Acute Myeloid Leukemia (AML) In Elderly: Cytogenetic Characteristics of Patients Treated At Hematology and Pediatric Oncology Center in Casablanca

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Abstract

AIM: The goals of this paper are: to report the incidence of AML in elderly, to describe cytogenetic characteristics of this population, to observe rare and novel cytogenetic abnormalities and lastly, to compare our finding with that previously reported in the literature.

METHODS: We conducted a retrospective analysis of 283 patients with acute myeloid leukaemia (AML) treated in our unit, we will report the incidence of AML in elderly, describe cytogenetic characteristics of this population, observe rare and novel cytogenetic abnormalities and compare our finding with that previously reported in the literature.

RESULTS: Among the 283 patients, 157 (54.4%) patients performed the karyotype, the cytogenetic analysis failed in 17 cases (11%). Prognostic group distribution was found to be favorable in 8 patients (6%) with 6 cases of t (8; 21) and 2 cases of inv (16), intermediate group in 94 patients (67%), including 58 cases (41,5%) with a normal karyotype, and an unfavorable group in 38 patients (27%) including complex karyotype (15%), -5 or del 5q (3%), -7 or del 7q (3.5%), t (9; 22) (2%). Some rare anomalies were observed.

CONCLUSION: However, the occurrence of a complex karyotype was more frequent than younger patients. In our unit, elderly benefit from supportive care, our study shows that it is a heterogeneous group and our treatment approach have to change especially for the patient from favourable risk group who can benefit from intensive chemotherapy. We have to improve our diagnosis with including molecular genetics that provides a documented substrate for a thoughtfully considered treatment plan.

Introduction

AML is an aggressive haematological malignancy, it's a rare disease, the incidence of AML increase with age, the median age at diagnosis is 67 years [1], the management of those patients is particularly difficult, both the nature of disease and the health of patient change with age, the take care of this fragile population is a veritable challenge for practician.

The cytogenetic profiles of elderly patients with AML are different from that of younger patients with more chromosomal abnormalities. The outcome in older adults is poor, high rates of good response translate into a 2-years survival of only about 15% to 20% [2], it's can be explained by a significant individual heterogeneity for those patients. Comorbid conditions, performance status, and decreased immune competence of elderly patients compromised the management of AML, and curative therapy as bone marrow transplantation cannot be proposed for those patients. On the other hand, AML in the elderly is not the same that younger people, a distinct gene expression profile noted for older compared with younger patients [3], [4].

A little is known about the cytogenetic profile of AML in Morocco, few studies are done, and no studies for elderly patients with AML are performed. In this article, we will try to describe the cytogenetic characteristics of patients having de novo acute

myeloid leukaemia AML aged more than 60 years treated in our unit from 2004 to 2016.

The goals of this paper are: to report the incidence of AML in elderly, to describe cytogenetic characteristics of this population, to observe rare and novel cytogenetic abnormalities and lastly, to compare our finding with that previously reported in the literature.

Patients and methods

The patients were identified by review of medical records at haematology and pediatric oncology centre of Casablanca, in Morocco during the period from January 2004 to July 2016. Informed consent was obtained from all individual participants included in the study. The patients had to have more than 60 years and should be followed for AML. Also, patients had to have a cytogenetic analysis at diagnosis. All samples were sent to a single reference laboratory who worked in collaboration with the university hospital.

Diagnosis of AML was confirmed by bone marrow aspiration and stained with May-Grunwald-Giemsa, and myeloperoxidase (MPO). The marrow blast count of 20% was required, and AML was classified into eight subtypes M0 to M7 according to the French American British (FAB) classification [5], [6].

Previously, the immunophenotyping was done in case of AML with minimal differentiation (AML-M0), acute megakaryoblastic leukaemia (AML-M7), erythroblastic leukaemia (AML-M6) and acute leukaemia of ambiguous lineage. To improve our diagnosis criteria, immunophenotyping has been done systematically for all young patients since 2011, for the elderly, few of patients benefited from immunophenotyping,

Cytogenetic analysis was done at diagnosis according to standard techniques with RHG banding. The bone marrow cells were cultured for 24 to 48 hours. Twenty cells were analysed, although examination of lower numbers of metaphases was also acceptable if an abnormal clone was detected. An abnormality was considered clonal when at least two metaphases had the same aberration in case of a structural abnormality or an extra chromosome. If there was monosomy, it had to be present in at least three metaphases. All the samples were sent at the time of diagnosis, to a single reference laboratory who worked in collaboration with the university hospital.

