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The Impact of Additional Chromosomal Abnormalities in Response to Imatinib Mesylate Therapy for Chronic Myeloid Leukemia

O Impacto das Alterações Cromossômicas Adicionais em Resposta ao Mesilato de Imatinibe na Leucemia Mielóide Crônica

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Abstract

Imatinib induces a complete cytogenetic response in more than 80% of newly diagnosed patients with chronic myeloid leukemia (CML) in the chronic phase (CP) and in 41% of patients in the first chronic phase after failure of interferon-α treatment. However, some patients do not respond completely. Therefore, according to most studies, drug resistance in CML patients treated with imatinib is correlated with cytogenetic abnormalities acquired during treatment. In this study we analyzed 48 CML patients treated with imatinib mesylate after interferon-α resistance in order to elucidate the impact of additional chromosomal abnormalities prior to imatinib in response to therapy. Cytogenetic abnormalities in addition to the Philadelphia chromosome (Ph) were detected in 33.3% of patients. Patients with Ph as the sole cytogenetic abnormality prior to imatinib therapy presented a major cytogenetic response and significantly longer median overall survival (p=0.006) than patients with additional chromosomal abnormalities. Therefore, in this group of patients, another choice of treatment should be considered, such as stem cell transplantation or combination regimens as appropriate. The present study indicates the importance of detecting a double Ph chromosome prior to imatinib therapy. Patients showing this abnormality did not respond to imatinib, thus indicating the abnormality's association with resistance. Our study suggests that classical cytogenetic analysis is still an important tool prior to and during follow-up of CML patients treated with imatinib.

Key words: Chronic myeloid leukemia, Imatinib, Chromosomal abnormalities

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INTRODUCTION

Philadelphia chromosome (Ph) is the cytogenetic hallmark of chronic myeloid leukemia (CML). It is characterized by a reciprocal translocation t(9;22)(q34;q11). The resulting molecular event is the creation of the bcr-abl fusion gene, which encodes a protein with increased kinase activity[1]. Cytogenetic analysis in CML serves three purposes: 1) presence of the Ph chromosome in conjunction with morphological findings establishes the diagnosis of CML; 2) sequential karyotyping is still the accepted standard technique for monitoring treatment efficacy; and 3) chromosomal abnormalities (in addition to the Ph chromosome) may be detected at diagnosis or during follow-up, adding important prognostic information[2,3].

Clinically, CML progresses through three distinct phases: chronic phase (CP), accelerated phase (AP), and blast crisis (BC). According to the International Bone Marrow Transplant Registry, the criteria for accelerated phase CML are: percentage of blasts in peripheral blood or marrow ≥ 10% and < 30%, percentage of blasts + promyelocytes in peripheral blood or bone marrow ≥ 20%, progressive splenomegaly, thrombocytopenia < 100 X 10^9/L unrelated to therapy, and karyotypic evolution (chromosomal aberrations in addition to single Ph chromosome)[4]. It remains controversial whether the acquisition of additional cytogenetic abnormalities heralds a transformation to blast crisis and should be considered a feature of the accelerated phase.

There is no single standard therapy for patients with CML. Advances in bone marrow transplantation and the effects of recombinant interferon-alpha (IFN-α) combined with chemotherapy with either hydroxyurea or cytarabine have proven clinically important[5]. The recent introduction of imatinib (Gleevec®, Novartis Pharmaceuticals, Basel, Switzerland) represents a new treatment option. Imatinib is a molecularly targeted therapy that inhibits the oncogenic fusion protein BCR-ABL and has demonstrated efficacy in the treatment of CML, particularly in the chronic phase[6].

Kantarjian et al.[7] described a complete cytogenetic response (CCR) in more than 80% of newly diagnosed patients with CML in CP and in 41% of patients in the first CP after failure of IFN-α treatment.

