with a median age of diagnosis of 11.1 years but also had a much wider 95% CI (4.3 to 14.8 years), due to the small sample size of 13 patients. The youngest patient for ALL was the youngest patient of the study with 3 months and diagnose of B-ALL. The oldest B-ALL patient was 17.3 years. For T-ALL the youngest patient was 3.5 years and the oldest 17.6 years. AML was diagnosed at a median age of 5 years (95% CI 2.6 to 9.3 years). The youngest age of diagnosis for AML was 9 months and the oldest 17.4 years. The two patients diagnosed with CML have been 10.7 years and 16.8 years.

As shown in Table 1, we collected the blood values of white blood cells, thrombocytes, and hemoglobin at the time of diagnosis.

Most patients had an increased white blood cell count. Patients with ALL had a median white blood cell count of 11.4 ($x10^9$/L) (95% CI from 8.1 to 31.6 ($x10^9$/L)). B-ALL patients had a slight lower median of 8.9 ($x10^9$/L) (95% CI 5.5 to 17.5 ($x10^9$/L)). The highest value for B-ALL is a white blood cell count of 784 ($x10^9$/L). Patients with T-ALL almost all had a high white blood cell count with a median count of 108.4 ($x10^9$/L) (95% CI from 47.6 to 296.2 ($x10^9$/L)). The highest value for T-ALL is 560 ($x10^9$/L). The median of patients with AML was 13 ($x10^9$/L) (95% CI from 9.1 to 20.4 ($x10^9$/L)).

Most of the patients showed a decreased thrombocyte level at the time of diagnoses as shown in Table 1. Patients with ALL showed a median count of 56 ($x10^9$/L) (95% CI from 47 to 80 ($x10^9$/L)). B-ALL patients had a median of 54 ($x10^9$/L) (95% CI from 42 to 80 ($x10^9$/L)) and patients with T-ALL slightly higher of 74 ($x10^9$/L) (95% CI from 43 to 184 ($x10^9$/L)). Data for 17 patients of AML where available and all had a decreased thrombocyte count with

a median of 34 (x10^9/L) (95% CI from 19 to 57 (x10^9/L)). One CML patient had thrombocytosis at the time of diagnoses.

Hemoglobin level was decreased in most of the patients. ALL patients had a median count of 82 (g/l) (95% CI from 78 to 90 (g/l)), B-ALL patients of 79 (g/l) (95% CI from 73 to 85 (g/l)) and T-ALL patients had a higher median of 99 (g/l) (95% CI from 87 to 120 (g/l)). AML patients had almost the same median count of 80 (g/l) (95% CI from 71 to 90 (g/l)) as the ALL patients.

Table 1. Distribution of all patients with different type/immunophenotype according to sex and hematological values

Parameter	**Total N=98 (%)**	**ALL N=76 (%)**	ALL subgroup: B-ALL N=63 (%)	ALL subgroup: T-ALL N=13 (%)	**AML N=20 (%)**	**CML N=2 (%)**
Gender						
Male	51 (52)	40 (53)	29 (46)	11 (85)	10 (50)	1 (50)
Female	47 (48)	36 (47)	34 (54)	2 (15)	10 (50)	1 (50)
WBC (x10^9/L)						
Low	26 (27)	23 (30)	22 (35)	1 (8)	3 (15)	0
Normal	26 (27)	16 (21)	16 (25)	0	8 (40)	2 (100)
High	45 (46)	36 (47)	24 (38)	12 (92)	9 (45)	0
Platelets (x10^9/L)						
Low	76 (78)	59 (78)	51 (81)	8 (62)	17 (84)	0
Normal	16 (16)	15 (20)	11 (17)	4 (31)	0	1 (50)
High	1 (1)	0	0	0	0	1 (50)
Hemoglobin (g/L)						
Low	85 (87)	65 (86)	51 (81)	10 (78)	19 (95)	1 (50)
Normal	12 (12)	10 (13)	11 (17)	3 (23)	1 (5)	1 (50)

ALL- acute lymphoblastic leukemia; AML- acute myelogenous leukemia; CML- chronic myelogenous leukemia; B-ALL- B cell lineage acute lymphoblastic leukemia; T-ALL- T cell lineage acute lymphoblastic leukemia; WBC- white blood cell count

As shown in Table 2, the most presenting symptoms of all subtypes were fever and pallor followed by lymphadenopathy. In patients with ALL and splenomegaly, the median increase in cm below the costal margin was 4 cm (95% CI from 3 to 5 cm). Patients with ALL and hepatomegaly had a median increase of 3 cm below the costal margin (95% CI from 2.5 to 4 cm), B-ALL patients had as well a median increase of 3 cm below the costal margin (95% CI from 2.5 to 4 cm) and T-ALL patients had a median of 4 cm below the costal margin (, 95% CI from 2 to 6 cm). AML patients had a median increase of 3 cm below the costal margin (95% 2 to 4 cm).

