Clonal Chromosomal Abnormalities in Philadelphia-Negative Cells and Their Clinical Significance in Patients with Chronic Myeloid Leukemia: Results of a Single Center

Kronik Miyeloid Lösemili Hastalarda Philadelphia Negatif Hücrelerde Görülen Klonal Kromozomal Anomaliler ve Bunların Klinik Anlamı: Tek Merkez Çalışması

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ABSTRACT Objective: Chronic myeloid leukaemia (CML) is a haematological disease characterised by the presence of reciprocal t(9;22) translocation, called Philadelphia (Ph) chromosome. Highly improved haematological and cytogenetic results were reported in patients with chronic phase CML after introduction of imatinib into the market. Recently a number of studies draw attention to the emergence of clonal chromosomal abnormalities (CCAs) in Ph (-) cells during cytogenetic follow-up of CML patients. The clinical significance of the CCAs has not yet been clearly defined. The present study aims to demonstrate the occurrence pattern of CCA in Ph (-) cells in our cohort of CML patients and to investigate the impact of CCAs on the course and prognosis of CML. **Material and Methods:** A total of 45 patients were evaluated. Thirty-five patients with clonal chromosomal abnormalities in Ph(-) cells constituted the first group (GI), which was compared to a second group (GII) of 10 patients with complete cytogenetic response but no CCAs in terms of survival and disease progression. **Results:** The most frequent CCAs were -21, -18 and -20, followed by -22, -10, -17 and -19. Trisomies of Y and 8 were seen in 2 patients. In 9 cases structural abnormalities, such as del(7)(q11), del(17)(q11q21) and different marker chromosomes were observed. There were no difference between the two groups in terms of survival and progression. Dysplasia to some extent seems to occur in both groups irrespective of presence or absence of CCAs. **Conclusion:** According to our results, there is no convincing evidence that CCAs can alter the natural course of CML on imatinib. We suggest that, regular cytogenetic monitoring with classical cytogenetic analysis is essential for CML patients on tyrosine kinase inhibitors, however it would be advisable to confirm and follow the most frequently observed numerical abnormalities by FISH technique as well.

Key Words: Leukemia, myelogenous, chronic, bcr-abl positive; prognosis; chromosome aberrations

ÖZET Amaç: Kronik miyeloid lösemi (KML), Philadelphia (Ph) kromozomu olarak adlandırılan, resiprokal t(9;22) translokasyonu ile karakterize hematolojik bir hastalıktır. İmatinibin piyasaya verilmesinden sonra, kronik fazdaki KML hastalarında yüksek oranlarda hematolojik ve sitogenetik tam yanıtlar elde edilmeye başlanmıştır. Yakın zamanlarda KML hastalarının takibinde Ph (-) hücrelerde klonal kromozomal anomalilerin (KKA) ortaya çıktığına dair bir dizi çalışma yayınlanmıştır. Bu KKA'ların klinik anlamı henüz açıklık kazanmamıştır. Bu çalışmanın amacı merkezimizde izlenen KML hastalarında KKA görülme kalıplarını ve bu anomalilerin klinik anlamını araştırmaktır. **Gereç ve Yöntemler:** Çalışmaya 35'i KKA'lı (GI) 10'u KKA'sız (GII) olmak üzere tam sitogenetik yanıtlı toplam 45 hasta dahil edilmiş ve bu iki grup sağkalım ve hastalık progresyonu açısından karşılaştırılmıştır. **Bulgular:** En sık görülen KKA'lar sırasıyla -21, -18, -20, , -22, -10, -17 ve -19'dur. İki hastada trizomi Y ve 8 görülmüştür. Dokuz hastada del(7)(q11), del(17)(q11q21) ve farklı marker kromozomlar olmak üzere yapısal anomaliler gözlenmiştir. GI ve GII arasında sağkalım ve hastalık progresyonu açısından anlamlı fark saptanmamıştır. KKA'dan bağımsız olmak üzere her iki grupta da displazinin düşük oranda ortaya çıktığı gözlenmiştir. **Sonuç:** Elde ettiğimiz sonuçlar, imatinib kullanan hastalarda görülen KKA'ların hastalığın doğal seyrine etki ettiğine dair yeterli kanıt sunmamaktadır. Tirozin kinaz inhibitör tedavisi altındaki hastalarda düzenli izlemin klasik sitogenetik analizle yapılması en uygun yoldur. Ancak, sık tekrarlayan sayısal anomalilerin FISH tekniği ile doğrulanması ve takibi de yapılmalıdır.