Conventional cytogenetic studies have been viewed as the standard follow-up method for patients on imatinib. Various studies demonstrate the prognostic value of the major cytogenetic and complete cytogenetic response, and the acquisition of additional cytogenetic abnormalities during this treatment has been correlated with resistance to imatinib[8]. However, most studies in patients treated with imatinib focus on the cytogenetic abnormalities acquired during treatment, and few studies have reported the effect of additional cytogenetic abnormalities prior to imatinib therapy.

The current study discusses the impact of specific types of cytogenetic abnormalities in addition to the Ph chromosome prior to imatinib mesylate treatment as a prognostic factor, regardless of disease phase, in response to therapy in patients with CML.

METHODS

PATIENTS AND SAMPLES

From January 2001 to December 2004, 48 patients with CML were treated with imatinib mesylate after INF-a resistance at the Hematology Unit of the Brazilian National Cancer Institute (INCa). CML diagnosis was based on morphologic and cytochemical studies of the initial peripheral blood films and bone marrow aspirates, as well as the presence of Ph chromosome in bone marrow cells. CP patients received 400mg daily of imatinib mesylate, while AP and BC patients received 600mg daily. Patients underwent cytogenetic evaluation before beginning treatment. There were 29 males (60.4%) and 19 females (39.6%), and median age was 47 years (range 17-75). The study was approved by the Research Ethics Committee, INCA (Protocol 58/05).

CYTOGENETIC STUDIES AND STATISTICAL ANALYSIS

Chromosomal analysis was performed before imatinib therapy and every three months. Bone marrow karyotypes were obtained from cultures in RPMI 1640 with 20% fetal calf serum (Gibco) at 37°C for 24 hours. Cell cultures were pulsed with colcemid to a final concentration of 0.06 μg/ml for the final hour of incubation. Cells were subsequently harvested by standard procedures (hypotonic shock: 0.075M) and fixed in methanol-acetic acid (3:1). GTG banding was performed as described by Seabright[9], and chromosomes were identified and arranged according to the International System for Human Cytogenetic Nomenclature (ISCN)[10]. The number of cells investigated for each patient at each analysis ranged from 20 to 50 metaphases. Cytogenetic response was defined according to the percentage of Ph-positive metaphases: complete cytogenetic response (CCR: 0% of Ph cells); partial cytogenetic response (PCR: from 1-34% of Ph cells); major cytogenetic response (MCR: CCR + PCR); minor cytogenetic response (mCR: 35-95% Ph cells); and no response (NR: > 95% of Ph cells). Clonal evolution was considered when some other abnormality was detected after beginning treatment. Cytogenetic response to therapy and significance of additional abnormalities were

compared by the χ^2 test. Survival rates and survival graphics were performed using the Kaplan and Meier methods (SPSS software, SPSS Inc., Chicago, USA).

RESULTS

HEMATOLOGICAL AND CYTOGENETIC FINDINGS PRIOR TO IMATINIB

Hematological evaluation at pre-treatment showed that of the 48 cases, 22 (45.8%) were in CP, 25 (52.1%) in AP, and 1 (2.1%) in BC. Of the 48 patients, 32 presented the classic t(9;22)(q34;q11) at the beginning of imatinib therapy and 16 (33.3%) presented additional abnormalities (Table 1). Additional cytogenetic abnormalities were detected in four patients with CP (25%) and twelve with AP (75%). The most frequent additional abnormality prior to imatinib was double Ph, observed in 6 cases (12.5%). Hematological studies immediately before initiating imatinib therapy showed that patients presenting only the Ph chromosome had a median of 118,000 cells/mm^3 in the white blood count (WBC), while patients presenting additional chromosomal abnormalities presented 176,500 cells/mm^3. Median platelet count was 319,000 cells/mm^3 in patients presenting only the Ph chromosome and 225,000 cells/mm^3 in patients with additional chromosomal abnormalities.

