This review focuses on the most recent advances in the diagnostic and prognostic work-up of adult acute lymphoblastic leukemia (ALL), and on their implications in the clinical management of the disease. Over the years, information obtained through extensive immunophenotyping, karyotyping, molecular genetics, multidrug resistance and, more recently, genomic profiling is progressively contributing to a better understanding of the biology of this complex disease, to the identification of subgroups of patients with different clinical outcomes, to a more precise monitoring of minimal residual disease, to the use of different therapeutic protocols based on prognostic indicators and, finally, to the design of innovative and specific treatment strategies. The next few years will tell us if this biologically-guided approach, which is progressively individualizing the management of adult ALL patients, will ultimately impact on the prognosis of a disease that has stagnated over many decade. Rev. Bras. Hematol. Hemoter. 2009;31(Supl. 2):41-47.

**Key words:** Acute lymphoblastic leukemia; chemotherapy; prognostic factors.

**Introduction**

Adult acute lymphoblastic leukemia (ALL) represents a biologically and clinically heterogeneous group of diseases characterized by the abnormal proliferation and accumulation of immature lymphoid cells within the bone marrow and lymphoid tissues. Increasing evidence suggests that chromosomal defects and molecular abnormalities are consistently present in patients with ALL, and progress in our understanding of the biological and genetic characteristics of ALL has not only improved our knowledge of leukemogenesis, but has also allowed the identification of prognostic groups with specific cellular and molecular features. For all newly diagnosed cases of ALL, a broad and integrated biological work-up, which includes immunologic, cytogenetic and molecular analysis, and seeks to identify the prognostic factors is needed as is the definition of suitable markers for the monitoring of minimal residual disease (MRD) during the course of the disease and to design therapeutic strategies tailored according to the biological characteristics of the leukemic cells.1-7

**Diagnosis**

Currently, the diagnosis and classification of ALL is a multistep procedure that relies on the simultaneous application of several techniques that investigate: morphology, cytochemistry, immunophenotype, cytogenetics, molecular genetics, immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements and genomic profiling, as an integrated research effort.8,9

**Morphology and immunophenotype**

According to the French-American-British (FAB) cooperative group classification system,10 ALL is characterized by the presence of more than 30% of lymphoblasts in the bone marrow (BM), while the World Health Organization (WHO) classification scheme11 established the cut-off at 20%. No specific morphologic/cytochemical tests are exclusive for ALL; however, by definition, ALL is negative for myeloperoxidase (MPO) both by cytochemistry and by anti-MPO monoclonal antibody (MoAb) staining.
The immunophenotype is an essential component in the initial diagnostic work-up of ALL and is also a valuable tool for monitoring disease during treatment and thus for the detection of MRD. The immunophenotypic characterization of blast cells at presentation has several objectives: a) lineage assignment, b) evaluation of cell maturation, and c) assessment of phenotypic aberrations. Flow cytometry is a powerful technique for the characterization of normal and neoplastic hematopoietic cells. The use of highly specific MoAbs capable of recognizing distinct epitopes of surface and intracellular antigens has improved the definition of the origin and level of differentiation of acute leukemias. It is customary to report percentage of blasts expressing each antigen tested and to consider any marker present on more than 20% of blasts as positive: the cut-off level of 20% is arbitrary, however.

