Prognostic impact of Additional Chromosomal Abnormalities in Egyptian Chronic Myeloid Leukemia Patients

Yasser H. ElNahass[1], Magda M. Assem[1], Magdy M. Saber[2], Sarah K. Abdalla[1,a*], Hossam K. Mahmoud[2], Fatma A. ElRefaey[1]

[1]*Department of Clinical Pathology, National Cancer Institute, Cairo University, Fom El Khalig square - El Manial, Cairo, Egypt;*
[2]*Department of Medical Oncology, National Cancer Institute, Cairo University, Fom El Khalig square - El Manial, Cairo, Egypt*
[a]*Present address: Moffitt Cancer Center, 12902 USF Magnolia Drive, Tampa, FL 33612 - USA.*

Abstract

BACKGROUND: Emergence of additional chromosomal abnormalities (ACAs) in chronic myeloid leukemia (CML) is associated with disease progression to advanced phases and reflects the genetic instability of CML.

AIM: Is to evaluate the frequency of ACAs in chronic phase (CP) and advanced disease (AP) CML patients and study their impact on patient's outcome, overall survival (OS) and event-free survival (EFS).

RESULTS: The studied group (n = 73) included 31 males (43%) and 42 females (57%). Median age of patients at diagnosis was 37 years (17–76). Median TLC was 208×109/L (2.1–784.2), median Hb was 9.4 g/dL (5.7–13), and median platelets count was 290.5×109/L (13–1271). We identified 32 patients (44%) with ACAs. ACAs emergence was significantly associated with advanced phases of CML (13/21, 62%) compared to CP (19/52, 36%) (p = 0.048). ACAs were associated with lower median OS and EFS in CP compared to AP (38 vs. 120 ms) and (58.3 vs. 77 ms) (p = 0.026 and p = 0.065, respectively). Early molecular responders (6/17, 35%) at 3 months, and 6 months (10/26, 38%) developed ACAs less than nonoptimal responders. Disease phase, hepatomegaly and bone marrow eosinophilia were significant predictors of OS (p < 0.001, p = 0.02, p = 0.04, respectively).

CONCLUSION: Early identification of ACAs in Ph+ metaphases at diagnosis and during therapy predicts CML outcome. ACAs emergence occurred at a higher frequency and at a younger age in our CML patients and are related to inferior EFS and OS.

Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm characterized by the presence of the Philadelphia (Ph) chromosome, produced by the reciprocal translocation t(9;22) (q34;q11) [1]. This translocation leads to the generation of the chimeric fusion gene *BCR-ABL1* [2]. At diagnosis, additional chromosomal abnormalities (ACAs) in Ph+ cells may appear in ~ 5% of patients. The proportion of patients with ACAs rises during the course of the disease to ~ 80% in the blast phase (BP), according to several series [3], [4]. Despite the efficacy of imatinib mesylate (IM) in CML – chronic phase (CP), treatment failure, or suboptimal response have been reported [5], [6]. The appearance of ACAs during tyrosine kinase inhibitor (TKI) treatment is commonly known as clonal evolution (CE) and seems to play an important role in IM resistance and is considered to be a poor prognostic feature [7]. The World Health Organization (WHO) suggests that CML patients showing ACAs emerging during treatment should be considered in accelerated phase (AP) [8] and European Leukemia Network (ELN) recommendations suggest that the presence of ACAs at diagnosis may represent a "warning" feature, requiring careful monitoring of the patient. Emerging ACAs during the course of treatment represent the failure of treatment [9], [10]. The aim of the current study is to investigate the frequency and prognostic impact of ACAs in a cohort of Egyptian CML patients, either at diagnosis or during the disease course, and to study their impact on response to TKI therapy and CML patient's survival.

Patients and Methods

Patients

This study was conducted on 73 CML Egyptian patients either newly diagnosed or under TKI treatment who presented to the outpatient clinic of Medical Oncology Department of National Cancer Institute from

January 2015 to January 2017. Median follow-up time was 24 ms (1–121 ms). Informed consent was provided by all included subjects and the study was approved by the Institutional Review Board. Eligible patients were >18 years fulfilling morphologic and cytogenetic criteria of Ph+ CML in (CP), (AP), or (BP) according to definitions in the revised 4th edition of the WHO classification [8].

Treatment

The included CML patients who were previously treated with a TKI received one or more of either a first-generation TKI (IM, IM/ GLIVEC®, 400 mg orally once per day or generic imatinib) or a second-generation TKI (Nilotinib/TASIGNA® or Dasatinib/SPRYCYL®). Hydroxycarbamide (1–6 g/day orally) was given to CML-CP patients as initial cytoreductive therapy until *BCR-ABL1* results by fluorescence *in situ* hybridization (FISH) were available.

