Leukemia: genetics and prognostic factors

Nelson Hamerschlak*

Abstract
Objective: To present the implications of genetics, particularly of cytogenetic techniques, for the diagnosis and prognosis of leukemia.

Sources: A survey of articles selected from MEDLINE, American Society of Hematology educational programs, the CAPES web portal, the National Comprehensive Cancer Network and textbook chapters.

Summary of the findings: Since the discovery in 1960 by Peter C. Nowel and David Hungerford of the 9:22 translocation (the Philadelphia chromosome), genetics has come to play an important role in hematology, in this case making it possible to diagnose chronic myeloid leukemia and opening doors to research avenues for the whole field of oncology. One point of great interest refers to the implications of these findings for the prognosis of a range of types of leukemia. In acute myeloid leukemia, the karyotype is of fundamental importance to postremission treatment decisions, and molecular factors determine the treatment of individuals with normal karyotypes. In chronic myeloid leukemia, clonal evolution is associated with progression to the blast crisis. Patients on imatinib who cease responding may have mutations on their ABL gene. Finally, in acute lymphoblastic leukemia, factors such as hyperdiploidy and t 12:21 are associated with good prognosis, whereas carriers of t 4:11 and t 9:22 are considered high risk patients.

Conclusions: Genetics has come to stay as far as hematology and, in particular, the management of leukemia and its prognostic factors are concerned. These tests should always be carried out and the appropriate treatment adopted in the light of their results, so that optimal patient outcomes can be achieved.


Introduction

In 1960, Peter C. Nowell, of the University of Pennsylvania School of Medicine, and David Hungerford, of the Fox Chase Cancer Center's Institute for Cancer Research described a translocation of chromosomes 9 and 22 (the Philadelphia chromosome, or Ph). This was the first gene described as causing cancer and was recently an important element in the development of the drug imatinib, which is the first medication to be developed with a genetic target.1-3 Since then, genetics has had an ever growing importance in the diagnosis, prognosis and treatment of leukemia.

Practically all types of leukemia have prognostic factors that are determined by cytogenetics; more specifically, by acquired mutations that, once detected, make it possible to define the appropriate treatment for a given patient.

This article is not intended to exhaust the subject, but to identify the principal cytogenetic mutations that are found in different types of leukemia. For the purposes of this article, the leukemia subtypes will be classified as acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL).

Acute myeloid leukemia

This form is characterized by uncontrolled and exaggerated growth of undifferentiated cells, called blasts, with myeloid characteristics. In the majority of cases of this form there is no obvious cause. However, in some patients the disease can be related to exposure to benzene, ionizing radiation, as was the case with Hiroshima victims, or exposure to chemotherapy. Fanconi anemia and Down Syndrome may be associated with the emergence of AML. Incidence is 1/150,000

* Coordenador, Programa de Hematologia e Unidade de Transplantes de Medula Óssea, Hospital Israelita Albert Einstein, São Paulo, SP, Brazil. Médico, Clínica de Especialidades Pediátricas, Hospital Israelita Albert Einstein, São Paulo, SP, Brazil.

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during childhood and adolescence. A variety of cell types can be observed in blood and bone marrow from AML patients. This makes it possible to arrive at a sub-classification comprised of eight subtypes: M0 and M1, immature myeloblastic; M2, mature myeloblastic; M3, promyelocytic; M4, myelomonocytic; M5, monocytic; M6, erythroleukemia; and M7, megakaryocytic. This morphological and immunophenotypical classification has prognostic implications, as do age, clinical conditions and, primarily, cytogenetic factors. The majority of patients report tiredness and shortness of breath during physical activity, pallor and signs of bleeding such as skin blemishes, bleeding from mucosa, the nose and other locations. Furthermore, fever and infections are common findings, together with painful bones. A diagnosis of AML is made by analyzing the appearance of cells under a microscope and identifying the blasts. The material obtained from blood and/or bone marrow should also undergo immunophenotyping and the number and appearance of the chromosomes should be analyzed (cytogenetic study). The chromosome analysis is particularly useful when prescribing the type of treatment and for analyzing the prognosis of each case. Two courses of treatment during this phase are cytarabine or Aracytin for 7 to 10 days and idarubicin or daunorubicin. Generally, two courses of treatment are given during this phase. In cases of promyelocytic leukemia or M3, a drug called all-trans retinoic acid is given orally as an additional treatment. The drug helps the leukemia cells of this AML subtype to mature. Postremission treatment depends on the age of the patient, clinical conditions and, primarily, on the results of cytogenetic studies, and can vary from increasing the intensity of the chemotherapy doses during one or more cycles to the use of one of several different donor marrow transplantation techniques (autologous or allogeneic).

