Acute Lymphoblastic Leukemia

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Creation date: December 2004

Scientific Editor: Professor Riccardo Riccardi

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Abstract
Acute lymphoblastic leukemia (ALL) is a malignant proliferation of lymphoid cells blocked at an early stage of differentiation and accounts for ¾ of all cases of childhood leukaemia. About 3,000 children in the United States and 5,000 children in Europe are diagnosed with ALL each year. The peak incidence of ALL occurs between age 2 and 5 years. ALL is a biologically heterogeneous disorder, so that morphologic, immunologic, cytogenetic, biochemical, and molecular genetic characterizations of leukaemia lymphoblasts are needed to establish the diagnosis or to exclude other possible causes of bone marrow failure and, finally, to classify ALL subtypes. ALL may be either asymptomatic or acute with life-threatening hemorrhage, infection, or episode of respiratory distress. Although ALL is a disease primarily of the bone marrow and peripheral blood, any organ or tissue may be infiltrated by the abnormal cells. The most frequent signs are lymphadenopathies, hepatosplenomegaly, fever, signs of hemorrhage, and bone pain. Biological findings include hyperleukocytosis due to circulating lymphoblasts, anemia and thrombocytopenia. Diagnosis is established by bone marrow biopsy, which evidences the leukemic cells infiltration. Most of the cases of ALL show chromosomal and genetic abnormalities, which occur spontaneously in important regulatory genes in a lymphoid cell population. The most common ALL translocation, the t(12;21), appears to have good prognostic implications. Four main treatment elements can be generally recognized in chemotherapy protocols adopted by international cooperative groups: induction with the aim of complete remission, CNS preventive therapy, consolidation/reinduction, and maintenance therapy. The survival rate for children younger than 15 years of age reaches about 75%, but, despite the significant improvement of outcome during the last decades, still roughly 25% of patients suffer from a relapse of the disease. Even if the management of relapse remains largely controversial, an increasing use of high dose chemotherapy blocks and stem cell transplantation is adopted in most cases. With the need to stratify patients in risk groups and to provide risk-adapted therapy, treatment requires high levels of organization, expertise and knowledge.

Key-words
Acute lymphoblastic leukemia (ALL), childhood leukaemia, t(12,21), Philadelphia chromosome

Definition/Diagnostic criteria

Acute lymphoblastic leukemia (ALL) is a malignant proliferation of lymphoid cells blocked at an early stage of differentiation. ALL is a biologically heterogeneous disorder, so that morphologic, immunologic, cytogenetic, biochemical, and molecular genetic characterizations of leukaemia lymphoblasts are needed to establish the diagnosis or to exclude other possible causes of bone marrow failure and, finally, to classify ALL subtypes. This heterogeneity reflects the fact that leukaemia may develop at any point during the multiple stages of normal lymphoid differentiation.

ALL Morphologic Classification

The French-American-British (FAB) Cooperative Working Group, defines three categories of lymphoblasts: L1 lymphoblasts are small cells characterized by a high nucleus-to-cytoplasm ratio. The pale blue cytoplasm is scanty and is limited to a small portion of the perimeter of the cell. The cells have indistinct nucleoli and nuclear membranes that vary from round to clefted; L2 lymphoblasts are larger, often in a more heterogeneous population, with a lower nucleus-to-cytoplasm ratio, prominent nucleoli (often with perinuclear chromatin condensation) and nuclear membranes that may be reniform or irregular. They may be indistinguishable from the M1 variant of myeloid leukaemia, and the differentiation must be made primarily by myeloperoxidase (MPO) staining; the M0 variant of myeloid leukaemia, which is MPO negative, may be indistinguishable from ALL without the immunophenotype. L3 lymphoblasts is a heterogeneous group of cells identical to Burkitt-like leukaemia and characterized by deeply basophilic cytoplasm and prominent cytoplasmatic vacuolization. Approximately 85% of children with ALL have predominant L1 morphology, 14% have L2, and 1% have L3, while the L2 subtype is more common in adults. While L3 lymphoblasts represent an immunophenotypically distinct population of mature B cells, there is no correlation between the various stages of pre-B cell differentiation or immunophenotype and L1 or L2 morphology.

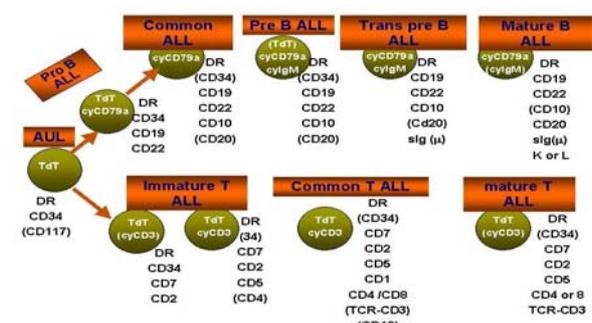### ALL immunophenotypic classification

Immunologic surface and cytoplasmic marker studies are of great significance for classification of acute leukemias. By using a selected panels of monoclonal antibodies (mAb) (**Table 1**) and the development of the multiparameter fluorescence-activated cell sorter (FACS) machine, it is possible to classify B and T-lineage ALL into discrete stages on the basis of the degree of differentiation or maturation of the normal B clone hit by neoplastic transformation, as shown in **Figure 1**. Mature B-cell ALL is rare and accounts for only 1 to 2% of all cases. This type of ALL is defined by the presence of surface immunoglobulin, most often IgM, which is monoclonal for κ or λ light chains. T-cell ALL has been subclassified into three stages of differentiation: early (stage I), intermediate (stage II), and late (stage III;). Frequently T-cell leukaemia presents with the antigen pattern of the early thymocyte (stage I), on the contrary of T-cell lymphoma, where usually malignant cells display an intermediate or a mature phenotype. Whether there is a correlation between sub-classification into stages of differentiation and clinical characteristics remains controversial, as well as controversial is the prognostic significance of this sub-classification. The development of immunophenotyping techniques, with regard primarily to the use of monoclonal antibodies, has allowed to confirm that in some instances individual leukemic cells simultaneously express both lymphoid and myeloid surface antigens, showing characteristics of more than one hematopoietic lineage. These leukaemia have been referred to as biphenotipic, mixed-lineage, or hybrid leukaemia, and, depending on the criteria applied, the incidence of this subgroup of ALL ranges from 7% to 25%.