A panel of antibodies is needed to establish the diagnosis (Table 1) and to distinguish among the immunological subclasses. The scoring system proposed by the European Group for the Immunological Characterization of Leukemia (EGIL) classified acute leukemias as B- or T-lineage ALL, or as acute myeloid leukemia (AML) by including the most specific markers of the lymphoid and myeloid lineage among those of early stages of differentiation, plus some non-specific but stem cell-associated markers. On the basis of these immunophenotypic analyses, a firm diagnosis can be made in 99% of cases. About 75% of cases of adult ALL are of B-cell lineage and 25% of T-cell lineage. Although the affiliation of ALL to the B- or T-cell lineage is relatively easy, a few cases remain difficult to classify as ALL or AML; these cases co-express lymphoid and myeloid antigens, either on the same cells (biphenotypic leukemia) or on two different populations (hybrid leukemia). There is no consensus regarding the diagnostic criteria for such cases. The EGIL group has suggested the use of a scoring system based on different combinations of B, T and myeloid antigen expressions. According to a strict scoring system, four groups might have been suggested the use of a scoring system based on different combinations of B, T and myeloid antigen expressions. According to a strict scoring system, four groups might have included the EGIL European Group.
single cell exceeds 46. In ALL, this process seems to be non-
random. Hyperdiploidy is seen in 15% of cases of adult ALL
and the association with a favorable outcome is less obvious
than in childhood ALL, where it occurs in about one third of
cases. Hypodiploidy (chromosomes < 46) is found in 2.8% of
cases of ALL and is associated with a poorer outcome. The
majority of chromosomal abnormalities found in ALL are
structural, usually translocations. More than 30 different
nonrandom translocations have been identified in ALL. As
only a relatively limited number of patients have so far been
studied and many of these translocations are uncommon, the
prognostic implications for most of them have still to be
cursively defined. Most of the more common karyotypic
structural rearrangements have been studied at the molecular
level. In molecular terms, chromosomal abnormalities or their
submicroscopic equivalents are of two general types: those in
which the breakpoint occurs within the involved genes leading
to the production of a fusion RNA transcript and a chimeric
protein (qualitative change), and those which represent Ig/
TCR rearrangement errors (quantitative change). Qualitative
abnormalities produce functional fusion genes: the most
common is the t(9;22)q(34;q11) translocation which forms the
BCR-ABL fusion gene; another is the t(1;19) q(23;p(13)
translocation, where the E2A gene fuses with PBX1. The
rearrangement involving the MLL gene on chromosome 11 in
the q23 region results in a fusion gene with AF4 on
chromosome 4q21; furthermore, several different partners
can fuse to the MLL gene. Quantitative abnormalities result
from Ig/TCR rearrangement errors which juxtapose the
proto-oncogene to regulatory Ig/TCR sequences, leading
to deregulated protein expression, for example the SIL-TAL1/
tald deletions on chromosome 1p32 in T-ALL. Qualitative
fusion transcripts predominate in B-lineage ALL and
recombinant errors are rare; in contrast, they are much more
frequent in T-ALL, where they represent the majority of
molecular abnormalities. Other lesions recently identified
in T-ALL include Notch1 and JAK1 mutations, TLX1
(HOX11), TLX3 (HOX11L2), TAL1 and LYL1 over-
expression, and ABL1 rearrangements. Intriguingly, the
role of mutations of the JAK genes (JAK1, JAK2 and JAK3)
is emerging in ALL, since JAK2 mutations were recently
described in B-lineage ALL; this finding has obvious
potential therapeutic implications, in light of the available
JAK2 inhibitors.

