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Cytogenetics of Leukemias: Identifying Genetic Abnormalities with Clinical and Prognostic Significance

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PT, GAP, RFMR and PRGZ designed the study, managed the literature searches and wrote the manuscript. Authors RCMR and DBK managed the literature searches, helped in the script of manuscript. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Introduction: The importance of cytogenetics in neoplastic processes such as leukemia is known. In 1914, Theodor Boveri suggested that chromosomal abnormalities were cellular alterations that cause the transition from normal to malignant proliferation. Over the course of several decades, different cytogenetic techniques were developed which led to the discovery of an increasingly broad spectrum of chromosomal abnormalities, resulting in a dramatic increase in the knowledge of human cancer.
Aim: This article aims to review the role of cytogenetics in leukemia, highlighting its

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importance for the clinical definition, treatment and prognosis of these neoplasms.
Methodology: For this, we carried out a search for scientific articles present in the electronic database PubMed, using the descriptors "Leukemia", "Cytogenetics", "Karyotype", "Fluorescent In Situ Hybridization'', "Prognosis", "Leukemia, Lymphoid ", "Leukemia, Myeloid" and "Leukemia, Chronic Lymphocytic". Books and specialized sites were also surveyed.
Discussion: Cytogenetic analysis not only helps to confirm a diagnosis but it also aids in obtaining data on prognosis, response to treatment and possibility of relapse. The analysis provides a better understanding of the pathways involved in leukemogenesis processes and the development of new types of therapy. This information is essential for the proper management of patients, underscoring the importance of joint work between the medical oncologist/hematologist and the cytogeneticist.

Keywords: Leukemia; cytogenetics; karyotype; fluorescent in situ hybridization; prognosis.

ABBREVIATIONS

GTG-Banding: G-banding technique using trypsin-Giemsa; FISH: fluorescent in situ hybridization; SKY: spectral karyotyping; CGH: comparative genomic hybridization; t: translocations; del: deletions; inv: inversions; dup: duplications; r: ring; mar: marker chromosome; GA: gene amplification; HSR: homogeneously stained regions; MRD: minimal residual disease; ISCN: International System of Human Cytogenetic Nomenclature; ALL: acute lymphocytic leukemia; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; CLL: chronic lymphocytic leukemia;

1. INTRODUCTION

The importance of cytogenetics within the neoplastic process of leukemia has been known for a long time. In 1914, in his book *Zur Frage der Entstehung Maligner Tumoren*, Theodor Boveri [1] suggested that chromosomal abnormalities were cellular changes that cause the transition from normal to malignant proliferation. However, it was only many decades later, in 1960, shortly after the advent of cell culturing methods and determining the correct diploid number of human chromosomes (2n=46) [2], that the first consistent chromosomal abnormality was identified in a human cancer. In 1960, Nowell and Hungerford detected a small marker chromosome in patients with Chronic Myeloid Leukemia [3], which was later called Philadelphia chromosome in tribute to the city where it was discovered.

Subsequently, the introduction of techniques for differentiated longitudinal staining of chromosomes by Caspersson et al. [4] and the development of techniques for high resolution chromosome by Yunis [5] in the 70's and 80's allowed multiple chromosomal abnormalities to be better characterized. Quickly, several reports describing cytogenetic findings in both hematological malignancies and in solid tumors were published, providing the first major review on the subject: The Chromosomes in Human Cancer and Leukemia by Sandberg in 1980 [6]. Next, an increasing number of malignancies, studied cytogenetically and presenting consistent and even specific chromosomal abnormalities, was being correlated with the clinical aspects of the disease, which showed that the use of cytogenetic analysis was a tool of great clinical value, not only for confirmation of the diagnosis, but also for prognostic subclassification [7].

The increasing discovery of new chromosomal abnormalities caused a dramatic increase in knowledge in the field of cytogenetics of human cancer, which led to the formation of a database by Dr. Felix Mitelman of the University of Lund in Sweden, the *Catalog* of Chromosome Aberrations in Cancer [8]. With the advent of genetic techniques and molecular cytogenetics in the 80's, new knowledge broadened the understanding of the molecular mechanisms involved in neoplastic initiation and progression. This occurred through the recognition of the existence of oncogenes, tumor suppressor genes [9], and the genes involved in cell cycle control [10-13].