Recently, a system was established for classifying cytogenetic risk, defining patient prognosis as favorable, intermediate or poor based on cytogenetic findings on diagnosis (Table 1).

Patients with t (15:17) have excellent prognosis with regimes using all-trans retinoic acid or arsenic trioxide with chemotherapy. Other cases with good prognosis are also not indicated for bone marrow transplantation. However, cases where prognosis is poor should be referred for transplantation. Patients with the normal karyotype are in the majority, and are classified as having intermediate prognosis. They do not respond uniformly to chemotherapy, even when more intensive. The probable reason for the diversity in response is the heterogeneous molecular nature of patients with AML and normal cytogenetic findings. Over the last 10 years, several different studies have demonstrated that the presence or

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absence of specific genetic mutations or changes in gene expression have a direct effect on patient prognosis.\textsuperscript{10,11} Although the majority of studies of the subject were undertaken with young adults, some of the characteristics they describe have also been demonstrated in children.\textsuperscript{12-14}

At the current point in time, the most significant prognostic factor found among patients with normal cytogenetic findings is a tandem duplication of the FLT3 gene. The presence of this mutation confers worse prognosis, with relation to its absence. Other molecular markers also influence the progress of AML patients with normal cytogenetic findings. The following genetic anomalies are favorable: NPM1 and CEBPA mutations; and, in addition to FLT3, MLL-PTD and overexpression of BAALC and ERG are unfavorable.\textsuperscript{11,13,14}

Another important factor to take into account, particularly in cases where there is \( t(8;21) \) and inversion of chromosome 16, is that other chromosome anomalies that are frequently found in combination with these mutations appear to modify prognosis. On the other hand, these patients can often express mutations to the KIT gene. Current studies have attempted to establish the true role of this abnormality in prognosis. These studies have demonstrated, primarily in adults, that the presence of mutations on c-kit is associated with a greater probability of relapse. These results still require further validation in children.

**Chronic myeloid leukemia**

As was mentioned in the introduction to this article, CML is characterized by the presence of an acquired genetic abnormality, which has been named the Ph chromosome. The Ph chromosome is an abnormality that involves chromosomes 9 and 22. On the molecular level, this merging of parts of different chromosomes is called BCR-ABL.\textsuperscript{1,2}

The causes leading to this anomaly are generally unknown. In a small proportion of cases, the disease may be related to radiation exposure. This was made relatively clear by studies carried out in Japan with survivors of the atomic bomb. It was found that this population had an increased risk of leukemia and other types of cancer.\textsuperscript{15}

In CML, in contrast with what we described with relation to AML, the abnormal cells generally function adequately, allowing a milder initial course of the disease, in comparison with acute cases.

The majority of CML cases are in adults. The frequency of this type of leukemia is 1 per 1 million children up to the age of 10 years. Among adults, the frequency is around 1 in 100,000 individuals.\textsuperscript{15}

The signs and symptoms of CLL generally have an insidious onset. Many patients are diagnosed by chance during clinical examinations or blood tests carried out for a range of reasons or as part of routine checkups.

Patients may describe tiredness, pallor, diaphoresis, weight loss and discomfort on the left side of the abdomen due to an enlarged spleen.

In the majority of patients, CML progresses to a more turbulent phase which is more difficult to control, known as the accelerated phase. In this phase the spleen becomes enlarged even further and there is an increase in the number of immature cells, i.e., blasts.

Finally, the disease evolves to what is known as the acute or blast crisis, when blast cells predominate in bone marrow and blood. In approximately 25% of the patients, this stage manifests itself as though it were ALL, whereas, in the remaining 75%, it manifests as AML.\textsuperscript{16}

This disease can be diagnosed on the basis of a blood test and can be confirmed by bone marrow studies. On observation of the cells, there is a large proportion of mature white cells with relation to the number of immature cells (blasts).