Definition of responses to TKI treatment

Hematologic, molecular, and cytogenetic remissions were defined according to the National Comprehensive Cancer Network guidelines [11]. Definitions of molecular response were defined according to ELN recommendations [12].

Measurements of survival

Event free survival (EFS) was defined as loss of hematologic or cytogenetic response or development of advanced phases of CML (AP or BP) and overall survival (OS) by absence of death from any reason [13].

Methodology

Cultivated bone marrow (BM) samples of all included patients were subjected to chromosomal banding analysis to detect the presence of ACAs in Ph+ cells. ACAs were classified as major route abnormalities that included: +8, a second Philadelphia chromosome (der 22), +19, i(17q10) [4], and minor route abnormalities that included: +21, −7, −17, del (Y) and 3q26.2, and other abnormalities [9]. The detected chromosomal abnormalities were confirmed using FISH molecular probes provided by Cytocell (UK) and MetaSystems (Germany). Diagnostic and serial measurements of *BCR-ABL1* fusion gene mRNA transcript level were done by real-time quantitative polymerase chain reaction according to IS on GeneXpert, Cepheid.

Conventional cytogenetics study

At least 20 metaphases were analyzed for each sample after trypsinization and staining slides with Giemsa. Metaphase capture and image analysis were performed using Metafer 4/MetaClient software analysis program (MetaSystems) and CytoVision (Applied Imaging). Chromosomal abnormalities were described according to the International System for Human Cytogenetics Nomenclature (ISCN, 2016) [14].

FISH

The following FISH probes were used: BCR/ABL translocation (Dual Fusion Probe), 17p subtelomere specific probe, 17q subtelomere specific probe, Del (20q) deletion probe and EVI1 (MECOM) break apart probe (Provided by Cytocell Aquarius) and XCP (XCyting Chromosome Paints) for chromosome 8, chromosome 19, chromosome 7, chromosome 21, and Del (7q) (q22q31) (MetaSystems Probes GmbH 1.Industriestr.7).

Statistical methodology

Statistical analysis was done using IBM SPSS® Statistics version 22 (IBM® Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Pearson's Chi-square or Fisher's exact tests were used to examine the relationship between qualitative variables. For not normally distributed quantitative data, a comparison between the two groups was done using the Mann–Whitney test (non-parametric t-test). Survival analysis was done using the Kaplan–Meier method and a comparison between two survival curves was done using the log-rank test. Multivariate analysis was done using the Cox-regression method for the factors affecting survival on univariate analysis. Hazard ratio (HR) with its 95% confidence interval (CI) was used for risk estimation. All tests were two-tailed. A $p < 0.05$ was considered statistically significant.

Results

Seventy-three CML patients were included, 31 (43%) males and 42 (57%) females. Fifty-two/73 (71%) patients were in CP, 6(8%) patients were in AP, and 15(20%) patients were in BP. Patients with AP and BP were grouped together as advanced phase disease (21, 29%). The median age of CP patients at diagnosis was 39 years (17–76) and 35 years (22–54) for AP. Median TLC was 225×109/L (2.1–784.2) for CP patients and 127×109/L (13.5–353) for AP, median Hb was 10 g/dL (5.7–13) for CP and 8.8 g/dL (7.1–11.5) for AP, and median platelets count was 319×109/L (123–1173) for CP and 237.5×109/L (13–1271) for

AP. Median PB blast % was 2% (0–9) for CP and 6% (0–92) for AP, median PB eosinophils was 3% (0–8) for CP and 2% (0–13) for AP, and median PB basophils was 3% (0–12) for CP and 3% (0–23) for AP. Median BM blast % was 2% (0–8) for CP and 25% (10–91) for AP, median BM basophils was 4% (0–11) for CP and 5% (0–17) for AP, and median BM eosinophils was 5% (0–14) for CP and 4% (0-16) for AP. Time to TKI treatment ranged from 6 to 150 days, with a median of 33 days. Sixty-eight patients (93%) were treated with 1st line TKI upfront (Gleevec® or generic IM) and 5 patients (7%) received a 2nd line TKI as upfront therapy (Nilotinib/Tasigna® or Dasatinib/Sprycel®). Forty three/52 (83%) were tolerant to TKI therapy, while 9 patients (17%) were intolerant. Ten/52 (19%) CP and 17/21 (81%) AP patients died by the end of study duration. Cumulative OS of CP patients at 12 months was 90% and at 24 months was 84% and was 71% and 42% for AP patients at 12 months and 24 months, respectively ($p < 0.001$). EFS of CP patients (38/52) was 73% by the end of 24 months follow-up duration.

