Chromosome aberrations in *de novo* acute myeloid leukemia patients in Kuwait

S. AL BAHAR, A. ZÁMEČNÍKOVÁ, R. PANDITA, K. BAVISHI, O. KREZE, S. NATH

Department of Hematology, e-mail: soleman97@hotmail.com, Kuwait Cancer Control Centre, Shuwaikh, Kuwait

Received September 17, 2003

Cytogenetic analysis was successfully performed at the time of diagnosis in 45 patients with *de novo* acute myeloid leukemia, including 10 children and 35 adults. In approximately 73% of AML patients (35 patients) clonal chromosome abnormalities were detected at the time of diagnosis. Twelve patients (22.8%) had apparently normal karyotypes. Recurring aberrations found in 22 of patients with abnormal karyotypes included t(15;17)(q22;q11), t(8;21)(q22;q22), inv(16)(p13q22), trisomy 8, monosomy 7 and del(5q). The highest frequency of chromosome changes was observed in AML-M3. The occurrence of the classical cytogenetic abnormalities was not a ubiquitous phenomenon. In 11 patients previously not described miscellaneous clonal chromosomal abnormalities were detected. Clonal chromosomal abnormalities detected in AML have shown correlations between specific recurrent chromosomal abnormalities and clinicobiological characteristics of the patients, therefore have been repeatedly shown to constitute markers of diagnostic and prognostic significance. Moreover, ongoing cytogenetic analysis can identify new nonrandom chromosome aberrations in AML and contribute to the identification of novel genes involved in the development of cancer, which can lead to better understanding of the disease pathogenesis.

Key words: acute myeloid leukemia, cancer cytogenetics, chromosomal abnormalities

Acute myeloid leukemia is a malignancy of immature bone marrow cells including high numbers of blasts with different degree of maturation. The disease is relatively infrequent; the annual incidence remains constant at 0.8-cases/10^5 population (between early childhood and age 45) and 15-cases/10^5 populations after the age of 45 [2]. The annual incidence of AML in Kuwait is 1.3-cases/10^5 population (32.4% of all leukemias in Kuwait) [1].

The classification of AML includes clinical data and biologic characteristics, which include morphological, cytochemical, immunophenotypic, cytogenetic and molecular characterization of the leukemic blasts [14]. Cytogenetic studies of acute myeloid leukemia have revealed that the majority of patients display acquired chromosome aberrations that are very closely, and sometimes uniquely associated with distinct subsets of leukemia. At present more than 5000 cases with clonal chromosomal aberrations have been reported worldwide and continue to be identified in AML. The observed particular chromosomal translocations in AML tend to be characteristic of particular stages of differentiation therefore the study of chromosomal aberrations has much importance in terms of the management of the disease [5, 11]. In addition to direct clinical use, recurrent chromosome abnormalities identified in hematologic malignancies have been essential in mapping and cloning of genes involved in the process of leukemogenesis. The detection of chromosome abnormalities, therefore, has a great clinical relevance for classification, treatment and outcome of the patient and will contribute to better understanding of disease pathogenesis and therefore to better approaches to treatment.

In our study, we present general cytogenetic characteristics to determine the type and frequency of chromosomal aberrations in leukemic cells of patients diagnosed with acute myeloid leukemia in Kuwait.

Patients and methods

The retrospective study was carried at Kuwait Cancer

Table 1. Distribution of karyotype findings in de novo AML patients compared with children

	Children No. (%)	Median age (years)	Adults No. (%)	Median age (years)	Total No. (%)	Median age (years)
Normal karyotype	3(6.6)	10.6	9(20)	39.6	12(26.6)	35.6
Abnormal karyotype	7(15.5)	5.2	26(57.7)	30.6	33(73.3)	25.2
Total	10(22.3)	6.9	35(77.7)	37.8	45(100)	30.2

Table 2. Frequency and percentage of cytogenetic abnormalities in children and adults compared with reported series