Anahtar Kelimeler: Lösemi, miyeloid, kronik, bcr-abl pozitif; prognoz; kromozom aberasyonları

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Chronic myeloid leukaemia (CML) is a clonal haematological disease characterised by the presence of t(9;22)(q34;q11) translocation which results in Ph chromosome, in about 95% of cases. This reciprocal translocation leads to the well-known rearrangement between the ABL gene on chromosome 9 and the BCR gene on chromosome 22. The resulting BCR-ABL fusion gene encodes a protein with increased tyrosine kinase activity, which plays the major role in CML pathogenesis.

Better understanding of molecular biology of the disease led to the introduction of imatinib mesylate, a potent ABL kinase inhibitor. With this new agent, high rates of cytogenetic response are achieved in chronic phase CML patients. Responses are mostly durable in those with major cytogenetic response.

Cytogenetic abnormalities other than Ph chromosome have been reported to occur during the natural course of the disease.[1] Occurrence of additional cytogenetic abnormalities in the Ph (+) cells is defined as clonal evolution (CE) and is considered a preceding sign for acceleration. Emergence of clonal chromosomal abnormalities (CCA) in Ph(-) cells has also been documented in patients using interferon (INF)-alpha and imatinib mesylate. However, the incidence and the clinical significance of those CCAs as well as their influence on prognosis have not been a major point of interest until recently. The aims of this study were to delineate the occurrence pattern of CCA in Ph (-) cells in our cohort of CML patients and to investigate the impact of CCAs on the course and prognosis of CML.

MATERIAL AND METHODS

DESIGN

A total of 183 Ph-positive chronic phase CML patients who were treated with INF and/or imatinib mesylate in the Haematology Department of Cerrahpaşa Medical Faculty between 1994 and 2007 were included in the study. The files of all enrolled patients as well as the database of the Genetics Department were retrospectively reviewed in terms of baseline and follow-up cytogenetic results. Informed consent was obtained before the review process. One hundred and six out of 183 patients had been monitored with cytogenetics. CCAs in Ph(-) cell population were detected in 35 of those 106 (33%) patients. The clinical data of the 35 patients with CCAs in Ph(-) cells were reviewed in detail and compared to 10 cases of CML patients (10/106 patients; 9%) with complete cytogenetic response and no other clonal chromosomal abnormalities. Dysplastic changes in bone marrow samples were also evaluated in relation to presence or absence of CCAs. For this purpose, bone marrow samples were reviewed for signs of dysplasia.

STUDY POPULATION

In total, 45 patients were evaluated in 2 groups. The first group included 35 patients with clonal chromosomal abnormalities in Ph(-) cells. At the time of imatinib treatment, their median age was 45 years (range: 15-78); 34 patients were in the chronic phase and one in an accelerated phase. The median follow-up duration was 49 months (range: 17-132). Male to female ratio was 24/11. Twenty-two patients had been previously treated with hydroxyurea (HU) alone and 8 with INF including 1 in combination with cytarabine (ARA-C). The median follow-up duration after imatinib therapy was 45 months (range: 10-66). Ten patients with CCR and no CCAs comprised the second group. The median age was 44 years (range: 28-56) and male to female ratio was 1. Three of the patients had received a prior course of INF before imatinib. The median follow-up in the second group was 28 months (range: 9-73). The characteristics of the patients are summarised in Tables 1-2.