Evaluation for emergence of ACAs

Thirty two/73 (44%) patients developed ACAs; 22 at diagnosis (16 CP and 6 AP patients) and 10 patients later during the course of the disease (3 CP and 7 AP patients). The reported 32 ACAs were 18 (56%) major route abnormalities, 4 (12%) minor route abnormalities, and 5 (16%) patients with more than one ACAs (major and minor) and 5 patients (16%) were found to express other (non-major/non-minor) secondary chromosomal changes. Ten/18 (56%) patients developed major route abnormalities at diagnosis and 8/18 (44%) patients during the course of disease, while 2/5 (40%) patients developed both major and minor route abnormalities during the course of disease and 3/5 (60%) at diagnosis. Minor route abnormalities were detected in 4 patients at diagnosis, while other chromosomal abnormalities were detected in 5 patients at diagnosis. Four/5 AP patients were identified with hyperdiploid karyotype and developed acute lymphoblastic leukemia BP and one patient developed mixed phenotype acute leukemia during disease course Table 1 describes ACAs in 32 CML patients.

Table 1: ACAs emergence in 32/73 CML patients

UPN	Age	Gender	Phase	Time of emergence	Karyotype	Major/Minor/others	TKI upfront
3	22	F	ABC	During course	51,XX,+8,+9,+10, +19,+22,der(22), t(9;22)(q34;q11) [16]/47,2q+,t(9;22)(q34;q11)[4]	Major	1st line
6	34	M	ABC	During course	52,XY,+8,+10,+11,11q-,+14,+21,+22, der(22), t(9;22)(q34;q11) [10]/46, XY, t(9;22)(q34;q11) [10]	Major	1st line
8	54	F	ABC	At diagnosis	45,XX,−7 , t(9;22)(q34;q11)[4]/46, XX, t(9;22) (q34;q11) [16]	Minor	1st line
9	26	F	CP	At diagnosis	46,XX, t(9;22)(q34;q11)[10]/44, XX, −10,-15, −20, +22, t(9;22)(q34;q11)[10]	Minor	1st line
10	58	F	CP	During course	48,XX,+19,+22,der(22), t(9;22)(q34;q11)[4]/47,XX,der(22), t(9;22)(q34;q11)[6]/46,XX t(9;22)(q34;q11)[10]	Major	1st line
11	38	F	CP	At diagnosis	48,XX, +8,+22,der(22), t(9;22)(q34;q11) [8]/47,XX,+19,t(9;22)(q34;q11) [6]/46,XX, t(9;22)(q34;q11) [6]	Major	1st line
13	33	F	CP	At diagnosis	46, XX, t(9;22) (q34;q11)[3]/46,XX, −7, +17 [7]/45, XX, −7 [10]	Minor	1st line
16	27	F	CP	At diagnosis	47,XX, −2,+8,+19, t(9;22)(q34;q22) [4]/46,XX, t(9;22)(q34;q22) [16]	Major	1st line
18	26	M	CP	At diagnosis	44, X, del (y), −17, t (9;22)(q34;q11) [8]/46, XY, t(9;22)(q34;q11) [12]	Minor	1st line
19	32	F	ABC	At diagnosis	47, XX, +22, der (22), t(9;22)(q34;q11) [18]/ 46, XX [2]	Major	1st line
20	23	F	CP	At diagnosis	47, XX, +22, der(22), t(9;22)(q34;q11) [3]/46, XX, t(9;22) (q34;q11) [17]	Major	1st line
27	40	M	AP	During course	47,XY, +8,11q-, t(9;22)(q34;q11) [15]/ 46,XY, t(9;22)(q34;q11) [5]	Major	1st line
39	26	M	ABC	During course	47, XY, +8, del (6q) ,t(9;22)(q34;q11) [15]/ 46, XY, t(9;22) (q34;q11) [5]	Major	1st line
40	31	M	CP	At diagnosis	45, XY, −9,t(9;22)(q34;q11) [14]/46, XY, t(9;22)(q34;q11) [6]	Other	1st line
41	35	F	ABC	At diagnosis	47, XX, +21, t(8;21)(q22;q22), t(9;22)(q34;q11) [15]/46, XX, t(9;22)(q34;q11) [5]	Other	1st line
42	18	M	CP	At diagnosis	47, XY, +8, t(9;22)(q34;q11) [16]/46, XY, t(9;22)(q34;q11) [4]	Major	1st line
43	26	F	CP	At diagnosis	46,XX, der(1q), t(9;22)(q34;q11) [20]	Other	1st line
45	23	M	CP	At diagnosis	48, XY,+8, der (22), t(9;22)(q34;q11)[18]/46, XY, t(9;22)(q34;q11)[2]	Major	1st line
46	48	M	CP	During course	47, XY, +4, t(9;22)(q34;q11)[6]/47,XY,+8, t(9;22)(q34;q11)[8]/47, XY,der (22),t(9;22)(q34;q11)[18]	Major	1st line
50	56	F	CP	At diagnosis	47,XX,+22, der (22), t(9;22)(q34;q11) [6]/47,XX,+19,t(9;22) (q34;q11) [4]/46,XX, t(9;22)(q34;q11) [10]	Major	1st line
51	29	M	ABC	During course	48, XY, +8, +22, der (22), t (9;22)(q34;q11) [16]/ 46,XY , t(9;22) (q34;q11) [4]	Major	2nd line
53	17	M	CP	During course	46, X , del (Y), +8, t(9;22)(q34;q11) [20]	Major and minor	1st line
54	18	M	CP	At diagnosis	46,XY, t(9;22)(q34;q11) [10]/59,XY, der(22), t(9;22)(q34;q11) [10]	Major	1st line
55	27	F	ABC	At diagnosis	46, XX, −7, +19, t(9;22)(q34;q11) [8]/47, XX, −7, +19, +22, der (22), t(9;22)(q34;q11) [4]/46,XX [8]	Major and minor	2nd line
60	42	M	ABC	During course	46, XY, −7,+22, der(22), t(9;22)(q34;q11) [12]/45,XY, −7, t(9;22) (q34;q11) [8]	Major and minor	1st line
62	44	F	AP	At diagnosis	46, XX, +4, −7, −17, der (22) , t(9;22)(q34;q11) [15]/46, XX, t(9;22)(q34;q11) [5]	Major and Minor	1st line
64	50	F	CP	At diagnosis	46,XX, , t (9;11;22)(q34;q23;q11) [20] , Variant Ph ch.	Other	1st line
65	54	M	CP	At diagnosis	45, XY, +13, −17, −20[5]/46, XY, t(9;22)(q34;q11)[15]	Major and minor	1st line
66	55	M	CP	At diagnosis	53, XY, +4, +8,+10,+12, +15, +19, +22, der(22), t(9;22)(q34;q11) [18]/48, XY,+4, +22, der(22), t(9;22)(q34;q11)[2]	Major	1st line
67	23	F	ABC	At diagnosis	47,XX, t(1;3),t(14;21), t(16;21), +22, der(22), t(9;22)(q34;q11) [20](complex karyotype)	Major	1st line
69	27	M	AP	During course	50, XY,+4,+8,+10,+22,der(22), t(9;22)(q34;q11) [18]/46,XY, t(9;22) (q34;q11) [2]	Major	1st line
72	31	F	CP	At diagnosis	46,XX, der(1q), t(9;22)(q34;q11) [20]	Other	1st line