A list of the main molecular genetic abnormalities
identified in ALL and currently used for molecular diagnosis
is reported in Table 2, even if this list is not exhaustive and
represents a compromise between the current most
appropriate molecular method to detect or exclude an
abnormality and the most widely used technique.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Abnormality</th>
<th>Genes involved</th>
<th>Incidence</th>
<th>Molecular detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-ALL</td>
<td>t(9;22)(q34;q11)</td>
<td>BCR ABL</td>
<td>Adults: 30% Children: 3%</td>
<td>RT-PCR</td>
</tr>
<tr>
<td></td>
<td>t(12;21)(p13;q22)</td>
<td>TEL AML1</td>
<td>Adults: &lt;1% Children: 20%</td>
<td>RT-PCR</td>
</tr>
<tr>
<td></td>
<td>t(4;11)(q21;q23)</td>
<td>MLL AF4</td>
<td>Adults: 5% Infants: 60%</td>
<td>RT-PCR</td>
</tr>
<tr>
<td></td>
<td>t(1;19)(q23;p13)</td>
<td>E2A PBX1</td>
<td>5%</td>
<td>RT-PCR</td>
</tr>
<tr>
<td></td>
<td>t(8;14)(q24;q32)</td>
<td>c-MYC IgH</td>
<td>1%</td>
<td>FISH</td>
</tr>
<tr>
<td></td>
<td>t(17;19)(q22;p13)</td>
<td>E2A HLF</td>
<td>&lt;1%</td>
<td>RT-PCR</td>
</tr>
<tr>
<td></td>
<td>t(11;19)(q23;p13)</td>
<td>MLL ENL</td>
<td>&lt;1%</td>
<td>RT-PCR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JAK1/2/3 mutations</td>
<td>10%</td>
<td>Sequencing</td>
</tr>
<tr>
<td>T-ALL</td>
<td>t(10;14)(q24;q11)</td>
<td>HOX11 TCR αβ</td>
<td>Adults: 31% Children: 7%</td>
<td>RT-PCR</td>
</tr>
<tr>
<td></td>
<td>t(7;10)(q34;q24)</td>
<td>HOX11 TCR β</td>
<td>Adults: 13% Children: 20%</td>
<td>RT-PCR, FISH</td>
</tr>
<tr>
<td></td>
<td>t(5;14)(q35;q32)</td>
<td>HOX11L2 TCR β</td>
<td>Adults: 31% Children: 7%</td>
<td>RT-PCR</td>
</tr>
<tr>
<td></td>
<td>t(1;14)(p32;q11)</td>
<td>TAL1 TCR αβ</td>
<td>1-3%</td>
<td>RT-PCR</td>
</tr>
<tr>
<td></td>
<td>Normal 1p32</td>
<td>SIL TAL1</td>
<td>9-30%</td>
<td>RT-PCR</td>
</tr>
<tr>
<td></td>
<td>inv(17)(p15q34), t(7;7)</td>
<td>HOXA genes TCR β</td>
<td>5%</td>
<td>FISH, RT-PCR</td>
</tr>
<tr>
<td></td>
<td>t(10;11)(p13;q14-21)</td>
<td>CALM AF10</td>
<td>10%</td>
<td>FISH</td>
</tr>
<tr>
<td></td>
<td>t(9;9)(q34;q34)</td>
<td>NUP214 ABL1</td>
<td>6%</td>
<td>FISH</td>
</tr>
<tr>
<td></td>
<td>t(9;14)(q34;q34)</td>
<td>EML1 ABL1</td>
<td>&lt;1%</td>
<td>FISH</td>
</tr>
<tr>
<td></td>
<td>NOTCH1 mutations</td>
<td>NOTCH1</td>
<td>50%</td>
<td>Sequencing</td>
</tr>
<tr>
<td></td>
<td>JAK1 mutations</td>
<td>JAK1</td>
<td>18%</td>
<td>Sequencing</td>
</tr>
</tbody>
</table>
Identification of recurring cytogenetic abnormalities and molecular alterations in ALL has had a major impact on risk assessment and a number of structural and chromosomal changes have been incorporated into existing classification systems.

