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Genetic Studies in Acute Lymphoblastic Leukemia, from Diagnosis to Optimal Patient's Treatment

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1. Introduction

In the recent years progress in the basic laboratory science has allowed for implementation of many advanced methods in the clinical practice. Thereupon French- American- British Group (FAB) classification of acute leukemias and the immunological classification, published in 1995 by European Group for the Immunological Characterization of Leukemias (Bene, 1995), were changed into WHO 2008 classification, which applied genetic investigations to differentiate subtypes of acute leukemia (WHO, 2008). In the former classifications, lymphoma and leukemia were diagnosed as distinct disorders. Apart from including lymphoma and leukemia in the WHO 2008 classification as a single disease, acute lymphoblastic leukemia (ALL) can be diagnosed if over 20% (in some investigations over 25%) lymphoblast infiltration is detected in bone marrow biopsy. In this new classifications precursor lymphoid neoplasms are divided into the B (about 80% of cases) or T cell lineage (about 15-25%). ALL diagnosis, based on antigen B or T investigation, uses flow cytometry method. Recent investigations suggest different molecular profiles for ALL-T type and T-lymphoblastic lymphoma especially an expression of CD47 in T -ALL, and over-expression of *MLL1* in T- lymphoblastic lymphoma (Hoelzer & Gokbuget, 2009; Raetz et al., 2006). The prevalence of ALL in children amounts to 30-35% of neoplastic diseases and its incidence is approximately 40 cases in a million per year. In adults ALL account for about 20% of all types of acute leukemia. The incidence in adults is estimated as 0,39 per 100 000 per year in 35-39 age range, and increases to 2,1 patients over 80 years (Anino et al., 2002). The distinction of various ALL subtypes characterized by recurrent genetic abnormalities was made possible thanks to specific genetic studies. The incidence of the diagnosed subtypes occurring in adult and children population varies and indicates differences in the clinical features (Harrison, 2008). Cytogenetic studies have become a routine procedure in clinical practice involved in acute leukemia treatment (Faderl et al., 1998). Carrying out of the above-mentioned methods is an indispensable condition to make a proper diagnosis according to WHO 2008 classification.

Cytogenetic studies implicate specific types of therapy in adults and children as well (Tomizawa et al., 2007).

However, the results of treatment of ALL patients improved not only because of better treatment modality standards and facilities but also thanks to proper and detailed diagnosis (Faderl et al., 2010).

I. B lymphoblastic leukemia/lymphoma	1. B lymphoblastic leukemia/lymphoma, not otherwise specified 2. B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities - B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); <i>BCR-ABL1</i> - B lymphoblastic leukemia/lymphoma with t(v;11q23);<i>MLL</i> rearranged - B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22);<i>TEL-AML1 (ETV6-RUNX1)</i> - B lymphoblastic leukemia/lymphoma with hyperdiploidy - B lymphoblastic leukemia/lymphoma with hypodiploidy (Hypodiploid ALL) - B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32);<i>IL3-IGH</i> - B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);<i>E2A-PBX1(TCF3-PBX1)</i>
II. T lymphoblastic leukemia/lymphoma	

Table 1. Precursor Lymphoid Neoplasms Classification WHO 2008.

2. Flow cytometry

Flow cytometry is a method, which is most commonly used for clinical diagnosis of ‘de novo’ acute leukemias, and as such does not require any additional preparation on the part of the patient. It is the method of sorting and measuring types of cells by fluorescent labelling of monoclonal antibodies on the surface or in cytoplasm of investigated cells. Types of an antigen or other markers present on the cell give further information about the immunophenotype of leukemic cells. In the method monoclonal antibodies are used for detecting antigens determined in CD classification. Despite that, it is not the method of cytogenetic examination; the frequent application of immunophenotyping to peripheral blood or bone marrow aspiration cells necessitates the description of the flow cytometry in this chapter too.

Flow cytometry investigation of neoplastic cells is commonly used for diagnosis of central nervous infiltration of ALL manifested as leptomeningeal disease. The National Comprehensive Cancer Network recommends the routine use of the flow cytometry for the diagnosis of the central nervous infiltration involvement in ALL (Brem et al., 2008).

The process of collecting data from samples is performed using a flow cytometer. The data generated by flow cytometers are presented as a 'plot' i.e. histogram. The histogram regions can be sequentially separated, based on fluorescence intensity, by creating a series of subset extractions, called 'gates'. For diagnostic purposes in hematology specific gating protocols exist. The WHO 2008 classification divided leukemias/lymphomas into B or T cell types and these types of leukemia can be distinguished both by immunophenotype and by molecular genetic studies.

The B lymphoblasts as well as the T lymphoblasts can express a panel of characteristic antigens (Tab.2).

Basic panel for diagnosis of acute leukemia	Panel of antigens for B derived ALL	Panel of antigens for T derived ALL	Minimal residual disease monitoring
CD 45 CD10 CD1a CD7 CD22 CD65 CD2 CD13 CD14 CD33 CD34 CD117 CD15 CD56 Additionally: CD16, CD66, CD36, CD64, CD 41, GlyA CD11b,CD11c, HLADR, CD38,	CD34 CD19 CD10 CD20 cyCD22 CD38 cyCD79a CD9 CD45 CD45RA IgG2a IgG3 CD52 CD58 Additionally: TdT, sIg, cyIgM, cyIgG1, IgG1, IgM, lambda, kappa For ALL CD19 positive: CD33, CD13, CD117, CD15BD, CD2, CD7, CD66, CD123	CD7 IgG1 CD2 CD3 CD5 CD4 CD8 cy/sCD3 CD1a CD34 CD38 CD45 IgG3 CD52 CD99 Additionally: cyIgG1, cyCD3 CD45RA, CD45RO, CD25, CD57, CD16 For ALL CD7 positive: CD33, CD13, CD117, CD15BD, CD56, CD65, CD117	Case nr 1, (B-cell ALL): Qu*: CD66c/19 and CD10/19/45RA; and CD45/19 or ES**: CD58/19, CD10/20/19, CD34/38/TdT, CD10/19/TdT, CD34/38/TdT, CD10/19/TdT Case nr 2, (B-cell ALL): Qu: Cd34/66c/19, ES: CD10/19/20, CD34/38/19, CD45/34/19, CD34/9/19, CD58/51/19, CD10/19/TdT Case nr 3, (B-cell ALL): CD19/22/34, CD19/TdT, CD33/HLADr Case nr 4, (T-cell ALL): sCD7/TdT/cCD3, CD7+/5+/3 negative, CD7+/1a+/3 negative, CD4+/8+/3 negative