Furthermore, it is generally possible to detect chromosome abnormalities in material taken from bone marrow. Techniques such as FISH or PCR are more sensitive, and not only important for diagnosis and assessment of response to treatment, but also for differentiation from other myeloproliferative diseases with similar presentation.\textsuperscript{16}

During recent years, a revolution has taken place in CML treatment with the emergence of tyrosine-kinase inhibitors. Imatinib was the first of these to be approved by the Food and Drug Administration (FDA), in the United States, and by the National Agency for Sanitary Vigilance (Agência Nacional de Vigilância Sanitária - ANVISA) in Brazil. It has elicited startling hematological and cytogenetic responses, only previously demonstrated after bone marrow transplantation. This medication is nowadays the standard treatment. It works best during the earliest phases of the disease, reducing in efficiency to the extent that leukemia progresses to the accelerated and blast phases.\textsuperscript{17,18}

Nowadays, and particularly with children, the decision making process on whether to treat this type of leukemia with imatinib or go for bone marrow transplantation should be made in conjunction by the doctor and the patient’s family, after the patient has received adequate information and an analysis has been made of donor availability and compatibility and of the risk factors involved.

Overexpression of BCR-ABL, clonal evolution and mutations of the ABL gene have been described as being primary and secondary resistance mechanisms to the action of imatinib.

Newer medications, dasatinib and nilotinib, both approved by the FDA, are used when patients are refractory or intolerant of imatinib, but their use with children is only just beginning and clinical trials are still being carried out. Their use as a first treatment option is also reserved, at least for the moment, to ongoing clinical trials.\textsuperscript{19,20}
The genetic features that are relevant to CML prognosis are based on clonal evolution, i.e., the possibility that new clones can appear, which generally determines progression to blast crisis and the mutations that confer resistance to imatinib. Of these mutations, T3 151 stands out because it does not allow patients to respond to the new medications either.21,22

Patients with CML should be monitored with karyotype studies until the Ph chromosome disappears, and thereafter using quantitative molecular tests for BCR-ABL with PCR. It is recommended that karyotyping be performed, even after normalization, at least once a year in order to detect clonal evolution that may represent an imminent blast crisis early enough. Such patients should be referred for bone marrow transplantation, even though it is not absolutely clear that progress to blast crisis is obligatory.

The isochromosome i (17q) is the most commonly occurring (20% of blast crises), causing loss of p53 (tumor suppressor gene). It is also believed that other genes may be involved in this mechanism, which is as yet little understood. Trisomy of chromosome 8 has also been described and related to overexpression of the Myc gene. Translocations are rare, but both t (3:21) and t (7:11) have been described. Anomalies have also been observed in the Ph chromosome. Duplication of Ph occurs in more than 30% of blast crises, and loss of chromosome 9 (der 9) occurs in 5 to 10% of cases. It is not yet clear whether the der 9 abnormality affects the prognosis of patients treated with imatinib or those given bone marrow transplantation.21-23

If follow-up with quantitative PCR and/or karyotyping demonstrates a decrease in the cytogenetic response of patients using imatinib, it is necessary to sequence the ABL gene in order to detect mutations. Among possible mutations, T315I does not respond to the newer tyrosine-kinase inhibitors. This being so, this prognostic factor is important for early indication of bone marrow transplantation, since specific medications are still undergoing clinical trials.24,25

Acute lymphoblastic leukemia

Acute lymphoblastic leukemia results from uncontrolled production of blasts with lymphoid characteristics and from a block on normal production of red and white cells and platelets. In the majority of cases the cause of ALL is not obvious. It is also believed that these cases have some type of relationship with radiation since they increased in number in Japan after the war.

Acute lymphoblastic leukemia develops from primitive lymphocytes, which may be found in varying stages of development. The main classification method is immunophenotyping. Cytogenetic studies are also an important methodology here.26,27

Complete treatment for ALL should take into account immunophenotyping, cytogenetic studies, initial blood cell counts, clinical conditions, and involvement or not of nervous system, testicles and glands.

The signs and symptoms of ALL are very similar to those of AML, such as tiredness, shortness of breath, signs of bleeding, infections and fever. Additionally, swollen glands, inflammation of the testicles, vomiting and headaches suggestive of nervous system involvement may occur.

With this type of leukemia, diagnosis is also made by microscopic analysis of blood and bone marrow, immunophenotyping and cytogenetic studies. Nervous system involvement should be investigated by means of cerebrospinal fluid assays.26,27

Treatment is with chemotherapy. Patients must be treated as soon as diagnosis is confirmed, and the initial objective is once more remission with restoration of normal production of red and white blood cells and platelets.