ACAs: Additional chromosomal abnormalities, CML: Chronic myeloid leukemia, CP: Chronic phase.

Emergence of ACAs and CML patient's characteristics

AP patients developed ACAs at a higher percentage (13/21, 62%) compared to CP patients (19/52, 36%) (p = 0.048). Patients <40 years had a higher incidence of developing ACAs than patients ≥40 years (p = 0.034). A higher percentage of patients with normo/hypocellular BM smears (7/8, 87%) developed ACAs compared to (25/65, 38%) with hypercellular BM at diagnosis (p = 0.018). A higher percentage of patients with splenomegaly at diagnosis (31/64, 48%) developed ACAs compared to (1/9, 11%) patients with no splenomegaly (p = 0.035).

ACAs and TKI response

CML patients who failed to achieve EMR had more ACAs (6/13, 46%) compared to (6/17, 35%) patients who achieved EMR (p = 0.547). A higher percentage of CML-CP patients (n = 52) who achieved an optimal molecular response were free of ACAs at diagnosis (13/18, 72%) compared to CP patients with ACAs at diagnosis (5/18, 28%) (p = 0.063). We also noted that CML-CP patients who were free of ACAs at diagnosis were tolerant to their received TKIs (20/34, 83%) compared to patients with ACAs (14/34, 41%) (p = 0.316).

OS and EFS in relation to ACAs

CML patients free from ACAs (41/73, 56%) had a significantly higher median OS (120 vs. 38 months) than those who developed ACAs (32/73, 44%) at diagnosis or during disease course (p = 0.026) (Figure 1).

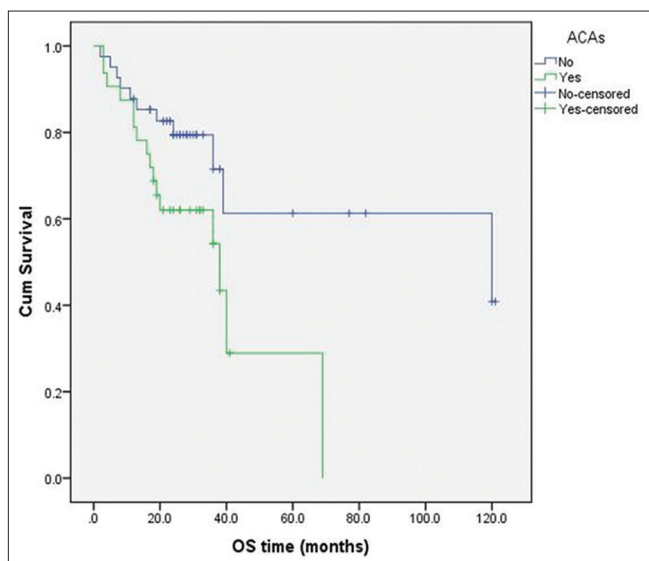

Figure 1: Additional chromosomal abnormalities and overall survival (p = 0.026)