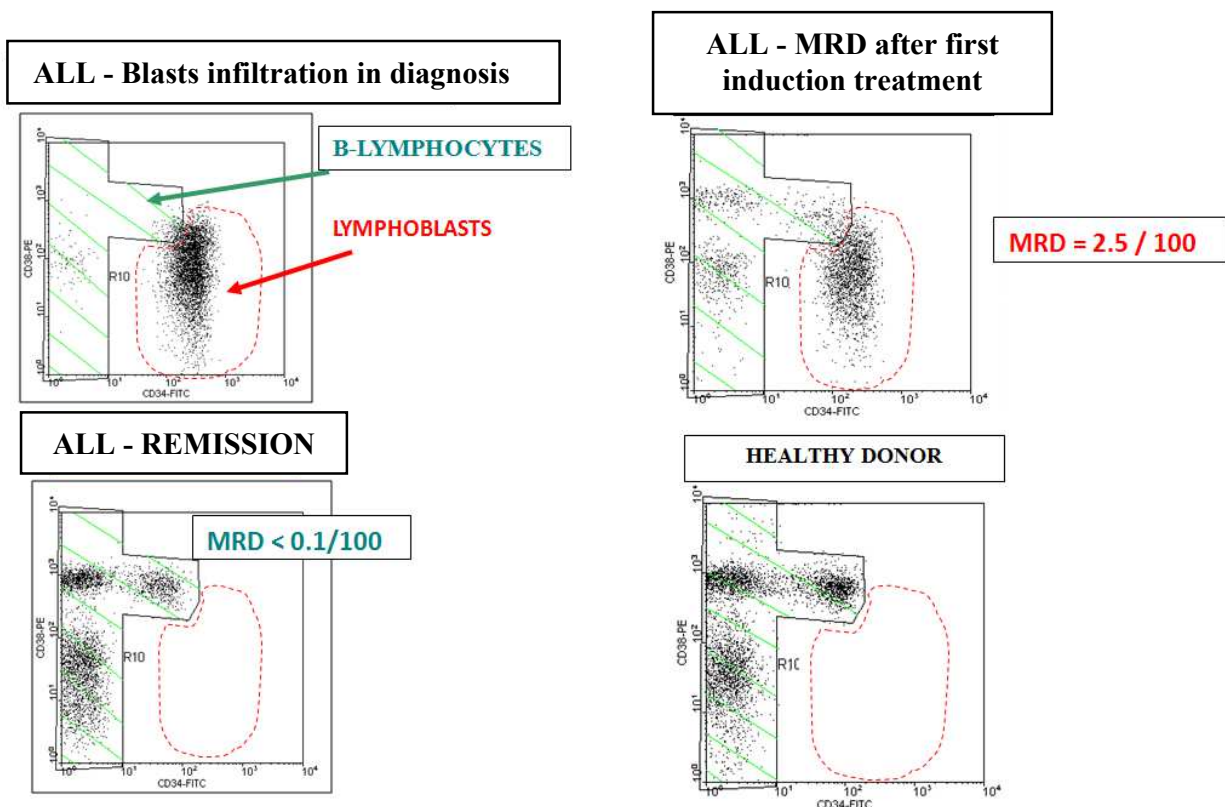
Table 2. The list of monoclonal antibodies for diagnosis and monitoring the treatment of acute lymphoblastic leukemia

The progress in the flow cytometry technique allowed to obtain information about 17-60 parameters of the investigated cells simultaneously (Wood et al., 2006).

This method, which is useful in diagnosis, can additionally detect a very small number of abnormal cells in bone marrow suspension or peripheral blood cells obtained from patients after the treatment, hence can detect the minimal (i.e. submicroscopic) residual disease (MRD) (Campana, 2009). The panel of antigens for MRD examination is matched individually on the basis of the results of immunophenotyping, which is performed during

diagnosis. At least two different aberrant phenotypes with the expression over 50% of leukemic blasts are used on the average. The following are necessary and useful criteria in monitoring MRD: coexpression of antigens from different than lymphoblastic cell lines, e.g. CD13, CD33, asynchronous antigens expression or overexpression within the same line and ectopic phenotypes. MRD can be evaluated using either the 'quadrant' method or 'empty spaces' technique. Usually, MRD is calculated as the percentage of total nuclear bone marrow cells and in 3-8 colour flow cytometry method, where the 0,1% -0,01% sensitivity is obtained. Flow cytometry is described as a widely applicable, rapid and accurate quantification method which provides additional information on normal hematopoietic cells and can differentiate these cells from neoplastic compartment (Figure 1). The potential weakness is phenotypic shifts and as a result there are multiple aberrant phenotypes required.

The results of immunophenotyping can be useful in clinical application for identifying antigens in targeted therapy, and as a minimal residual disease monitoring during treatment (Giebel et al., 2010; Rhein et al., 2010). Targeted therapy involves the use of monoclonal antibodies as follows: anti CD20 (rituximab), anti CD52 (alemtuzumab), anti CD22 (epratuzumab), anti CD 33 (+toxin) (gemtuzumab ozogamycin), and Blinatumomab used recently (Nijmeijer et al., 2010; Raetz et al., 2008; Topp et al., 2009; Topp et al., 2011).



Bone marrow samples were stained using MoAb: CD34-FITC / CD38-PE / CD19-PC5

Diagram 'dot plot' showed only CD19(+) cells

Normal B-Lymphocytes – shaded area

Lymphoblasts – dash area

Fig. 1. Empty Spaces method for MRD monitoring during ALL CD19+ treatment. The figure shows the bone marrow samples examination.

3. Cytogenetic methods used in acute lymphoblastic leukemia

Cytogenetic changes underline leukemogenesis targeted and are closely associated with the type neoplasm developing. Several cases of precursor lymphoid neoplasms have characteristic genetic abnormalities that are important in determining their biologic and clinical features (Harrison, 2001; Mulligan, 2009). Some of mutations occurred both in ALL and in AML, e.g. *FLT3* mutations (point mutations and internal tandem duplications (Chang P., 2010). Chromosomal abnormalities are detected in about 80% of ALL cases but in about 40% numerical abnormalities exist and in 40% there are structural alterations (Witt et al., 2009). Over the years, methods of cytogenetic analysis evolved and became a part of routine laboratory testing, providing valuable diagnostic and prognostic information in children and adult patients (Park et al., 2008). The reference material for cytogenetic investigation the cells obtained during bone marrow aspiration. Peripheral blood cells are used only if bone marrow cells are unavailable or if special methods of cytogenetic investigation are used e.g. fluorescence in situ hybridization or polymerase chain reaction instead of conventional cytogenetics.