Table 2. Distribution of patients with pediatric leukemia according to clinical manifestations

Parameter	**Total N=98 (%)**	**ALL N=76 (%)**	ALL subgroup: B-ALL N=63 (%)	ALL Subgroup: T-ALL N=13(%)	**AML N=20 (%)**	**CML N=28 (%)**
Asthenia;	1 (1)	1 (1)	1 (2)	0	0	0
Fever	70 (71)	54 (71)	47 (75)	7 (54)	14 (70)	2 (100)
Pallor	68 (69)	52 (68)	45 (71)	7 (54)	15 (75)	1 (50)
Bleeding	33 (34)	27 (36)	21 (33)	6 (46)	11 (55)	0
Lymphadenopathy	58 (59)	43 (57)	34 (54)	9 (69)	14 (70)	1 (50)
Splenomegaly	48 (49)	38 (50)	30 (48)	8 (62)	9 (45)	1 (50)
Hepatomegaly	55 (56)	46 (61)	37 (59)	9 (69)	8 (40)	1 (50)
CNS involvement	2 (2)	2 (3)	1 (2)	1 (8)	0	0

ALL- acute lymphoblastic leukemia; AML- acute myelogenous leukemia; CML- chronic myelogenous leukemia; B-ALL- B cell lineage acute lymphoblastic leukemia; T-ALL- T cell lineage acute lymphoblastic leukemia; CNS- Central nervous system

Molecular/cytogenetic examinations were performed successfully in 94 out of 98 patients (96%) and chromosomal changes were detected in 67 of the 94 patients (71%).

Table 3 shows the distribution of patients according to their chromosomal aberrations including patients with Down syndrome. Among aberrations, the most frequent were structural aberrations (46%).

Table 3. Distribution of 94 patients according to characteristics of chromosomal aberrations

Chromosomal aberrations	Number of patients	%
Normal karyotype	27*	29
Numerical aberrations	12*	13
Structural aberrations	43	46
Numerical and structural aberrations	12*	13

*includes patients with Down syndrome;

ALL

The molecular/cytogenetic analysis was successful in 73 out of 76 patients with ALL. The most frequent chromosomal aberration in ALL subgroups divided according to WHO classification was translocation t(12;21) with *ETV6/RUNX1* fusion detected by FISH in 20 (27%) patients, including cases of deletion 12p (Figure 3), followed by hyperdiploidy in 11 (15%) patients (Figure 4). Of the 11 patients in the hyperdiploidy group, 9 had more than 50 chromosomes. There was no patient with Down syndrome included in this group. There were 3 patients whose chromosomal abnormalities involved band 11q23 (*MLL*) and one of them had t(4;11). Other recurrent structural rearrangements detected were 9p aberrations in 5 patients (7%) and del(6q) in 2 patients (3%).There were no children with t(9;22) in our study. Nonspecific structural chromosomal aberrations were detected in 6 cases.

Table 4. The frequency of acquired chromosomal aberrations in 73 pediatric acute lymphoblastic leukemia cases compared with literature

Parameter	**Our ALL patients N=73 (%)**	**WHO classification† (%)**	**Prognostic group**
Normal karyotype	23 (32)*		intermediate
Hyperdiploidy	11 (15)	20-25	favorable
Hypodiploidy	3 (4)	1	unfavorable
t(12;21) *ETV6/RUNX1*	20 (27)	20-25	favorable
t(9;22) *BCR-ABL*	0	3	unfavorable
11q23 *MLL*	3 (4)	2-10	unfavorable
9p aberrations	5 (7)	10	un/favorable§
6q deletion	2 (3)	10	intermediate
Other abnormalities	6 (8)		

ALL- acute lymphoblastic leukemia; * includes patients with Down syndrome; § favorable in T-cell lineage ALL/ unfavorable in B-cell lineage ALL
† Reference: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (35)

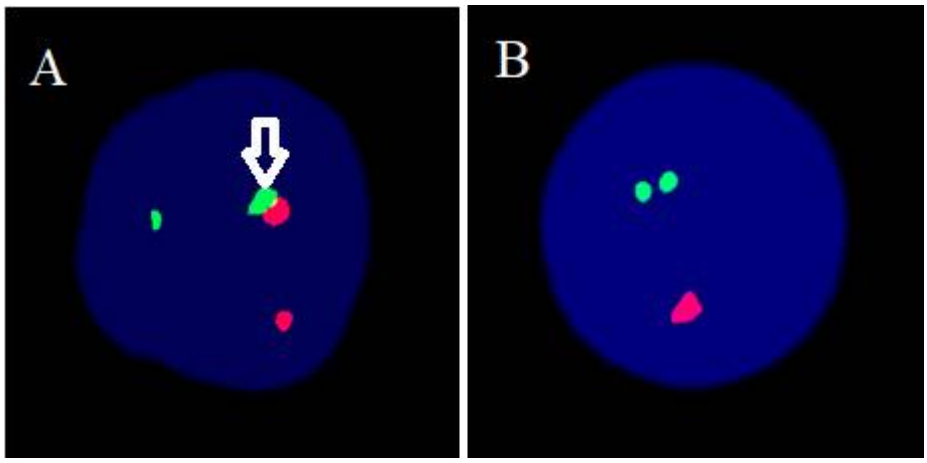


Figure 3. FISH analysis using Vysis LSI *ETV6/RUNX1* Dual Fusion FISH Probe Kit with orange-labeled *ETV6* (12p13) and green-labeled *RUNX1* (21q22) probes. (**A**) Interphase nuclei with 1 orange, 1 green and 1 fusion signal (arrow pointed) indicating the t(12;21). (**B**) Interphase nuclei with 2 green and 1 orange signal indicating the del12p.

