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Pregled
Review

CURRENT DIAGNOSIS AND TREATMENT OF CHILDHOOD LEUKEMIA

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In the past ten years, great success has been made in the diagnosis and treatment of malignant diseases in children, in particular in the management of childhood leukemia, so that most of these children can now be cured. The principles of diagnosis and treatment of childhood leukemia are presented.

Descriptors: LEUKEMIA – classification; DIAGNOSIS; THERAPY; CHILD, PRESCHOOL; CHILD; ADOLESCENT

INTRODUCTION

Leukemias are the most common malignant diseases in children accounting for 25%-30% of all childhood malignancies. Leukemias are classified as acute (95%-97%) or chronic (3%-5%) leukemia (1, 2), and subclassified according to their morphological, cytochemical, immunologic, cytogenetic and molecular genetics characteristics. Morphological classification of acute lymphatic leukemias (ALL), known as FAB (French-American-British) classification, distinguishes three morphological types of ALL: L1 (85%), L2 (14%) and L3 (1%). Acute myeloid leukemias (AML) are morphologically divided into 8 types, M0 to M7. Cytochemical tests are helpful in the subclassification of acute leukemias and include nonenzymatic methods such as PAS and Sudan black and enzymatic methods, i.e. peroxidase, alkaline phosphatase, esterases and acid phosphatase. ALL are mostly PAS positive, less commonly acid phosphatase positive (mostly immunologically T-ALL), and very infrequently undifferentiated (3). According to immunologic classification, ALL are divided into B-ALL (80%) and T-ALL (15%-20%); B group is subdivided into pro-B, common, pre-B and B-ALL, and T group into immature T, cortical T and mature T-ALL (4). Cytogenetic analysis of leukemia cells is essential during initial evaluation to establish the diagnosis and risk classification, and to help define the biology of the disease. Repeated analyses are recommended as needed after diagnosis to judge therapeutic response or to detect genetic evolution. The primary approach is conventional karyotyping, which can demonstrate chromosomal number (ploidy) and structural changes such as amplifications, deletions, translocations, or inversions. Additional genetic studies should be guided by the results of the initial karyotype and by the diagnosis suspected based on the clinical, morphological and immunophenotyping studies. New technologies have resulted in a large number of potential methodologies that may have clinical utility. Fluorescent in situ hybridization (FISH) uses fluorescently labeled nucleic acid probes to detect chromosomal abnormalities in metaphase and interphase cell chromosomes. This technique complements karyotyping through its ability to analyze many more leukemic cells (≥500 cells analyzed by FISH) than conventional karyotyping (≈20 to 40 metaphases in routine karyotyping). FISH probes designed specifically for chromosomes and translocations commonly implicated in neoplasms are a useful technique in monitoring for the presence of MRD. Polymerase chain reaction (PCR) techniques allow for selective amplification of defined DNA regions, and thus can be used to identify unique sequences specific to genetic abnormalities such as inversions, deletions, or translocations. When coupled with DNA sequencing, this technique can detect single base pair mutations in DNA and is highly sensitive, identifying a mutation in 100,000 BM cells. Abnormal RNA transcripts, resulting from chromosomal translocations, can be detected with a high level of sensitivity using reverse transcription PCR (RT-PCR). In addition, subclassification using genomic and RNA expression microarray and proteomic technologies will clearly have a major impact on the ability to classify leukemias and determine appropriate molecularly directed therapies in the future (4, 5). Cytogenetic variations have a major prognostic role in ALL patients; the expected rate of 5-year relapse free survival is 80% in patients with hyperploidy >50 chromosomes, 90% with 47-50 chromosomes, probably good with triploidy 66-73 chromosomes, 60% with tetraploidy 82-94, 80% with normal diploidy 46 chromosomes, <71% with hypodiploidy, 73% with pseudodiploidy, 53% with t(1;19), 53% with t(4;11) and 14% with t(9;22). Most authors agree that the major

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"prognostic" factors include age, leukocyte count, immunophenotype, cytogenetic finding, DNA index, organomegaly, presence or absence of central nervous system (CNS) disease, response to induction steroid therapy, and blast percentage in bone marrow on day 15 and 33 of treatment (5).