Table 1. Monoclonal antibodies commonly used to immunophenotype leukaemia
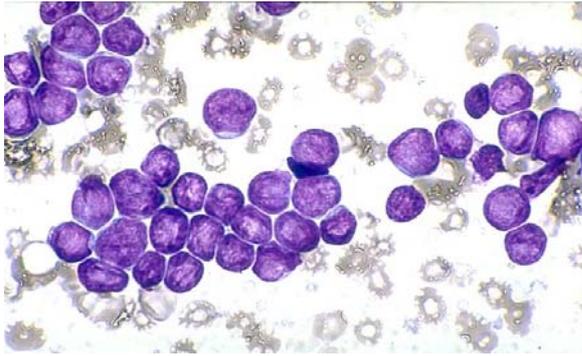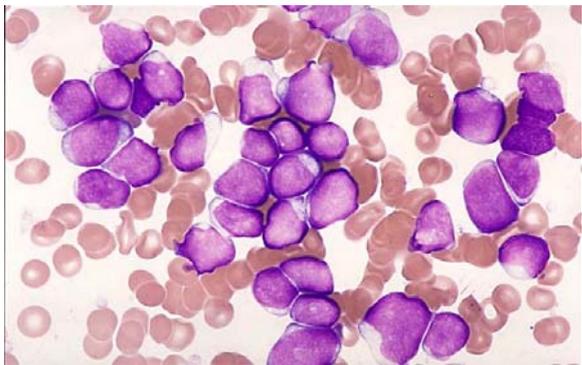

Figure 1 Representation of stages of lymphoid differentiation and immunophenotyping in B and T–cell precursor ALL of childhood

Conter V, Rizzari C, Sala A, Chiesa R, Citterio M and Biondi A. Acute Lymphoblastic Leukemia. Orphanet Encyclopedia. December 2004. http://www.orpha.net/data/patho/GB/uk-ALL.pdf
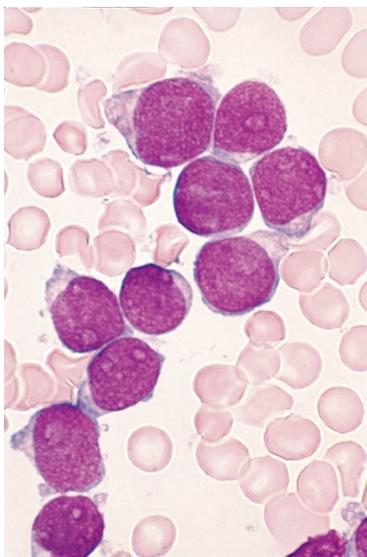
ALL1 lymphoblasts

ALL2 lymphoblasts

ALL3 lymphoblasts

Immunoglobulin and T-cell receptor gene rearrangements
Somatic rearrangement of Ig and T-cell receptor (TcR) gene loci occurs during early differentiation of any B and T cell, by joining the germline variable (V), diversity (D) and joining (J) gene segments. By this process, each lymphocyte gets a specific combination of V-D-J

segments that codes for the variable domains of Ig or TcR molecules. The uniqueness of each rearrangement further depends on random insertion and deletion of nucleotides at the junction sites of V, D, and J gene segments, making the junctional regions of Ig and TcR genes as "fingerprint-like" sequences. This combined sequence constitutes a specific signature of each lymphocyte. Due to the clonal origin of the neoplasm, each malignant lymphoid disease will represent the expansion of a clonal population with a specific Ig/TcR signature. Therefore, junctional regions can be used as leukemia-specific targets for PCR analysis of Minimal Residual Disease.

Conventional cytogenetics and molecular genetics
Conventional cytogenetic analysis detects only in mitotically active (metaphase) neoplastic cells. Nowadays, the recent development of molecular cytogenetic techniques, including newer methods of chromosomal banding and standard fluorescent *in situ* hybridization (FISH) with the molecular genetic techniques of spectral karyotyping (SKY) and comparative genomic hybridization (CGH), allows to recognize chromosomal abnormalities in the leukaemia cells of most of the cases of pediatric ALL. The cytogenetic abnormalities reported in ALL involve both chromosomal number (ploidy) and structural rearrangement.
ALL can be classified into 4 subtypes based on the modal number of chromosomes: hyperdiploid with more than 47 chromosome (35-45% of cases, defined by a DI greater than 1.0), pseudodiploid (46 chromosome with structural or numeric abnormalities: about 40% of cases; DI of 1.0), diploid (46 chromosome: 10-15% of cases; DI of 1.0) and hypodiploid (fewer than 46 chromosome: about 8% of cases; DI less than 1.0). DI becomes a statistically significative prognostic factor when ≥ 1.16, which corresponds to a modal number of 53 chromosomes: children with higher ploidy (greater than 50 chromosomes) have the best prognosis; on the contrary, those in the pseudodiploid category have a relatively poor prognosis. An exception to the general rule that hyperdiploid ALL cases have good prognoses is the relatively rare group of hyperdiploid ALL cases with the near-tetraploid subtype (82 to 84 chromosomes), which appears to have a poorer prognosis.
Translocations are the most common structural chromosomal changes in ALL (**Figure 2**), particularly frequent in the pseudodiploid and hypodiploid groups. They are assumed to play a fundamental role in the leukemogenic process and in most cases [*i.e.* translocations t(9;22),

t(4;11), and t(1;19)] are associated with elevate risk of early treatment failure; the most common ALL translocation, the t(12;21), however appears to have good prognostic implications.