DEFINITIONS

Clonality assessments were made according to the rules of International System for Human Cytogenetic Nomenclature (ISCN) 1995/2005.[2,3] Structural chromosome abnormalities and trisomies were defined as "clonal" if they were present in at least 2 metaphases. Monosomies considered clonal only if they exist in at least three cells. Samples carrying

TABLE 1: Characteristics of CML Patients with CCAs in Ph (-) Cells.

Patient No.	Gender	Age at Dx (years)	Sokal Score at Dx	Interferon prior to imatinib	Age at imatinib onset (years)	Months on imatinib	Best CR	Time to best CR (months)	Overall survival (months)	Dysplastic changes on BM
1	F	46	0,746	Yes	51	64	CCR	18	132	No
2	F	25	0,783	No	29	49	CCR	36	98	No
3	M	52	0,648	Yes	55	51	CCR	12	82	Yes
4	M	42	1.464	No	43	48	MCR	24	64	Yes
5	M	57	2.333	Yes	61	57	CCR	18	103	Yes
6	M	36	0.754	Yes	40	47	CCR	36	98	Yes
7	F	21	0,621	Yes	24	58	CCR	12	91	Yes
8	M	39	0.901	Yes	42	66	MCR	30	102	No
9	M	46	0.780	No	46	48	CCR	18	52	No
10	M	25	1.535	No	25	25	CCR	7	26	No
11	M	18	0.691	No	18	49	CCR	28	49	Yes
12	M	30	0.855	No	30	35	CCR	12	38	No
13	F	15	1.672	No	15	41	CCR	6	42	No
14	M	28	0.887	No	28	29	MCR	12	30	No
15	M	51	0,784	No	51	44	CCR	18	49	No
16	M	38	1.338	No	38	50	CCR	18	51	No
17	M	49	1.088	No	52	47	MCR	18	82	Yes
18	M	50	1.034	Yes	54	56	CCR	12	99	No
19	F	68	0.798	No	68	13	CCR	3	30	No
20	M	54	0.858	No	54	25	CCR	18	25	No
21	F	53	0.698	No	53	29	CCR	6	30	No
22	M	33	0.943	No	33	15	CCR	6	17	No
23	F	42	1.338	No	42	13	CCR	18	31	No
24	F	45	0.682	Yes	46	44	CCR	4	61	No
25	M	25	1.225	No	25	48	CCR	17	49	No
26	M	53	2,190	No	53	45	CCR	5	46	Yes
27	M	30	0.498	No	31	47	CCR	9	64	No
28	F	63	1.852	No	63	33	CCR	3	35	No
29	F	61	1.148	No	61	10	CCR	7	23	No
30	M	40	0.633	No	41	48	CCR	17	58	No
31	M	78	0.885	No	79	25	CCR	9	40	NA
32	F	56	1.133	No	56	31	CCR	6	34	No
33	M	50	3.331	No	51	42	CCR	12	50	No
34	M	36	0.564	No	36	21	MCR	9	23	No
35	M	57	1,171	Yes	58	45	MCR	29	56	Yes

Abbreviations: F: female, M: male, CR: cytogenetic response, Dx: diagnosis, CCR: complete cytogenetic response, MCR: major cytogenetic response, BM: bone marrow, NA: not available

TABLE 2: Characteristics of CML Patients with CCR and No Other CCAs.

Patient No.	Gender	Age at Dx (years)	Sokal Score at Dx	Interferon prior to imatinib	Age at imatinib onset (years)	Months on imatinib	Best CR	Time to best CR (months)	Overall survival (months)	Dysplastic changes on BM
36	M	51	0.631	Yes	51	34	CCR	20	73	No
37	F	45	0.716	Yes	47	50	CCR	10	72	No
38	F	49	1.091	Yes	50	52	CCR	19	65	No
39	M	30	1.303	No	33	9	CCR	4	9	No
40	F	43	1.262	No	43	27	CCR	27	27	No
41	M	28	0.537	No	28	33	CCR	14	35	No
42	M	56	0.796	No	57	11	CCR	6	24	No
43	M	49	5.689	No	49	28	CCR	24	29	Yes
44	F	31	1.210	No	32	11	CCR	3	25	Yes
45	F	38	0.821	No	38	12	CCR	6	13	Yes