CYTOGENETIC AND HEMATOLOGICAL RESPONSE AFTER IMATINIB

Patients were analyzed prior to imatinib and two to five times (median three) during imatinib therapy, with a follow-up of 6 to 42 months (median 20 months). Cytogenetic responses varied in this patient cohort. MCR was observed in 17 patients (37.5%), of whom 15 had Ph as the sole abnormality and 2 had additional abnormalities: trisomy 8 and der(9)del(9p)t(1;9;22), patients 10 and 15 respectively (Table 1). The difference between patients with the single Ph chromosome and those with additional abnormalities was 88.2 vs 11.8% (p=0.0048). Nine patients showed complete cytogenetic response. In this group, 8 patients (18.7%) presented only the Ph chromosome prior to treatment and one presented trisomy 8. Median CCR was 9.5 months, ranging from 6 to 42 months. The hematological response was observed prior to cytogenetics in all the cases. Minor remission was observed in 6 patients, of whom 3 presented the Ph chromosome and 3 showed additional chromosomal abnormalities: del(3p), t(1;3), and t(6;6)(Table1).

Finally, no cytogenetic response to imatinib was observed in 25 patients (14 with Ph chromosome and 11 with additional chromosomal abnormalities). All patients with double Ph had no cytogenetic response to imatinib. The same was true for patients who presented del(17p), add(2p), t(2;9;22), complex karyotype, add(13p) and add(20p), and del(5q).

Clonal cytogenetic evolution after therapy was observed in 10.4% of patients. Trisomy 8 was detected in 2 patients, one of whom presented only the Ph chromosome prior to imatinib. The abnormalities observed in the other three patients were monosomy X, add(19)(p13), and a complex karyotype (case 12, Table 1).

CLINICAL FOLLOW-UP

During clinical follow-up, patients with additional abnormalities showed significantly shorter median overall survival than those with only Ph chromosome (p= 0.006; Figure 1), regardless of the specific type of cytogenetic abnormality. The correlation between median overall survival and cytogenetic response showed that patients who reached MCR had a significantly longer median overall survival than those with no response (p= 0.007; Figure 2). Eight patients evolved to death, six of which presented additional abnormalities prior to imatinib. None of them responded to imatinib.

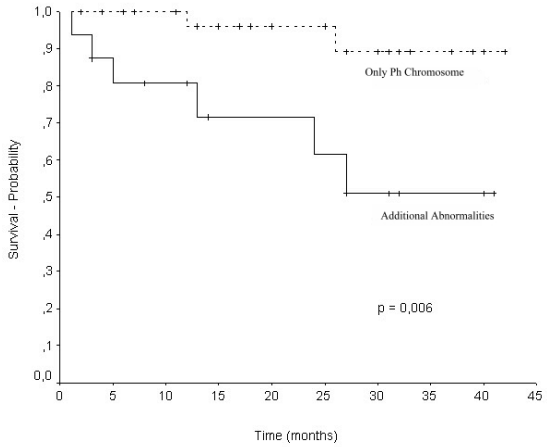

Figure 1. Overall survival curves of patients on imatinib according to pre-treatment karyotype (Kaplan and Meier)

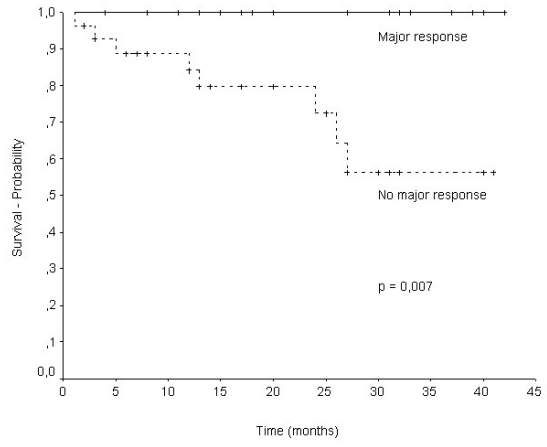

Figure 2. Overall survival curves of patients on imatinib according to cytogenetic response (Kaplan and Meier)

Table 1. Detailed cytogenetic and clinical data of 16 CML patients with abnormalities in addition to the Ph chromosome prior to imatinib therapy