The first fusion gene described in ALL was BCR-ABL, created by the del(22) of the t(9;22)(q34;q11), also referred to as the Philadelphia (Ph) chromosome, and has been identified as the translocation with the worst prognosis in pediatric ALL. The Ph chromosome has been demonstrated in about 95% of cases of chronic myelogenous leukaemia (CML), 25% of adult ALL cases, and 3% to 5% of paediatric ALL cases. Clinically, patients with Ph-positive blasts are older, have higher leukocyte counts, larger percentages of circulating blasts, a higher frequency of FAB L2 morphology, a higher frequency of CNS leukaemia and more prevalent pseudodiploid karyotypes than are found in Ph negative cases. Most Ph+ blasts have a B-lineage immunophenotype, although isolated cases with a T-cell or mixed phenotype have been reported. The consistent lack of success in treating this form of ALL has prompted most investigators to consider BMT during first remission as a therapeutic option. In addition, it was recently noted that children with Ph+ALL and low initial blood cell counts may have a durable response to chemotherapy.

One of the most exciting development in ALL was the identification of TEL-AML1 as the most common genetic alteration in this disease. The TEL-AML1 fusion is created by the translocation t(12;21); modern molecular techniques have demonstrated this translocation in approximately one-fourth of childhood ALL cases. TEL-AML1 expression has been associated with an excellent prognosis, with event free survival (EFS) approaching 90%.

Two recent studies have demonstrated that the incidence of TEL-AML1 in relapsed Ph-negative B-cell precursor ALL was about 20-25%, a result similar to the reported incidence at diagnosis. In addition, these two reports highlighted that the period of remission was significantly longer in children expressing TEL-AML1 and that the majority of relapses in this group appeared late (>2 years after diagnosis). This could mean that TEL-AML1 positivity predicts a favourable short-term outcome, while long-term results are still unknown. Interestingly the outcome of TEL-AML1 positive relapsed ALL patients is significantly better than the outcome of negative patients, but it remains to be verified whether TEL-AML1 is an independent risk factor.

With an overall incidence of 5% to 6,5%, the t(1;19)(q23;p13) is the most common translocation detected by conventional cytogenetics in childhood ALL. This translocation is found in 15% to 25% of pre-B (cIg+)

immunophenotype cases and in 1% of early pre-B (cIg-) or T-cell immunophenotype. It was reported that t(1;19) accounted for the majority of treatment failures in pre-B ALL.

The q23 region of chromosome 11 is a relatively frequent site of structural rearrangements in children with ALL; these abnormalities (including translocation, deletion and partial duplication) are detected in 4.5% to 5.7% of blasts cells, 80% of infant leukemia (*i.e.*, ALL and AML in patients younger than 1 year), and 85% of secondary leukemias in patients who have received epipodophyllotoxin therapy. The patients with 11q23 abnormalities are usually young, lack hyperdiploidy, and have high leukocyte counts, organomegaly, central nervous system (CNS) involvement, an early pre-B cell immunophenotype, myeloid-related antigen expression, and a poor prognosis.

The majority of cases with translocations involving the 11q23 region results from exchanges with chromosome 4. The t(4;11)(q21;q23) has been reported in up to 5% of pediatric ALL cases, is mostly observed in children younger than 1 year, commonly newborns; the t(4,11) fusing MLL and AF-4 accounts for about 70% of MLL translocations in infant ALL.

MLL rearrangements are associated with long-term EFS rates of less than 20% despite treatment with aggressive multiagent chemotherapy.

Figure 2: Distribution of chromosomal abnormalities in pediatric ALL
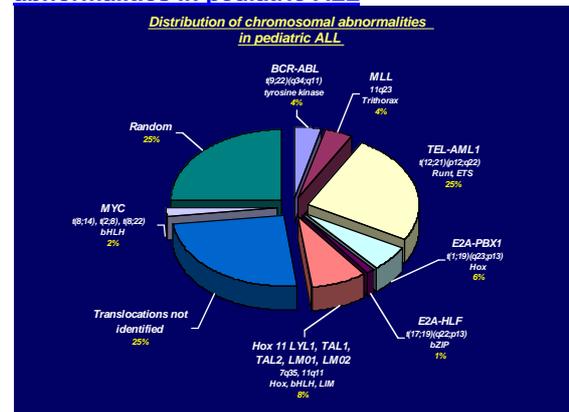
Pathogenesis

In most cases of ALL, as in other lymphoid malignancies, a single damaged progenitor cell, capable of expansion by (theoretically) indefinite self-renewal, gives rise to malignant, poorly differentiated precursors. It is not completely clear where in the normal course of differentiation the leukaemia "clonal event" occurs. In pediatric ALL there is evidence that these events occur in committed lymphoid precursors, whereas in acute myeloid leukaemia

(AML) and Philadelphia chromosome–positive ALL, it appears that they may occur at an earlier stage of blood cell development because there is evidence of mutation in multiple cell lineages. Many of the described molecular mutations bear evidence of immunoglobulin joining region (VDJ) and T-cell receptor (TcR) recombinase activity. Greaves hypothesized a model, analogous to that proposed by Knudson, that one or, more likely, two sequential mutations (one initiational and the other promotional), occurring spontaneously in important regulatory genes in a lymphoid cell population undergoing significant proliferative stress, could account for most ALL cases. It has been suggested that first "hit" occurs *in utero* based on concordance studies of twins with leukaemia. Although these studies provide evidence that the development of leukaemia may begin *in utero*, the question of whether these genetic changes are the "accelerating" events or merely incidental genetic changes in patients who later developed leukaemia remains unresolved.

Several congenital disorders are associated with an increased risk of leukaemia. Children with trisomy 21 (*i.e.*, Down syndrome) are up to 15 times more likely to develop leukaemia than normal children. Other less common pre-existing chromosomal abnormalities have been linked to leukemia. Included among these are Klinefelter's syndrome, Bloom syndrome, and Fanconi's anemia. Lymphoid malignancies, with a predominance of T-ALL, have been reported in patients with ataxia-telangiectasia (AT), an autosomal recessive disorder characterized by increased chromosomal fragility.