Today, direct access is available on the Internet to the Mitelman Database of Chromosome Aberrations in Cancer [14] - http://cgap.nci.nih.gov/Chromosomes/Mitelman - as well as to the *Atlas of Genetics and* Cytogenetics *in Oncology and* Haematology [15] - http://atlasgeneticsoncology.org/. These websites contain information about chromosomal abnormalities identified in tumors, as well as genes involved in the abnormalities and their functions in cells. Furthermore, these websites have updated information concerning clinical diagnoses, prognoses and survival rate.

2. CHROMOSOMAL ANALYSIS METHODS

Currently, several methods are proposed in existing literature for karyotypic studies in leukemia and the best type of sample for karyotypic study is bone marrow aspirate. In this tissue, cells are in active mitosis, and it is possible to obtain cells in metaphase without the addition of mitogens, unlike peripheral blood samples. There are exceptions to this rule, especially in chronic lymphoproliferative processes (as in cases of Chronic Lymphocytic Leukemia). In these situations, stimulated cultures with addition of specific mitogens or specific mitogenic agents to stimulate B lymphocytes are necessary to obtain methaphases.

Although it is possible to perform cytogenetic preparations for karyotypic studies immediately after bone marrow aspiration, the best results are obtained after short periods of cell culture (24 to 48 hours). Next, colcemid is added to the culture, which arrests cells in metaphase. The cells are then treated with hypotonic solution. This allows for better visualization and identification of the chromosomes upon application to microscope slides for subsequent staining. There are several methods for chromosome staining but the most widely used is the G-banding technique using trypsin-Giemsa (GTG-Banding) [16].

We can also apply this suspension of cells, used traditionally for karyotyping, to the technique of fluorescent *In situ* hybridization (FISH). Slides are prepared from this suspension so that specific DNA probes labeled with fluorescent material will be used for hybridization experiments. These probes target regions that can map not only on metaphase chromosomes, but also on interphase nuclei. Spectral karyotyping (SKY) is a variant of the FISH methodology which uses different fluorophores, allowing simultaneous identification of all 24 chromosomes [17].

Unlike the techniques described above, studies of comparative genomic hybridization (CGH) use extracted DNA from bone marrow aspirates or blood of the patient instead of cultured cells. Using this methodology, the addition and loss of chromosomal segments can be detected in a single experiment without prior knowledge of specific abnormalities [18].

3. BASIC CONCEPTS, NOMENCLATURE AND INTERPRETATION OF CYTOGENETIC STUDIES IN LEUKEMIA

Chromosomal abnormalities found in leukemias are variable and can be either numerical or structural. Numerical abnormalities are those with an altered normal number of 2n=46 chromosomes. These include trisomies (additional presence of an entire chromosome), tetrasomy (additional presence of two copies of the same chromosome), polysomy (presence of multiple copies of a given chromosome) and monosomies (lack of a specific chromosome). Triploidy are those where the total number of chromosomes is 69 (3n) and tetraploidy, 92 (4n).

Structural abnormalities, in turn, consist of abnormalities where there are changes in chromosome structure. These include translocations (t) (transfer of a chromosomal segment to another), deletions (del) (loss of part of a chromosome), inversions (inv) (chromosomal rearrangement in which a segment is inverted), insertions (ins) (part of a chromosome is inserted interstitially into a chromosome or on a different chromosome) and duplications (dup) (duplication of part of a chromosome). Structural abnormalities also include *ring* chromosomes (r) which are formed by deletion of the telomeric regions of each arm and joining its ends to form a ring structure, and marker chromosomes (mar) for chromosomes whose origins can not be identified.

Gene amplification (GA) is a structural abnormality observed through the techniques of molecular cytogenetics, particulary FISH. GA is identified as an increase in the copy number of a gene resulting from repeated replication of a DNA region. Double minutes and homogeneously stained regions (HSR) are examples of gene amplification that can be identified by normal staining methods. Double minutes are characterized by extrachromosomal circular DNA structures, while HSR refers to amplified intra chromosomal structures.

Clones are defined as a population of cells that has the same chromosomal abnormality. An operational definition asserts that a clone exists if two or more cells are found with the same structural change or the same supernumerary chromosome. Moreover, if a chromosome is absent, the same change must be detected in at least three or more cells.