Patients with no ACAs (41/73, 56%) had a higher median OS than patients with major route abnormalities (23/73, 31%) (120 vs. 40 months) in
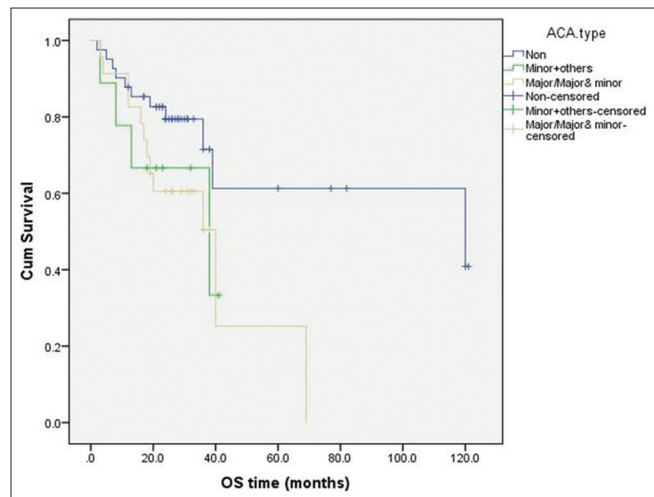

Figure 2: Overall survival by additional chromosomal abnormalities category (p = 0.084)

pairwise comparison analysis (p = 0.084) (Figure 2). CML-CP patients (n = 52) who were free of major route ACAs by end of follow-up time (33/52, 63%) had a superior cumulative EFS at 24 months than patients who developed major route abnormalities (12/52, 23%) (77% vs. 58.3%) (p = 0.065) (Figure 3).
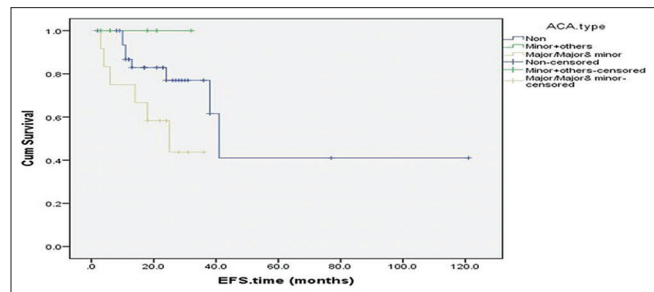

Figure 3: Relationship between additional chromosomal abnormalities category and event-free survival (p = 0.065)

Correlations between TKI response and patients survival

The patterns of molecular response and its relation to OS and EFS in 52 CML-CP patients are described in Table 2. We also report that CML-CP patients (n = 52) who shifted to generic IM had a higher cumulative OS (85% vs. 66%) and EFS (88% vs. 66%) at 24 months than patients who had frequent treatment interruptions due to unavailability of Glivec® (p = 0.087 and p = 0.100, respectively).

Correlations between patients characteristics and OS and EFS

Female patients had a higher median OS than males (p = 0.033). CML-CP patients had a higher cumulative survival at 24 months (84%) than patients presenting with CML-AP (42%) (p < 0.001) (Figure 4).

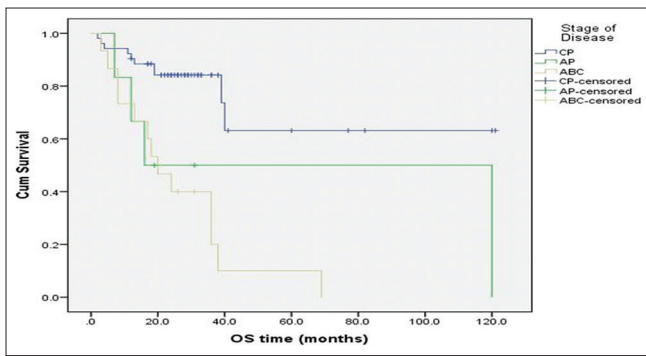Patients presenting with thrombocytopenia had a lower median OS (20 months) than patients

Figure 4: Overall survival according to chronic myeloid leukemia phases at presentation (p < 0.001)

who presented with higher platelet counts (40 months) (p = 0.070). Patients with normo/hypocellular BM had a lower median OS than patients presenting with hypercellular BM (36 vs. 69 months) (p = 0.074). Patients with BM eosinophilia (≥5%) had a higher median OS than patients with BM eosinophils count <5% (36 vs. 69 months) (p = 0.01). OS was higher in patients with splenomegaly (73%) (p = 0.978) and hepatomegaly (85%) (p = 0.079) compared to their normal counterparts (67% and 60%, respectively). Table 3 illustrates the subgroups of variables considered for the OS comparison between CP and AP groups of CML patients.