Cytomorphological, cytochemical, immunophenotyping, cytogenetic and molecular genetics analysis of cells obtained by bone marrow puncture is crucial in diagnostic work-up. Along with some other tests such as cerebrospinal fluid (CSF) analysis, it enables patient classification according to risk level into standard, medium and high risk groups. Each of these patient groups requires different, more or less intensive therapy (6-9).

TREATMENT

Current therapeutic protocols for ALL (BFM, COG, POG, AIEOP) consist of the following: (a) induction, i.e. introduction of remission (vincristine, pronisone or dexamethasone, L-asparaginase with or without anthracycline); (b) prophylaxis of CNS leukemia (intrathecal administration of methotrexate with or without cytosine arabinoside and hydrocortisone with medium (2 g/m^2) or high (5 g/m^2) doses of methotrexate, and prophylactic CNS irradiation (1200 Gy) for T-ALL and HR-ALL); (c) second induction for adjunctive remission reinforcement (attempting to destroy residual leukemia cells); and (d) remission maintenance therapy (continuing suppression of leukemia cell growth, further reduction of leukemia mass, and trying to prevent development of a resistant clone of leukemia cells). The treatment takes 24-36 months, depending on the therapeutic protocol applied (in male children usually 36 months due to the greater likelihood of the disease relapse). In high risk ALL patients (HR-ALL) / t(9;22) (BCR/ABL), t(4;11) (MLL/AF4), IKZF1 deletion, hypodiploidy, remission not achieved on day 33 of therapy; poor response to pronisone, high doses of cytostatics are used, followed by allogeneic transplantation of hematopoietic stem cells; if there is no compatible donor, then another induction therapy with prophylactic CNS irradiation and remission maintenance therapy (mercaptopurine, methotrexate) is continued for one more year. Currently, first remission is achieved in 99% of ALL and cure in 85% of standard risk, 75% of medium risk and 55% of high risk ALL patients (10, 11).

T-ALL accounts for 15%-20% of acute childhood leukemias. History is usually quite short, as initial symptoms occur 10-15 days before making the diagnosis. Older children (age 8-16 years) are usually involved, more commonly male. The disease is characterized by massive hepatosplenomegaly, tumor mass in anterior mediastinum, and an increased risk of CNS disease (at the time of diagnosis or in relapse) and testicular disease. Leukocyte count is significantly elevated, hemoglobin is frequently >10 g/dL; morphologically, it is usually of L2 type; acid phosphatase positive; immunologically T type (immature, cortical or mature); t(11;14), t(10;14), t(8;14), t(1;14), t(8;21) and t(15;17) translocations; molecular genetics variations: MYC-IGH, TTG2-TCRD. The patients enter first remission, however, with a high rate of systemic and extramedullary relapses. The prognosis is poor, yet significant improvement in therapeutic outcome has lately been reported with the use of aggressive chemotherapy and radiotherapy (12, 13).

B-ALL is extremely rare, accounting for 1%-2% of ALL. Morphologically, the L3 type is most common. Cytogenetically, the t(8;14), t(8;22) and t(2;8) translocations are detected. Until recently, the prognosis was very poor but has improved lately, now being comparable to other high risk ALL types. Treatment is the same as for Burkitt's lymphoma, i.e. a combination of monoclonal antibodies (anti CD20) and aggressive cytostatic therapy. In patients with ALL and t(9;22) translocation with extremely poor prognosis, protocols with a combination of cytostatic therapy, imatinib mesylate (Glivec, ST1571 inhibiting ABL tyrosine kinase) and transplantation of hematopoietic stem cells are used (14).

Infantile ALL (2%-3% of childhood ALL) is a biologically specific entity significantly varying from ALL in older children. Blasts have fetal characteristics; myeloid antigens are usually present, along with a relatively high resistance to cytostatics. On immunophenotyping, it is usually of the early pre-B, CALLA (CD10) negative type; mostly with t(4;11)(q21;q23) translocation associated with unfavorable prognosis, and MLL-AF4 genetic variation. Children younger than 12 months have poor prognosis and those younger than 6 months extremely poor prognosis. The rate of poor prognostic factors such as high leukocyte count, massive organomegaly, CNS leukemia and failure of complete remission after 14-day therapy (slow induction of remission) is high in these patients; almost all patients enter first remission (relatively late), however, with a high rate of systemic and extramedullary relapses. Infants with ALL require very intensive therapy. Most centers advocate allogeneic transplantation of peripheral stem cells once remission has been achieved. The rate of long-term event free survival (EFS) is approximately 30% (15).