Abbreviations: F: female, M: male, CR: cytogenetic response, Dx: diagnosis, CCR: complete cytogenetic response, MiCR: minor cytogenetic response, MCR: major cytogenetic response, BM: bone marrow, NA: not available.

additional chromosomal abnormality only on 1 metaphase were considered non-clonal and were excluded.

Cytogenetic responses to therapy were defined as follows:[4]

Complete cytogenetic response (CCR) indicated that there were no Ph+ cells at all.

Presence of less than 35% Ph+ cells was designated as *major cytogenetic response (MCR)*.

Partial (PCR) and *minor cytogenetic responses (MiCR)* were considered if the frequency of Ph+ cells lied between 35-65% and 66-95%, respectively.

No cytogenetic response was defined as the presence of 96-100% of Ph+ cells.

CONVENTIONAL CYTOGENETICS

Conventional cytogenetic analyses were performed in the Cytogenetic Laboratory of Medical Biology Department of Cerrahpaşa Medical Faculty after 24 h or overnight unstimulated culture of bone marrow. GTL banding was applied and chromosome abnormalities were described according to the ISCN 1995/2005.[2,3] At least 20 metaphases were evaluated whenever possible.

STATISTICAL ANALYSIS

All descriptive and comparative statistical analyses were carried out using SPSS v.11 for Windows. The groups were not normally distributed; we therefore used the non-parametric Mann-Whitney U test to compare different parameters in the 2 groups. A p value < 0.05 was considered statistically significant.

RESULTS

CYTOGENETICS IN PATIENTS WITH CCAS

In 35 patients the emergence of a cytogenetically abnormal clone in Ph(-) cells was observed after a median of 13 months (range 3-35 months) following imatinib onset. All patients had complete haematological response (CHR) to imatinib, and 1 partial, 10 major and 24 complete cytogenetic response when clonal chromosomal abnormalities were noticed in Ph(-) cells. Four of 10 cases (patients 4, 20, 21, 23) with MCR at the time when the CCAs were discovered, improved their cytogenetic response to CCR, although one of these patients (patient 4) returned to MCR after 2 successive samples with CCR. One of the 10 MCR cases (patient 35), regressed to MiCR. One patient with partial cytogenetic response (patient 14) at the time of first CCA occurrence progressed to MCR. Another patient (patient 10) lost his CCR that had been achieved at the time of emergence of the first CCAs and went back to MiCR. This patient underwent allogeneic stem cell transplantation from his HLA full match brother but died 1 month after transplantation due to transplant related infection.

Up to 9 cytogenetic analyses of bone marrow were regularly performed every 6 to 12 months according to cytogenetic results for each case during the follow up period after imatinib. Twenty-nine different kinds of cytogenetic abnormalities were observed (monosomies of all chromosomes, trisomies of chromosomes 8 and Y, structural abnormalities involving chromosomes 7 and 17, and different marker chromosomes) in a total of 165 events. Numerical chromosome abnormalities, especially monosomies were predominant in our series. Monosomies of all chromosomes were observed clonally at least in one case. The most prominent one was -21 which was observed in 14 cases. Monosomies of 18 and 20 were seen in 13 cases each, -10, and -22 was observed in 11 cases each, and -17 and -19 were present in 10 cases each. Trisomies of Y and 8 were seen in 2 patients. In 9 cases structural abnormalities, such as del(7)(q11), del(17)(q11q21) and different marker chromosomes were observed. CCAs in the study population were shown in Table 3. Figure 1 shows the chromosome involvement in 35 patients with CCAs in Ph(-) metaphases.