The modal number is the most common chromosome number in a population of tumor cells, while the term stem cell indicates the most frequent chromosomal constitution. The modal number is described as hypodiploidy when the number of chromosomes is less than 46 and hyperdiploidy when the number of chromosomes is greater than 46. When the number of chromosomes is normal (2n=46) and there is some structural and/or numeric change, the karyotype can be described as pseudodiploid.

Chromosomal aberrations can present in leukemia in two ways: primary and secondary. The primary abnormalities are those considered to be related to the initial steps of tumorigenesis, whereas the secondaries arise during the course of the neoplasia. Other chromosomal aberrations include cryptic chromosomal abnormalities, which are usually small and are not detected by conventional cytogenetic analysis and minimal residual disease (MRD), which refers to the small number of cancer cells that remain in a patient in remission that can only be detected by sensitive tests [19].

Standards for identification and description of cytogenetic abnormalities observed in leukemia are in the International System of Human Cytogenetic Nomenclature (ISCN - 2013) [20]. These are globally accepted standards and reports of cytogenetic samples studied should follow these specifications.

4. MOLECULAR CONSEQUENCES OF CHROMOSOMAL ABNORMALITIES

In hematological malignancies, translocations are the most common structural rearrangements (the majority of which are balanced) and these can cause changes in function in one or more genes through one of the following mechanisms:

1) Deregulation: generally characterized by increased expression of an apparently normal gene located at a translocation breakpoint. This mechanism is apparent in Burkitt lymphoma t (8;14), where the rearrangement leads to the juxtaposition of the MYC gene located in band 8q24 with the constitutively active immunoglobulin (IG) gene *IGH,* located in 14q32, which results in abnormal activation of the MYC gene, a gene with a critical role in cell cycle progression.

2) Creation of a hybrid gene: the fusion of sections of two genes, one from each of the respective breakpoints of the translocation. An example of this mechanism is the Philadelphia chromosome in Chronic Myeloid Leukemia. This is an abnormality secondary to a reciprocal translocation between the long arm segments of chromosomes 9 and 22 that leads to the formation of the hybrid gene BCR/ABL1, resulting in the production of an abnormal protein.

Other structural abnormalities, such as deletions, result in the loss of oncogenes and tumor suppressor genes located in the deleted regions. In the case of numerical chromosomal abnormalities and gene amplification, increases in gene expression are observed due to the presence of extra copies of a particular gene [19,21].

5. INFLUENCE OF CYTOGENETICS IN CLINICAL PRESENTATION, PROGNOSIS AND TREATMENT OF LEUKEMIA

Throughout the 60's and 70's, cytogenetic changes were observed in specific subtypes of leukemia. However, the most important discoveries were those that connected cytogenetic changes with their clinical manifestations in patients [22]. Now the World Health Organization includes the use of cytogenetics to classify and characterize leukemias [23].

The following contains a description of the cytogenetic abnormalities observed in most major types of leukemia, along with their clinical significance and prognosis.

5.1 Acute Lymphocytic Leukemia

Currently, cytogenetic changes in Acute Lymphocytic Leukemia (ALL) are well known and classified, and clonal abnormalities are identified in 50-70% of patients [24]. Although chromosomal abnormalities are similar in children and adults, their distribution and, possibly, their biological significance are not. In children with ALL, the most chromosomal abnormalities are balanced translocations that are associated with certain immunological subgroups [25,26]. The most common structural change is t(12;21) (TEL/AML1 or ETV6/RUNX1) observed in 20-25% of cases Fig. 1, followed by t(11;v) (q23;v) (involving the

MLL gene) and t(v;14) and t(7;v) (involving the TCR gene), which are found in 10% of cases (where v corresponds to a variable chromosome). The t(1;19) (E2A/PBX1) and t(9;22) (BCR/ABL1, the Philadelphia chromosome) structural abnormalities are identified in 5% and 4% of patients, respectively Fig. 1.