Most of the cases of ALL show chromosomal and genetic abnormalities (described in **Conventional cytogenetics and molecular genetics)**. Many of these molecular changes occur at the location of immunoglobulin, TCR, and transcription factor encoding genes. In addition to chromosomal translocations, there is a variety of genetic events that appear to be leukemogenic but are undetectable with classic cytogenetic methods. Other gene expression modalities are beginning to be used to characterize leukaemia. These include complementary DNA (cDNA) micro-array, real-time and other modifications of reverse transcriptase-polymerase chain reaction (RT-PCR), which examine messenger RNA expression levels, and new mass spectroscopy techniques for proteins (proteomics). These and other new techniques allow researchers to examine large patterns of gene expression at either the RNA or protein level. If specific patterns can be correlated with clinical response, these new characterization methods should allow increased refinement of current prognosis (risk)-based stratification systems.

Predisposing Factors

Radiation exposure
Ionizing radiation can play a role in the development of acute leukaemia; in fact a high incidence of leukaemia was seen after the atomic bomb explosions in Hiroshima and Nagasaki with ALL being more frequent in children and AML more common in adults. However the cases of leukaemia attributable to radiation are rather few. Controversy persists about the risks from exposure to ionizing radiation from routine emissions from nuclear power plants or as a result of fallout from atmospheric nuclear testing. More recently it has also been suggested that exposure to electromagnetic fields (EMF) may be related to the development of childhood ALL. Conflicting studies exist in the literature, but the most recent data have concluded that EMF exposure does not cause childhood ALL.

Chemical exposure
The role of toxic chemical exposure (*e.g.*, to benzene) in the development of childhood ALL is questionable, although it has been shown that NAD(P)H:quinone oxidoreductase 1, one of the enzymes responsible for benzene and other quinone metabolism, has a mutation with decreased enzymatic activity that has been linked to the development of both AML and ALL in adults. Other factors that could be involved in the development of ALL include parental cigarette smoking; paternal herbicide and pesticide exposure; maternal use of alcohol, contraceptives, and diethylstilbestrol; household radon exposure; and chemical contamination of ground water. Definitive causal relationships between these factors and childhood ALL have not been demonstrated.

Other possible predisposing factors
The role played by viral infection in the pathogenesis of human leukaemia has been investigated intensively. This reflects the fact that the age distribution of ALL at diagnosis corresponds with a time when the immune system is developing and is perhaps more vulnerable to the oncogenic effects of some viruses. Some authors have suggested an increased risk of ALL in children born to mothers infected recently with influenza, varicella, or other viruses, but no definitive link between prenatal viral exposure and leukaemia risk has been confirmed. The only link found is the association between the Epstein-Barr virus (EBV) and cases of endemic Burkitt's

lymphoma-leukaemia, the L3 morphologic subtype of ALL.

The GSTs represent a set of xenobiotic detoxifying enzymes with a known series of polymorphic mutations that affect function. GST deficiency, rather than high GST activity, has been associated with an increased risk of cancer, although deficiency of GSTT1 and GSTM1 could enhance chemotherapeutic efficacy in some patients. Conflicting results have been reported on the association between GSTM1 and GSTT1 genotypes and outcome as well as on the association between the risk of relapse in childhood ALL and GSTs polymorphisms.

Clinical Presentation

ALL may present insidiously or acutely, as an incidental finding on a routine blood count of an asymptomatic child or as a life-threatening hemorrhage, infection, or episode of respiratory distress. Although ALL is a disease primarily of the bone marrow and peripheral blood, any organ or tissue may be infiltrated by the abnormal cells. The duration of symptoms in children presenting with ALL may vary from days to months. The first symptoms are usually non-specific and include anorexia, irritability and lethargy. Fever is the most common finding, occurring in approximately 60% of patients. Progressive bone marrow failure leads to pallor (anemia), bleeding (thrombocytopenia) and susceptibility to infections (neutropenia). Over one third of patients may present with a limp, bone pain, arthralgia or refusal to walk due to leukaemia infiltration of the periosteum, bone or joint, or to the expansion of the marrow cavity by leukaemia cells. Less common signs and symptoms include headache, vomiting, respiratory distress, oliguria and anuria. At initial diagnosis, 60 to 70% of children have enlargement of the liver or spleen, usually asymptomatic, with organs palpable more than 2 cm below the costal margin. Lymphadenopathy (usually painless, localized or generalized) due to leukaemia infiltration is an equally frequent presenting sign (see **Table 2**).

Laboratory findings

Anemia, abnormal leukocyte and differential counts, and thrombocytopenia are usually present at diagnosis, reflecting the degree to which bone marrow has been replaced with leukemic lymphoblasts. The presenting leukocyte counts range widely, from 0.1 to 1500 x 10^9/L (median 15 x 10^9/L) and are increased (> $10x10^9$/L) in slightly over one half of the patients (**Table 2**). Hyperleukocytosis (> $100x10^9$/L) occurs in 10% to 15% of the patients. The degree of leukocyte count elevation at diagnosis is a very strong predictor of prognosis in ALL.

Neutropenia (less than 500 granulocytes per mm^3) is a common phenomenon and is associated with an increased risk of serious infection. Hypereosinophilia, generally reactive, may be present at diagnosis. Decreased platelet counts (median, $50x10^9$/L) are usually present at diagnosis and can be readily distinguished from immune thrombocytopenia, as isolated thrombocytopenia is rare in leukemia. Severe hemorrage is uncommon, even when platelet counts are as low as $20x10^9$/L, provided that infection and fever are absent. Coagulopathy, usually mild, can occur in T-cell ALL and is only rarely associated with severe bleeding. More than 75% of patients presents with anemia, which is usually normochromic and normocytic and associated with a normal to low reticulocyte count. Anemia or thrombocytopenia is often mild (or even absent) in patients with T-cell ALL. Pancytopenia followed by a period of spontaneous hematopoietic recovery may precede the diagnosis of ALL in rare cases and must be differentiated from aplastic anemia.