3.1 Conventional cytogenetics and fluorescence in situ hybridization

The gold standard for cytogenetical investigation is still conventional cytogenetics (Figure 2) but now often combined with analyses using fluorescence in situ hybridization (FISH), and polymerase chain reaction (PCR) technique.

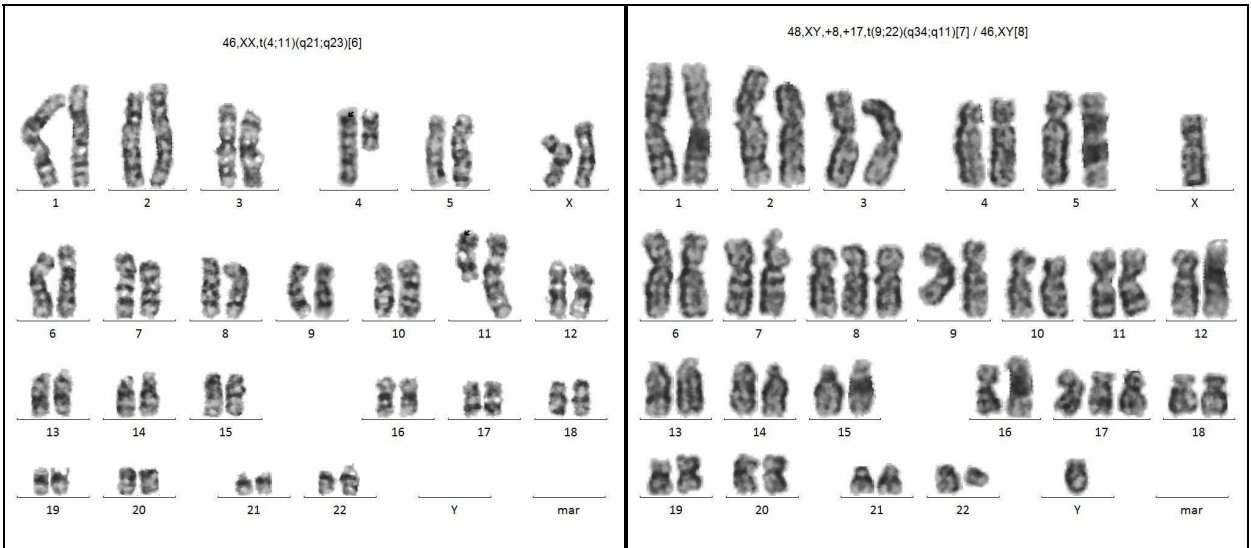


Fig. 2. Results of conventional cytogenetic investigation in ALL patients (GTG method). Images show the t(4;11) and t(9;22).

For cytogenetical investigation the sample of 2-3ml volume from bone marrow aspiration or 5-10ml from peripheral blood, with the addition of heparin, must be delivered to cytogenetic laboratory in 24 hours, and can be transported in a room temperature. This diagnostic procedure must be carried out before the start of any antineoplastic treatment. The main elements of the cytogenetical methods are cell cultures which are mandatory for obtaining metaphases for chromosomal analysis. The duration time of cells cultures oscillates from 0,4-2 hours (called 'immediate') to 24, 48 and 96 hours. Then cytogenetical analysis of cells is

performed in metaphases and the rest of cell suspension can be left in minus 20 Celsius degree for additional investigations. According to the guidelines of European Cytogeneticists Association (ECA), prepared as a quality framework for cytogenetic laboratories, diagnostic metaphases must be obtained from at least 90% of cell cultures (Bricarelli et al., 2006). The minimum number of metaphases required to obtain the result, is 20 in normal karyotype. If the karyotype is abnormal the number of metaphases may be lower (sometimes only few metaphases) providing that clonal aberrations are recurring (Haferlach et al., 2007). Cytogenetic findings were reported up to 3 weeks after the sample had been received in laboratory. There are some limitations of conventional cytogenetic analysis such as sometimes morphologically insufficient quality of metaphases or the mitotic index. The paper presents results of investigation of 70 ALL in children revealed, that karyotypes were obtained in 84% (Soszynska et al., 2008).

Fluorescence in situ hybridization method (FISH) is a cytogenetic technique providing detection of characteristic chromosomal DNA sequences, by painting them. FISH is a method of supplementing the classical cytogenetic studies, but can also be an independent method for cytogenetic analysis in ALL. Principle of FISH is the use of DNA fragments precisely defined sequence, that is, molecular probes of complementary hybridizing appropriately prepared DNA test. Special locus-specific probe mixtures are used to count chromosomes. Fluorescence microscopy can be used to find out where the fluorescent sound is bound to the chromosome. To determine the percentage of cells with genetic abnormalities sought is indicated in the analysis of at least 100 interphase or metaphase cells. There are some modifications of FISH technique that were implemented in hematology laboratories e.g. multiple colour FISH (M-FISH) is widely applied for detection of *BCR/ABL* translocation and for *MLL* gene rearrangements. Employed ratios of probe mixtures are supposed to create secondary colours that are useful in differentiating subtypes of ALL.

M-FISH elucidated complex karyotypes (Broadfield et al., 2004; Harrison et al., 1999).

Results of FISH and conventional cytogenetics should be determined in accordance with the International System for Human Cytogenetic Nomenclature (ISCN, 2005).

In ALL diagnostic procedures FISH testing should always be done if:

1. In the conventional cytogenetic study, no metaphases were obtained or the quality is not suitable for release as a result,
2. The outcome of conventional cytogenetics GTG technique suggests the presence of aberrations, but does not confirm it,
3. In conventional cytogenetics was not found chromosome aberrations characteristic for the type of leukemia (e.g. the CD10 positive leukemia with Ph cryptic or masked Ph).