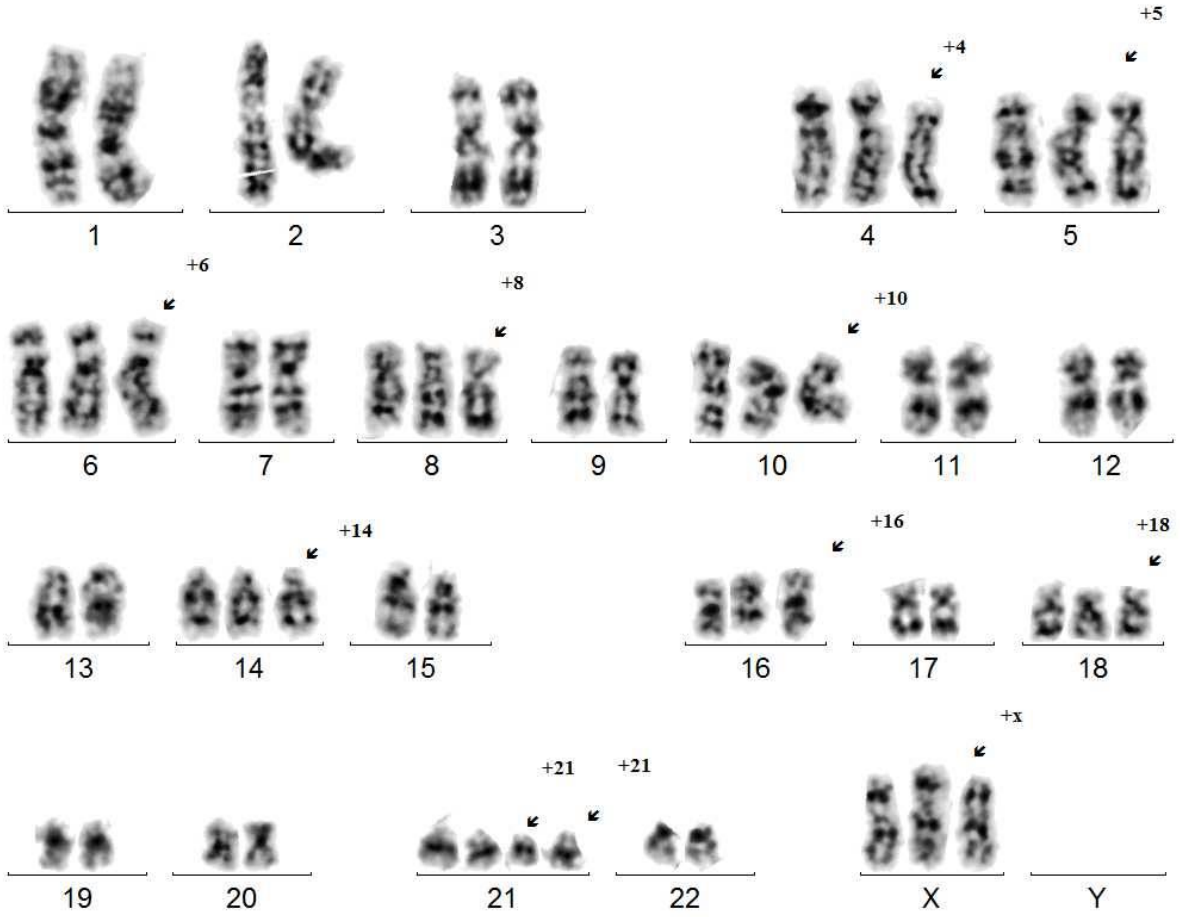


Figure 4 Conventional cytogenetic analysis of the bone marrow G- banding of metaphase spreads showing a higher hyperdiploidy with 57 chromosomes. Karyotype: 57, XX,+4,+5,+6,+8.+10,+14,+16,+18,+21,+21,+X

The majority of patients in favorable prognostic subgroups which include hyperdiploidy and *ETV6/RUNX1* were below 10 years of age, and females.

On examination, hepatosplenomegaly was observed in 80% of patients with 9p aberration, fever in 80% *ETV6/RUNX1* fusion gene subgroup of patients and splenomegaly in a subgroup of patients with *MLL* rearrangements (100%).

The lowest hemoglobin level was noted in ALL with *MLL* rearrangements, with a mean value of 73.7 g/L while the highest level was seen in ALL with 9p aberration with a mean value of 120 g/L. The lowest WBC count was seen in patients with hyperdiploidy with a mean value of 13.9 $x10^9$/Land the highest count was found in ALL with *MLL* rearrangements, with a mean value of 358 $x10^9$/L. The immunophenotypes of leukemic blasts from patients with 9p aberrations showed in 4 patients T-cell ALL and in 1 patient with B-cell ALL.

