original research report

Additional chromosomal abnormalities in Philadelphia-positive chronic myeloid leukemia

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BACKGROUND AND OBJECTIVE: The emergence of non-random chromosomal abnormalities is a well-recognized occurrence in chronic myeloid leukemia (CML) and detection of these abnormalities is important in prognostic stratification. The frequency and types of additional chromosomal abnormalities in CML patients has not been determined in our region.

PATIENTS AND METHODS: We conducted a descriptive, prospective study of additional chromosomal abnormalities in patients with an established diagnosis of Philadelphia-positive CML from May 2001 to June 2007. Cytogenetic studies were repeated every three months with the conventional G-banding technique and described according to the international system for Human Cytogenetic Nomenclature. All patients received imatinib mesylate.

RESULTS: In 219 patients with Philadelphia-positive CML, 34 (15.5%) (median age, 38 years) developed 51 additional chromosomal abnormalities. Five cases had variant translocations prior to starting imatinib; the remaining 29 cases acquired chromosomal abnormalities after starting imatinib, including 8 cases that received prior interferon-alfa. Twenty-one patients were in chronic phase, 10 in accelerated phase and 3 were in blast crisis. Trisomy 8 was the most frequent abnormality followed by random chromosomal abnormalities and variants of the Philadelphia chromosome.

CONCLUSIONS: The overall frequency of additional chromosomal abnormalities was similar to that in previous reports. Early identification of these abnormalities may help in adapting to a more appropriate therapeutic approach.

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder characterized by massive proliferation and accumulation of myeloid cells that differentiate normally.[1,2] Almost all patients carry a specific translocation [t(9;22)(q34;q11.2)][3] and derivative [der(22)]— the Philadelphia chromosome (Ph)— that results in the juxtaposition of the DNA sequence from the *BCR-ABL* genes,[4] and encodes a 210-kilodalton dysregulated tyrosine kinase, which is necessary and sufficient for leukaemogenesis.[5,6] Although this Ph chromosome is thought to be the initial event in CML, the acquisition of additional cytogenetic abnormalities are likely responsible for disease progression.[4]

The emergence of non-random chromosomal abnormalities in addition to the Ph chromosome is a well-recognized occurrence in CML and is referred to as clonal evolution.[4] It is a marker of disease progression and is thought to reflect the genetic instability of the highly proliferative CML progenitors.[4] The frequency of clonal evolution increases with advancing stage of CML, and is reported in about 30% of cases with accelerated phase[7] and up to 80% in blast phase.[8] About 5% to 10%[9] patients showed variant Ph translocation by the involvement of one or more chromosome regions in addition to chromosomes 9 and 22.[4,10] The clinical and prognostic significance of these cases have not been well described.[8] Other chromosomal abnormalities including an extra Ph chromosome, trisomy 8, trisomy 19, and isochromosome 17q (with loss of p53)[11] or 20q,[12] which occur as the disease transforms to the aggressive phase. Regardless of the underlying mechanisms, the net result of additional genetic abnormalities is the potential for a more malignant phenotype and, possibly, less dependence on *BCR-ABL* for proliferation and survival.[4,7]

The detection of these cytogenetic abnormalities may be important in stratifying patients into good and bad prognostic groups and to offer them appropriate

treatment options. Furthermore, until now there has been no data on the prevalence of these translocations from our region. Our objective was to determine the frequency and type of additional chromosomal abnormalities other than (9; 22) (q34; q11) in patients with CML. To the best of our knowledge the impact of these abnormalities on the response to imatinib has not been studied from our region.

PATIENTS AND METHODS

This study was a descriptive, prospective analysis of 219 patients with Ph-positive CML (chronic, accelerated and blast phase according to WHO criteria),[12] in all age groups and both sexes treated at Aga Khan University Hospital over a period extending from May 2001 to June 2007. The diagnosis of CML was based on characteristic peripheral blood smear and bone marrow examination findings and was confirmed by presence of the Philadelphia chromosome on bone marrow cytogenetic studies or detection of the *BCR/ABL* translocation by polymerase chain reaction (PCR).