Twenty-seven of 165 (16%) cytogenetic abnormalities were observed in a median of 2 analyses (range 2-4), although not always consecutively, in 12 cases. Monosomy of -21 was observed in at least 2 different bone marrow samples in 3 cases (patients 8, 12, 24), -6 in 2 cases (patients 3, 12), -8 in 2 cases (patients 3, 4), +8 in 2 cases (patients 17, 21), -9 in 2 cases (patients 3,4), -13 in 2 cases (patients 3,12), and -20 in 2 cases (patients 28,31). The following cytogenetic abnormalities were detected more than once in one case each: del(7)(q11) (patient 35), –Y (patient 4), +Y (patient 8), -3 (patient 24), -10 (patient 4), -14 (patient 4), -15 (patient 4), -16 (patient 4), -17 (patient 9), -18 (patient 4), -22 (patient 1) and a marker chromosome (patient 29).

CLINICAL COMPARISON BETWEEN CARRIERS AND NON-CARRIERS OF CCAS

In order to evaluate the impact of occurrence of CCAs on the prognosis and natural course of CML during imatinib treatment we compared

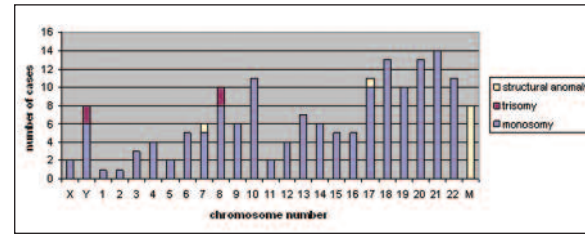

FIGURE 1: Chromosome involvement in 35 patients with CCAs in Ph (-) metaphases.
M: marker chromosome.

CCA carriers (group I) with non-carriers (group II) in terms of overall survival and treatment response. There were no statistically significant differences in basic characteristics between the 2 groups except that the duration of treatment with imatinib was shorter in group II (27.5 months vs 44.5 months) (Table 3). Dysplasia to some extent seemed to occur in both groups irrespective of presence or absence of CCAs. The overall survival durations were not significantly different since all patients were alive at the end of the follow-up period, except one patient that lost the complete cytogenetic response and was transplanted for this reason but died due to transplant related infection.

DISCUSSION

CML patients with CCAs in Ph(-) cells have recently been reported in a couple of series.[5-7] The frequency of CCAs in Ph(-) cells differ between 2% and 24% in different reports.[8,9] We found a frequency of 33% in our patients. This may seem higher than the results in related literature. However, the incidence of CCAs in Ph (-) cells in the literature could have been underestimated because CML screening has often been performed using FISH or by scoring only the certain abnormalities like Ph chromosome, -7, or +8 on G-banded preparations.[5,9] Bumm et al indicated the need for further studies with classical cytogenetics to establish the correct frequency of CCAs.[10] Our results totally depend on classical cytogenetics, which might be an explanation for the higher rate of CCAs. If only the trisomies and structural abnormalities are considered,

TABLE 3: Clonal Chromosomal Abnormalities in Ph (-) clone.