Table 5. Correlation between the most frequent acquired chromosomal aberrations and clinical features in the group of 73 ALL patients

Parameter	**Hyperdiploidy** **N=11 (%)**	***ETV6/RUNX1*** **N=20 (%)**	***MLL*** **N=3 (%)**	**9p aberrations** **N=5 (%)**
Frequency (%)	15	27	4	7
Gender M/F*	4 (36)/7 (64)	9 (45)/11 (55)	2 (67)/1 (33)	4 (80)/1 (20)
Age (range)	6 (3.9-9.5)	6.2 (4.8-8.1)	8.8 (1.4-16.2)	3.7 (2.2-5.2)
B-cell ALL	11 (100)	17 (85)	3 (100)	1 (20)
T-cell ALL	0	3 (15)	0	4 (80)
Fever	10 (91)	16 (80)	2 (67)	3 (60)
Pallor	6 (55)	13 (65)	3 (100)	4 (80)
Bleeding	4 (36)	5 (25)	0	2 (40)
LAP	7 (64)	12 (60)	1 (33)	2 (40)
Hepatomegaly	7 (64)	8 (40)	2 (67)	4 (80)
Splenomegaly	6 (55)	6 (30)	3 (100)	3 (60)
CNS	0	1 (5)	0	1 (20)
WBC ($x10^9$/L)	13.9 (2.9-63.2)	46.2 (0.6-512.9)	358 (179-501)	244 (31.1-784)
Haemoglobin (g/L)	87.4 (68-117)	84.7 (53-138)	73.7 (55-91)	120 (102-149)
Platelets ($x10^9$/L)	87.6 (12-243)	86.3 (24-375)	106 (32-240)	144.2 (26-306)

* male/female; B-cell ALL- B cell lineage acute lymphoblastic leukemia; T-cell ALL- T cell lineage acute lymphoblastic leukemia; LAP- lymphadenopathy; CNS- central nervous system; WBC- white blood cells

AML

The molecular/cytogenetic analysis was successful in 19 out of 20 patients with AML. The most frequent chromosomal aberrations in AML subgroups divided according to WHO classification were translocation t(8;21) with *RUNX1-RUNX1T1*fusion detected by cytogenetic examinations and confirmed by FISH in 4 patients (21%) (Figure 5) followed by t(9;11) in 2 patients(11%) (Figure 6) and inv(16) or del(16)(q22) in 2 patients. Other abnormalities include complex karyotypes were found in 3 children with Down syndrome.

Table 6. The frequency of acquired chromosomal aberrations in 19 pediatric acute myeloid leukemia cases compared with literature

Parameter	**Our AML patients N=19 (%)**	**WHO classification† (%)**	**Prognostic group**
Normal karyotype	4* (21)	20-30	intermediate
t(9;11*) MLLT3-MLL*	2 (11)	5-12	favorable
t(15;17)*PML/RARA*	1 (5)	1	favorable
t(8;21)*RUNX1-RUNX1T1*	4 (21)	5-12	favorable
inv(16) or del(16q) *CBFB-MYH11*	2* (11)	5-10	favorable
Deletion-7/del(7q)	3 (15)	4	unfavorable
Other abnormalities	3* (16)		

AML- acute myelogenous leukemia; * includes patients with Down syndrome;
† Reference: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (35)

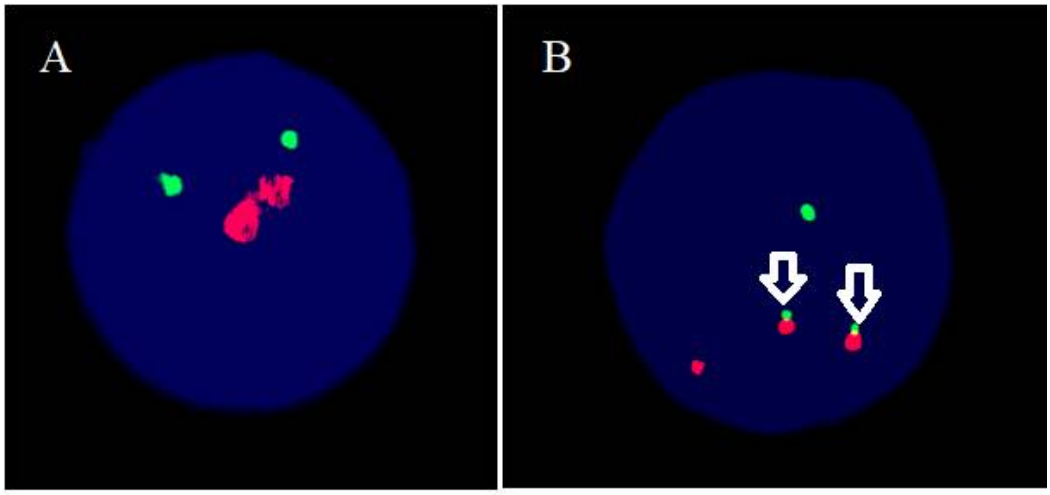


Figure 5. FISH analysis using Vysis *RUNX1/RUNX1T1* Dual Fusion FISH Probe Kit with orange-labeled *RUNX1T1* (8q21.3) and green-labeled *RUNX1* (21q22) probes. (**A**) Interphase nuclei without translocation, showing 2 orange and 2 green signals. (**B**) Interphase nuclei with 1 orange, 1 green and 2 fusion signals (arrow pointed) indicating the t(8;21).