The new technique, which detected chromosomal imbalances, introduced by Kallioniemi (Kallioniemi et al., 1994) is comparative genomic hybridization (CGH), which is a method of molecular cytogenetics. CGH gives a global overview of chromosomal deletions and amplifications throughout the whole genome with one step analysis (McGrattan et al., 2008). CGH can detect submicroscopic deletions 5-10Mbp in size and detect extra-chromosomal fragments of chromatin size 2-3Mbp. The major limitation of CGH, however, is the failure to detect balanced chromosome abnormalities such as translocations, inversions, and clonal heterogeneity (Ness et al., 2002). This method can be performed as a complementary test

GTG and FISH. CGH could be established as a routine method of analysis for screening patients with ALL (Kowalczyk et al., 2010).

Test result conventional cytogenetics and FISH help determine the specific subtype of ALL diagnosis, identify risk factors, establishing prognosis, treatment selection, is also to monitor the disease and the effectiveness of treatment.

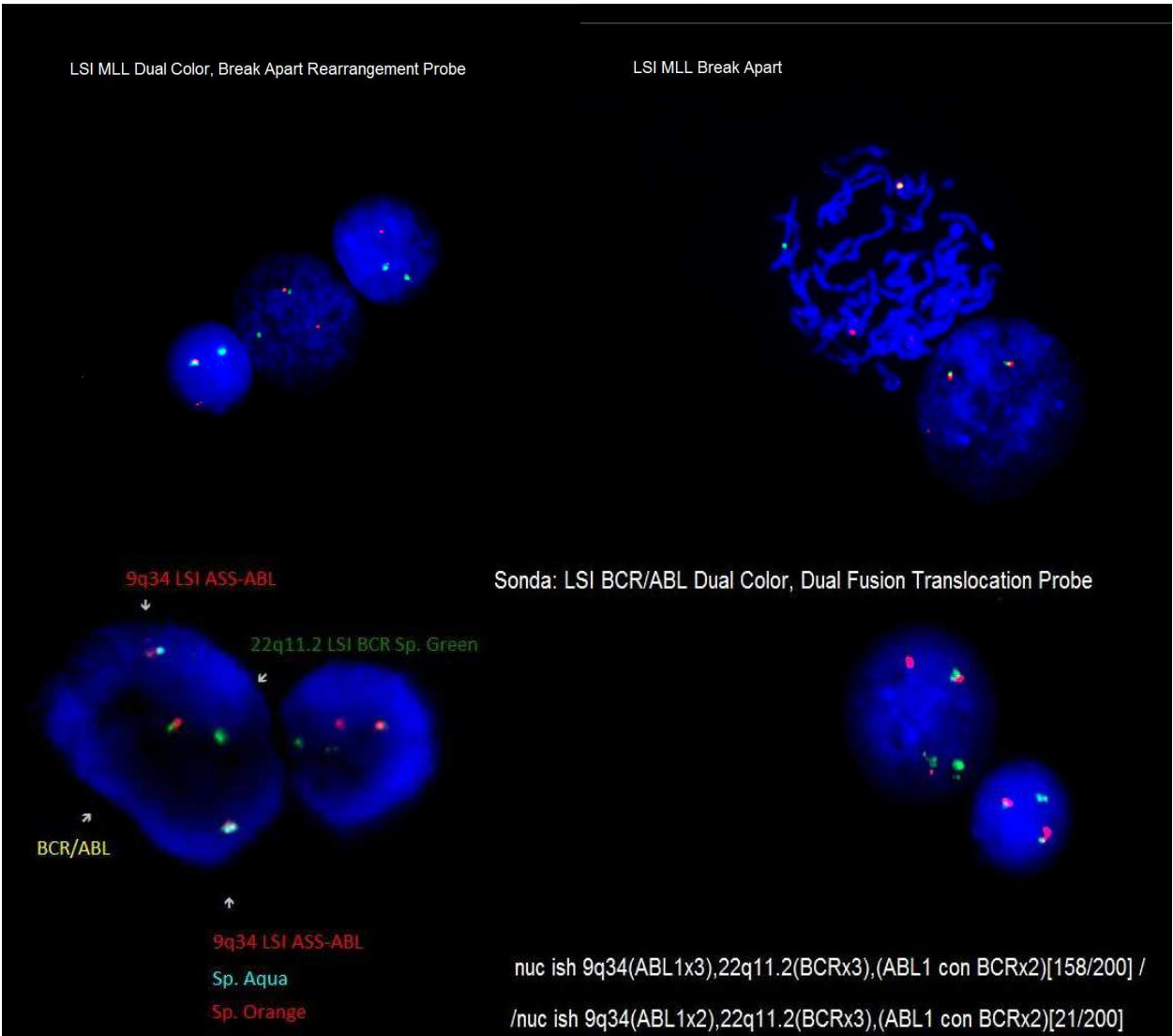


Fig. 3. Results of FISH method detecting translocations in All patients: t(9;22) and t(4;11).

3.2 Polymerase chain reaction

The polymerase chain reaction (PCR), a technique of molecular biology now is a routinely used method for ALL diagnosis as well as for categorizing ALL subtypes according to WHO2008 classification, monitoring the treatment results and measurement of minimal residual disease too.

For PCR investigation the sample of 1-5ml volume from bone marrow aspiration or 5-10ml from peripheral blood, with the addition of EDTA, must be delivered to cytogenetic laboratory in 24 hours, and must be transported in +4°C.

PCR has a very high sensitivity and detects known fusion events (Wesołowska et al., 2011). Suitably selected set of primers can detect aberrations at the RNA level (the method of RT-PCR). There are some variants of PCR technique which are very useful in hematological investigation e.g. single-nucleotide polymorphisms (SNPs), Asymmetric PCR, Multiplex-PCR, Nested PCR, Quantitative PCR (Q-PCR), Quantitative real-time PCR, Reverse Transcription PCR (RT-PCR). The RT-RQ-PCR method can detect translocation-specific malignant lymphoblasts at a sensitivity determined as 10^{-4} - 10^{-6} . The method is used for monitoring MRD and its advantage is the stability of gene fusion in the course of the disease, and high sensitivity determinations. The disadvantage of RT-RQ-PCR is the lability of RNA and the possibility of its application only in a small group of patients because of the limited frequency of gene fusions in ALL.

In the monitoring of MRD may be used the following gene fusions: *BCR-ABL1*, *MLL-AFF1*, *TCF3-PBX1* and *ETV6-RUNX1*, present in approximately 40% of pediatric and adults patients (Campana, 2009; Bruggemann et al., 2010), and *IGH@-CRLF2* or *P2RY8-CRLF2* abnormalities, which are detected in about 15% of adult or high risk pediatric B-ALL (Yoda et al., 2010).