**Genome-wide approaches**

Genomic profiling is becoming a reality that may affect our approach to ALL patients. The potential exploitation of microarray analysis can be summarized in 5 points: 1) it can define the genetic signature of neoplastic populations; 2) it can define the lineage affiliation; 3) it can identify sets of genes that characterize subsets of patients with distinct responses to treatment and, ultimately, have a prognostic impact; 4) it may identify potential therapeutic targets; finally, 5) it may allow the identification of drug susceptibility or resistance. In both childhood and adult ALL, gene expression profiling has revealed distinct gene expression patterns in specific subtypes.\(^{28,29}\) Hierarchical clustering of all adult ALL samples based on gene expression profile has identified two well-defined groups, which correlate with the T- or B-cell lineage of the leukemic cells. Within B-lineage ALL, further analyses have identified gene expression profiles associated with the presence of the ALL1-AF4, BCR-ABL or E2A-PBX1 gene rearrangements.\(^{30}\) Remarkably, gene signatures are extremely similar between cases that harbor specific rearrangements regardless of age. Within T-lineage ALL, gene expression profiling has recently allowed the identification of specific signatures that are associated with molecularly defined aberrations, and in particular with MLL rearrangements, SIL-TAL1 aberrations, HOX11 and HOX11L2 aberrations, CALM-AF10, inv(7) and SET-NUP214; the three latter resulting in the deregulation of HOXA genes. Furthermore, in T-ALL the wide use of gene expression profiling is proving helpful in identifying a handful of distinct subsets, which may have important prognostic implications. The identification of sets of genes associated with induction treatment and overall survival highlights the role of this approach in patient stratification. In addition, gene profiling can identify genes that may be the target of specific therapeutic strategies. Finally, gene expression profiling may be useful in a diagnostic setting, as shown by the international MILE (Microarray Innovations in LEukemia) project, that enrolled more than 3000 patients with acute and chronic leukemias worldwide, and proved highly sensitive in disease classification (95.6% median sensitivity and 99.8% median specificity).\(^{31}\)

Additional important findings are being seen with the use of SNP (Single Nucleotide Polymorphism) arrays, which allow the identification of copy number alterations and, in some instances, Loss of Heterozygosity (LOH). Among the lesions that have been identified using this approach, it is worth mentioning that deletions of Ikaros, that have been reported in both adults and children, are associated with BCR/ABL1 rearrangements and with a dismal prognosis in both cohorts of patients.\(^{32,33}\)

**Minimal residual disease**

One of the most important challenges in leukemia treatment is to accurately distinguish patients who require more intensive (and potentially more toxic) therapy from those for whom cure rates can be achieved with less intensive therapy. MRD studies can provide a direct measurement of the degree and rapidity of leukemic cell responses to chemotherapy and to estimate the amount of residual tumor rather than just to establish its presence; this information can be used to improve strategies of risk assessment and treatment selection in the management of ALL patients.\(^{34-38}\)

Leukemia cells can be potentially distinguished from normal hematopoietic progenitors on the basis of morphological and cytochemical properties, immunophenotype, karyotypic or genetic abnormalities, and Ig/TCR gene rearrangements. These different characteristics have been exploited in an attempt to detect small numbers of blasts within normal cells and a variety of techniques have been developed for the detection of residual disease.\(^{12,13,24,39}\) The greatest limitation in the routine use of MRD studies in ALL therapy protocols is that none of the techniques currently available for MRD detection can be applied to all patients. Because PCR may detect residual leukemic cells in cases not amenable to flow cytometric investigation, and vice-versa, it is possible to apply the two techniques in tandem.

In ALL, MRD can be studied utilizing techniques that enable the identification of leukemic cells with a sensitivity of \(10^{-6}\):

1) flow-cytometric immunophenotyping, using aberrant or patient-specific phenotypes;
2) RT-PCR and RQ-PCR analysis of breakpoint fusion genes;
3) RT-PCR and RQ-PCR analysis for the detection of clone-specific Ig and/or TCR gene rearrangements.

In adult ALL, prospective studies with MRD-based risk stratification are ongoing. As in children, a very good response documented by an early and rapid decrease of MRD during induction, may be associated with a low(er) relapse risk in adult ALL patients. However, in general the decrease of MRD occurs slower in adults than in children and few patients reach a negative MRD status. Nevertheless, before using MRD data to guide therapy, further analyses are required to conclusively establish the predictive value of MRD findings.