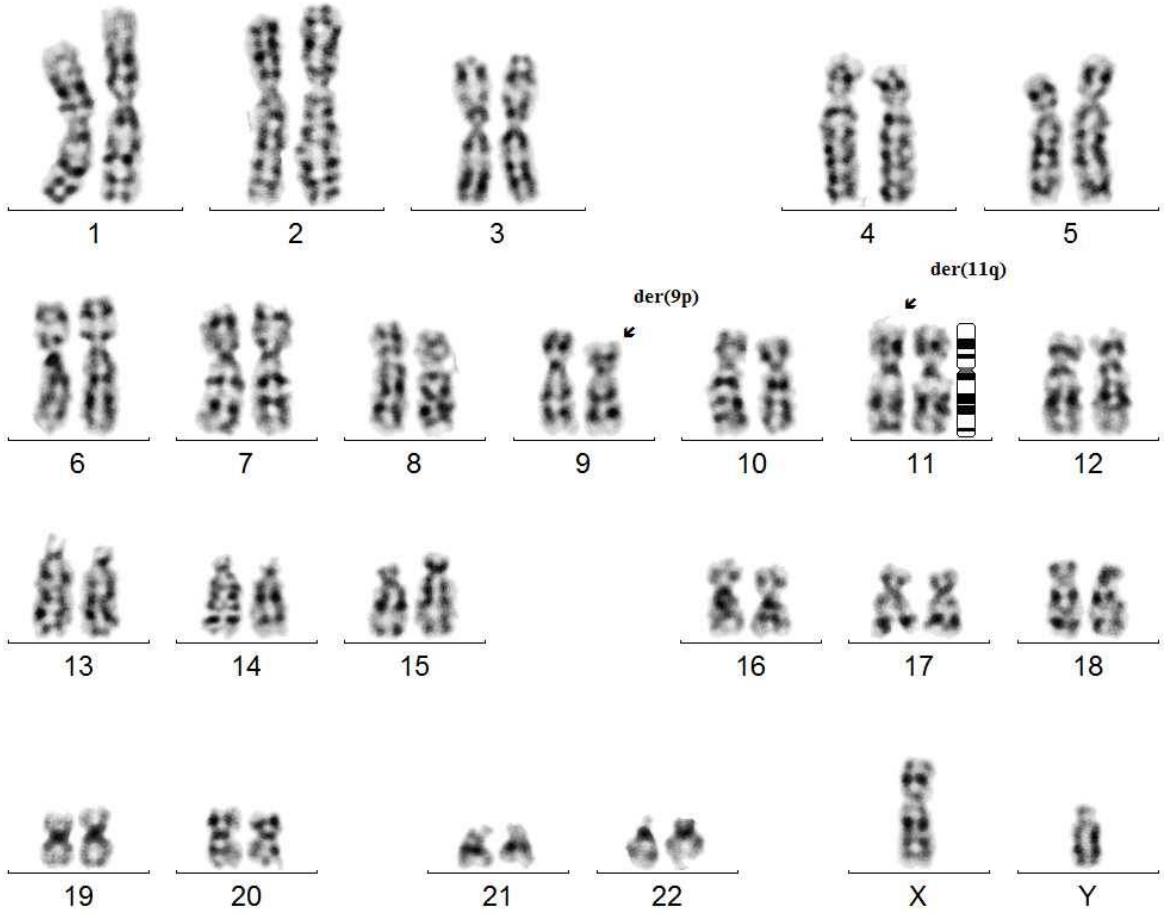


Figure 6. Conventional cytogenetic analysis of the bone marrow G- banding of metaphase spreads showing a translocation between chromosome 9 and 11. Karyotype: 46, XY, t(9;11)(p22;q23)

The majority of patients in the favorable prognostic subgroup with t(8;21) were below 10 years of age (mean age was 6 years), and female. In the same group, physical examination observed lymphadenopathy in 75% of patients and bleeding and fever in 50% of patients. Hepatomegaly was found in all patients with t(9;11) and splenomegaly in all patients with inv(16)/del16q.

The lowest hemoglobin level was noted in AML with t(9;11) with a mean value of 73.5 g/L. The lowest platelet count was seen in patients with del 7q/-7 with a mean value of 26.7 $x10^9$/L. The highest count of WBC was found in the subgroup of AML with inv(16)/del 16q, with a mean value of 45.3 $x10^9$/L.

Table 7. Correlation between the most frequent chromosomal aberrations and clinical features in 19 AML patients

Parameter	**t(8;21) N=4 (%)**	**del 7q/-7 N=3 (%)**	**t(9;11) N=2 (%)**	**inv(16)/del 16q N=2 (%)**
Frequency (%)	21	16	11	11
Gender M/F*	1 (25)/3 (75)	2 (67)/1 (33)	1 (50)/1 (50)	1 (50)/1 (50)
Age (range)	6 (3.9-9.5)	6.2 (4.8-8.1)	8.8 (1.4-16.2)	3.7 (2.2-5.2)
Fever	2 (50)	1 (33)	ND	1 (50)
Pallor	1 (25)	3 (100)	ND	0
Bleeding	2 (50)	2 (67)	ND	0
LAP	3 (75)	3 (100)	ND	1 (50)
Hepatomegaly	1 (25)	1 (33)	2 (100)	1 (50)
Splenomegaly	0	2 (66)	1 (50)	2 (100)
CNS	0	0	0	0
WBC ($x10^9$/L)	24.3 (8.4-57.7)	9.2 (5.8-12.5)	29 (3.2-54.8)	45.3 (19.5-71)
Haemoglobin (g/L)	83.8 (70-108)	84.7 (76-95)	73.5 (57-90)	86.5 (78-95)
Platelets ($x10^9$/L)	39.5 (19-55)	26.7 (15-52)	114 (93-135)	39.5 (19-60)