Predictors of survival in Egyptian CML patients

Multivariate analysis of CML patients categorical variables included factors affecting OS in univariate analysis (gender, thrombocytopenia [platelets count <100×10^9/L]), BM cellularity, BM eosinophilia (≥5%), disease stage (CP vs. AP), presence of ACAs, and presence of hepatomegaly). Factors that were predictors of OS were the phase of CML disease, BM eosinophilia, and hepatomegaly. AP patients (n = 21) were 7 times more liable to death than CP patients (n = 52) (HR = 6.97 95%, CI: 3.03–15.96, p < 0.001), patients with no hepatomegaly (39/73) were 2.8 times more liable to death than patients with hepatomegaly (33/73) (HR = 2.8, 95% CI: 1.20–-6.52, p = 0.017), and a finding that denotes presence of hepatomegaly was protective in the studied CML group and patients with no BM eosinophilia (≥5%) were 2.37 times more liable to death than patients with BM eosinophilia (HR = 2.37, 95% CI: 1.053–5.376, p = 0.037).

Discussion

Mechanisms of resistance to TKIs in CML include genomic amplifications, point mutations of the

Table 2: Patterns of molecular responses and OS and EFS in 52 CML-CP patients

Type of molecular response	Frequency (%)	Major route ACAs (n=10/52) (%)	ACAs (n=19/52) n.(%)	Cum OS at 24 ms (%)	p	Cum EFS at 24 ms (%)	p
EMR (3 months) *BCR-ABL1* ratio: (n=52)							
<10% (optimal response)	30 (58%)	3 (30%)	10 (33%)	76	0.656	90	0.051
≥10%	22 (42%)	7 (78%)	9 (41%)	77		61	
6 months *BCR-ABL1* ratio: (n=52)							
<1% (optimal response)	36 (68%)	6 (46%)	13 (36%)	92.3	0.204	89	0.049
≥ 1%	16 (31%)	4 (57%)	7 (44%)	83.3		70	
MMR (12 months) *BCR-ABL1* ratio: (n=52)							
<0.1% (optimal response)	25 (48%)	3 (37%)	8 (32%)	95	0.276	89	0.241
≥ 0.1%	27 (52%)	7 (64%)	11 (41%)	90.5		65	
DMR (18 months) *BCR/ABL1* ratio: (n=52)							
<0.01% (optimal response)	20 (38%)	4 (67%)	6 (30%)	NR	0.424	89	0.244
≥0.01%	32 (61%)	6 (46%)	13 (40%)	NR		60	
At 24 months (n=52):							
<0.0001% (undetectable *BCR-ABL1* ratio) (optimal response)	12 (23%)	4 (100%)	4 (33%)	NR	NR	67	NR
≥0.0001%	40 (80%)	6 (40%)	15 (37%)	NR		83	
Type of response to TKI therapy:							
Optimal	18 (35%)	3 (60%)	5 (28%)	94.4	0.125	94	0.065
Suboptimal (warning)	10 (19%)	2 (50%)	4 (40%)	NR		79	
Failure	24 (46%)	5 (50%)	10 (42%)	70.8		57	

EMR: Early molecular response, MMR: Major molecular response, DMR: deep molecular response, NR: Not reached, CML-CP: Chronic myeloid leukemia-chronic phase, ACAs: Additional chromosomal abnormalities, OS: Overall survival, EFS: Event-free survival, TKI: Tyrosine kinase inhibitors.

Table 3: Predictors of OS in CML-CP and AP phases of CML

Parameter	n (%) CP	AP	No of deaths (%) CP	AP	Cum survival at 12 months (%) CP	AP	Cum. survival at 24 months (%) CP	AP	Median survival (months) CP	AP	p
Totals	52	21	10 (19%)	17 (81%)	90	71	84	42	*	20	
Age (years)											
<40	26 (50)	14 (67)	3 (11)	11(79)	92	71	88	36	*	18	<0.001
≥40	26 (50)	7 (33)	7 (27)	6 (86)	89	71	80	57	*	36	0.066
Gender											
Male	22 (42)	9 (43)	7 (32)	9 (100)	86	78	71	22.2	40	17	0.004
Female	30 (58)	12 (57)	3 (10)	8 (67)	93	67	*	57.1	*	36	<0.001
ACAs											
Present	19 (36)	13 (62)	6 (32)	10 (77)	84	77	73	45	40	20	0.075
Absent	33 (63)	8 (38)	4 (12)	7 (87)	94	63	91	37	*	13	<0.001
ACAs category											
None	33 (63)	8 (38)	4 (12)	7 (87)	94	63	91	37	*	13	<0.001
Major + (major and minor).	12 (23)	11 (52)	4 (33)	8 (73)	83	81	75	44	40	20	0.172
Minor + others	7(14)	2 (10)	2 (29)	2 (29)	86	50	71	*	*	8	------
Hepatomegaly											
No	28 (54)	11 (52)	7 (25)	10 (91)	86	45	78	18	*	12	<0.001
Yes	24 (46)	10 (48)	3 (13)	7 (70)	96	*	92	69	*	38	0.019