Table 2: Clinical and laboratory features at diagnosis in children with ALL

Clinical and laboratory features	Percentage of patients
Symptoms and physical findings	
Fever	60
Hepatosplenomegaly	70
Paleness	55
Bleeding (e.g., petechiae or purpura)	50
Lymphadenopathy	50
Bone pain	25
Abdominal pain	20
Weight loss	15
Laboratory features	
Leukocyte count (mm^3)	
<10,000	53
10,000–49,000	30
>50,000	17
Hemoglobin (g/dL)	
< 7.0	43
7.0 - 11.0	45
> 11.0	12
Platelet count (mm^3)	
< 20.000	28
20.000 – 99.000	47
> 100.000	25

Diagnostic methods

To definitively establish the diagnosis of leukaemia, inspection of smears of bone marrow aspirates is essential. Whereas normal bone marrow has fewer than 5% blasts, leukaemia marrow generally is almost completely infiltrated by leukaemia blasts. The marrow specimen is usually hypercellular and characterized by an homogeneous population of cells. Leukaemia must be suspected in patients whose marrows contain greater than 5%, but the diagnosis should not be made on the basis of a single marrow smear with fewer than 25% blasts. A bone marrow aspirate may be difficult to obtain at the time of diagnosis. This is usually due to the density of blasts in the marrow, but it may be caused by bone infarction, fibrosis, or necrosis. The distinction between ALL with lymph-node involvement and non-Hodgkin's lymphoma (NHL) with bone marrow invasion (stage IV) is arbitrary. Commonly, the disease is classified as ALL when there are ≥ 25% lymphoblasts in the marrow and as NHL Stage IV when there are ≥ 5 and < 25 per cent lymphoblasts in the marrow. In some situations (*e.g.*, to differentiate an aplastic presentation of ALL from aplastic anemia) multiple bone marrow aspirates and biopsy specimens are necessary.

Epidemiology

Childhood cancer incidence is around 120-150/million/year in subjects 0-14 years old and the number of new cases/year worldwide is approximately 250,000. Leukaemia is the most common malignancy in children and accounts for one-third of all childhood cancers. Approximately 3/4 of all cases of childhood leukaemia are ALL. About 3,000 children in the United States and 5,000 children in Europe are diagnosed with ALL each year. The peak incidence of ALL occurs between age 2 and 5 years. The incidence of ALL is higher among boys than girls, and this difference is greatest among pubertal children. T-cell ALL represents approximately 10-15% of ALL cases in developed countries and compared with B-cell precursor ALL, it is characterized by male predominance, mediastinal mass (approximately one-half of patients), higher median age, higher white blood cell counts (one-third to one-half have initial leukocytes counts greater than 100,000 per mm^3) and normal hemoglobin levels at the time of diagnosis. In general, T-ALL associates with higher risk features and prednisone poor response in 30% versus 10% in B-cell precursor ALL In the United States, ALL is more common among white children than black children, and this is related probably to geographical variation in biology or different environmental exposure.

Treatment

Four main treatment elements can be generally recognized in chemotherapy protocols adopted by international cooperative groups: induction, CNS preventive therapy, consolidation /reinduction, and maintenance therapy. The biological heterogeneity, which characterizes childhood ALL has determined an increasing need to stratify patients in risk groups and to provide risk-adapted therapy. Treatment has thus become increasingly complex and high levels of organization, expertise and knowledge are nowadays requested to achieve optimal results. For these reasons children with ALL should be treated in centres which can offer specialized personnel and provide up-to-date diagnostic tools and treatment strategies.

Induction

The treatment planned in this phase is aimed at eradicating signs and symptoms of the disease and to re-establish a normal hematopoiesis. This goal is generally indicated with the term of "complete remission" (CR). Children in CR must have no physical evidence of leukaemia, normal complete blood cell count and normally regenerating bone marrow (with < 5% leukemic blasts). Information on CR status also includes the absence of detectable CNS or extramedullary disease as evaluable with physical examination and cerebrospinal fluid (CSF) findings. Induction treatment intensity has increased over the years, consisting of a combination of two (vincristine + steroids), three (+ anthracycline) or four (+ asparaginase) drugs, which can induce a complete remission rate comprised from 85% to approximately 95%. CNS preventive therapy is administered in this phase and mainly consists of intrathecal methotrexate (see following section). Conventionally, the systemic chemotherapy is given in 4-6 weeks and allows a complete remission rate ≥ 95%; some patients (<5% of the overall population) will not achieve CR because of death (approximately 2%) or persistent disease (about 2-3%).

Central Nervous System Preventive Therapy

The prevention of CNS recurrence has been a well-established concept since late 60's. Since then in fact it became clear that CNS recurrence could represent the first sign of leukaemia resistance and progression. Leukaemia cells, undetected in the CSF at the time of diagnosis, may proliferate because systemically administered antileukemic agents do not penetrate the blood–brain barrier. With modern therapy regimens, which include different modalities of CNS preventive therapy, the incidence of CNS relapse is overall below 5%.

Cranial irradiation is generally no longer used for patients with a good prognosis; intrathecal methotrexate alone or triple intrathecal chemotherapy, given periodically throughout maintenance chemotherapy, provide adequate CNS preventive therapy for these patients.

Consolidation/Reinduction

It is well known that the achievement of remission is not a sufficient goal to obtain the cure of ALL and that a significant amount of additional therapy is necessary before leukaemia is totally eradicated. Consolidation/Reinduction therapy is defined as one/more periods of intensified treatment administered after remission induction and is considered a relevant component of many chemotherapy protocols, particularly for higher-risk patients. Each cooperative group has its own tradition in the type of consolidation/reiduction to be delivered. Commonly, agents and schedules are designed to minimize the development of drug resistance onset.

Maintainance therapy and duration of treatment

Drugs particularly effective as induction agents are not generally used for maintenance therapy. In particular the use of low dose methotrexate and 6-MP, administered continuously, is widely accepted and constitutes the principal element in most maintenance therapy regimens. In general, weekly methotrexate and daily 6-MP appears the optimal schedule. The dosage of the drugs used in this phase seems to have a key role in determining its efficacy. Patients who receive maintenance therapy on a continuous rather than an interrupted schedule have longer remission durations. Compliance problems also may diminish the efficacy of maintenance therapy. The optimal length of maintenance chemotherapy has not yet been definitely established. However most groups treat patients for a total of 2 years (thus including the maintainance phase).