* male/female; LAP- lymphadenopathy; CNS- central nervous system; WBC- white blood cell count; ND- no data

CML

In our study, CML was diagnosed in only 2 children. The two patients diagnosed with CML have been 10.7 years and 16.8 years. Clinical characteristics of these patients are shown in Table 1. Both of them had a translocation between chromosome 9 and 22 which leads to the *BCR/ABL1* hybrid gene known as "Philadelphia chromosome" (Figure 7).

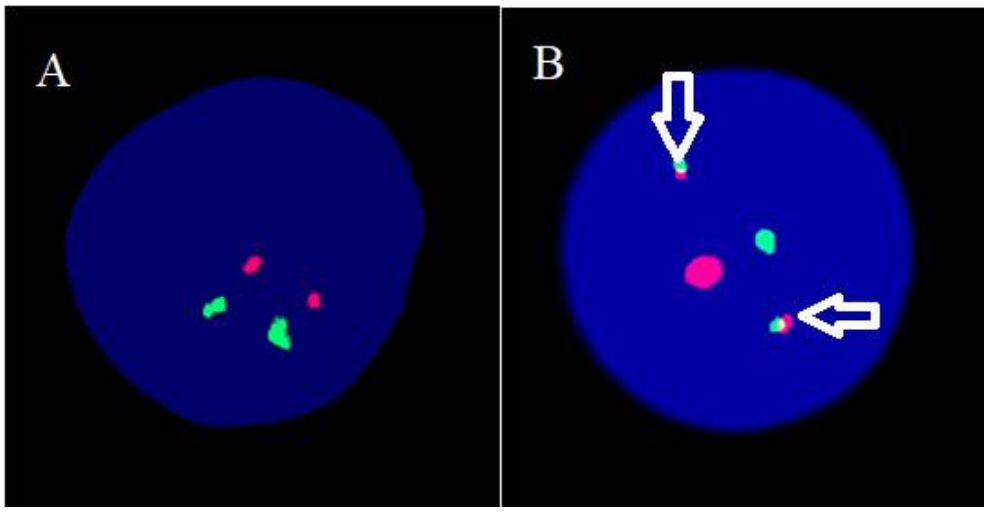


Figure 7. Fluorescence *in situ* hybridization (FISH) analysis using Vysis LSI *BCR/ABL* Dual Color, Dual Fusion Translocation Probe Kit with orange-labeled ABL (9q34) and green-labeled BCR (22q11.2) probes. (**A**) Interphase nuclei without translocation, showing 2 orange and 2 green signals. (**B**) Interphase nuclei with 1 orange, 1 green and 2 fusion signals (arrow pointed) indicating the t(9;22).

5. DISCUSSION

In this retrospective analysis, we included 98 patients with newly diagnosed pediatric leukemia. Molecular/cytogenetic examinations were performed successfully in 94 out of 98 patients, acquired chromosomal aberrations were found in 67 children (Table 3). Among the patients with abnormal karyotypes, the most frequent aberrations were structural (46%) (Table 3). Normal cytogenetic analysis was obtained in 27 patients (29%) (Table 3), consistent with the results of other studies (35). The percentage of normal karyotypes varies for different types of pediatric leukemia. In this study, the percentage of children with AML having normal karyotypes was smaller (21%) (Table 6) compared to the ALL group of children (32%) (Table 4). The percentage of children in ALL group having normal karyotypes in this study is also higher than in previously reported one (23% Petković *et al.*) (31). Since it has been previously reported that the frequency of chromosomal aberrations varies among populations, especially among geographically restricted ones, that could explain the discrepancy between these results obtained from ALL children coming from the southern part of Croatia and the ones reported in Petković *et al.* obtained from ALL children coming from the northern part of Croatia (30,31). Also, available methods of analysis can contribute to the variation in frequencies. Last 20 years, above mentioned chromosomal changes were detected using conventional cytogenetics, complemented with FISH and RT-PCR. Using these techniques in this study success of karyotyping was much higher (96%) than for Petkovic *et al* (1995.) who had a smaller success, 55 of 70 patients (79%) because only conventional cytogenetics examination were available (31).

In our cohort, the most prevalent type of pediatric leukemia was ALL (77%), followed by AML (21%), and only two children had CML (2%) which is consistent with distribution in pediatric populations in Europe and US reported in the literature (1).