ACAs: Additional chromosomal abnormalities, CML-CP: Chronic myeloid leukemia-chronic phase, AP: Advanced phase, CP: Chronic phase. P value < 0.05 is considered significant.

ABL tyrosine kinase domain, and emergence of ACAs [15]. The median age of patients at diagnosis was 37 years which is comparable to previous studies in Pakistan, Nigeria, Mexico [16], [17], [18], and previous Egyptian reports [19], [20]. In our study, we found that patients <40 years tended to develop ACAs more than patients ≥40 years (55% vs. 30%), respectively (p = 0.034). Similarly, median age (27 years) of patients who developed trisomy 8, a major route abnormality was higher than the median age of patients (40 years) who were free from the same abnormality (p = 0.023). The same observation was detected when comparing the median age of CML patients with an extra Philadelphia chromosome (17/73, 32 years) and without the same abnormality (56/73) (38 years) (p = 0.225). These findings matched those of GIMEMA working group who reported a lower median age of 45 years in patients with ACAs compared to 50 years in patients without ACAs [21]. Our findings also matched those reported by the German CML study Group IV who reported a lower median age of 48 years in patients with ACAs compared to 53 years in patients with the sole standard t (9;22) abnormality [22]. In our study, the incidence of ACAs was 44% (32/73) and this was higher than the highest previously reported incidence by others (15%, 6%, 7%, and 24%), respectively [16], [21], [22], [23]. In addition, the incidence of major route abnormalities was 18/73, 25%. Our observed incidence was comparable to two other reports showing an ACAs incidence of 42% and 30%, respectively [24], [25]. Incidence of emergence of ACAs in our CML-CP patients was 36% and 62% in AP (p = 0.048). These findings were in agreement with previously reported data by others [23], [26]. We found that a significantly higher percentage of patients with splenomegaly (48%) developed ACAs during their disease course compared to 11% of patients with no splenomegaly (p = 0.035). This finding was in accordance with a previous study [21], who reported a higher median increased spleen size (4 cm) in patients with ACAs compared to a median of 2 cm in those without ACAs. This finding could be explained by the fact that CML patients with a higher Sokal prognostic score at diagnosis have a higher probability to develop ACAs [13]. We also describe an association between hepatomegaly at presentation and a higher median OS (p = 0.086) in univariate survival analysis as the presence of hepatomegaly was found to be protective and a favorable independent predictor of OS in the multivariate model (HR = 2.80, 95% CI: 1.20–6.52, p = 0.017). The protective role of hepatomegaly could be explained by the fact that liver represents an extramedullary site of hematopoiesis that takes over this process in the event of BM infiltration by myeloid elements as observed in CML, thus maintaining other blood lineages physiological counts and providing tumor infiltrating lymphocytes to carry on an effective anti-tumor immune response. In this work, CML patients with hypercellular BM smears at presentation developed less ACAs than those who presented with a normal or reduced BM cellularity (p = 0.018), explained in light of the presence of concomitant BM fibrosis in patients with reduced BM cellularity at the presentation which would classify these patients as an advanced disease (AP) CML even before any evidence of CE, an explanation confirmed by BM trephine specimens examination of included CML patients [8]. BM basophils <3% in our CML-CP patients (n = 52) was significantly associated with a higher median EFS than patients with BM basophils ≥3% (p = 0.049). This finding was in agreement with that reported by Cortes *et al.*, 2003 [23], who used a 5% cutoff for BM basophils and observed the same relationship between the two groups of CP patients and achieving MCyR. However, we found a different relationship between BM eosinophilia (≥5%) and OS in our Egyptian CML patients describing eosinophilia to be a favorable prognostic factor and independent predictor of survival in Egyptian CML patients upon univariate (p=0.014) and multivariate analyses models (p = 0.037, HR = 2.38, 95% CI = 1.05-5.38). This was different to what was reported by the Hasford and EUTOS scoring systems to evaluate risk groups in CML patients [27]. The mentioned scoring systems utilize PB eosinophils percentage in its formula, denoting that a higher PB eosinophils % results in a higher patient score and higher risk category. Although PB eosinophils percentage and not BM eosinophils are included in Hasford and EUTOS scoring systems [27], the current finding in this study introduces BM eosinophils percentage as an independent predictor of OS in Egyptian CML patients and a variable that could be potentially introduced in risk stratification of CML in middle-Eastern populations, although larger cohort studies are needed to justify this attempt. Patients with thrombocytopenia (<100×109/L) had a lower median OS than patients who presented with higher platelet counts (p = 0.07) and was found to be an independent poor prognostic factor in Egyptian CML patients upon the log-rank statistical test of the impact of variables on OS (p=0.082). This correlation was similar to a previously reported significant correlation between thrombocytopenia at presentation and unresponsiveness to TKI therapy and lack of MCyR which was consequently and significantly correlated with OS and EFS [28]. A higher percentage of CML-CP patients who failed to achieve optimal response to TKI therapy had ACAs (42%) compared to (28%) of optimal responders. These findings were comparable to reported findings by Cortes *et al.*, 2003, who concluded that either CP or AP CML patients who developed ACAs had inferior hematologic and cytogenetic responses to TKI than their ACAs free counterparts. Our presented findings also matched those of the GIMEMA working party who reported a lower percentage of optimal responders (major molecular response at 12 months) among patients with ACAs (67%) compared to 86% among patients without ACAs. In the current work, median OS of CP patients at the end of 24 months follow-up duration could not be reached as more than half of patients were still alive and median survival was