All 219 patients received hydroxyurea as an initial treatment, which also included 10 patients who were pre-treated with interferon. Imatinib mesylate was started at doses of 400 mg daily for the chronic phase and 600 mg daily for the accelerated and blast phase of the disease. We studied the cytogenetic response of this group. Cytogenetic analysis on cultured bone marrow aspirate samples anti-coagulated with heparin was performed using conventional trypsin-giemsa G-banding technique, and described according to the International System for Human Cytogenetic Nomenclature (ISCN). At least 20 metaphases were analyzed where possible. Cytogenetics was performed every 3 to 6 months while on imatinib mesylate. Clonal cytogenetic evolution was defined as gains in at least two metaphases or losses in at least three metaphases.

RESULTS

In 219 cases of Ph+ CML, we identified 34 (15.5%) cases that developed 51 additional chromosomal abnormalities (Table 1). Prior to starting imatinib 5 cases of the 34 had a variant translocation, including two who were pre-treated with interferon alfa. The rest of the 29 cases acquired chromosomal abnormalities after starting imatinib mesylate, which also included 8 cases that received interferon prior to imatinib.

Karyotype analysis

We identified trisomy 8 as the commonest additional chromosomal abnormality, followed by variant Ph chromosome and other random abnormalities (Figure 1). Trisomy 8 was detected as an isolated abnormality in nine cases, and it was noted in combination with other chromosomal aberrations in four cases. During a median follow-up of 18 months (range, 3-40 months) only one patient died in this group. Variants of Philadelphia chromosome were found in seven cases; five at diagnosis and two acquired later. Chromosomes 1, 3, and 5 were involved with one in each case and two cases had a chromosome 12 and 17 translocation in addition to t(9;22). Median follow-up of this group was 13 months (range, 2-36 months). Other abnormalities included monosomy 7 in combination with other aberrations (n=4), double Ph+ (n=7) and deletion Y (n=2) (Table 2). Overall, seven patients had more than one chromosomal abnormality.

Table 1. Baseline characteristics of the 34 Ph+ CML patients with additional chromosomal abnormalities.

Characteristics	Value (n)
Phase*	
Chronic	21
Accelerated	10
Blast	03
Gender	
Male	27
Female	07
Age, median (years)	38
Range	19-62
Prior interferon therapy	10

*defined according to WHO criteria.

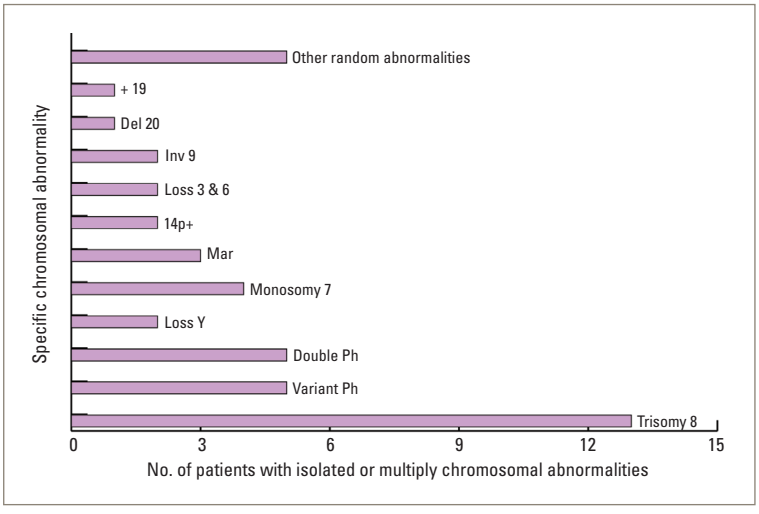

Figure 1. Frequency of various cytogenetic aberrations in 34 cases of Philadelphia-positive chronic myeloid leukemia.

Table 2. Philadelphia-positive chronic myeloid leukemia patients with additional chromosomal abnormalities.