Congenital leukemia is a very rare disease. It is diagnosed from the time of birth to 6 weeks of life; its etiology remains unknown. It is usually associated with trisomy 21, Turner syndrome, mosaic trisomy 9 and mosaic monosomy 7. Several cases of congenital juvenile myelomonocytic leukemia have been described. Clinical picture is predominated by subcutaneous leukemic nodules (leukemia cutis), hepatosplenomegaly, lethargy, pallor, purpura (petechiae) and respiratory distress. It is usually a monocytic subtype of AML and occasionally ALL (pre-B immunophenotype). In case of congenital leukemia in Down syndrome or congenital leukemia with normal karyotype, therapy should be delayed for as long as possible because spontaneous remission may occasionally occur; in case of disease exacerbation, chemotherapy should be introduced. Chemotherapy should be administered for congenital leukemia with chromosomal aberrations in tumor cells because this type of leukemia is associated with rapid progression and very poor prognosis (16, 17).

Relapse ALL - Some patients develop relapse of the disease, either as systemic or isolated relapse (CNS or testes), or as early (during cytostatic therapy or within 12 months of therapy completion) or late (more than 12 months of cytostatic therapy completion) relapse; it is more common in medium risk and high risk subtypes, and all this influences the choice of therapy and prognosis of disease. Protocols for ALL relapse are used in the management of relapse; the patients receive blocks of high doses of cytostatics and CNS radiotherapy, and in high risk group allogeneic transplantation of hematopoietic stem cells from a related compatible donor or, if unavailable, from unrelated compatible donor, or haploidentical transplantation(18-21).

AML - The age incidence of AML is constant except for the peak incidence in the neonatal period and slight increase in the incidence during adolescence. Many of the clinical features of AML are similar to those in ALL. WHO classification of AML defines that 20% blasts are required for the diagnosis of AML. Antibodies to cell surface proteins are useful in the diagnosis of AML and can be correlated with FAB subtypes. Fifteen percent of AML have a t(8;21)(q22q22), which is associated with FAB M2. The translocation creates an AML1-ETO fusion gene. The translocation seems to interfere with the expression of a myeloid-specific gene. A related myeloid transcription factor is also altered by the cytogenetic inv(16) and t(16;16), which occurs in 15% of AML cases. These cases are associated with myelomonocytic differentiation with abnormal bone marrow eosinophils and favorable progression. They result in a chimeric protein (CBFB-MYH11). Acute promyelocytic leukemia (APML) is associated with balanced translocation of the retinoic acid receptor α (RARα gene at 17q21) and the PML gene at 15q21. RARα is a transcription factor that binds retinoids and interacts directly with DNA. These molecular abnormalities have clinical importance. APML can be treated with trans-retinoic acid due to its binding to the RARα receptor. Detection of AML-ETO or CBFB-MYH11 is associated with a high rate of long-term remission after treatment with high-dose cytosine arabinoside. Gene expression profiling by microassay technology has been able to define distinct expression profiles of leukemias based on their genetic mutations. These profiles may in the future be able to uncover links between molecular subclass and clinical outcome that cannot be identified by standard cytogenetic analysis and clinical variables at present. Receptor tyrosine kinase mutations (FLT 3 mutations) have been described in pediatric AML. FLT 3 is a receptor tyrosine kinase that is highly expressed on myeloid blasts. FLT 3 internal tandem duplications (FLT 3-ITD) have been identified in up to 16.5% of pediatric AML patients. Patients with FLT 3-ITD mutations have a poorer prognosis. Patients with AML are classified in the standard or high risk group according to cytomorphological and cytogenetic subtype and therapeutic response (reduction of blast count in bone marrow after 15-day therapy). The following cytostatics have been most widely used in the management of AML: cytosine arabinoside (AraC), idarubicin (Ida), mitoxantrone, etoposide (VP) and thioguanine. Therapeutic protocol for AML consists of two inductions, consolidation, intensification, prophylactic CNS radiotherapy and remission maintenance therapy. In high risk AML, induction of remission and consolidation are followed by allogeneic transplantation of hematopoietic stem cells; if compatible donor is not available, intensive cytostatic therapy is continued, along with prophylactic CNS radiotherapy and remission maintenance therapy for another year (thioguanine with periodical monthly reinduction with cytosine arabinoside). The treatment takes 18-24 months, depending on the therapeutic protocol applied. Currently, first remission is achieved in 95% and cure in about 55% of children with AML, and in 65% of standard risk and 40% of high risk patients (22, 23). In case of disease relapse, protocols for disease relapse are used (combinations of high doses of cytostatics/mitoxantrone/AraC or HD AraC/Lasp, Ida/AraC, FLAG, Ida/FLAG and transplantation of hematopoietic stem cells, mostly allogeneic from related compatible donor or, if unavailable, from unrelated compatible donor, or haploidentical); in case of resistant disease, experimental therapy with new cytostatics and monoclonal antibodies (lestaurtinib/Ida-AraC; HDAC or clofarabine/AraC or bortezomib/Ida-AraC; bortezomib/AraC-etoposide) is administered (24).