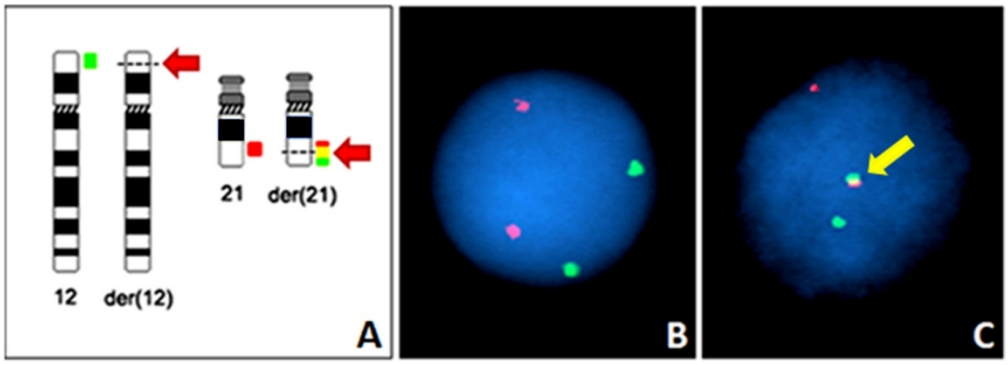

Fig. 1. Ideogram showing the translocation (12;21)(p13;q22), together with the binding sites of DNA probes used in the FISH technique (A). This translocation involves the reciprocal exchange of segments of distal regions of the short arm of chromosome 12 and the long arm of 21 (dashed lines and red arrows indicate the chromosomal breakpoints). Because these segments are formed by light bands of the chromosomes 12 to 21, the translocation usually is undetected by conventional karyotyping (GTG-banding). The result is a molecular fusion of TEL and AML1 genes on derivative chromosome (21). FISH with the ETV6/AML1 Translocation Probe - Two Colour Direct Labeled Probe - Aquarius Probes (Cytocell Ltd., Cambridge, UK) probe allows the identification of (B) two green fluorescent signals and two red signals in a normal interphase nucleus and of (C) a yellow signal in the presence of TEL/AML1 fusion, indicating a translocation on chromosome derivative (21) (yellow arrow), in a positive nucleus for translocation. (der: derivative chromosome)

Other types of translocations are detected in 20% of cases, while the absence of these chromosomal rearrangements is observed in 30% of patients [27,28]. In infants, translocations involving the 11q23 region (involving the MLL gene) are identified in 70% of patients and these abnormalities likely occur during pregnancy [24].

On the other hand, numerical chromosomal abnormalities occur in clones with more or less copies of one or more chromosomes [29]. In ALLs, hyperdiploidy cells have a specific chromosome constitution that depends on its modal number. They are observed in about 25% of cases of childhood ALL. It is interesting to note that the gain of chromosomes is not random and 8 chromosomes count for approximately 80% of all additions: +4, +6, +10, +14, +17, +18, +21 and + X. Chromosome additions usually present as trisomies. The exceptions are chromosomes 21, 14 and 18, which can be tetrasomic. Structural abnormalities are also present in approximately 50% of cases, and the changes in 1q are the most frequent [30].

In turn, the hypodiploid is observed in less than 2-6% of pediatric patients with ALL [31]. Based on the number of chromosomes and cytogenetic findings, the hypodiploid cases are divided into three distinct subgroups: quasi-haploid (25 to 29 chromosomes), low hypodiploid (30 to 39 chromosomes), and high hypodiploid (40 to 45 chromosomes). Most cases of

hypodiploids show 45 chromosomes and only a small number of cases have less than 45 chromosomes [32].

In relation to phenotypic subtypes of ALL, the cases that affect T cells have a smaller proportion of chromosomal abnormalities (60-70%) than the cases that affect B cells (~80%). However, several abnormalities observed in T-ALL are cryptic chromosomal abnormalities and are difficult to detect using standard karyotyping methods [33]. More recently, with the development of new techniques of molecular cytogenetics, FISH and CGH, different submicroscopic rearrangements such as intrachromosomal amplification of chromosome 21 (iAMP21) or RUNX1 gene (AML1) have also been discovered [34].

In ALL, chromosomal changes are traditionally used as diagnostic and prognostic markers. Their significance is similar to or even more important than other factors such as age at diagnosis and leukocyte count [31,32] (Table 1).

Table 1. Prognosis related to cytogenetic abnormalities observed in pediatric ALL [31,32,35].