21 months for AP patients. As expected, the presenting phase of CML at diagnosis was found to be an independent poor prognostic factor in univariate and multivariate analyses with CML-CP patients having a significantly higher cumulative OS at 24 months than patients with AP (p < 0.001) (HR = 7,95% CI = 3.03–15.9, p < 0.001).We also found that CML patients who were free of ACAs had a significantly higher OS than patients who developed ACAs (p = 0.026) and a higher cumulative EFS at 24 months (p = 0.065). This finding was in accordance with that reported by others [21], [23]. We were able to identify a negative impact of major route ACAs in a total of 18 patients (11 in CP and 8 in AP of CML) on OS and EFS compared to patients with the minor route or other abnormalities (p = 0.084 and p = 0.065), respectively. We also attempted to evaluate the relationship between the type of TKI received upfront whether 1st line (IM) or 2nd line (Nilotinib and Dasatinib), time to TKI treatment and shift to generic IM and the frequency of developing ACAs, molecular response, patient's tolerance to TKIs, and their OS and EFS. We found that among patients who developed ACAs (n = 32), a larger number of patients had a > 15 days' time to TKI (25/30, 83%) compared to (5/30, 17%) with shorter time to TKI (≤15 days) (p = 0.130). This finding necessitates earlier therapy from time of diagnosis to eliminate CE. We also found that a larger number of patients who received 1st line TKI upfront tended to develop ACAs (30/32, 94%) compared to those who received 2nd line TKI as an upfront therapy (2/32, 6%), a finding proving a deeper molecular response achieved by 2nd line TKI, affecting survival and elimination of CE. In addition, a larger number of patients who had interruptions to Glivec® therapy without shifting to other generic IM (23/32, 72%) developed ACAs compared to those who shifted to a different generic IM without treatment interruption (9/32, 28%). We also report that CP patients who were tolerant to their received TKIs tended to develop ACAs less (14/34, 17%) than their counterparts who were defined as intolerant to TKIs (2/6, 33%). These observations could be matched with previous studies [10], [28], [29]. An interesting observation that we report in this study is the near significance higher median OS and EFS of CML-CP patients who had a shift to a different generic IM during their treatment course due to the unavailability of Glivec® (p = 0.087 and p = 0.100, respectively). However, due to the small number of patients in the crossover to generic IM group (n = 22) compared to their counterparts who did not receive the same generic drug (n = 43), we could not reach a statistically significant difference.

Conclusion

We report a higher incidence of ACAs in our Egyptian CML patients at diagnosis and during the course of therapy than western populations. The appearance of ACAs, particularly, major route abnormalities at diagnosis or their emergence during treatment plays an important role in defining response to TKI therapy and have a negative impact on CML patients response to TKI therapy in terms of cytogenetic and molecular responses as a major mechanism of resistance to TKI therapy; this finding also applies to CML patients presenting in AP of the disease. CML patients who developed ACAs, particularly major route abnormalities, had a lower OS and EFS than their counterparts without ACAs, which proves the negative impact of CE in this disease. We conclude that the impact of ACAs on response to TKIs therapy in CML patients has important clinical and biological implications. Our study suggests that the detection of ACAs, besides *BCR-ABL1* kinase domain mutation, is an important determinant of CML patients prognosis and survival. Earlier identification of these abnormalities may help in adopting more appropriate therapeutic approach.

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