**Prognostic factors**

The diagnostic methods aimed at defining ALL subgroups with a different prognostic likelihood have
substantially broadened in recent years. Adult ALL are generally divided into only two groups: a standard risk group and a high-risk group. The presence of BCR-ABL and of MLL rearrangements represents a well-established unfavorable prognostic factor. At variance, there are still different issues to be clarified in a further attempt to incorporate risk stratification in adult cases: 1) the time point of risk stratification, 2) the combination of MRD and conventional risk factors, and 3) treatment decisions based on risk stratification. A useful measure in risk assessment is the rate of clearance of leukemic cells from the bone marrow during the early phase of therapy. Another approach is to use PCR or immunological methods to measure MRD soon after the induction of clinical remission. Thus, while risk classifications are today based on clinical features of patients and on the characteristics of the leukemic cells, the new risk stratification algorithm of adult ALL is more complex and is based on:

- earlier "conventional parameters", such as clinical characteristics including age, white blood cell count, organ involvement (e.g. CNS, mediastinum, extramedullary involvement);
- immunophenotype;
- cytogenetic aberrations and molecular genetics;
- monitoring of MRD.

**Young adult patients with ALL**

Young adult patients between 15 and 20 years of age with ALL represent a unique epidemiologic group in that they may be treated by either adult or pediatric hematologists. Recent data suggest that the outcome of this subgroup of patients is markedly improved if they are treated on intensive pediatric ALL protocols rather than on less intensive adult ALL protocols. There seems to be no significant differences in presenting clinical features, immunophenotypic characteristics, or cytogenetic abnormalities for young adult patients treated on pediatric or adult protocols. Nevertheless, adolescents treated in adult trials appear to have a significantly higher risk of treatment failure resulting, in most cases, in resistant disease. Why this occurs is still unclear; it could be explained by the different treatment modalities or differences in therapeutic practices. A reasonable strategy for this category of patients is to develop and implement age-unrestricted but disease-specific treatment protocols, or simply to utilize pediatric protocols.

**Conclusions**

Clearly, the overall approach to the management of ALL in all ages of life has changed remarkably over the last few years and is continuously evolving. This largely stems from advances in the biological characterization of the leukemic cells. This is gradually leading to a more targeted and individualized clinical management of patients. This has translated, among other things, into: a) an always better prognosis for younger children; b) stratification of childhood ALL based on the degree of MRD response; c) an improved outcome for adolescents and young adults treated with aggressive pediatric-like protocols; d) investigation of the use of a pediatric like approach in older adult patients (up to what age?); e) an incorporation of MRD monitoring into adult ALL protocols; f) use of tyrosine kinase inhibitors as front line treatment for Ph+ ALL; g) the possibility of managing elderly Ph+ ALL patients with such compounds alone; h) a broader clinical use of MoAbs; i)...

It is easy to foresee that the continuous refinement of technological tools, aimed at an ever more sophisticated characterization of ALL cells, will progressively lead to an ever greater use of targeted strategies in the management of patients of all ages suffering from ALL.

**Resumo**

Esta revisão focaliza os mais recentes avanços no diagnóstico e prognóstico da leucemia linfoblástica aguda do adulto e suas implicações no manuseio clínico desta doença. Com o passar dos anos, informações obtidas através de extensa pesquisa em imunofenotipagem, citogenética, genética molecular, resistência a múltiplas drogas e, mais recentemente, perfil genômico têm contribuído progressivamente para o melhor entendimento da biologia desta doença complexa, na identificação de sub grupos de pacientes com evolução clínica distintas, no mais preciso monitoramento da doença residual mínima, no uso de diferentes protocolos baseados em indicadores prognósticos e, mais recentemente, também no desenho de tratamentos inovativos e específicos. Os próximos anos nos dirão se abordagens baseadas guiadas biologicamente, que será uma individualização progressiva do manuseio dos pacientes adultos com LLA podem causar um impacto favorável em uma doença estagnada há várias décadas. Rev Bras Hematol Hemoter 2009;31(Supl. 2):41-47.

**Palavras-chave**: Leucemia linfoblástica; quimioterapia; fatores prognósticos.

**References**


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