Recent results of the most important international cooperative groups

During the 80's and 90's results obtained by most large cooperative or institutional pediatric oncology groups have become rather similar. Interestingly, improvement of outcome was obtained with markedly different therapeutic strategies, all of them having however a common approach toward treatment intensification. The Berlin-Frankfurt-Münster (BFM) group has treated more than four thousand children in four consecutive trials between 1981 and 1995. The probability for EFS at 8 years improved from 65.8% in study ALL-BFM 81 to 75.9% in study ALL-BFM 90. The incidence of isolated CNS relapses was reduced from 5.3% in study ALL-BFM 81 to 1.1% in study ALL-BFM 90. These studies have shown that reintensification is a crucial part of treatment, even in low risk patients, that presymptomatic cranial radiotherapy can be safely reduced to 12 Gy, or even be eliminated if it is replaced by early intensive systemic and intrathecal methotrexate applied and that inadequate response to an initial 7-day prednisone window (combined with one intrathecal injection of methotrexate on day 1) defines about 10% of the patients with a high risk of relapse. The leading cause of failure in childhood ALL is still recurrence of disease which is more frequently associated to clinical (*e.g.* age, WBC), and biological characteristics (such as immunophenotype and cytogenetics) and to poor early *in vivo* treatment response. A very important information has been obtained from the study 90 too, which showed, in the context of an european intergroup study, that the response evaluated with the detection of minimal residual disease (MRD) at defined timepoints helps to define the patient at high risk to relapse more specifically. In study 95 treatment strategy was further refined; in particular an increase of treatment intensity was planned for HR patients (intensive use of polichemotherapy blocks followed by a protocol II). In this group of patients major benefits derived to PPR patients (EFS 56+/- 4 in study 95 vs 34+/-3 in study 90). In the current study ALL 2000, MRD evaluation performed with a semiquantitative method at 5 and 12 weeks after treatment start allows the allocation of patients according to "molecular response" to treatment and is one of the main stratification criteria.

The Children's Cancer Group (CCG) has reported marked improvements in trials conducted during two successive series of studies (1983-1988 and 1989-1995). Overall, 10-year EFS was 62% +/- 10% for the 1983-1988 series and 72% +/- 1% for the 1988-1995 series (P< 0.0001). Five-year cumulative rates of isolated CNS relapses were 5.9% and 4.4%. Therapy based on the BFM 76/79 study improved outcomes for intermediate and higher risk patients in the first series. For intermediate risk patients, delayed intensification (DI) was most crucial to improve outcome and cranial irradiation was safely replaced with maintenance intrathecal methotrexate, providing patients intensified systemic therapy. In the second series, randomized trials showed better outcome with one vs no DI phase for lower risk patients, with two vs one DI phase for intermediate risk patients, and with the CCG 'augmented regimen' for higher risk patients defined by a slow day 7

marrow response. Cranial irradiation was safely replaced with additional intrathecal methotrexate for higher risk patients with a rapid day 7 marrow response. In a subsequent study, substitution of dexamethasone in place of prednisone in induction and maintenance improved outcome for standard risk patients. All patients received dexamethasone in DI. These successful treatment strategies form the basis for our current ALL trial.

The St. Jude Children's Research Hospital (SJCRH) has reported long-term results of the most recent trials (Total Therapy studies 11, 12 and 13A). In particular the recent Total 13A was started to patients recruitment in1991 and closed in 1994. Early intensive intrathecal therapy in this study has yielded a very low 5-year isolated CNS relapse rate of 1.2 +/- 0.9%, with a 5-year event-free survival rate of 76.9 +/- 3.3%. Factors consistently associated with an adverse prognosis included male sex, infant or adolescent age group, leukocyte count >100 x 10(9)/l, nonhyperdiploidy karyotype and poor early response to treatment. Risk classification based on age and leukocyte count had prognostic significance in B-lineage but not T-lineage ALL. Early therapeutic interventions or modifications for patients with specific genetic abnormalities or persistent minimal residual leukemia may further improve long-term results.

Relapse of acute lymphoblastic leukemia

Although the treatment of childhood ALL has been gradually intensified during the last 30-40 years, leading to a significant improvement of the outcome, still roughly 25% of patients suffer from a relapse of the disease. This percentage of children on its own represents the fourth most common malignancy in children.

The management of relapse remains controversial but increasingly involves the use of high-dose chemo/radiotherapy and stem cell infusion and despite recent improvements, the overall results remain unsatisfactory and relapsed ALL continues to make a major contribution to the morbidity and mortality of childhood cancer.

Diagnosis

ALL can recur in the bone marrow (isolated bone marrow relapse), in an extra-medullary site like the central nervous system (CNS), testis or ovary (isolated extra-medullary relapse) or in two or more locations (combined relapse). Diagnosis is made by the morphological demonstration of lymphoblasts on smears obtained from the site of relapse. Medullary ALL relapse is defined as the presence of ≥ 25% lymphoblasts in a bone marrow aspirate following the first complete remission (CR).

CNS relapse is defined as the presence of morphologically identified lymphoblasts on smears of CSF cytocentrifugated preparations with a CSF mononuclear cell count > 5 per microliter, or as the evidence of tumor infiltration in the CNS following the first CR. Testicular relapse is defined as the histological evidence of lymphoblastic infiltration in one or both testes; relapses occurring in different organs than CNS or testis must be necessarely addressed after bioptic examination. Combined ALL relapse is defined as the presence of extramedullary lymphoblastic infiltration and ≥ 5% blasts in a bone marrow aspirate following the first CR.

Prognostic factors

During the last 20 years the major international study groups have analysed the clinical and biological features of patients with relapsed ALL, in order to determine which have the strongest impact on EFS. The identification of features able to predict the response to second-line therapy may help to assign patients to treatment protocols with either reduced toxicity or a more aggressive approach (e.g. stem cell transplantation).

Duration of first remission

Duration of first remission is the strongest predictor for the achievement of second CR and survival; several reports confirm that late relapses, occurring at least 30 months after initial diagnosis are associated with a better EFS, while early (≥ 18 months from diagnosis but < 6 months from the end of front-line therapy) or very early (< 18 months from initial diagnosis) relapses have poor outcome. A study on 600 children with ALL relapse treated with chemotherapy documented prolonged second remissions in fewer than 5% of children who relapsed within 18 months after achieving first remission; in contrast, sustained second remissions were observed in approximately 25% of children whose relapses occurred 18 months after primary diagnosis.