The most common method of MRD detection is based on the sequence of rearranged Ig gene joints/TCR, encoding the immunoglobulin and T cell receptors in response real-time quantitative polymerase chain reaction. The method can be used in more than 95% of ALL patients and has a sensitivity of 10^{-4} to 10^{-5} . The Ig/TCR RQ-PCR method is complicated, time-consuming and cost-intensive, but nevertheless is regarded in Europe as the 'gold standard' in the monitoring of MRD. This is due to the large stage of standardization, comprising the steps research, primers and probes, as well as guidelines on the interpretation of the data obtained this allows receive high reproducibility of results in the determination of risk groups and assessment of MRD that is necessary in conducting multicenter studies (Witt et al., 2009).

3.3 Microarray analysis of genetic abnormalities in ALL

Microarray platforms for analysis of genetic alterations include cDNA array (detected large alterations, often over 100kB) and oligonucleotide arrays (oligo array, used short 20-50kB nucleotide probes). The potential of high-density microarray of specific cDNA sequences allows for hybridization of fluorescently labelled mRNA of leukemic cells. The value of this technique for the diagnosis and follow-up of ALL specific genetic abnormalities is enormous. Protein microarrays were used to investigate Notch-transduced signals in the development of T-cell ALL (Chan et al., 2007). The identification of novel miR genes expressed in different types of ALL forms the basis for further studies of the biology of ALL (Schotte et al., 2011).

4. Genetic differences and similarities amongst B and T derived ALL

4.1 B-cell acute lymphoblastic leukemia (B-ALL)

During the B-cell ontogenesis the V,D and J segments are rearranged to generate a unique gene sequence for each cell. The distinctive genetic abnormalities occurring in B-derived ALL are as follows: clonal DJ rearrangements of *IGH@* gene, T-cell receptor rearrangements noticed in 70% of B-ALL, which are not helpful in differentiating from T-cell ALL, t(9;22)

mainly found in adults patients, t(12;21) and hyperdiploidy (usually without structural abnormalities) occurring mainly in children. In B-type of ALL del(6q), del(9p), del(12p), t(17;19) and intrachrmosomal amplification of chromosome 21 (iAMP21) are often detected.

The t(12;21) occurs most frequently in children, and probably arose early in pregnancy. This genetic abnormality causes the fusion of two genes *AML1* and *TAL*, resulting disorder in an early stage of B cell development.

Adults most often found in t(9;22), which causes the fusion of *BCR* and *ABL* genes and leads to the fusion protein *BCR-ABL1* (tyrosine-kinase) that interacts with multiple signal paths (eg RAS).

Translocation	Gen	Function	Prevalence % adults/children	Detection for MRD monitoring
t(9;22)(q34;q11)	<i>BCR-ABL</i>	Enhanced tyrosine kinase activity, which function in intracellular signalling pathways	<25>/5-8	mRNA
t(4;11)(q21;q23)	<i>MLL-AF4</i>	Transcription factor in the regulation of differentiation pathways	6/2-7 mainly in infants	mRNA
t(1;19)(q23;p13)	<i>E2A-PBX</i>	Transcription factor	/ <6 (25-30% in pre-B-ALL)	mRNA
t(12;21)(p13;q22)	<i>TEL-AML1</i>	Transcription factor	<2/1-2	mRNA
11q23 aberrations	<i>MLL</i> and any one of fusion partners	Transcription factor	3-4/5-6	mRNA

Table 3. Structural chromosome abnormalities in B type of ALL

4.2 T-cell acute lymphoblastic leukemia (T-ALL)

Clonal rearrangements of T-cell receptor (TCR) genes, an abnormal karyotype, translocations and chromosomal deletions almost always occurred in T-derived ALL (Table 4). About 20 % of patients diagnosed as T-ALL displayed immunoglobulin gene rearrangements as well (Szczepański et al., 1999). The abnormal karyotype is present in about 50-70% of cases and mainly involves the alpha and delta TCR(14q11.2), the beta locus (7q35) and the gamma locus (7p14-15).

Translocations

These genetic abnormalities are surrounded by translocations of the partner gene. The most frequently involved genes are as follows: *HOX11* (*TLX1*) (10q24) occurred in 30% of adults and in 7% of pediatric patients, *HOX11L2* (*TLX3*) (5q35) found in 10-15% of adults and in

20% of children. There are transcription factors. The other transcription factors engaged in translocations are *MYC* (8q24.1), *TAL1* (p32), *RBTN1* (*LMO1*) (11p15), *RBTN2* (*LMO2*) (11p13), *Lyl1* (19p13) and the cytoplasmic tyrosine kinase *LCK* (1p34.3-35). In about 10-8% of patients other translocations occurred as follows: *PICALM-MLLT10* [*CALM-AF10*; t(10;11)(p13q14)] and *MLL* most often with the partner gene *ENL* (19p13). The translocations are often not detected by conventional cytogenetic methods hence PCR must be used (WHO, 2008).

Deletions

Del(9p) is the most frequently occurring deletion (in about 9% of cases), detected mainly by PCR method, only 30% can be detected by conventional cytogenetics (Brett-Gardiner et al., 2011).

Gene mutations

In over 50% of T-ALL cases *NOTCH1* gene mutations were found. The *NOTCH1* signalling pathway has three components and the mature *NOTCH1* protein which is essential for early T-cell development (Liu et al. 2011; Palomero & Ferrando A., 2009).

Brain and Acute Leukemia, Cytoplasmic (*BAALC*) gene expression

BALLC gene is located on chromosome 8q22.3. Its high expression (overexpression) in the T-cell ALL is associated with worse overall survival and relapse-free survival. Kuhn et al. demonstrated, that high *BAALC* expression is associated with inferior overall survival also in adults B-precursor ALL patients. *BAALC* overexpression can be regarded as an additional negative prognostic factor in adult ALL patients (Kuhnl et al., 2010).

5. Treatment of acute lymphoblastic leukemia patients

The main treatment rule for patients with acute lymphoblastic leukemia is to adjust the intensity of treatment to the level of disease aggression. This fact accounts for a wide application of protocols for risk-adapted therapy in everyday clinical practice. Clinical and biologic features which were defined as risk factors formerly (Table 5) are now replaced by minimal residual disease estimation (Attarbaschi et al., 2008; Bassan et al., 2009; Conter et al., 2010).