The gender distribution of ALL was quite equal with a mildly higher prevalence of boys (52%) (Table 1), which is also seen in the general epidemiologic data(1). In our study, the median age of diagnoses of ALL was about 1 to 2 years higher (1). Even though we only had a small sample size of 13 patients with T-ALL there has been a significant difference in the gender distribution of T-ALL patients compared to B-ALL patients (P=0.0126). In our study, T-ALL affects boys more often than girls (11 out of 13) (Table 1) and occurs at an older age (11 years at time of diagnosis) than B-cell ALL (4.2 years at time of diagnosis). These data are consistent with the general epidemiologic data (9). The median WBC count was higher in the T-ALL patients (median count of 108.4 $x10^9$/L) than in the B-ALL (median count of 8.9 $x10^9$/L) patients (1). Thrombocyte and hemoglobin count were decreased in most of the patients as suspected (15). Fever, pallor, lymphadenopathy, and hepatomegaly were the

most common symptoms at the time of diagnosis and they are all known to be common clinical manifestations of leukemia (1). CNS involvement was only seen in two patients, one with T-ALL and one with B-ALL immunophenotype.

The most frequent chromosomal aberration in the group of 73 children with ALL was the translocation t(12;21) with *ETV6/RUNX1* fusion gene seen in 20 patients (27%), (Table 4, Figure 3) consistent with data from the literature (27). The most frequent presenting symptoms at the time of diagnosis in that subgroup of children were fever (80%), pallor (60%) and lymphadenopathy (60%) and most of the children (17 out of 20) had B-ALL immunophenotype (85%) (Table 5). The *ETV6/RUNX1+* patients were studied with regard to their gender, 11 of them were females and 9 males (Table 5). The present study revealed lower frequency of hyperdiploidy (11 out of 73 patients, 15%), compared to the frequency reported in the 2008 WHO classification (20 to 25%) and in Petković *et al.*(20%) (Table 5) (31,35). Both hyperdiploidy and *ETV6-RUNX1* subtypes are associated with favorable outcomes in pediatric ALL (Table 4) (27). Patients with recurrent 11q23 abnormalities are usually younger and have higher leukocyte counts (1,23). In our study, 3 patients with B-lineage ALL had 11q23 *MLL* rearrangements (Table 4) with high leukocyte counts (Table 5), two of them were infants and one with t(9;11;22) had disease onset at 13.5 years old which is consistent with data from the literature (1,23). There were no children with t(9;22) Ph-positive ALL in our study (Table 4). It is known that this type of ALL is associated with poor prognosis and is relatively uncommon in childhood ALL (3%)(35). The relationships between certain cytogenetic findings and specific immunophenotypes in ALL are well established and our study showed that all patients with hyperdiploidy and the majority of patients (85%) with t(12;21) had B-cell lineage ALL (Table 5) which is compatible with the finding from literature (1). Aberration 9p is not associated with specific lineage which is also confirmed in our study. 4 patients had T-cell ALL and 1 patient B-cell ALL (Table 5) (36).

AML was as expected the second most common type diagnosed. The median age was 5 years at the time of diagnosis but the 95% CI was much wider than in ALL (2.6 to 9.3 years). Three patients were diagnosed during adolescence (15 to 19 years) when AML is getting more common (1). Median WBC count was at the higher normal values, this is lower than described by Redner *et al*. (18). Thrombocytopenia was seen in all of our patients which is in accordance with Redner *et al*. (18). The median hemoglobin was also decreased. Signs of bleeding were seen in more than half of the patients. Fever, pallor, lymphadenopathy were as expected the most presenting symptoms (Table 2) (1).

The most frequent chromosomal aberration in AML subgroup was translocation t(8;21) with *RUNX1-RUNX1T1* fusion gene detected in 4 patients (21%) (Table 6, Figure 5). The WHO Classification from 2008 describes a frequency of 5 to 12% for the t(8;21) translocation (35). This was found to be higher in our study group but it should be considered that we had a small sample size. It is most common and usually connected with a favorable outcome (8,18). The majority of patients were below 10 years of age (mean age was 6 years), most of them have been female (3 out of 4). Physical examination observed lymphadenopathy in 75% of patients (Table 7). Also, we detected 4 out of 19 patients with normal karyotype who are classified into the intermediate risk group which we expect to have various prognoses (Table 6). The *MLL*-rearranged subgroup with t(9;11) has been seen in 2 out of 19 patients (11%) (Figure 6, Table 6) and these findings agree with the 2008 WHO Classification (35). In the same group, patients had the lowest hemoglobin level with a mean value of 73.5 g/L and both patients presented with hepatomegaly (Table 7) Both symptoms are frequently seen in these patients (8). The lowest platelet count was seen in patients with del 7q/-7 with a mean value of 26.7 $x10^9$/L (Table 7). This cytogenetic subgroup has a poor prognosis (8).

In our cohort of 98 patients, there have been 8 children with Down syndrome (DS): 3 of them were diagnosed with ALL and 5 of them with AML. It is well known that children with DS have a 10 to 20 fold increased risk of developing acute lymphoblastic or myeloid leukemia than children without DS (37). Our finding showed that AML is also more common in children with DS as previously reported in the literature (6). All patients had different chromosome abnormalities than patients with non-DS AML (Table 6).

This study has shown the significance of cytogenetic analysis in pediatric leukemia. It shows in detail, the types, and frequencies of chromosomal aberrations, the correlation of each type with clinical and biological features and their prognostic stratification (favorable, intermediate or unfavorable). This stratification is clinically important for choosing the appropriate treatment protocols.