Chromosome identification and classification of chromosomal abnormalities were made according to the International System for Human Cytogenetic Nomenclature 2013 (ISCN) [7]. The cytogenetic findings were classified into three prognostic risk categories: favourable, intermediate and adverse, according to the classification proposed by Mrozek in 2006 [8]. The favorable group included patients with t (8; 21)(q22; q22), t (15; 17)(q24; q21) and inv (16) (p13·1q22)/t(16; 16)(p13·1; q22), whether alone or in combination with other abnormalities. The intermediate group included patients with normal karyotype and other aberrations excluded in the favourable or adverse group. The adverse one included those with complex karyotype defined with 3 or more abnormalities, inv (3) (q21q26)/t (3; 3) (q21; q26), t (6; 9) (p23; q34), t (6; 11) (q27; q23), t (11; 19) (q23; p13.1), del (5q) and monosomies 5 and 7.

Cytogenetic abnormalities such as t(8;21), t(15;17), inv(16)/t(16;16), 11q23,+8, t(9;11), -5/del(5q) and -7/del(7q) were further evaluated as sole or in combination with other anomalies. For the t(8;21) the characteristics and associated abnormalities were detailed.

To investigate the frequency of monosomal karyotype defined by the presence of at least 2 autosomal monosomies or single autosomal monosomy associated with at least one structural abnormality, we studied the distribution of autosomal chromosomal monosomies among patients with cytogenetic abnormalities other than core-binding factor.

Rare and novel abnormalities were also detailed. The research was done in the Atlas of Genetics and Cytogenetics in Oncology and Hematology [9] and Pubmed. All statistical analyses were evaluated using SPSS 16·0 software.

Results

From January 2004 to July 2016, 1483 patients aged more than 19 years old, with the novo AML were followed in our department. One thousand and two hundred (80%) were aged between 20 and 60 years old and 283 (20%) more than sixty years old. The total number of patients with de novo AML having more sixty than ears old entered the hospital per year varied between 12 patients on 2006 and 31 patients on 2011 with a median of 21 new patients per year. On the 283 patients, 150 (53%) were male, and 133 (47%) were female; the sex-ratio was at 1:12. The median age was 69 years (61-99) old and distribution of patients' ages per decade shows some difference of frequency: 151 (53.35%) patients were aged between 60 and 69 years, 100 (35.5%) between 70 and 79 years, 32 (11.3%) had more than 80 years old. Immunophenotyping was performed in 46% of cases. AML-M2 was the most frequent subtype with 157 (54.4%) patients. Patient's characteristics are showed in Table 1.

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Table 1: characterisation of clinical, biological and immunologic presentation of AML, by age (WBC: white blood cell; FAB: French-American-British)

	60-69 years	70-79 years	Older than 80 years
No patients	151	100	32
Novo	151	93	32
Myélodysplasie. No	0	3	0
Sex. Male No (%)	76	55	11
Laboratory values, median (range) element/mm3			
WBC count	29953 (1000-184300)	77330 450-347000	22278 1100-229300
FAB classification, No (%)			
M1	41 (27.15)	21	5
M2	43 (28.48)	35	5
M3	4 (2.65)	0	0
M4	9 (5.96)	7	2
M5	2 (1.32)	4	3
M6	5 (3.31)	2	2
M7	0	2	0
M0	9 (5.96)	5	1
Unknown	38 (25.16)	24	14

Among the 283 patients, 157 (54.4%) patients performed the karyotype, the cytogenetic analysis failed in 17 cases (11%). The frequency of the most cytogenetic abnormalities detected at diagnosis among 157 cases of AML arising in older adults with cytogenetic study and their associated clinical, biologic and immunologic features are presented in table 2. Clonal abnormalities were observed in 82 (58.5%) of the 157 patients.

Prognostic group distribution was found to be favorable in 8 patients (6%) with 6 cases of t(8; 21) and 2 cases of inv (16), intermediate group in 94 patients (67%), including 58 cases (41.5%) with a normal karyotype, and an unfavorable group in 38 patients (27%) including complex karyotype (15%), -5 or del 5q (9%), -7 or del 7q (5%), t(9,22) (2%). In our analyze we found some rare anomalies, like t(6,12)(q12; p12) ,+3, +7, +16, -21, and t (5.16).