There are the same treatment protocols for B and T derived 'de novo' acute lymphoblastic leukemia. However, during the relapse of T-ALL other medications are recommended such as: Nelarabine, Forodesine and Clofarabine (De Angelo, 2009). Most European Groups use regimens containing prednisolone/dexamethasone, vincristine, daunorubicin and asparaginase in the induction phase of treatment of adult All patients (Conter et al., 2010; Bassan et al., 2009; Patel et al., 2010).

During consolidations additional chemotherapy using cyclophosphamide, cytarabine is administered, and including intensive intrathecal chemotherapy. Maintenance chemotherapy lasting 2-3 years consists of low-dose antineoplastic drugs, mainly of mercaptopurine and methotrexate (Holowiecki et al., 2006).

Hyper CVAD (cyclophosphamide, vincristine, adramycin and dexamethasone, without L-asparaginase) is a reasonable alternative for induction therapy and gives results similar to

Translocation	Gen	Function	Prevalence % adults/children	Detection for MRD monitoring
t(1;14)(p32;q11)	TAL1(SCL)	Transcription factor	/3	DNA (TAL1- TCRD)
t(11;14)(p15;q11)	RBTN1(i1)	Transcription factor	/9	DNA (LMO1-TCRD)
t(11;14)(p13;q11)	RBTN2(LMO2)	Transcription factor	/4-5	DNA (LMO2-TCRD)
t(10;14)(q24;q11) t(7;10)(q35;q24)	HOX11 HOX11	Transcription factor	/10	DNA (HOX11- TCRD or TCRB)
inv(7)(p15q34)	HOXA	Transcription factor	/5	mRNA (HOXA-TCRB)
t(5;14)(q35;q32)	HOX11	Transcription factor	10-15/20	mRNA (HOX11L2- BCL11B)
t(1;14)(p34;q11)	LCK	Transcription factor	3/3	DNA (TAL1-TCRD)
t(11;19)(q23;p13)	MLL-ENL	Transcription factor	/5	mRNA
t(7;9)(q34;q32) t(7;9)(q34;q34)	TAL2 NOTCH1	Transcription factor	~30/ ~50	mRNA
Del(9)(p21)	CDKN2A,CDKN2B	Loss of control cell cycle	/65-80	DNA/mRNA
del(1)(p32)	SIL-TAL1	Transcription factor	/10-25	DNA/mRNA (SIL-TAL1)
t(10;11)(p13- 14;q14-21)	CALM	Transcription factor	10/8	mRNA (CALM-AF10)
MLL	MLL-ENL/ AF10 etc	Transcription factor	8/5	mRNA (MLL- ENL/ AF10 and other)
9p34 episomal amplification	NUP214-ABL	Enhanced tyrosine kinase activity, which function in intracellular signalling	/6	mRNA
inv(7)(p15q34)	HOXA	Transcription factor		mRNA

Table 4. Structural chromosome abnormalities in T type of ALL

	High risk factors (Hoelzer et al., 1988)	High risk factors PALG (Holowiecki et al., 2006)
Age	>35 years	≥ 35 years
WBC count	>30x10e9/l for B-type ALL >100x10e9 for T-type ALL	≥ 30x10e9/l for B-type ALL ≥ 100x10e9/l for T-type ALL
Immunophenotype	Prepre-B, early T, mature T	Prepre-B, early T, mature T
Genetics		t(4;11) or t(9;22)
Time to remission	CR after >4 weeks	MRD positivity post induction or during or post consolidation treatment

Table 5. Clinical and immunological prognostic factors for newly diagnosed adult ALL patients

the traditional induction protocols (Kantarjian et al., 2004). Various protocols for induction and consolidation as well as post remission treatment refer to pediatric patients and adults. In adult group of patients aged below 30 years some collaborative groups preferred more intensive treatment compatibility to pediatric protocols. This more intensive treatment gives better results defined as better complete remission rate (e.g. 83 vs 94% in the study of LALA-94 (adult) and FRALLE-93 (pediatric), respectively), disease free survival (e.g. 5 year EFS amounting 38% for adult (HOVON) and 71% for DCOG (pediatric) study, respectively), or overall survival (e.g. 5 year OS amounting respectively 56% (UKALLXII/E2993, adult) and 71% (ALL97, pediatric) (DeAngelo et al., 2007; Barry E. et al., 2007; Boissel et al., 2003; De Bont et al., 2005; Ramanujachar et al., 2007; Rijneveld et al., 2009; Testi et al., 2004).

For more advanced aged patients reduced intensity chemotherapy protocols are used generally due to lower tolerance to very intensive therapy (Giebel et al., 2010; Marks, 2010). In this age group significantly more frequently occurs high risk factors such as complex cytogenetic abnormalities, low hypodiploidy, t(4;11) and t(9;22). Philadelphia chromosome positivity (t(9;22)) is a very high risk factor but also allows the conduct of targeted therapy with the use tyrosine kinase inhibitors. In these cases, the cytogenetics methods allow the use of targeted therapy (Foa et al., 2011).

ALL occurs in children about five times more frequently than in adults. In recent years significantly increased the effectiveness of ALL treatment in the pediatric group of patients (Freyer et al., 2011). Surveillance, Epidemiology and End Results register (SEER) analysis showed the biggest improvement in survival of patients aged 15-19 years because the 5-year overall survival increased from 41 to 62%. Long term survival in between the ages 2 and 10 years are found in more than 90% (Stock, 2010). These good results of treatment may be due inter alia to the fact that children are usually treated within the prospective multicenter clinical trials, which included the optimal diagnostic methods and used most effective treatment.

Although many genetic abnormalities are important as prognostic factors, only a few have an influence on the choice of treatment (Tomizawa et al., 2007). There are some rules allowing for the introduction of certain particulars from diagnosis to modifications of

High risk factor	
WBC count	≥20G/l
Age	Infants or children ≥10 years
7-day response to steroid pretreatment	Presence of >1 × 10 ⁹ /l blasts in peripheral blood
Ploidy	< 45 chromosomes
Translocations:	t(9;22)/BCR/ABL t(4;11)/MLL/AF4
MRD after induction treatment	≥10 ⁻³
CR after induction of treatment at the expected time (33 day of treatment)	No

Table 6. Adverse prognostic factors in acute lymphoblastic leukemia in children, which are most commonly used in everyday clinical practice.

patients' treatment and the results of cytogenetic findings are found to be useful. The first paper claiming cytogenetic abnormalities to have given important prognostic information was published by Secker-Walker (Secker-Walker et al., 1978). The impact of cytogenetics on the treatment results was published by Southwest Oncology Group 9400 study (Pullarkat et al., 2008). The authors presented the four different karyotype categories (Table 7) in which the most important prognostic factor for overall survival and relapse-free survival is cytogenetics instead of age.