Prognosis	Chromosomal Abnormality*	Overall survival at 5 years (95% CI)*
Good	Hyperdiploidy (>50 chromosomes)	93% (91-95)
	t(12;21)(p13;q22)	96% (94-98)
Intermediate - good	Normal chromosomes	-
	t(1;19)(q23;p13.3)	84% (71-92)
Intermediate	Pseudodiploidia	-
	Hyperdiploidy (47-50 chromosomes)	-
	Hypodiploidy (45 chromosomes)	-
Intermediate – poor	Hypodiploidy (< 45 chromosomes)	50% (15-78)
Adverse	Almost tetraploid	-
	Monosomy of chromosome 7	87% (65-96)
	del(7p) / del(7q)	-
	del(5q)	-
Adverse / no prognostic[+]	9p changed	86% (79-90)
Poor	t(4;11)(q21;q23)	-
	Quasi-haploid	-
Rather poor	t(9;22)	58% (42-7)
	t(17;19)	-
	Amplification iAMP21	69% (49-82)
No prognostic	12p changed	-
	del(6q)	-
	14q11	-
Unknown	+8	-

**t: translocation; del: deletion; +: presence of additional chromosome; p: short arm; q: long arm; CI: confidence interval*

[+]These possibilities were associated with different abnormalities involving 9p

Importantly, the prognosis of hyperdiploidy groups depends on the presence or absence of associated structural changes. Generally, if these structural changes are also present, the prognosis is made based on the structural abnormality [36].

This information has permitted the sub-classification of ALL in different risk groups with different treatment options. The detection of subgroups of poor prognosis led to the search for new treatment protocols and to the introduction of more aggressive alternative therapies. This subsequently led to a change in the rates of remission and survival [25].

5.2 Acute Myeloid Leukemia

Unlike ALL, Acute Myeloid Leukemia (AML) is most common in the elderly population, with an incidence of approximately 3.4 per 100,000 per year and a median age of 70 years at diagnosis [37]. Furthermore, in contrast to patients diagnosed with chronic myeloid leukemia (CML) who are positive for t(9;22) or its variants, the cytogenetic profile of AML is much more complex. Currently, over 2,000 numerical or structural changes have been described in AML which reflects a highly heterogeneous cytogenetic profile [32]. The proportion of abnormal karyotypes reaches 50-80% [38] and varies according to the age group. Abnormal karyotypes are more common in pediatric AML (68-77%) than adult AML (52-59%). In addition, the frequency and type of chromosomal abnormalities can be influenced by other factors such as gender, previous chemotherapy treatment, and ethnicity/geographic origin Fig. 2 [32].

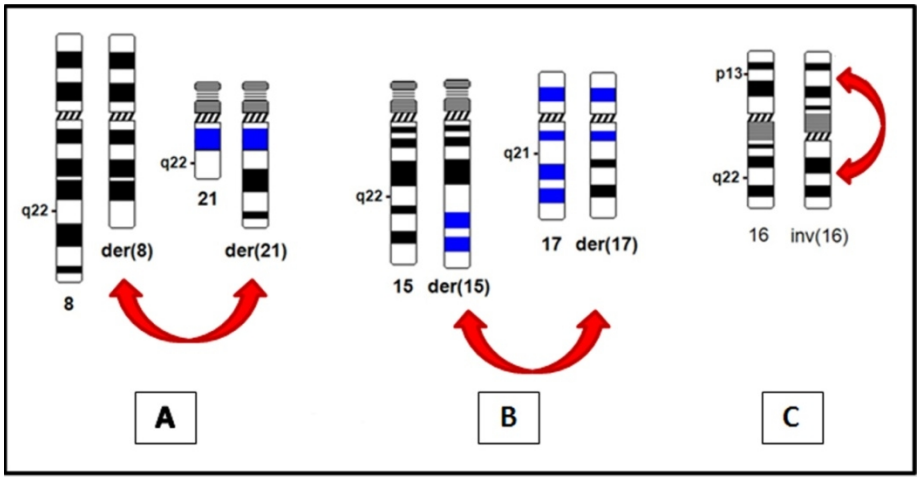

Fig. 2. Structural abnormalities observed in AML: (A) translocation between chromosomes 8 and 21 [t (8;21)]; (B) translocation between chromosomes 15 and 17 [t (15: 17)] and (C) inversion of chromosome 16 [inv(16)] (der: derivative chromosome)

In AML, a significant proportion of patients presents a single chromosomal rearrangement in their leukemic blasts, unlike many types of solid tumors and malignant lymphomas which often contain both multiple numerical and structural abnormalities. Heim and Mitelman estimate that only one change is detected in approximately 55% of all cases of abnormal karyotypes in AML [32]. These changes reflect well established associations between identified chromosomal abnormalities and the resulting clinical subtypes [39,40].