Abnormality	Children No. (%)	Median age (years)	Adults No. (%)	Median age (years)	Total No. (%)	Median age (years)	Reported series (%) Children	Adults
t(8;21)	1(2.2)	6	3(6.6)	22	4(8.8)	18	8	12
t(15;17)	0(0)	0	8(17.7)	31	8(17.7)	31	11	12
inv(16)	0(0)	0	3(6.6)	38.3	3(6.6)	38.3	4	6
–7/del(7q)	0(0)	0	3(6.6)	35	3(6.6)	35	6	9
del(5q)	0(0)	0	1(2.2)	61	1(2.2)	61	1	2
+8	0(0)	0	2(4.4)	43.5	2(4.4)	43.5	2	9
+21	0(0)	0	1(2.2)	64	1(2.2)	64	1	4
miscellaneous	6(13.3)	5.1	5(11.1)	36.6	11(24.4)	19.4	19	23
Total	7(15.5)	5.2	26(57.7)	30.6	33(73.3)	25.2	23	78

Control Centre at the Department of Hematology from October 2001 to July 2003. The study population consisted of 42 adults and 10 children with acute myeloid leukemia. Cytogenetic analysis was successfully performed at diagnosis in 45 patients with *de novo* acute myeloid leukemia. Seven of the patients had insufficient number of metaphases (less than 20 metaphases). Routine cytogenetic studies were performed on peripheral blood and/or bone marrow aspirates at diagnosis and during subsequent course of the diseases on unstimulated direct and/or short-term cultures, using the GTG-banding technique. A patient was classified as having normal karyotype only after 20 normal metaphases were analyzed. Karyotype analysis was performed according to the International System of Human Cytogenetic Nomenclature [8].

Results

Fifty two cases of newly diagnosed *de novo* acute myeloid leukemia were investigated between October 2001 and July 2003. Of the 45 patients diagnosed with *de novo* AML with an evaluable chromosome analysis, 12 patients (26.6%) had an apparently normal karyotype. The median age was 39.6 years (range 18–64) in adults and 10.6 years in children (range 1–14). In the remaining 33 cases (73.3% of the study population), clonal chromosome aberrations were detected (Tab. 1). The overall incidence of clonal chromosome abnormalities was higher in adults (74.2%) than in children (70%). Twenty two patients with abnormal karyotype had consistent or recurrent abnormalities (Tab. 2). The remaining 11 patients had miscellaneous clonal abnormalities (Tab. 2).

The chromosomal abnormalities of 33 patients with aberrant karyotype were classified into cytogenetic subgroups which closely correlated with FAB subtypes: t(8;21) (n=4, 8.8%), t(15;17) (n=8, 17.7%), inv (16) (n=3, 6.6%), trisomy 8 alone (n=2, 4.4%), monosomy 7/del (7q)/del (5q) (n=4, 8.8%), non-Down associated trisomy 21 (n=1, 2.2%) and complex abnormalities (n=11, 24.4%).

Recurrent chromosomal abnormalities. Consistent or recurrent abnormalities were detected in 66% of patients with an abnormal karyotype (Tab. 2). The distribution of chromosome abnormalities was uneven, according to the categories of the FAB nomenclature. The highest frequency of chromosome changes was observed in AML-M3 and the lowest in M1 and M6. Three chromosomal translocations were closely associated with specific clinical-pathologic subsets of AML. The translocation t(8;21), t(15;17) and the inv(16) were closely associated with M2, M3 and M4 subtypes, respectively.

t(15;17)(q22;q12-21). The t(15;17) was the most frequently observed chromosome change in our study observed in 22% patients with aberrant karyotype. All the patients with t(15;17) were adult cases (median age 36.5 years range 26–37). This rearrangement was the sole karyotypic abnormality in 7 of the patients in this subgroup, one patient had a complex chromosome rearrangement in addition to the t(15;17). All the 8 patients with t(15;17) had acute promyelocytic leukemia; this translocation was not found in any other subset of AML. Complete cytogenetic remission was achieved in all of the examined cases (median 30 days). One of the patients with t(15;17) died 8 months after the

diagnosis, the other 7 patients are alive and still in clinical remission.

t(8;21)(q22;q22). This translocation found in 4 patients with AML-M2 (12% of the patients with clonal abnormality) was the second most frequently observed translocation. Interestingly both male patients with this translocation had a loss of a sex chromosome in the sideline with a modal number of 45 chromosomes. The loss of the sex chromosome was the only additional change in 1 case and in 1 patient an additional clone with extra chromosome 8 was detected (Tab. 3). Complete cytogenetic remission (CCR) was observed in all the examined cases (median 40 days) and the patients are still in remission.

inv(16)(p13q22). The inversion of chromosome 16 was observed in 3 adult patients with AML-M4. The inv(16) was the sole abnormality in 1 case and in 2 cases extra chromosome 22 was detected as an additional anomaly (Tab. 3). In 2 of the examined cases CCR was achieved (median 30 days), in 1 patient no cytogenetic study was performed after diagnosis.