Patient No.	Clonal Chromosome Abnormalities in Ph(-) cells (%)	Time on Imatinib (months)
1	-17 (%18), -21 (%14), -22 (%32)	23
	-22 (%17)	50
	-18 (%15)	58
2	-10(%15), -16 (%15)	4
	-1 (%15), -17 (%15), -22 (%20)	53
3	-Y (%20), -4 (%16), -5 (%12), -6 (%12), -8 (%12), -9 (%24), -12 (%12), -13 (%24), -15 (%28), -16 (%12), -18 (%12), -19 (%28), -20 (%20), -21 (%24), -22 (%12)	25
	-6 (%12), -8 (%20), -20 (%16)	31/3
	-8 (%11), -9 (%15), -13 (%11), -20 (%11)	39/9
4	-Y (%14)* , -10 (%14)* , -14 (%18), -15 (%14), -16 (%11)*	8
	-Y (%10), -3 (%10), -7 (%13), -8 (%10), -9 (%10), -10 (%13), -11 (%10), -14 (%10), -15 (%10), -18 (%13), -20 (%13), mar1 (%10),mar2 (%7)*	19
	-4 (%15), -8 (%20), -9 (%15), -10 (%25), -13 (%30), -14 (%15), -15 (%25), -16 (%20), -18 (%15), -21 (%15), -22 (%15)	25
5	-Y (%25)	33
	-10 (%15), -12 (%15	42
6	-17 (%14), -19 (%14)	23
	-10 (%11)	34
	-17 (%20)	42
7	-X (%21)	14
8	+Y (%30), -17 (%20)	29
	+Y (%10), -Y (%16)*, -7 (%10)*, -10 (%10)*, -13 (%13)*, -15 (%13)*, -18 (%27)*, -21 (%23)*, -22 (%10)*	37
	-21 (%10)*	43
	+Y (%83)	50
9	-12 (%17), -20 (%13)	
10	-22 (%19)	7
	mar (%25)	18
	mar (%12)*	25
11	-7 (%14), -18 (%19), -21 (%14)	28
	-8 (%14), -13 (%14), -15 (%14)	34
	-19 (%14)	41
12	-9 (%23), -17 (%31)*, del(17)(q11q21) (%23)*, -21 (%23)*	5
	-6 (%15), -13 (%15), -15 (%20), -19 (%15), -21 (%15)	19
	-6 (%15), -10 (%15), -13(%15), -18 (%20)	26
13	-5 (%16), -17 (%32)	12
	-21 (%12), mar (%8)	29
14	-7 (%15)*, -20 (%27)	16
15	-16 (%22), -18 (%17), mar (%11)	22
16	-10 (%15), -20 (%15)	19
	-9 (%20)	25
17	-20 (%16)	20
	+8 (%7)	26
	+8 (%16)	34
18	-6 (%15), -14 (%15), -21 (%25)	12
	-4 (%12,5), -8 (%12,5), -10 (%12,5), -17 (%12,5), -20 (%21)	45
19	-20 (%13)	9
20	+Y (%16), +8 (%10,5)	3
21	-8 (%17), -16 (%10), -18 (%10), -19 (%10)	4
	-9 (%10), -18 (%13)	7
	-6 (%15)	13
22	-19 (%18), mar (%23,5)	8
23	-18 (%16)	9
24	-3 (%15), -14 (%15), -21 (%15)	4
	-2 (%14), -3 (%14), -4 (%19),-8 (%19), -9 (%14), -10 (%14), -21 (%24)	31
25	-17 (%11,5), -21 (%19)	17
	-19 (%18)	36
26	mar (%10)	5
27	-6 (%15), -10 (%15), -14(%15), -17(%20), -20(%25), -21(%25)	35
28	-19 (%11), -20 (%11)	13
	-3 (%13), -20 (%10)	21
29	-10 (%16), -12 (%16), -13 (%16), -17 (%16), -18 (%16), -19 (%21), -21 (%16), -22 (%16), mar (%11)	7
	mar (%4)	14
30	-Y (%19), -8 (%19), -11 (%10), -13 (%12), -14 (%12), -18 (%10), -19 (%16) , -20 (%19), -21 (%19), -22 (%10)	23
	mar(%15)	39
31	-18 (%17), -22 (%13)	9
32	-X (%20), -21 (%25), -22 (%15)	6
33	-Y (%26), -8(%16), -14(%16), -18(%16), -20(%32), -22(%16)	27
34	-20 (%7,5)	11
35	del(7)(q11) (%33), -7 (%11)*	29
	der3? (%21)**, del(7)(q11) (%21), -12 (%21), der12? (%12,5)**, -19 (%25)**, mar (%12,5)**	40

*Observed both in Ph+ and Ph- metaphases,**Observed in Ph+ cells, repeating abnormalities are bold typed.