Risk group	Cytogenetics markers
I. Standard risk (5 years overall survival ≥50%)	Hyperdiploid (>50 and < 66 chromosomes)
II. Intermediate risk (5 years overall survival 40-50%)	Normal diploid, 11q23 deletions without MLL rearrangements del9(p), del6(q), del(17p), del(12p), 13/ del(13q), t(14q32),t(10;14) low hypodiploidy (47-50 chromosomes) TCR translocations Tetraploidy (>80 chromosomes)
III. High risk (5 years overall survival 30-40%)	t(1;19), 7(Ph-), del(7p), +8, 11q23/MLL gene and any one of fusion partners t(17;19), t(5;14)/TLX3, CALM-AF10
IV. Very high risk (5 years overall survival ≤30%)	t(9;22) BCR-ABL, t(4;11) AF4-MLL, t(8;14) MYC-IGH Complex (≥5 abnormalities without known translocations) Low hypodiloidy (30-39) Triploidy (60-78)

Table 7. Risk groups defined using karyotype and genetic categories based on SWOG analysis (Pullarkat et al., 2008)

Philadelphia chromosome positivity, low hypodiploidy (near triploidy) and complex cytogenetic abnormalities (more than five chromosomal changes) influenced shorter overall survival (Marks et al., 2009). Sometimes more intensive treatment e.g. modified Hyper-CVAD regimen gives better results in very high risk ALL with t(4;11) (Li et al., 2009).

The translocation between chromosome 9 and 22 results in the formation of the Philadelphia chromosome (Ph) and generates the expression of a p 190 protein or encoding a chimaeric p210 protein. Ph positivity is more often present in adult patients and the incidence increases with the age from 20% in 30 years to 39% in over 60 years (Moorman A.V., 2010). In these cases, targeted therapy using tyrosine kinase inhibitors such as imatinib or dasatinib is combined with chemotherapy both in young and elderly patients (Foa et al., 2011; Ravandi et al., 2010; Laport et al., 2008; Ottmann et al., 2007; Tanguy-Schmidt et al., 2009). These types of combined therapy give better results mostly if allogeneic bone marrow transplantation was performed in the first complete remission (Yanada et al., 2008). In patients aged above 60 years chemotherapy should be reduced, and limited to administer monotherapy, imatinib 600-800mg/day, or in combination with glucocorticoids and/or vincristin (Ottmann, Wassmann et al., 2007).

5.1 Minimal residual disease testing

Many publications have shown that the MRD positivity has prognostic value for the treatment results of children and adults (Moricke et al., 2008; Stow et al., 2010; Hoelzer et al., 1988; Holowiecki et al., 2008). Result of the MRD examination is important in monitoring the induction and consolidation treatment (Figure 4), and in terms of eligibility for bone marrow transplantation (Couston-Smith et al., 2011; Giebel et al., 2010). MRD testing can be performed using the flow cytometry method or PCR and/or FISH.

In our paper published by Giebel et al. (Giebel et al., 2009) we documented that MRD measured by flow cytometry combined with cytogenetics replaces conventional risk criteria in adults with Ph-negative acute lymphoblastic leukaemia as the relapse rate is significantly lower in standard risk karyotype or intermediate risk karyotype and MRD lower than 0,1% after induction treatment according to the PALG 4-2002 protocol (Figure 5).

6. Allogeneic transplantation in ALL patients

Provide for the implementation of EBMT recommendations, bone marrow transplantation (the family donor and from an unrelated donor, and autotransplantation) during the second remission. For the decision on the application of allogeneic bone marrow transplantation (BMT) is to monitor MRD. Many research groups recommends allogeneic BMT in first remission period already, in patients at a high risk of leukemia, which is defined of t(9;22), t(4;11) occurrence and also hipodiploid (near haploid) karyotype (Ferra et al., 2010; Marks et al., 2008; Marks et al., 2010).

The presence of MRD after induction and/or consolidation treatment is also classified as high risk and is an indication for early performing allogeneic bone marrow transplantation. The sensitivity of MRD at a level of 10^{-5} can justify reducing the strength of the treatment in some specific cases. Patients of the latter group needs to step up therapy and eligibility for allogeneic bone marrow transplantation.