In ALL treatment, a combination of several different drugs is used to control the disease. It is important to correctly choose the best treatment regime and sequence in order to guarantee the best possible chances of cure. Nowadays, more than 70% of children with this type of disease can be cured, as can around 50% of young adults. However, best results are achieved by chemotherapy regimes based on age, clinical status, laboratory results and response to initial treatment.26,27

The presence of unfavorable prognostic factors or relapse of the disease should lead to a more aggressive approach, meaning the different bone marrow transplantation modalities.28 One cause of unfavorable prognosis, which occurs in 5% of childhood ALL and 25% of adult cases, is presence of the Ph chromosome. In these cases, using tyrosine-kinase inhibitors together with chemotherapy and transplantation may be useful, since in isolation they have demonstrated poor results.

The initial phase of treatment is called induction and should include treatment or prevention of the disease in the central nervous system, which involves intrathecal chemotherapy.

Once remission has been achieved, patients undergo cycles of postremission chemotherapy and will go on to use chemotherapy medication, generally orally, as maintenance for 2 to 3 years.

To give an idea of the complexity of treating ALL, we shall give some examples of drugs and treatment used during induction and postinduction: intravenous doxorubicin, intramuscular asparaginase, intravenous vincristine, oral prednisone, intrathecal hydrocortisone, oral, spinal and intravenous or intramuscular methotrexate, intravenous and spinal cytarabine, oral 6 mercaptopurine, nervous system irradiation and tyrosine-kinase inhibitors in cases where the Ph chromosome is present.
The diversity in prognosis of children with ALL can be primarily attributed to resistance to the drugs employed or specific resistant blast cells with specific genetic abnormalities.28

Favorable genetic abnormalities that are associated with B precursors involve hyperdiploidy (more than 50 chromosomes) and the fusion of TEL-AML1 or t (12;21). Hyperdiploidy makes blasts more sensitive to chemotherapy. It is believed that this fact is related to in vitro findings of these cells undergoing spontaneous apoptosis and being sensitive to accumulation of high doses of methotrexate. Hyperdiploid patients have three to four copies of chromosome 21, which carries a gene which codes reduced folate transport. Patients with TEL-AML1 fusion are highly sensitive to asparaginase for reasons that are not yet clear. Studies carried out by the US Children’s Oncology Group have shown that trisomy of chromosomes 4, 10 and 17 may also be associated with good prognosis. The UK Medical Research Council found associations between good prognosis and trisomy of chromosomes 4 and 18. Another interesting fact is related to t(1;19) / E2A-PBX1 fusion, which is associated with poor prognosis when conventional chemotherapy doses are used, but with excellent results when aggressive regimes are used (event-free survival > 90%).

Patients with Ph chromosome or t (4:11)/MLL-AF4 fusion have extremely high-risk disease. There is, however, a marked influence of age. In the case of the Ph chromosome, prognosis is very bad for adolescents and young adults who are not treated with a combination of chemotherapy and tyrosine-kinase inhibitors, but relatively mild in children aged 1 to 9 years of age with low leukocyte counts on diagnosis. The MLL-AF4 fusion is indicative of much worse diagnosis for children less than 1 year old, compared with those over 1 year.

Recently, gene expression profile studies have shown that almost all cases of type T ALL can be classified based on the involvement of one or more oncogenes: LYL1 with LMO2, HOX11, TAL1 with LMO1 or LMO2, HOX11L2 and MLL-ENL. Favorable prognosis is associated with subtypes HOX11 or MLL-ENL. The HOX11L2 subtype has different prognostic implications depending on the chemotherapy regime chosen.29,30

Overexpression of the oncogene HDM2 and mutations and anomalies on p53 are associated with very bad prognosis, both in B and T ALL.29,30

Conclusions and recommendations

Cytogenetic studies are a tool of fundamental importance to diagnosis and to establishing prognostic factors in acute forms of leukemia and CML. Nowadays, when dealing with these patients, it is unacceptable not to perform these studies routinely. After treatment, patients lose the initial characteristics of the disease, making it impossible to recover initial findings.

References


Correspondence:
Nelson Hamerschlak
Hospital Israelita Albert Einstein
Av. Albert Einstein, 627
CEP 05652-900 - São Paulo, SP - Brazil
E-mail: hamer@einstein.br