Cytogenetic abnormalities detected at clinical presentation are one of the main predictors of outcome in AML. Conventional cytogenetic studies have defined certain chromosomal rearrangements as favorable, standard and unfavorable risk [37,41]. Examples of these

chromosomal aberrations associated with a favorable risk designation of adult AML are t(8;21), inv(16)/t(16;16) and t(15;17) and aberrations with an unfavorable risk are abnormalities involving 3q and 9q. Although the prognostic impact of autosomal monosomy in AML has been well described to -5 or -7, the lack of any autosome is indicative of poor prognosis. Moreover, extra copies of one or more chromosomes, as well as trisomies, tetrasomies or the presence of marker chromosomes or ring chromosomes have a minimal prognosis impact when compared to monosomies [40,42] Table 2.

Table 2. Cytogenetic abnormalities with prognostic relevance in AML (Based in Ferrara et al., 2008 [37,40,43])

Prognosis	Cytogenetics*
Favorable	t(8;21)
	inv(16)/t(16;16)
	t(15;17)
	t(21;21)
Intermediate	Normal
	t(9;11)
	Abnormalities in 11q23
	del(9q)
	+6, +8, +21, +22 and –Y
	Complex karyotype
Unfavorable	-5, -7
	del(5q) and del(7q)
	Abnormalities in 3q, 9q, 11q, 17p, 20q and 21q
	t(6;9), t(8;16) and t(9;22)

**t: translocation; del: deletion; inv: inversion: +: presence of additional chromosome; -: absence of chromosome; p: short arm; q: long arm*

Initially, the presence of secondary abnormalities associated with primary abnormalities in AML was considered as a possible modifier of the prognosis. However, in chromosomal abnormalities previously associated with a favorable prognosis, recent studies were unable to confirm these assertions [38]. Cytogenetic analysis also identified the existence of cases of AML induced by previous use of alkylating agents and related drugs such as chlorambucil, busulfan, and cyclophosphamide. More recently, studies found that drugs with interactions with topoisomerase-II are associated with the development of AML [44].

Although we know a great deal about the molecular consequences of translocations and gene fusion in AML, our current knowledge of the underlying mechanisms and pathogenetic consequences of unbalanced abnormalities (which are more frequent than balanced abnormalities) is still rudimentary.

5.3 Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) is a unique disease, universally characterized by the presence of the Philadelphia chromosome in 85-90% of cases [45] Fig. 3. CML occurs in all age groups, but is most common in the elderly. After a variable time in the chronic phase of the disease, patients progress to the acute phase with accelerated or blast crisis. At this time, about 75-80% of cases show additional chromosomal abnormalities as +8 and i(17q) [32]. Interestingly, these changes appear weeks or months before clinical diagnosis of the

acute phase, meaning that although patients can be clinically in the chronic phase, they are cytogenetically in acute phase [46].