Abnormalities of chromosomes 7 and 5. The –7/del(7) abnormality was observed in 3 male patients (9% of patients with abnormal karyotype). Monosomy 7/del(7q) as a sole abnormality was detected in 2 patients and in 1 patient with a complex karyotype. The patient with monosomy 7 achieved CCR after 45 days of therapy, however the patient with monosomy 7 and complex karyotype died 10 months after diagnosis. The patient with del(7q) achieved CCR after 1 month of diagnosis and relapsed 10 months after the diagnosis. One patient in this subgroup had a deletion of 5q as the sole chromosomal abnormality (2.2% of patients with abnormal karyotype) and he is still in CCR.

Trisomy 8 and trisomy 21. Trisomy of chromosome 8 as a sole abnormality was observed in 2 patients (6% of patients with chromosome aberrations) and the extra chromosome 21 in 1 patient. One patient with +8 achieved CCR after 40 days of therapy, on other no cytogenetic data are available after the diagnosis. The patient with +21 achieved CCR 1 month after the diagnosis and is still in remission.

Miscellaneous clonal abnormalities. Various non-recurrent chromosomal abnormalities were detected in 33% of patients with abnormal karyotypes (Tab. 4). Complex clonal abnormalities were higher in children (60% of children) than in adults (14.3%). CCR was achieved in all patients in this subgroup (median 43 days) and are till in remission except 1 patient with Down syndrome who died 1 year after the initial diagnosis. Among the 5 adult cases only 3 patients achieved CCR (median 52 days), 1 patient relapsed 3 months and 1 patient died 1 year after the diagnosis.

Table 3. Additional chromosome anomalies in a group of patients with recurrent chromosomal abnormalities

No.	Sex/Age	Chromosome abnormality
1.	M/6	46,XY,t(8;21)(q22;q22)/45,X,-Y,t(8;21)(q22;q22)/ 46,X,-Y,+8,t(8;21)(q22;q22)
2.	M/27	46,XY/45,X,-Y,t(8;21)(q22;q22)/46,XY, t(8;21)(q22;q22)
3.	M/30	46,XY/46,t(15;17)(q22;q21),t(17p;?)/ 47,XY,+8,t(15;17)(q22;q21),t(17p;?)/ 48,XY,+8,t(15;17)(q22;q21),t(17p;?),+mar
4.	M/31	46,XY/47,XY,inv(16)(p13;q22),+22
5.	F/44	46,XX,inv(16)(p13;q22)/47,XY,inv(16)(p13;q22),+22/ 48,XY,+15,inv(1 6)(p13;q22),+22
6.	M/45	46,XY/46,XY,t(2;3)(p21;qter)/ 45,XY,t(1;12)(p?;p?),t(2;3)(p21;qter),-7

Table 4. Miscellaneous chromosome abnormalities

No.	Sex/Age	Chromosome abnormality
1.	M/57	46,XY,t(9;22)(q34;q11)/46,XY, t(2;14)(q?,q23),t(9;22)(q34;q11),t(19;?)+mar
2.	M/24	46,XY/46,XY, abn 11q, mar1, mar2
3.	F/2	46,XX/47-55,XX, +2,+abn3q,+4,+19,+20,+mar1,+mar2
4.	F/52	46,XX/46,XX,t(1;X)(p32;q13)
5.	M/27	46,XY/46,XY,17p+, mar
6.	F/23	46,XX/48,XX,+6,+8,+8,-18,-19,+mar/ 50,XX,+6+8+8,+mar
7.	M/4	46,XY/46,XY,t(3;5)(q25-27;q34)
8.	M/14	46,XY/47XY,+8/47,XY,+8,del(9q)
9.	F/1 DS	47,XX,+21c/46,XX,1q+,17q+,-20,+21c
10.	F/8	46,XX/46,XX,t(5;12)(q33;p13)
11.	F/2	46,XX/46,XX,t(15;20?)(qter;q?)