Patient no.	Phase	Pretreatment karyotype	New additional abnormalities during imatinib	Last cytogenetic response to imatinib
1	AP	46,XY,t(9;22)(q34;q11),del(17)(p11)[18]/ 46,XY,t(9;22)(q34;q11)[12]	-	NR
2	CP	46,XY,add(2)(p25),t(9;22)(q34;q11)[6]/46, XY,t(9;22)(q34;q11)[43]	-	NR
3	AP	46,XY,del(3)(p12p14),t(9;22)(q34;q11)[5]/ 46,XY,t(9;22)(q34;q11)[31]	-	mCR
4	CP	46,XY,t(2;9;22)(p15;q34;q11)[28]	+8	NR
5	AP	47,XY,t(9;22)(q34;q11),+der(22)t(9;22) (q34;q11)[7]/46,XY,t(9 ;22)(q34;q11)[14]	-	NR
6	AP	47,XX,t(9;22)(q34;q11),+der(22)t(9;22) (q34;q11)[20]	-	NR
7	CP	46,XY,t(1;3)(q25;q29),t(9;22)(q34;q11)[3] /46,XY,t(9;22)(q3 4;q11)[34]	-	mCR
8	AP	46,XX,t(9;22),-13,add(18)(p11), +der(22)t(9;22)[1]/45,XX,t(9;22),- 13,add(18)(p11)[23]	-	NR
9	AP	46,XY,t(6;6)(q21;p25),t(9;22)(q34;q11)[4] /46,XY,t(9;22)(q34;q11)[29]	-	mCR
10	CP	47,XY,+8,t(9;22)(q34;q11)[3]/46,XY, t(9;22)(q34;q11)[23]	-	MCR
11	AP	46,XX,t(9;22)(q34;q11),add(13)(p11), add(20)(p13)[21]	-	NR
12	AP	47,XY,t(9;22)(q34;q11),+der(22)t(9;22) (q34;q11)[31]/46,XY[20]	45,XY,dic(3;9)(q11;q11) ,del(5)(q31;q35),- 7,t(9;22)(q34;q11), -17,+2der(22)(t(9;22) (q34;q11)[18]/45,XY, t(9;22)(q34;q11), dic(17;20) (q11;q11), -18,+2der(22)t(9;22) (q34;q11)[4]	NR
13	AP	46,XX,del(5)(q22q33),t(9;22)(q34;q11)[9]/ 46,XX,t(9;22)(q34;q11)[18]	-	NR
14	AP	47,XY,t(9;22)(q34;q11),+der(22)t(9;22) (q34;q11)[1]/46,XY,t(9;22)(q34;q11)[10]	-	NR
15	AP	46,XY,der(9)del(9) (p21)t(1;9;22)(q32;q34; q11)[7]/46,XY[38]	-	MCR
16	AP	47,XY, t(9;22)(q34;q11),+der(22)(q34; q11)[5]/ 46,XY,t(9;22)(q34;q11)[15]	-	NR

CP: chronic phase; AP: Accelerated phase; BP: Blastic phase; NR: no response; mCR: minor cytogenetic response; MCR: major cytogenetic response

DISCUSSION

Imatinib is the first molecularly targeted therapy for CML. In cases with complete and major response, it produces a rapid and dramatic resolution of the peripheral blood abnormalities associated with CML. Nevertheless, the cytogenetic response is delayed in comparison to the hematological response[11].

Karyotype abnormalities in addition to Ph translocation are associated with acceleration of the disease and have also been shown to play a role in CML resistance to imatinib[12]. In the present study, we show evidence suggesting different responses to imatinib according to the additional chromosomal abnormalities acquired during evolution.

Some authors have considered the presence of additional chromosomal abnormalities as the sole criterion for the disease acceleration. O'Dwyer et al.[13] observed that patients with clonal evolution before therapy show excellent responses to imatinib mesylate.

However, another study[14] concluded that the main prognostic factors for relapse in patients in the chronic phase are the presence of cytogenetic clonal evolution and failure to achieve a major cytogenetic response.