Pediatric leukemia is a cytogenetically heterogeneous hematologic disease and the possible limitations of our study were a small number of patients in each chromosomal subgroup and lack of available data for statistical analysis. Further research should include a larger number of cytogenetically homogenous patients.

6. CONCLUSIONS

- Acquired chromosomal changes were detected in 71% of successfully karyotyped patients
- The most frequent chromosomal aberration in ALL was t(12;21) with *ETV6/RUNX1* fusion gene
- The most frequent chromosomal aberrations in AML was translocation t(8;21) with *RUNX1-RUNX1T1* fusion gene
- Only 2 children have been diagnosed with CML, both Ph-positive
- The most common presenting features of leukemia, in more than 50% of children, were four symptoms: hepatomegaly, lymphadenopathy, fever, and pallor

In conclusion, our study showed frequencies of various types of acquired chromosomal aberrations for a sample of south Croatian children with pediatric leukemias. Our results are similar results reported in the literature.

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8. SUMMARY

Objectives: Pediatric leukemias are generally characterized by recurrent genetic aberrations, which are thought to be specifically associated with diagnosis and prognosis. The aim of the study was to investigate the frequency and types of acquired chromosomal aberrations and correlation with other biological characteristics and prognostic risk factors.

Materials and methods: We retrospectively reviewed clinical features and results of cytogenetics and molecular analysis for patients younger than 18 years with newly diagnosed pediatric leukemia from January 2000 to December 2017.

Results: In an 18-year period, 98 children with pediatric leukemia were hospitalized, 47 girls (48 %) and 51 boys (52 %). We had 76 patients diagnosed with ALL (77%), 20 with AML (21%), and 2 with CML (2%). Fever, pallor, lymphadenopathy, and hepatomegaly were the four most common presenting symptoms of leukemia in children (more than 50%). Among all patients, 29% had normal karyotype while 71% had acquired numerical and/or structural chromosomal aberrations. The most frequent chromosomal aberrations in pediatric ALL were t(12;21) *ETV6/RUNX1* fusion gene (27%) and hyperdiploidy (15%), both associated with prognostic favorable outcomes and B cell lineage immunophenotype. In AML subgroup, the most frequent chromosomal aberration was translocation t(8;21) with *RUNX1-RUNX1T1* fusion gene (21%), slightly higher than reported in the 2008 WHO Classification. On the other hand, the frequency of 11q23 *MLL*-rearrangements, t(15;17) with *PML-RARA* fusion gene, inv(16) or del(16q), deletion -7/del(7q) was in accordance to the literature. Two patients were diagnosed with CML, Ph-positive.

Conclusion: In this study, the frequency of various acquired chromosomal aberrations as well as their correlation with clinical and biological risk factors in a group of children with newly diagnosed leukemia are similar to previously published studies at pediatric population in Europe and US.

9. CROATIAN SUMMARY

Uvod: Pedijatrijske leukemije općenito su karakterizirane ponavljajućim genetičkim aberacijama, a za koje se smatra da su specifično povezane s dijagnozom i prognozom. Cilj istraživanja bio je ispitati učestalost i vrste stečenih kromosomskih aberacija i usporediti ih s drugim biološkim karakteristikama i prognostičkim čimbenicima rizika.

Materijali i metode: Retrospektivnim istraživanjem prikazali smo kliničku sliku i rezultate citogenetičke i molekularne analize kod pacijenata mlađih od 18 godina sa novodijagnosticiranom pedijatrijskom leukemijom u periodu od siječnja 2000. do prosinca 2017. godine.

Rezultati: U osamnestogodišnjem razdoblju leukemija je dijagnosticiranau 98 djece, 47 djevojčica (48%) i 51 dječaka (52%). Od toga 76 pacijenata imalo je dijagnozu ALL (77%), 20 AML (21%), i 2 CML (2%).Više od 50% djece imalo je četiri najčešća simptoma leukemije:vrućicu, bljedilo, limfadenopatiju i hepatomegaliju.Od svih pacijenata uredan kariotip imalo je 29% pacijenata dok je 71% imalo stečene numeričke i/ili strukturne kromosomske aberacije.Najučestalije aberacije u pedijatrijskoj ALL bile su t(12;21) s fuzijom *ETV6/RUNX1* gena (27%) i hiperploidija (15%), a obje su povezane sa prognostički povoljnim ishodom i B staničnim imunofenotipom. U grupi djece sa AML najučestalija kromosomska aberacija bila je t(8;21)s fuzijom gena *RUNX1/RUNXT1*(21%), nešto viša nego što je objavljeno u klasifikaciji SZO 2008. S druge strane učestalost 11q23 *MLL*- preuređenja, t(15;17) s fuzijom *PML-RARA* gena, inv (16) ili del (16q), -7/del(7q) bile su u skladu s podacima iz literature. . Philadelphiapozitivnu CML leukemiju imalo je dvoje djece.

Zaključak: Učestalost i raznolikost stečenih kromosomskih aberacija upedijatrijskim leukemijama iz našestudijekao i njihova povezanost s kliničkim i biološkim prognostičkim čimbenicima slična je podacima iz literature za pedijatrijsku populaciju u Europi i SAD-u.

10. CURRICULUM VITAE

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