Discussion

Numerous recurrent karyotypic aberrations have been and continue to be discovered as primary pathogenetic changes in AML, suggesting that in many cases, cytogenetic abnormalities reflect basic differences in leukemia biology [3]. In this study, we present the results of the cytogenetic analysis of 45 patients with *de novo* AML identified at the first presentation, among over 250 patients with acute and

chronic myeloid and lymphoid leukemias cytogenetically investigated. The present study delineates the type and the incidence of chromosomal abnormalities and of their associated changes investigated in the same center between October 2001 and July 2003. To date, karyotypic findings of AML patients in Kuwait have not been previously described.

In the present study, 26.6% of our patients had apparently normal karyotypes. The overall incidence of normal karyotypes was higher in children (30%) than in adults (25.7%). The reason, why in a substantial number of patients with AML only cytogenetically normal metaphases can be detected, remains an enigma. The possible explanation can be that normal results may be attributed to the existence of non-neoplastic cells dividing preferentially *in vitro*, however, the fact that in these patients the normal karyotype found at diagnosis remains normal at relapse, argue against this hypothesis. More likely patients with cytogenetically normal karyotype display submicroscopic genetic changes undetectable by cytogenetic techniques and recent advances in molecular genetic techniques support this theory [9, 13].

Clonal chromosomal abnormalities were detected in approximately 73% of patients with AML. Our data are consistent with the results from several large prospective studies where the frequency of abnormal clones in untreated patients with *de novo* AML varies between 60% to 80% [4, 5, 9, 11]. The differences regarding the incidence of chromosomal abnormalities identified between pediatric cases and adults were minor (70% in children and 74.3% in adults). The type of abnormalities identified in children is similar to those seen in adults, although surprisingly differences were noted between the distributions of chromosomal abnormalities in children compared with adult cases. While the majority of the adults with AML can be assigned to a known cytogenetic subgroup with recurrent chromosomal abnormality, recurrent chromosome translocation was detected only in 10% of the children population. The remaining 60% of children with abnormal karyotype had nonrecurring complex miscellaneous chromosomal anomalies. The median age at diagnosis for all children patients was 6.9 years (7.8 in reported series). Our pediatric patients with normal karyotype tended to be older (median age 10.6) compared to those with miscellaneous abnormalities (median age 5.1), while only minor differences were seen between the age of adult patients with normal (median age 39.6) and complex karyotypes (median age 36.6). Further differences were seen in a clinical outcome of the patients. While 5 of 6 children in this subgroup are still in CCR, 2 of 5 adults relapsed shortly after CCR and 1 patient died 5 months after the initial diagnosis confirming the poor outcome of patients with complex karyotypic abnormalities.

The frequency of recurrent chromosome abnormalities in our study are similar to those seen in reported series, although our patients with t(8;21) tend to be younger (median age 18 years) than in reported series (median age 29.5) and while patients with inv(16) tend to be older (median age in our study 38.3 and 26 years in reported series) [5, 9, 11]. The chromosome t(15;17) was the most frequently observed genetic abnormality in our study, represented by 17.7% of patients with abnormal karyotype. All patients with t(8;21) and t(15;17) are alive and still in CCR, except 1 patient with t(15;17) confirming the previously described favorable outcome of patients in this subgroup. Interesting finding in our study was the detection of trisomy of chromosome 22 in 2 of our patients with inv(16). Additional anomalies associated with inv(16) are reported in 25% of patients where the frequency of +22 is approximately 15% [5, 11], therefore the frequency of trisomy of chromosome 22 was surprisingly high in our subgroup (66.6%). The highest median age was observed in the group of 4 male patients with chromosome 5 and 7 abnormalities (median age 41.5 years; comparing with median age of 30.6 in adults with abnormal karyotype). Contrary to this frequency in adult patients (8.8%), these abnormalities were not detected in childhood AML in our study. Previously observed reports indicate, that the reduced incidence of abnormalities of chromosomes 7 and 5 in pediatric patients may be the result of their limited environmental exposures and that these abnormalities in adults result from exposure to leukemogenic agents [7, 10]. In our group of patients, the patient with the monosomy of chromosome 7 and del(5q) as a sole anomaly achieved CCR and are still in remission, however the patient with chromosome 7 monosomy and complex karyotype died 10 months after the diagnosis suggesting the correlation between the complexity of genome changes and disease evolution. Use of cytogenetic studies in AML has permitted identification of specific chromosomal aberrations which correlate closely with distinct morphological features and characteristics, and at the same time has the potential to provide independent prognostic information as has documented by other studies [6, 7, 12].