No.	Age	Prior therapy	Phase	Initial karyotype at diagnosis	Time of CE (mo)	Karyotype with additional abnormalities	Follow-up (mo)
1	24	HU	CP	46XY, t(9;22)(20)	24	46XY, t(6;7), t(9;22) (17)	36
2	38	HU	CP	46XY, t(9;22) (20)	18	47XY, +8, t(9;22) (10)	19
3	24	HU, IFN	CP	46XY, t(9;22) (20)	10	46XY, -3, -7, t(9;22), 14p+, mar (8)	33
4	39	HU, IFN	CP	46XY, t(9;22) (20)	8	47XY, +8(2), 45XY, -7, t(9;22), (9)	18
5	24	HU	CP	46XY, t(9;22) (20)	11	47XY, +8(2)	20
6	35	HU, IFN	CP	46XY, t(9;22) (20)	25	45XY, -7(9), 47XY, +8(5), 46XY, t(9;22) (22)	18
7	35	HU	CP	46XX, t(9;22) (20)	11	47XX, t(9;22), +Ph (11)	36
8	27	HU, IFN	CP	46XY, t(9;22) (20)	8	47XY, t(9;22), +Ph (05)	28
9	19	HU	CP	46XY, t(9;22) (20)	27	47XY, +8(5)	19
10	34	HU, IFN	CP	46XX, t(9;22) (20)	16	47XX, +8(12)	17
11	47	HU, IFN	CP	46XY, t(3;9;22) (20)	Diag	46XY, t(3;9;22) (20)	17
12	54	HU	CP	46XY, t(9;22) (20)	21	47XY, +8(3), inv9, t(9 ;22) (12)	16
13	51	HU	CP	46XX, t(9;22) (20)	5	46XX, t(9;22), inv9(15)	11
14	26	HU	CP	46XY, t(9;22) (20)	11	45X-Y(6), 46XY, t(9;22) (20)	12
15	40	HU	CP	46XY, t(9;22) (20)	6	47XY, t(9;22), +Ph (11)	12
16	37	HU, IFN	CP	46XY, t(9;22) (20)	10	45X-Y(10)	38
17	37	HU	CP	46XY, t(5;9;22) (20)	Diag	46XY, t(5;9;22) (10)	6
18	40	HU	CP	46XX, t(1;9;22) (20)	Diag	46XX(16)	13
19	58	HU, IFN	CP	46XY, t(9;12;22) (20)	Diag	46XY, t(9;12;22), +Ph (2)/ 46XY, t(9;12;22) (15)	8
20	48	HU	CP	46XY, t(9;22) (20)	8	47XY, t(9;22), +Ph (3)	14
21	62	HU	CP	46XY, t(9;22) (20)	4	47XY, +8, t(9;22)(2)	3
22	30	HU	AP	46XX, t(9;22) (20)	14	46XX, t(3;11), t(9;22), mar (10)	5
23	39	HU	AP	46XY, t(9;22) (20)	12	46XY, t(9;22), del 20(20)	24
24	43	HU	BC	46XY, t(9;22) (20)	24	46XY, t(1;2), t(9;22) (20)	43
25	37	HU	AP	46XY, t(9;22) (20)	30	46XY, t(9;12;22) (11)	40
26	38	HU	AP	46XY, t(9;22) (20)	22	47XY, +8 (14)	40
27	30	HU	BC	46XY, t(9;22) (20)	20	47XY, +8, t(9;22), +Ph (10)	26
28	31	HU	AP	46XY, t(9;22) (20)	20	47XY, +8 (3)	39
29	46	HU	AP	46XY, t(9;22) (20)	9	47XY, +8 (7)	17
30	48	HU	BC	46XY, t(9;17;22) (12)	Diag	46XY(26)	13
31	44	HU	AP	46XX, t(1;6), t(9;22) (20)	18	50XX, t(1;6), t(9;22), +8, +19, +21, +Ph (4)	15
32	24	HU, IFN	AP	46XY, t(9;22) (20)	18	46XY, -3, -6, -7, t(9;22), 14p+, mar(14)	24
33	51	HU	AP	46XY, t(9;22) (20)	6	46XY, t(9;17;22) (20)	16
34	45	HU, IFN	AP	46XX, t(9;22) (20)	13	46XX, t(7;9), t(21;22)	36

CE: clonal evolution; HU: hydroxyurea; IFN: interferon-alfa; CP: chronic phase; AP: accelerated phase, BC: blast crisis; Diag: at diagnosis.

A complete cytogenetic response was observed in 5 of 34 (14.7%) patients with additional cytogenetic abnormalities. These included three cases with trisomy 8, who developed trisomy 8 in their Ph-negative clone while on imatinib at 17, 39 and 40 months of follow-up. The other three cases with trisomy 8 in a Ph negative clone by conventional cytogenetics showed either signs of progressive disease or *BCR/ABL* positivity by FISH technique. Two patients that had a complete cytogenetic response with imatinib had a variant chromosome at the time of presentation. At the median follow-up of 19 months (range, 3-43 months), 25 patients were alive, 8 had died with progressive disease and 2 were lost to follow-up.