Chronic myeloid leukemia (CML) is a rare disease of childhood; 3% to 5% of 100 leukemia affected children develop chronic leukemia that is nearly always of myeloid type. Two forms occur in children, i.e. juvenile and adult type, the latter being more common and similar to CML in adults. The adult type of CML develops through 3 stages: chronic, acceleration and blast crisis. In chronic stage of CML, imatinib mesylate (Glivec, ST1571, inhibiting ABL-tyrosine kinase) is most widely used for providing best results (hydroxyl urea and interferon used to be employed in the past); however, upon achievement of stable remission, transplantation of hematopoietic stem cells is necessary. Protocols for AML in combination with transplantation of hematopoietic stem cells have been used in children with juvenile type, unfortunately, with quite modest results (25).

Secondary tumor diseases are relatively rare in children treated for leukemia. The likelihood of such a disease is 0.9% in children treated with cytostatic therapy alone and up to 4% in children receiving cytostatic therapy and prophylactic CNS radiotherapy.

CONCLUSION

Although there has been dramatic progress in the treatment of ALL in the past half century, overall approximately 10% to 15% of patients with ALL and 35% to 45% of those with AML who have access to the most advanced therapies still die from their disease. In addition, a number of those who are cured have suffered significant acute toxicities and/or long-term adverse sequels. Hope for future progress lies in the improved understanding of the biology of leukemias that is likely to come from the application of new molecular and genomic technologies to the study of this disease, which will allow for more individualized therapy. As new therapies emerge, international cooperative trials will likely be required to confirm their efficacy with statistical certainty.

ABBREVIATIONS

ALL acute lymphatic leukemia
AML acute myeloid leukemia
CML chronic myeloid leukemia
FAB French-American-British
PAS acid phosphatase
CNS central nervous system
CSF cerebrospinal fluid
BM bone marrow
FISH fluorescent in situ hybridization
MRD minimal residual disease
PCR polymerase chain reaction
BFM Berlin-Frankfurt-Munster
AIEOP Associazione Italiana Ematologia Oncologia Pediatrica
COG Children's Cancer Group
POG Pediatric Oncology Group
HR high risk
SR standard risk
EFS event free survival
WHO World Health Organization
APML acute promyelocytic leukemia
AraC cytosine arabinoside
Ida idarubicin
VP etoposide
HD high dose
Lasp L-asparaginase
IZKF1 Ikaros Zinc Finger protein I

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S a ž e t a k

SUVREMENA DIJAGNOSTIKA I LIJEČENJE LEUKEMIJA U DJECE

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Posljednjih desetak godina došlo je do velikog napretka u dijagnostici i liječenju dječjih zloćudnih bolesti. Najveći napredak postignut je u liječenju dječjih leukemija, pa se danas većina djece s leukemijom može izliječiti. U radu se opisuju načela dijagnostike i liječenja dječjih leukemija.

Deskriptori: LEUKEMIJA – klasifikacija; DIJAGNOZA; TERAPIJA; DIJETE, PREDŠKOLSKO; DIJETE; ADOLESCENT

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