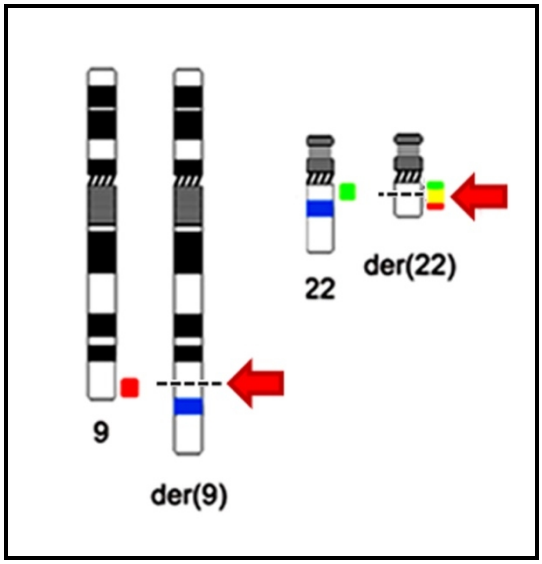

Fig. 3. Ideogram showing the reciprocal translocation between the long arms of chromosomes 9 and 22, leading to the formation of the Philadelphia chromosome [der(22)] (dashed lines and red arrows indicate the chromosomal breakpoints). The result is the formation of molecular chimeric gene BCR/ABL1, in the derivative (22). The colors red (BCR gene) and green (ABL gene), indicate the binding sites of DNA probes used in the FISH technique. The fusion of both red and green signals can be observed in the derivative (22). (der: derivative chromosome)

In this sense, it is interesting to differentiate the cytogenetic signs of the acute phase of complex translocation that leads to the formation of the Philadelphia chromosome, and involves not only chromosomes 9 and 22, but also a third, and possibly more chromosomes. Variant translocations are found in 5-10% of cases of CML. In these cases, translocation should be considered as a complex 3-way translocation t(9;22;v). In other cases the 3-way translocation is more evident upon identification of a larger chromosomal segment translocated to chromosome 9. Less frequently, the translocation can involve the sequential translocation of more chromosomes (resulting in a complex 4, 5 or 6 way translocation).

It is also important to note that the t(9;22) translocation seems balanced when observed by karyotyping, but FISH analysis has shown that deletions on chromosome 9 are present in 10-15% of cases [47]. In addition, patients with CML and normal karyotype (which are a minority) may present with fusion of *BCR/ABL1* secondary to insertions or other cryptic complex chromosomal rearrangements [32].

CML is perhaps the most dramatic example of how therapies with a specific molecular target may change the natural course of the tumor, representing a landmark for molecular medicine and pharmacogenetics [45]. Imatinib mesylate, a specific inhibitor of the BCR-ABL1 chimeric protein, is currently the standard therapy for CML. Its implementation caused a dramatic decrease in the use of allogeneic stem cell transplants, significantly increasing patient survival [48].

Despite the favorable rates of complete hematologic and cytogenetic response, residual disease remains detectable in the majority of patients treated with imatinib mesylate, suggesting that the drug is not able to completely eradicate leukaemic stem cells [49]. On the other hand, the emergence of drug resistence cases of leukemia has led to an attempt to develop a range of novel inhibitors that are currently being tested in preclinical and clinical studies [50,51].

5.4 Chronic Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL) is considered the most common form of leukemia in the Western world and primarily affects individuals in late adulthood. Of all leukemias, CLL contains the hematological abnormality most difficult to analyze cytogenetically, due mainly to its low mitotic index and poor response to mitogenic agents [24]. However, the recent discovery of a new group of mitogens (such as CpG oligonucleotides, CD40L and IL-2), which primarily stimulate B cells, resulted in improved CLL cytogenetic analysis [52].

Clonal chromosomal abnormalities were detected by karyotyping techniques in 30-50% of cases. With the use of new and adequate mitogenic stimulants, these changes have been noted in up to 80% of patients [52]. The advent of molecular cytogenetic techniques such as FISH has also caused an increase in the identification of these abnormalities. This was determined mainly by the ability of the study to conduct experiments not only in metaphase cells, but also in interphase nuclei. The use of FISH has a high rate of detection of cytogenetic changes in CLL for about 65-90% of patients [53] (Fig. 4).

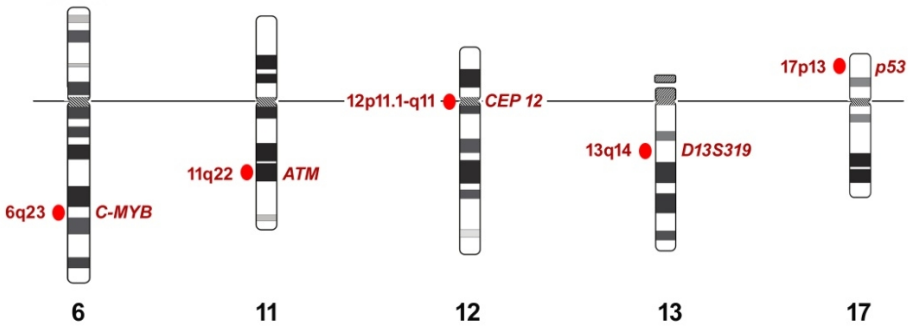

Fig. 4. Ideogram showing the binding sites of DNA site-specific probes used in the FISH technique to assess CLL patients (p = short arm q: long arm)