There are recurrent cytogenetic changes that define the so-called "routes" as clonal evolution. Thus, the acquisition of a second Ph chromosome, trisomy 8, isochromosome 17, and trisomy 19 constitute the main routes, accounting for approximately 70% of cases[3]. In addition, molecular abnormalities such as *TP53* mutations can arise[15].

In the current study, we considered additional chromosomal abnormalities as the sole prognostic factor, regardless of the disease phase, and observed that these abnormalities were associated with lower cytogenetic response rates and decreased overall survival.

Trisomy 8, del(3), and Ph variant were associated with major and minor cytogenetic responses, while double Ph, del(17), and add(2) were associated with resistance. To our knowledge, the present study is the first to highlight the importance of detecting double Ph chromosome prior to imatinib, since all the patients who presented this abnormality failed to show a cytogenetic response. For these patients, other therapeutic approaches may be indicated, such as stem cell transplantation or combination regimens as appropriate, for patients presenting certain additional abnormalities.

We also observed that patients who only presented one Ph chromosome prior to imatinib showed increased overall survival rates as compared to those with additional abnormalities (40 months versus 28 months, p = 0.006). Other authors have found similar data. In a study on imatinib as treatment for CML blastic transformation, Sureda et al.[10] observed that the presence of additional cytogenetic abnormalities was significantly associated with shorter event-free survival. We also observed that patients who achieved MCR had higher overall survival rates, similar to the results described by O'Brien et al.[16]

Schoch et al.[17] also described an association between response to imatinib and chromosomal abnormalities other than Ph chromosome. Their results were similar to ours in several ways. They observed that patients with additional abnormalities had lower cytogenetic response rates. However, in our study, the main additional chromosome was the double Ph chromosome, whereas their sample showed trisomy 8. In our study, the patient with +8 reached CCR and presented +8 disappearance, whereas they observed one case with a Ph-positive clone that reached CCR but still presented trisomy 8 in 35% of metaphases.

Clonal evolution after treatment was observed by us and others (Cortez et al.[18] In our study 10.4% of the patients developed clonal cytogenetic evolution during imatinib, similar to the results presented by Schoch et al.[15] (12.9%). The most frequent chromosomal abnormality during imatinib therapy was trisomy 8, a finding consistent with other studies[2,17].

Some authors have suggested that imatinib may favor the emergence of clonal chromosomal abnormalities in Ph-negative cells[19], an event not observed in our study.

Thus, patients on imatinib therapy should be monitored systematically by conventional karyotyping, even in the case of CCR. Despite current molecular techniques, our study indicates that classical cytogenetic analysis is still an important tool for follow-up of patients treated with imatinib.

REFERENCES

1. Jacob RT, Gayathri K, Surath, Rao DR. Cytogenetic profile of chronic myeloid leukemias. Indian J Cancer. 2002;39:61-65.
2. Mohamed AN, Pemberton P, Zonder J, Schiffer CA. The effect of imatinib mesylate on patients with Philadelphia chromosome-positive chronic myeloid with secondary chromosomal aberrations. Clin Cancer Res. 2003;9:1333-337.
3. Deininger MWN. Cytogenetic studies in patients on imatinib. Semin Hematol. 2003;40:50-55.
4. Speck B, Bortin MM, Champlin R, Goldman JM, Herzig RH, McGlave PB, et al. Allogeneic bone-marrow transplantation for chronic myelogenous leukemia. Lancet. 1984;1:665-68.
5. Silver RT. Chronic myeloid leukemia. Hematol Oncol Clin North Am. 2003;17:1159-173.
6. Hochhaus A, La Rosee P. Imatinib therapy in chronic myelogenous leukemia: strategies to avoid and overcome resistance. Leukemia. 2004;18:1321-331.
7. Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-pesserini C, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med. 2002;346:645-52.
8. Braziel RM, Launder TM, Druker BJ, Olson SB, Magenis E, Mauro MJ, et al. Hematopathologic and cytogenetic findings in imatinib mesylate-treated chronic myelogenous leukemia patients: 14 months' experience. Blood. 2002;100:435-41.
9. Seabrigth M. A rapid banding technique for human chromosomes. Lancet. 1971;2:971-72.
10. Mitelman F (ed). ISCN: an international system for human cytogenetic nomenclature. Basel: Karger; 1995.
11. Sureda A, Carrasco M, Miguel M, Martinez JA, Conde E, Sanz MA, et al. Imatinib mesylate as treatment for blastic transformation of Philadelphia chromosome positive chronic myelogenous leukemia. Haematologica. 2003;88:1213-220.