TABLE 4: Comparison of Characteristics between Carriers and Non-Carriers of CCAs.

	Group I [Ph(-) with CCA]	Group II [Ph(-) without CCA]	P
Number of patients	35	10	>0.05
Age at diagnosis (years)	45 (15-78)	44 (28-56)	>0.05
Sokal score	0.887 (0.498-3.331)	0.820 (0.537-5.689)	>0.05
Spleen size at diagnosis (cm)*	6 (0-27)	3.5 (0-25)	>0.05
WBC at diagnosis (/mm^3)	126000 (17400-375000)	99300 (5500-573000)	>0.05
Hb at diagnosis (g/dl)	11.4 (5.9-15.8)	11.8 (9-14.3)	>0.05
Platelet count at diagnosis (/mm^3)	316500 (62000-1845000)	241000 (196000-482000)	>0.05
Months on imatinib	44.5 (10-66)	27.5 (9-52)	0.047
No. of patients with a prior history of interferon	8 (8/34; 23%)	3 (3/10; 30%)	>0.05
Overall survival (months)	49 (17-132)	28 (9-73)	>0.05
No of patients with dysplasia in BM	8 (8/34; 23%)	3 (3/10; 30%)	>0.05

Abbreviations: BM: bone marrow, Hb: haemoglobin, WBC: white blood cell count.

* measured in cm as palpable portion of spleen beyond left costal margin.

the frequency of CCAs in our series also comes down to 13%.

The median time to first detection of CCA in Ph(-) cells on imatinib has been reported to differ between 6 and 18 months.[6,11] In our patient group it was 13 months (range 3-35 months). O'Shea et al, and O'Dwyer et al also reported a similar median time of approximately 13 months.[9,12]

We observed in Ph (-) cells of 11 patients some clonal chromosome abnormalities (-Y, -5, -7 and +8) that have been reported to occur in myelodysplastic syndrome. Of those 11 patients, six had dysplastic changes in their bone marrow samples. Deininger et al recently reported 2 cases of MDS/AML (6.7%) in 30 patients with CCAs in Ph(-) cells in a median follow-up of 51 months.[13] Considering the low number of reported cases in the literature one might assume that the overall incidence of MDS is not high. In our study, none of the six patients with dysplastic bone marrow changes had clinical findings of a concurrent MDS.

The most frequently reported autosomal abnormalities in Ph(-) cells of CML patients are -7/7q- and +8.[2,3,6,8,9,14-22] We found persistent del(7)(q11) abnormality only one patient. There are three reported cases with this abnormality in CML patients. Terre et al reported del(7)(q11) in Ph(-) cells in one of their cases, whereas Miyamoto and Schoch et al found the same abnormality in the Ph(+) cells of their cases.[7,21,23] Our patient with del(7)(q11) achieved only major cytogenetic response following 45 months on imatinib. Almost all of the -7 and +8 abnormalities in our series were transient and did not have any clinical significance.

We detected del(17)(q11q21) in one patient. As far as we know, this abnormality was not reported in CML, but there are two reported acute promyelocytic leukemia cases and two diffuse large B-cell lymphoma cases.[14,22,24] Our del(17)(q11q21) patient had a low Sokal score and achieved complete cytogenetic response within 12 months of imatinib treatment. He had been in CCR for about three years at the time of enrolment.

Monosomies 21, 20 and 18 were the most frequently observed chromosome abnormalities in our series. O'Shea D et al reported -20 in one of their 7 cases.[9] -X was detected in two of our cases; in one patient, as a sole abnormality and in the other together with -21 and -22. Lin et al also reported co-occurrence of -X and -22 in one patient.[19]

Loss of the Y chromosome is known to be an age-related phenomenon. Several groups studied the loss of Y chromosome in haematological diseases both as an aging phenomenon and as part of

the leukemic process and they agreed that the loss of the Y chromosome was present in both normal and malignant marrows and its frequency increases with age in both situations. It is rare below the age of 50, but increases up to 25% after 80 years.[18,25,26] The overall frequency of –Y in bone marrow samples has been reported between 3.4% and 8.2%.[25,27] If –Y is observed in a minority of cells, it is assumed to be related to age, but when it is observed in a higher proportion of cells (>75%), it is usually considered a secondary event associated with the underlying pathology.[25,26] However, -Y seems harmless in terms of disease progression.[11,13,27] We observed -Y in 6 of our cases. In none of our cases –Y was present in more than 26% of the cells. None of those 6 patients were older than 70 and none had a poor clinical outcome.