Cytogenetic abnormalities identified in CLL primarily consist of deletions and/or amplifications. The main abnormalities are detected rearrangements of 13q, 11q, 17p, and 6q and trisomy of chromosome 12. More recent studies using the new mitogenic agents have also shown that translocations appear to be common. In general, approximately 65% of patients with CLL have a single chromosomal abnormality, 25% with two abnormalities and the remaining (6%) with more complex changes [24].

Cytogenetic changes identified in CLL have provided some insights into the pathogenesis of this neoplasia by pointing out candidate genes, such as p53 (located at 17p13) and ATM (at 11q22-q23). In addition, certain abnormalities are considered to be predictive factors

associated with characteristics of disease. For example, deletions of 11q have been associated with marked lymphadenopathy, and deletion of 17p confers resistance to conventional chemotherapy [54]. Thus, cytogenetic analyses have been useful in predicting the clinical course of patients with CLL. Chromosomal aberrations are considered independent predictors in relation to disease progression and survival, and accordingly, a new classification system has been created to correctly categorize them [24,55] Table 3.

Table 3. Chromosomal abnormalities in CLL, showing the genes involved, and their correlation with prognosis and median survival presented by patients [24,55]

Alteration*	Genes involved	Prognosis	Median survival (in months)
del(13q14)	Rb1, Leu-1, Leu-2, Leu-5, CLLD6-CLLD8, KPNA3, miR15 and miR16	Good	79 to 133
+12	CDK2, CDK4, STAT6, APAF-1, MDM-2 and CCLU1	Intermediate	33 to 114
del(6q)	?		33 to 114
del(11q22-23)	FDX, ATM, MLL, PZLF, Mre11, RDX, NPAT, CUL5 and PPP2R1B	Poor	13 to 79
del(17p13)	p53		9 to 32

** del: deletion; p: short arm; q: long arm; +: additional chromosome*

Recent studies based on next generation sequencing have revealed new genes implicated in CLL that may potentially have clinical relevance, NOTCH1, SF3B1, BIRC3 and MYD-88. NOTCH1 encodes a transmembrane protein that acts as a ligand-activated transcription factor and regulates multiple target genes. *SF3B1* is a core component of the spliceosome, a complex of five small nuclear ribonucleoproteins involved in the splicing of precursor messenger RNA and the formation of mature mRNA through the removal of introns in protein-encoding genes. *BIRC3* is a negative regulator of the MAP3K14 serin-treonine kinase, the pivotal activator of non-canonical NF-κB signaling [56] Table 4. Furthermore, the last gene, myeloid differentiation primary response gene 88 (MYD-88) has been studied, revealing its predominance in cases of CLL with mutated immunoglobulin genes [57].

Table 4. Genetic mutations in CLL, showing the genes involved and overall survival [56]

Mutation*	Overall survival (years)
NOTCH1	4-8
SF3B1	4-9
BIRC3	3.5

6. FINAL CONSIDERATIONS

Chromosomal aberrations identified by cytogenetic analysis techniques have provided a better understanding of the pathways involved in leukemogenesis and in the development of new types of therapy [58]. All of this information is critical to the proper management of patients, which emphasizes the importance of joint work between the medical oncologist/hematologist and the cytogeneticist.

7. CONCLUSION

Cytogenetic analysis helps to confirm the diagnosis of leukemias, and it also aids in obtaining data on prognosis, response to treatment and possibility of relapse. Cytogenetic results are already considered part of several treatment protocols of leukemia. Bone marrow aspirate is the tissue usually used in this analysis and the cells are in active mitosis. Thus it is possible to obtain cells in metaphase without the addition of mitogens. Several chromosomal abnormalities have been identified associated to leukemia and the strong association between Philadelphia chromosome and CML is considered an exception to the rule. The cytogenetic analysis may also provide a better understanding of the pathways involved in leukemogenesis processes and aid in the development of new types of therapy for leukemias.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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