Site of relapse

Different sites of relapse are characterized by different outcome: bone marrow (BM) relapse is the main cause of treatment failure in children with ALL and generally implies a poor prognosis for most patients. The results of a number of studies suggest that prolonged second remissions (greater than 2 years) can be obtained with aggressive chemotherapy in approximately 10% of patients who relapse in the BM on therapy and in up to 40-50% of patients who relapse after cessation of therapy.

Many studies suggest that combined (medullary and extra medullary) relapses have a better

outcome compared to isolated BM relapses; combined relapses in fact tend to be later and to display better response to chemotherapy. Data published by the BFM study group evidenced an EFS at 7 years of 42% versus 15% for combined BM and extramedullary relapses versus isolated marrow relapses.

Despite the success of front line CNS preventive therapy in reducing the incidence of CNS recurrence, CNS relapse remains a relevant cause of treatment failure in ALL; CNS recurrence is in fact observed in about 10% of patients. In the past, the outcome for these patients was generally poor, with most patients suffering of a subsequent CNS relapse or recurrence at other sites, such as BM and testis. Intensive treatment plans have recently led the results for patients with an isolated CNS relapse to an EFS of about 70%.

The outcome for patients with an isolated overt testicular recurrence appears to vary with the time of presentation; an isolated testicular relapse occurring in a patient on treatment is associated with the worst prognosis; nevertheless a CCG study suggests that with local irradiation and intensive systemic retreatment, prolonged EFS can be obtained in nearly one-half of such patients. In contrast, a late, isolated overt testicular relapse that occurs off therapy has a better prognosis, with a prolonged DFS obtained in more than two-thirds of patients.

Immunophenotype

T-cell immunophenotype represents an independent negative prognostic factor; data from the BFM group demonstrate that T lineage ALL children relapsing at any site and treated with a chemotherapy schedule obtain a 5 years EFS of 5-10%.

Peripheral blast count

According to the POG or BFM group experience the number of peripheral blood blast cells (≥ 10.000 µL) at the time of relapse is a biological predictor of negative outcome.

Cytogenetic aberrations

The translocation t(9;22)(q34;q11), occurring in about 10% of B-cell precursor ALL first relapses, has been shown to be an indipendent risk factor associated with an adverse prognosis. In contrast, the prognostic value of TEL-AML1 fusion resulting from the cryptic translocation t(12;21)(p13;q22) is still not entirely clear. General characteristics of TEL-AML1 positive childhood ALL, both at diagnosis and at relapse, include confinement to B-cell lineage, good response to combination chemotherapy, and a

low WBC count, as well as a favourable age distribution. The majority of relapses (80%) occur off-therapy and achieve and mantain long-lasting 2nd CR when treated with chemotherapy only.

Minimal residual disease

Several studies have demonstrated the prognostic value of MRD during the early phases of front line treatment in ALL. In particular, a study conducted within the I-BFM group on 240 children affected by ALL has shown a significant correlation between different MRD levels and different risk of relapse: analysis of MRD at two different time points (end of induction treatment and beginning of consolidation) has shown the most significant relevance in the identification of patients with different risk of relapse, independently from other biological and clinical parameters since then utilized as stratification criteria.

MRD analysis represents a predictive parameter even for patients presenting a recurrence of ALL. In particular, a retrospective study conducted on 30 patients with relapsed ALL and stratified in the BFM intermediate risk group has shown that the persistance of MRD during the early phases of second line treatment was significantly related to the prognosis.

Children presenting MRD levels below 10^{-3} after 36 days of Induction therapy had an EFS of 86%, while patients with MRD level above 10^{-3} presented a subsequent recurrence of the disease.

These results suggest that in children with ALL relapse, the extent of early response can be used to predict long-term outcome. Patients whose MRD rapidly decreases are likely to have a very good outcome even if treated with chemotherapy. Thus, transplant procedures that have a high risk for morbidity and mortality might not be necessary in these children.

Another aspect of potential clinical relevance in the study of MRD is the evaluation of remission in patients undergoing bone marrow transplantation after ALL relapse. It has been recently demonstrated that the detection of MRD levels just before the accomplishment of stem cell transplantation is linked to the probability of relapse after transplant procedure.

However the benefits of a more intensive treatment in patients showing a slow reduction in MRD remain unclear.

Treatment

The optimal treatment for relapsed childhood ALL is still controversial since the overall results remain unsatisfactory worldwide, especially in

early bone marrow relapses and in heavily pre-treated patients.

Despite continuing uncertainty about the best treatment approach there have been no successful randomized trials comparing chemotherapy and BMT.

Whether a child with ALL relapse should receive a chemo/radiotherapy schedule or a stem cell transplant is thus in many instances still a matter of intensive debate and the choice is mainly performed on policies adopted in each single cooperative group.

Chemotherapy and Radiotherapy

The chemotherapy approach to the relapsed patient should include aggressive multidrug reinduction therapy followed by intensive systemic consolidation and maintenance chemotherapy. The combination of vincristine, prednisone, and L-asparaginase produces complete remissions in approximately 70% to 75% of patients. The addition of an anthracycline can increase the remission rate to >80%, even in children who have been treated on modern intensive trials; however the use of anthracyclines may be restricted by the risk of cardiotoxicity, given that these drugs are often extensively used during first-line therapy. Following induction, most protocols include further consolidation and intensified continuing therapy for a total of about two years.

Further administration of intensive courses of cytarabine and teniposide, or high-dose ifosfamide with etoposide may induce a complete remission in approximately one-third of patients not achieving complete responses with the four-drug reinduction regimen.

Further CNS-directed treatment is essential to avoid overt CNS relapse as a second event and radiation therapy is normally given to sites of extramedullary relapse. In spite of the efforts performed, the overall results of chemotherapy treatment of ALL relapse remain disappointing: large series of patients treated consistently with modern intensified protocols have an overall EFS after BM relapse of 30% to 60%. No regimen is clearly superior, and the results mainly reflect the selection of patients. In all studies, the reported EFS after chemotherapy for early relapses involving the bone marrow is only 5-10%. Furthermore, despite encouraging results after intensive chemotherapy for later marrow relapse, there is evidence that the risk of second relapse continues for many years.