Table 2: Frequency and percentage of cytogenetic abnormalities (Percentage do not add to 100 because patients with more than one abnormality are counted more than once)

Abnormality	All patients (no:140) No	60-69 years	70-79 years	Older than 80 years
Normal	58	40	11	7
complexe	21	17	4	0
T(8,21)	6	6	0	0
Inv(16)	2	2	0	0
-5 /del(5q)	14	11	3	0
-7 /del (7q)	8	7	1	0
11q23	5	2	1	2
T(9,22)	3	2	1	0
Trisomy:				
+8		6	5	0
+21		3	0	0
+7		2	1	1
+19	24	0	1	0
+16		0	1	0
+18		1	0	0
+13		1	1	0
+3		0	1	0
Monosomy				
-8	5	0	2	0
-21		1	0	0
-Y		0	1	0
-X		1	0	0
Other abnormalities	11	6	5	0

Other abnormalities: del 12q ,del 20q, del 9q, del 8q, t(7,11)(p15 ;p15), t(9,11)(q23 ;q23), t(5,16)(q33,q22), t(3,5)(q26;q34),t(13,13)(q10 ;p10),t(13,14)(p11 ;q11), t(6,12)(q12 ;p12)

However, the occurrence of a complex karyotype was more frequent than younger patients. t(8; 21) and t(15; 17) were seen less than younger patients. No significant variation in frequency of particular abnormalities across the age range was noted. Frequency and percentage of cytogenetic abnormalities among cases are shown in Table 2.

In the favourable risk group t(8,21) was detected in 6 cases and it was accompanied by additional changes in 5 cases, the inversion of chromosome 6, it was found in 2 cases, it was presented as a sole abnormality. For the intermediate risk group, the majority of cases classified within the intermediate risk group had a normal karyotype (41.5%), the abnormalities that were detected were represented by trisomy 8 which was sole in 9 cases, and associated with trisomy 10 and trisomy 12 in one case. Trisomy 21 was observed in 3 cases, del 11q23 was noted in 4 cases as a sole abnormalities, and associated with other changes in 1 case. Trisomy 7, trisomy 13 and trisomy 3 were found in 4, 2, and 2cases respectively. Many single aberrations were detected like trisomy 18, trisomy 19, del 12q, del 9q, del 20q, del17q, monosomy 8, monosomy 20, loss of chromosome X and loss of chromosome Y.

Overall, 38 of 157 cases were assigned to the unfavourable risk group, in 15%, this was based on the presence of complex karyotype, monosomy 5/del5 was found in 12 cases sole, and in combination in 2 cases, monosomy 7/del 7 was sole in 6 cases, and combined in 3 cases.

Some new and rare abnormalities were noted like trisomy 3, trisomy 7, trisomy 16, monosomy 21. t(3,5)(q26;q34) as a sole anomaly was described in 68 years old patient; we also found t(5,16)(q23,q22) in 61 years old man. t(6,12) (q12;p12) was isolated in patient having 61 years old, other rare abnormalities were detected as t(13,14) (p11;q11), t(13,13) (q10;p10).

To further characterise the cytogenetic features of ML in older adults, we summarise the frequency of additional changes occurring in combination with primary chromosomal aberrations in Table 3.

Table 3: The frequency of additional changes occurring in combination with primary chromosomal aberrations

	Total	Cytogenetic abnormalities							
		T(8, 21)	Inv(16)	-7/del7	-5/del5	11q23	monosomies	+8	+21	Other abnormalities
Total		6	2	8	14	5				
Alone		1	2	4	2	4				
T(8,21)					1					
Inv(16)										
-7/del7				1						
-5/del5		1		1						
11q23					1					
Complexe				2	5	1				
+8		1								
+21										
-Y		1								
-X		1								
Other abnormalities		1			4					

Discussion

Acute myeloid leukaemia is a rare disease occurring in adults older than 55 years of age. It's affecting annually 3-4 persons per 100000 individuals [10]. The median age of patients with AML is around 70 years. AML is an aggressive haematological malignancy, with extremely poor prognosis with overall survival (OS) of less than 20% at 5 years [2].

The diagnosis of AML depends primarily on detection of leukemic blasts of myeloid lineage (more than 20%) in the bone marrow. The World Health Organization classifies AML into 4 major categories (each with 2 or more subtypes) using morphologic, immunophenotypic, genetic and clinical features. The main categories are (1) AML with recurrent genetic abnormalities, (2) AML with myélodysplasie-related features, (3) therapy-related AML and MDS, and (4) AML not otherwise specified. Genetic and molecular abnormalities highlight the heterogeneity of AML and identify subsets associated with better or worse prognosis.

In Morocco, cytogenetic analysis was done systematically since 2004 for all young patients, but not all elderly patients benefited from the assessment at diagnosis. The number of patients how had cytogenetic and immunophenotyping analysis decrease with increasing ages.