DISCUSSION

In chronic phase CML, Philadelphia chromosome and *BCR/ABL* translocation is supposed to be the sole acquired genetic abnormality, so imatinib mesylate was assumed to be an ideal agent to block the defect, because it specifically inhibits the tyrosine kinase activity of the *BCR/ABL* protein.[1,12] The acquisition of secondary cytogenetic abnormalities may represent the changing natural history of CML in an era of tyrosine kinase inhibitors, treatment-related effects or the manifestation of an underlying stem cell defect.[13,14] Emergence of these cytogenetic abnormalities after interferon are a well known phenomenon in 50% to 60% cases; however, recently it has been recognized in patients receiving imatinib as well.[14,15] We found the overall frequency of additional chromosomal abnormalities in 34 (15.5%) cases, including Philadelphia-negative clones in 6 cases. A similar incidence is observed in previous reports.[14] Of the 34 patients, 13 (38%) had trisomy 8, making it the most common chromosomal aberration in this group. This is in comparison with other studies conducted by O'Dwyer et al[14] and Medina et al (32%).[16] In the present study, variants of Ph chromosome, which involves t(9;22) in association with another chromosome, were seen in 22% of cases, which is in contrast to Mohamed et al[1] who reported variants in 8.6% of such cases. Chromosomes 1, 3, 5 (n=1 in each), 12 (n=2) and 17 (n=2) were involved in addition to 9 and 22 in our study. The frequency of monosomy 7 and double Philadelphia is less in our series, however, and the incidence of the loss of the Y chromosome is comparable to previous reports.[17] The significance of the loss of the Y chromosome is unclear; it has been observed in healthy males.

Random chromosomal abnormalities found in individual patients were loss of chromosome 3 and 6 in combination with monosomy 7 (n=4); del 20q (n=1), mar (n=3), all in combinations, 14p+ (n=2), and inversion 9 (n=2). Other random abnormalities included t (1; 2), t (3; 11) in combination with mar, t(7; 9) in addition to t(21; 22), t(1; 6) and t(6; 7). Marker (mar) chromosome is a structurally abnormal chromosome in which no part can be identified; the clinical significance of this abnormality is not known.[18]

Clonal evolution in interferon-treated patients is a well documented phenomenon and usually an indication of disease progression,[10] but recently this phenomenon has been recognized in patients treated with imatinib mesylate.[15] The extent to which these aberrations affect the prognosis and response to the therapy has only been studied in a small number of cases.[10,15]

The mechanism of the emergence of these abnormal clones is unclear.[14] The assumed causes of these additional chromosomal abnormalities include the inherent ability of a CML clone to transform into another malignant disorder such as myelodysplasia,[16] or there may be a multi-step leukemogenic pathogenesis apart from the *BCR/ABL* dependant mechanism, or it could be due to imatinib mesylate, which blocks the activity of *BCR/ABL* proteins so the remaining cells proliferate and develop random abnormalities in a Philadelphia- negative clone.[14-16] Another possibility is the inherent instability of the CML clone that predisposes to other abnormalities.[4]

In this series we identified a very small subset of patients whose Ph+ clone remained sensitive to imatinib mesylate despite the presence of additional chromosomal abnormalities. A complete cytogenetic response was reported in 26% cases earlier;[1] in contrast we observed this response in only 14% of patients. This supports a role for a monitoring algorithm that includes periodic bone marrow karyotyping of CML patients receiving imatinib mesylate to exclude the unrecognized coexisting myelodysplastic clones and/or clinical evolution in the absence of Ph+ cells. The early recognition of these abnormalities will lead to earlier and appropriate therapeutic intervention, which may include increasing the dosage of imatinib mesylate, combination therapy, other investigational agents and the choice and timing of allogeneic stem cell transplantation before progressing to the accelerated or blast phase.[13]

In conclusion, acquisition of additional chromosomal abnormalities in CML is a well known phenomenon that can occur with tyrosine kinase inhibitors as well. There are some postulated mechanisms, but the exact pathogenesis and the underlying biology in acquisition of these abnormalities remains unclear, and requires molecular insights to clarify the details. Early identification of these abnormalities may help in adapting a more appropriate therapeutic approach. However, large multi-center studies are needed for further clarification of the clinical relevance.

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