12. Hochhaus A. Cytogenetic and molecular mechanisms of resistance to imatinib. Semin Hematol. 2003;40:69-79.
13. O'Dwyer ME, Mauro MJ, Kurilik G, Mori M, Balleisen S, Olson S, et al. The impact of clonal evolution on response to imatinib mesylate (STI571) in accelerated phase CML. Blood. 2002;100:1628-633.
14. O'Dwyer ME, Mauro MJ, Blasdel C, Farnsworth M, Kurilik G, Hsieh YC, et al. Clonal evolution and lack of cytogenetic response are adverse prognostic factors for hematological relapse fo chronic phase CML patients treated with imatinib mesylate. Blood. 2004;103:451-55.
15. Otero L, Cavalcanti Jr GB, Klumb CE, Sheiner MAM, Magluta EP, Fernandez TS, et al. Chromosome 17 abnormalities and mutation of thr TP53 gene: Correlation between cytogenetics, flow cytometry and molecular analysis in three cases of chronic myeloid leukemia. Genet Mol Biol. 2005;28:40-43.
16. O'Brien SG, Guillot F, Sarson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003;348:994-1050.
17. Schoch C, Haferlach T, Kern W, Schnittger S, Berger U, Hehlmann R, et al. Occurrence of additional chromosome aberration in chronic myeloid leukemia patients treated with imatinib mesylate. Leukemia. 2003;17:461-63.
18. Cortez JE, Talpaz M, Giles F, O'Brien S, Rios MB, Shan J, et al. Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. Blood. 2003;101:3794-800.
19. Terre C, Eclache V, Rousselot P, Imbert M, Charrin C, Gervais C, et al. Report of 34 patients with clonal chromosomal abnormalities in Philadelphia-negative cells during imatinib treatment of Philadelphia-positive chronic myeloid leukemia. Leukemia. 2004;18:1340-346.

Resumo

Imatinibe induz à resposta citogenética completa em cerca de 80% dos pacientes diagnosticados com leucemia mielóide crônica (LMC) em fase crônica (FC), e em 41% dos pacientes em 1ª FC após falha do tratamento com interferon-α. Alguns pacientes, entretanto, não respondem completamente. Em muitos estudos, a resistência à droga em pacientes tratados com imatinibe é correlacionada a alterações cromossômicas adquiridas durante o tratamento. No presente estudo, foram analisados 48 pacientes tratados com imatinibe após resistência ao interferon-α, com o objetivo de verificar o impacto das alterações cromossômicas adicionais ao Philadelphia (Ph), prévias à terapia com imatinibe. Alterações adicionais foram detectadas em 33,3% dos pacientes. Pacientes com somente o cromossomo Ph apresentaram melhor taxa de resposta citogenética e sobrevida global significativa maior quando comparados com os pacientes que apresentavam alterações cromossômicas adicionais antes do início da terapia com imatinibe. Assim, nesse grupo de pacientes, a escolha de outra conduta terapêutica, como o transplante de células tronco-hematopoéticas ou regime de combinação de drogas, pode ser indicada. O presente estudo indica a importância do duplo Ph antes do início da terapia com imatinibe. Todos os pacientes com esta alteração não responderam ao tratamento, sendo a mesma associada à resistência à droga. Este estudo sugere que a citogenética clássica permanece como uma ferramenta importante no monitoramento de pacientes portadores de LMC tratados com imatinibe.

Palavras-chave: Leucemia mielóide crônica, Imatinibe, Anomalias cromossômicas