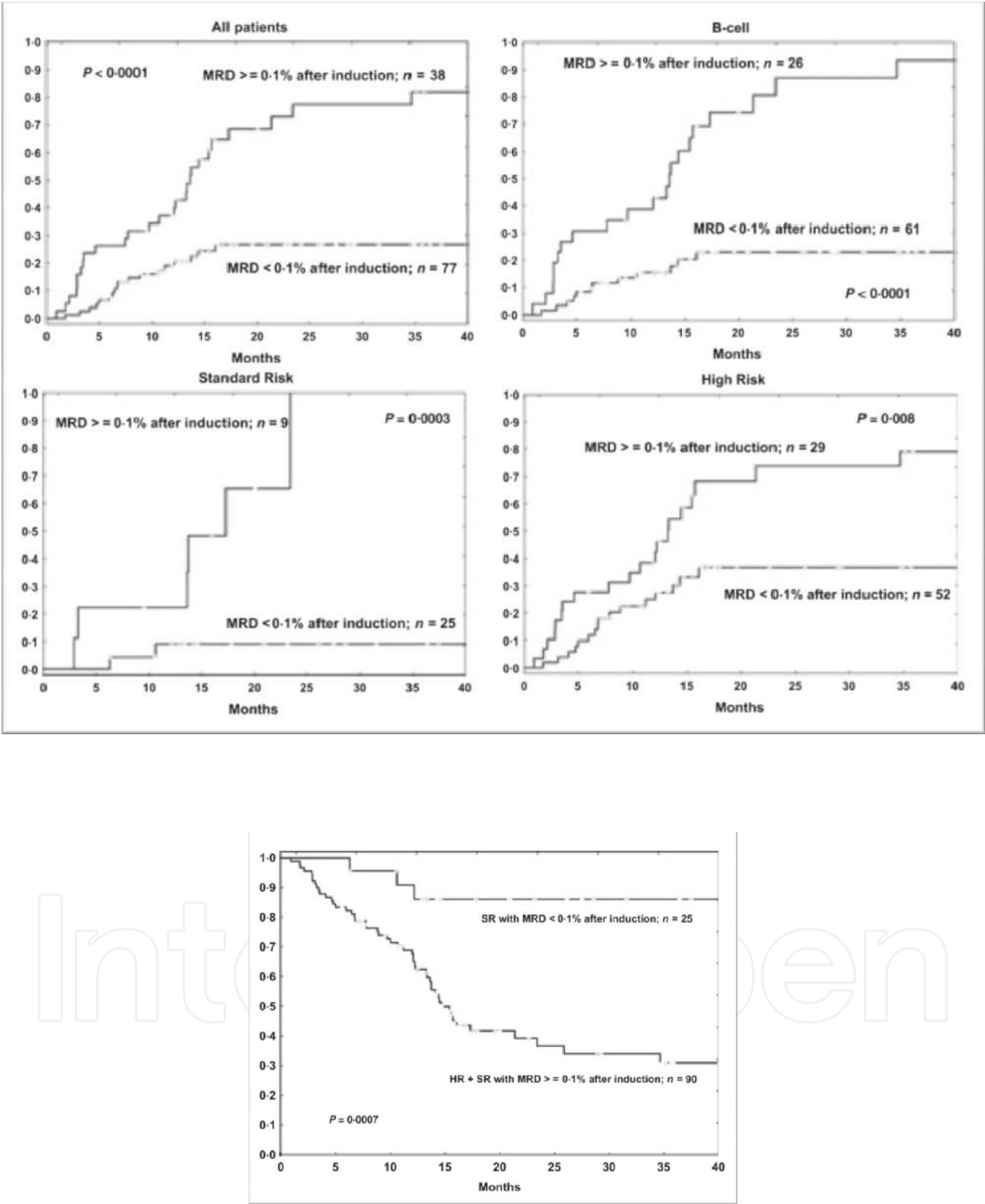


Fig. 4. The influence MRD test result on the effectiveness of treatment of adult ALL patients according to the protocol PALG 4-2002 (Holowiecki et al., 2008).

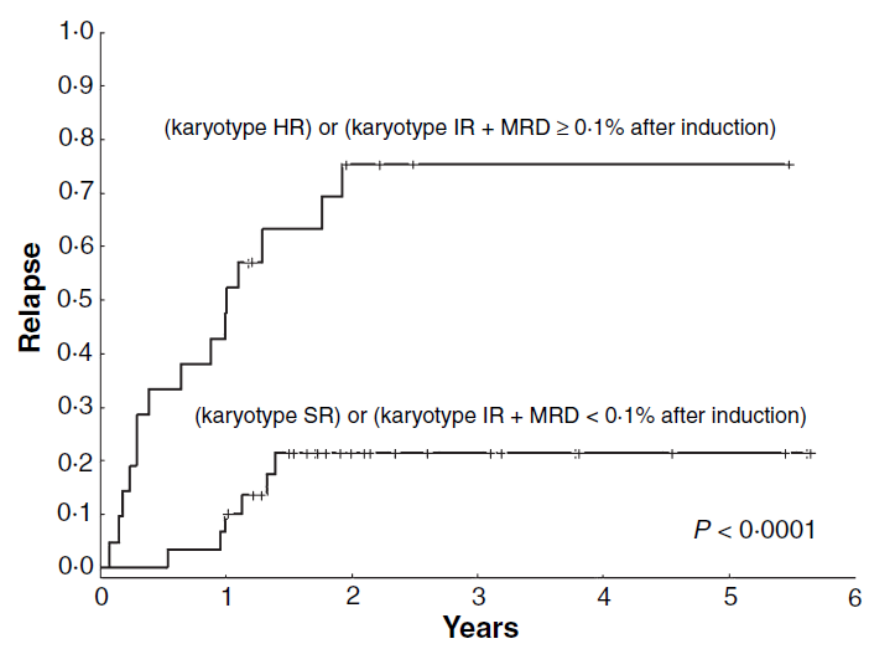


Fig. 5. Relapse incidence for Ph-negative ALL according to stratification criteria based on the combination of karyotype and minimal residual disease status. Karyotype risk groups were defined as proposed by Moorman et al (2007) SR indicates standard risk i.e. the presence of either del(9p) or high hyperdiploidy; HR, high risk i.e. the presence of t(4;11), t(8;14), hypodiploidy/almost triploidy or complex karyotype; IR, intermediate risk i.e. all remaining patients (Giebel et al., 2009).

7. Conclusions

The introduction of the WHO 2008 classification forces implementation of cytogenetic methods into the diagnostic procedures. Conventional cytogenetic and genetic aberrations analysis must be conducted in each case of acute lymphoblastic leukemia. Some genetic abnormalities cause the application of targeted therapy, the main example is the use of tyrosine kinase inhibitors in cases of the t(9;22). Cytogenetic methods, evaluating the minimal residual disease, are useful for optimization of the treatment strategy especially for recommendation of the allogeneic hematopoietic stem cell transplantation which is performed in high risk patients during the first complete remission.

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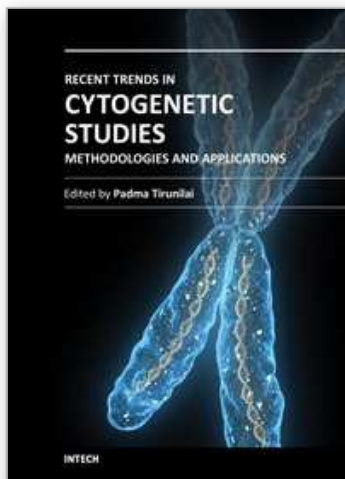
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Recent Trends in Cytogenetic Studies - Methodologies and Applications

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Recent Trends in Cytogenetic Studies - Methodologies and Applications deals with recent trends in cytogenetics with minute details of methodologies that can be adopted in clinical laboratories. The chapters deal with basic methods of primary cultures, cell lines and their applications; microtechnologies and automations; array CGH for the diagnosis of fetal conditions; approaches to acute lymphoblastic and myeloblastic leukemias in patients and survivors of atomic bomb exposure; use of digital image technology and using chromosomes as tools to discover biodiversity. While concentrating on the advanced methodologies in cytogenetic studies and their applications, authors have pointed out the need to develop cytogenetic labs with modern tools to facilitate precise and effective diagnosis to benefit the patient population.

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