Management of the 5-10% of children who develop isolated CNS relapse remains controversial and most reports have very small numbers of patients. Now that few children receive cranial irradiation in first remission, intensified systemic and intrathecal chemotherapy and cranial or craniospinal irradiation could provide an appropriate and effective treatment.

Intrathecal methotrexate alone induces CNS remissions in more than 90% of patients but, unless followed by maintenance intrathecal therapy or craniospinal irradiation, a further relapse occurs within 3 to 4 months. Therapy for CNS relapse must also contain intensive systemic therapy to prevent further relapses of all types. A common approach is to induce a CSF remission with intrathecal chemotherapy first and then to administer craniospinal irradiation at doses of 2,400 to 3,000 cGy to the cranial vault and 1,200 to 1,800 cGy to the spinal axis. However, an early CNS relapse in conjunction with other adverse prognostic factors, has a poor prognosis, with reported EFS in recent CCG and MRC studies < 25%. The Pediatric Oncology Group (POG) has reported that in relatively large trials for isolated CNS relapse and in one early study incorporating systemic intensification, triple intrathecal therapy and cranial irradiation, an overall EFS of 46% at 4 years was achieved. More recently in a series of 83 children who received intensified chemotherapy and delayed craniospinal irradiation for CNS relapse, the 4-year EFS was 46.2% for those with initial CR of less than 18 months and 83.3% for those with longer first remissions.

With the increased intensity of many protocols, the incidence of testicular relapse appears to be decreasing from the 10-15% seen in the 1970s and 1980s to 2-5% of more recent trials. Optimal therapy for testicular relapse includes the administration of local radiotherapy and the use of systemic chemotherapy. Radiation dose appears to be a crucial factor in local disease control. Doses less than 1,200 cGy are generally suboptimal; doses of 2,400 cGy to both testes have been considered adequate. Reports of local recurrence in patients treated with 2,400 cGy, however, suggest that higher doses may be better. Bilateral testicular radiotherapy is indicated for all patients; unilateral treatment may be followed by relapse in the contralateral testis. Radiation therapy adversely affects normal testicular function. Sterility is an expected consequence at the radiation doses used. Studies also indicate that testicular endocrine function may be impaired at doses of 2,400 cGy. Elevated follicle-stimulating hormone and luteinizing hormone levels, decreased testosterone levels, and delayed sexual maturation have been observed after gonadal irradiation. For this reason, such patients must be carefully followed for signs of delayed sexual maturation and may require androgen replacement therapy.

The impact of a testicular relapse on prognosis depends on whether it was overt (clinically detectable) or occult (detected on routine testicular biopsy), whether the recurrence was an isolated event or accompanied by a simultaneous hematologic relapse, and whether the relapse occurred during or after initial treatment. Because isolated testicular relapse frequently heralds a systemic relapse, treatment must include intensification of systemic therapy in addition to bilateral testicular irradiation. Most centers systemically "reinduce" patients who suffer an overt testicular relapse with intensive systemic chemotherapy. This strategy has dramatically improved the prognosis for patients with testicular relapse. A better prognosis is associated with a testicular relapse occurring as an isolated event and appears to vary with the time of presentation. An isolated testicular relapse occurring in a patient on treatment is associated with the worst prognosis, although a CCG study suggests that with local irradiation and intensive systemic retreatment, prolonged EFS can be obtained in nearly one-half of such patients. In contrast, a late, isolated, overt testicular relapse that occurs off therapy has an even better prognosis. Prolonged DFS can be obtained for more than two-thirds of such patients.

Stem Cell Transplantation

Although successful BMT in patients with end-stage leukaemia have been performed for 30 years, there is still no clear agreement about the indications for BMT in second remission. Furthermore, relapses rates after BMT remain high. As with chemotherapy, the chance of EFS after BMT is influenced by length of first CR, site of relapse and immunophenotype.

The risks of allogeneic BMT and the paucity of HLA-compatible siblings have lead to the use of autologous BMT (ABMT) in second remission. Despite preliminary encouraging results, comparative studies from Germany and the UK have shown that ABMT is not superior to conventional chemotherapy. It has been suggested that ABMT may have a role in management of patients with early CNS relapse who lack an histocompatible sibling donor.

Now that alternative donors are increasingly available BMT could theoretically become possible for any child with relapsed ALL. A large European survey including both adults and children with ALL compared the results of ABMT and transplants from unrelated donors. Leukemia-free survival was similar in the two groups, but transplant related mortality (TRM) was higher in the BMT group (42% vs 17%) while relapse was lower (32% vs 61%).

However, TRM from selected single centers is already similar in sibling and unrelated donor transplants and further general improvements in transplant related mortality (TRM) can be expected. Use of partially mismatched related donors further extends the potential access to allogeneic transplants.

There are many difficulties in comparing the outcome of BMT and chemotherapy for relapsed ALL, including the lack of randomized trials, selection biases and duration of time to transplant.

The largest study involved a comparison of data from the IBMTR and a cohort of patients treated with chemotherapy by the POG. A careful attempt was made to match chemotherapy and BMT patients: irrespective of the duration of first remission, BMT resulted in superior leukaemia-free survival when compared to chemotherapy.

Comparative data from Germany and Italy show similar results with a statistically significant benefit for BMT over chemotherapy in early marrow relapses. Review of the outcome for all unselected children relapsing after MRC UKALL X in the UK demonstrated that BMT reduced the risk of a second relapse with an absolute increase in 5-year EFS of 14%. However, when the outcome of BMT was compared with standardized intensive chemotherapy in UKALL R1, the overall EFS rates for chemotherapy, sibling donor BMT and unrelated donor BMT were similar.

A reasonable conclusion is that BMT is associated with a modest increase in leukaemia-free survival and that this may be most evident in children with a short early remission. The benefits may be reduced, or in some studies fully counter-balanced by the TRM- a factor assuming clear relevance with the increasing use of unrelated donors.

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