The cut-off of 60 years old is arbitrarily used to define "older" patients; it is unknown if with this age limit we can discriminate patients subgroups with different outcomes. In our unit, for adult patients with AML, treatment is proposed only for young patients, they are treated by a uniform protocol called AML-MA 03 which included two inductions (7 + 3), two consolidations and a maintenance treatment without any stratification, this protocol was proposed from 2003 to 2010. On 2011 another protocol, AML-MA 11 was developed with two risk groups stratification based on the age (more or less than thirty years old) and cytogenetic finding as favourable, core binding factor (CBF) leukaemias with t(8;21) or inv(16)/t(16;16) versus all the others groups, the favourable group was receiving intensive chemotherapy involving cytarabine at a range of doses. Patients with acute promyelocytic leukaemia were treated by the APL-2004 protocol.

Age was considered from many years one of the factors prognostic; it's associated with poor outcome. Because the worst survival in AML elderly patients, the lack of resources and beds availability, we concentrate all our efforts to treat patients aged between 20 and sixty years old by improving the diagnosis, risk stratification and supportive care. Patients aged more than sixty years old receive systematically palliative care, we propose a low dose of aracytine, hypomethylating agents, and best supportive care with oral cytostatic drugs like hydroxyurea. Patients also benefited from transfusions, antibiotics and analgesics.

In our study we found 283 patients aged more than 60 years old, with 53% of them aged between 60 and 69 years.

Some factors are implicated in the adverse outcome of elderly in comparison with younger individuals. In this age group, AML has a particularly dismal outcome with less than 5% of the patients being alive 5 years after the diagnosis, as compared to 40% in the young [11], [12]. Advanced age is often accompanied by frailty and comorbidities [13], with poorer tolerance of combination chemotherapy regimens leading to the use of less intensive treatment protocols. Come of AML in the elderly; it is necessary to distinguish subgroups of patients with the paramount curable disease, who can receive treatment and those with incurable disease who can benefit from supportive care [14].

Age is not the unique factor of poor outcome; physical condition is very important; it can vary considerably among older people of the same age. Polypharmacy also constitutes an important prognostic factor [15]. Furthermore, poor prognosis in this group is associated with increased frequency of adverse cytogenetic features. Higher frequencies of adverse cytogenetics and unfavourable molecular aberrations are more common among the ageing populations. Distinct gene expression profiles noted for older compared with younger patients explain the poor outcomes in older individuals [16], [17].

Some previous studies have identified diagnostic cytogenetic as a key determinant of outcome in AML. Recent studies have revealed that the disorder arises from a series of recurrent hematopoietic stem cell genetic alterations accumulated with age [18]. The incidence of chromosomal abnormalities in AML differs according to geographical regions in the world. In this article, we analyse the largest cohort of patients with AML in the elderly done in Morocco. This study aims to describe the profile of our patients and destining subgroups. We defined prognosis subgroup, only 8 patients (6%) were in a favorable risk group, the most frequency of the common cytogenetic abnormalities detected at diagnosis permit to classified patients in the intermediate risk group with 94 patients (67%), and unfavourable risk group include 38 patients (27%). In light of our result, our diagnosis approach has to change with including molecular studies in our routine, and some of our patients will benefit from intensive chemotherapy.

In our study, some rare abnormalities were detected as t(3,5)(q26;q34) as a sole anomaly on 68 years old patient, this translocation was described on two cases, a 48 years old female patient and an unknown male age both with M2 AML, we also found t(5,16)(q23,q22) on 61 years old man, it's a rare abnormality, only two cases were reported in

literature. t(6,12) (q12;p12) was isolated on man having 61 years old, it's very rare abnormality in AML, other rare abnormalities were detected as t(13,14)(p11;q11), t(13,13)(q10;p10), and trisomy 16 or trisomy 3 which are very rare in AML [10]. Our finding is very important; it can help to define novel genes and mutations involved in the leukemic process.

In fact, recent molecular studies including next-generation sequencing (NGC) testing of myeloid neoplasms (MNs) have shown that acquired mutational events that can involve FLT3, NPM1, CEBPA, DNMT3A, IDH1, IDH2, KIT, MLL-PTD, TET, RUNX1, ASXL1, and TP53 are frequent in the novo AML or MDS and can be used for risk stratification, especially in patients with normal karyotype [19], [20], [21], [22].

In conclusion, the outcome in elderly with AML continuously declines with progressively increasing age. Comorbid conditions, performance status, adverse cytogenetic and unfavourable molecular aberrations are among the most critical determinant factors. This paper furnishes clinically and biological information, this background information can be useful in our treatment approach especially for the patient from favourable risk group who can benefit from intensive chemotherapy. Early referral to palliative medicine and the use of this subspecialty as a supportive care service it's not always the best proposition for those patients. This work sheds light on some missing practice on our routine like molecular studies which can offer useful guidance during the treatment.

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