Our results are not in line with those of the relevant literature in terms of high monosomy frequencies. This can be explained by the fact that in most of the above-cited studies cytogenetic analysis was performed using FISH to scan some pre-determined abnormalities or by scoring only those particular abnormalities on G-banded preparations. However, in our study, we solely, depended on classical karyotyping and therefore we were able to see all the abnormalities.

On the other hand, it is difficult to interpret monosomies in cytogenetics because of the risk of "creating" them in the process of slide making. However, not all of the monosomies are to be considered technical errors. There are, for example, persistent monosomies that were observed in two or more marrow samples of the same patient in our series. Overall, we could not demonstrate any significant difference in terms of disease progression and disease free survival in Ph(-) CML patients with or without monosomies.

Despite the advances in molecular cytogenetic techniques, classical cytogenetics is still regarded as the "golden standard" in the follow-up of haematological malignancies. The reason for preferring FISH and RT-PCR to karyotyping is the fast and easy application of these techniques. However, they are only confined to certain parts of the genome and although they are good in tracing specific abnormalities, they do not give information about additional abnormalities. Consequently, they do not put new information into the data pool of the subject, but classical cytogenetics provide a whole view of the genome and therefore, must be performed whenever possible. Molecular cytogenetic techniques should rather be used as an adjunctive test. In respect to our data, we recommend that especially monosomies of chromosomes 20, 22, 18 should be checked by FISH as well. Due to the retrospective nature of this study, we could not confirm our classical cytogenetic results by FISH.

Etiologic factors associated with the development of CCAs in Ph(-) cells are diverse. Several mechanisms, including the effect of drugs such as interferon and imatinib have been suggested. As in some other studies, we could not demonstrate any definitive causative relationship between imatinib and the emergence of CCAs.[13]

Due to low number of patients, no subgroup analysis could be performed; thus, there are no robust data to comment on the clinical or prognostic significance of a certain clonal abnormality in our cohort of patients. We observed that a CCA, alone or in combination with other abnormalities, could vanish and recur during follow-up without any clinical relevance. This makes the whole issue difficult to interpret.

With the limited data we obtained from our study, we conclude that the emergence of CCAs must be a multifactorial phenomenon. Imatinib may play a role as shown in other studies but we were not able to demonstrate any negative effect of imatinib.[7] These clonal abnormalities seem to occur randomly on a rather unstable background of dividing cells. We could not associate CCAs with MDS development in our patients though several of them had minor dysplastic changes in their marrows. Our data clearly indicate that occurrence of CCAs in Ph(-) cells does not have a major impact on the clinical outcome of patients who had achieved at least a MCR. The overall sur-

vivals as well as the sustained response rates were not different in carriers and non-carriers of CCAs in our study. Although the duration of imatinib treatment in the non-carrier group was significantly shorter than in the carrier group, its median was far beyond the median time to first appearance of CCAs (27 vs. 13 months). We therefore considered the difference negligible. But we still think that multicenter studies with large numbers of patients are required to identify the development pattern and clinical consequences of CCAs in Ph(-) cells. Currently, there is no convincing evidence that CCAs can alter the natural course of CML. Therefore, one may not consider the emergence of CCAs to redesign a treatment plan. However, accumulating data may change our concept of handling patients with CCAs; therefore we still suggest regular cytogenetic monitoring with classical cytogenetic analysis for CML patients on tyrosine kinase inhibitors.

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