In conclusion, chromosomal abnormalities are frequent in *de novo* AML in Kuwait. Recurring chromosomal aberrations were present in 66% cases with abnormal karyotype and correlated with subtypes of AML. However, the occurrence of recurrent chromosome anomalies is not a ubiquitous phenomenon. The present study confirms previous reports demonstrating the identity of unusual complex clonal chromosome aberrations in AML, suggesting that this disease is heterogeneous in its pathogenesis. Continued characterization of these abnormalities can lead to the identification of novel genes involved in the development of leukemia which may ultimately lead to use of new therapeutic protocols.

References

[1] AL-BAHAR S, PANDITA R, AL-MUHANNAHA A, AL-BAHAR E. The epidemiology of leukemia in Kuwait. Leukemia Research 1994; *4*: 251–255.

[2] Atlas of Genetics and Cytogenetics in Oncology and Haematology 1998. www.infobiogen.fr//services/chromcancer/Anomalies/Anomliste.html.

[3] CALIGURI AM, STROUT MP, GILLILAND DG. Molecular biology of acute myeloid leukemia. Semin Oncol 1997; *24*: 32–44.

[4] GILES FJ, KEATING A, GOLDSTONE AH, AVIVI I, WILLMAN CL, KANTARJIAN HM. Acute myeloid leukemia. Hematology 2002; *1*: 73–110.

[5] GRIMWADE D, WALKER H, OLIVER F, WHEATLEY K, HARRISON CJ et al. On behalf of the MRC adult and children's leukemia working parties, the importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. Blood 1998; *92*: 2322–2333.

[6] GRIMWADE D, WALKER H, HARRISON G, OLIVER G, CHATTERS S et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 Trial. Blood 2001; *98*: 1312–1320.

[7] MAURITZSON N, ALBIN M, RYANDER L, BILLSTORM R, AHLGREN T et al. Pooled analysis of clinical and cytogenetic features in treatment-related and de novo adult acute myeloid leukemia and myelodysplastic syndromes based on a consecutive series of 761 patients analyzed 1976–1993 and on 5098 unselected cases reported in the literature 974–2001. Leukemia 2002; *16*: 2366–2378.

[8] MITELMAN F. ISCN 1995. An International System for Human Cytogenetic Nomenclature. Basel, Karger, Switzerland, 1995.

[9] MROZEK K, HEINONEN K, DEL LA CHAPELLE A, BLOOMFIELD CD. Clinical significance of cytogenetics in acute myeloid leukemia. Semin Oncol 1997; *24*: 17–31.

[10] PEDERSEN-BJERGAARD J, ANDERSEN MK, CHRISTIANSEN DH, NERLOV C. Genetic pathways in therapy-related myelodysplasia and acute myeloid leukemia. Blood 2002; *99*: 1909–1912.

[11] RAIMONDI SC, CHANG MN, RAVINDRANATH G, BEHM FG, GRESIK MV et al. Chromosomal abnormalities in 478 children with acute myeloid leukemia: Clinical characteristics and treatment outcome in a Cooperative Pediatric Oncology Group study–POG 8821. Blood 1999; *94*: 3707–3716.

[12] SLOVAK ML, KOPECKY KJ, CASSILETH P, HARRINGTON DH, THEIL KS et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group study. Blood 2000; *96*: 4075–4083.

[13] SWEETSER DA, CHEN CS, BLOMBERG AA, FLOWERS DA, GALIPEAU PC et al. Loss of heterozygosity in childhood de novo acute myelogenous leukemia. Blood 2001; *98*: 1188–1194.

[14] VARDIMAN JW, HARRIS NL, BRUNNING RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002; *7*: 2292–2302.