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Core binding factor acute myeloid leukemia (CBF-AML) in México: A single institution experience

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ABSTRACT

Twenty one patients with CBF-AML presented prospectively in the Centro de Hematología y Medicina Interna de Puebla (Puebla, México) between February 1995 and March 2010, 14 with the t(8;21)(q22;q22) and 7 with the inv(16)(p13;q22)/ t(16;16)(p13;q22); they represent 13% of all cases of AML. The median age of the patients was 24 years (range 1 to 61). Seven of 14 patients with t(8;21)(q22;q22) had an M2 morphology whereas 3/7 with the inv(16) had an M4 morphology; in addition to the myeloid markers identified by flow-cytometry (surface CD13, surface CD33, and cytoplasmic myeloperoxidase) lymphoid markers were identified in the blast cells of 8/14 cases of the t(8;21) patients, but in no patient with the inv(16). Nineteen patients were treated with combined chemotherapy and 16 (84%) achieved a complete molecular remission. Seven patients were auto or allografted. Relapses presented in 10/16 patients. The median probability of overall survival (OS) has not been reached being above 165 months, whereas the 165-month probability of OS and leukemia-free survival was 52%; despite a tendency for a better outcome of patients with the t(8;21), there were no significant differences in survival of patients with either the t(8;21) or the inv(16). In this single institution experience in México, we found that the CBF variants of AML have a similar prevalence as compared with Caucasian populations, that the co-expression of lymphoid markers in the blast cells was frequent in the t(8;21) and that these two AML subtypes were associated with a relatively good long-term prognosis. Further studies are needed to describe with more detail the precise biological features of these molecular subtypes of acute leukemia.

Leucemias agudas mieloblásticas con mutaciones en el factor de unión al "core" (core binding factor) en México: Experiencia de una sola institución

RESUMEN

Veintiún pacientes con leucemia aguda mieloblástica (LAM) con mutaciones en los genes del factor de unión al "core" (core binding factor, CBF) se estudiaron de manera prospectiva en el Centro de Hematología y Medicina Interna de Puebla (Puebla, México) entre febrero de 1995 y marzo de 2010, 14 con t(8;21)(q22;q22) y 7 con inv(16)(p13;q22)/t(16;16)(p13;q22); esta cifra corresponde a 13% de todos los casos de LAM. La mediana de edad de los pacientes fue de 24 años (rango 1 a 61). Siete de los 14 pacientes con t(8;21)(q22;q22) tuvieron una morfología M2, en tanto que 3/7 con inv(16) se presentaron con morfología M4. Además de los marcadores mieloides identificados por citometría de flujo (antígenos CD13 y CD33 de superficie y mieloperoxidasa citoplasmática), se identificaron marcadores linfoides en los blastos de 8/14 pacientes con t(8;21), pero no en los que tuvieron inv(16). Diecinueve pacientes fueron tratados con quimioterapia combinada y 16 (84%) lograron la remisión completa, hematológica y molecular; a siete pacientes se les hicieron trasplantes de células hematopoyéticas. Diez de 16 pacientes tuvieron recaídas leucémicas. La mediana de probabilidad de supervivencia (SV) global no se ha alcanzado y es mayor de 165 meses, en tanto que la SV global y libre de enfermedad a 165 meses es de 52%; se observó una tendencia a mayor SV en los pacientes con t(8;21), aún cuando las diferencias no fueron significativas. En esta experiencia de una sola institución, hemos encontrado que las variantes CBF de las LAM tienen una prevalencia similar a la descrita en poblaciones caucásicas, que la expresión aberrante de marcadores linfoides en los blastos es frecuente en aquellas con t(8;21) y que estas variantes de LAM tienen un pronóstico mejor que la

LAM sin estos marcadores. Se requieren estudios adicionales para describir con más detalles y precisión las características de estas variantes moleculares de LAM.

Key words. Acute leukemia. Myeloblastic. CBF. Core-binding factor. inv16, t(8;21). Treatment. Mexico.

***Palabras clave.** Leucemia aguda. Mieloblástica. CBF. Core-binding factor. inv16. t(8;21). Tratamiento. México.*

INTRODUCTION

Core-binding factors (CBFs) are a class of hematopoietic transcription factors that are crucial for the regulation of hematopoietic ontogeny, and are frequent targets of mutation and gene rearrangement in human leukemia. CBF acute myeloid leukemia (CBF-AML) corresponds to two distinct subtypes of AML characterized by recurrent chromosome translocations, namely t(8;21)(q22;q22)) and inv(16)(p13;q22)/t(16;16)(p13;q22); it is the type of leukemia most responsive to cytarabine (ara-C) therapy and is of relative favorable prognosis as compared with other types of AML, although in this type of leukemia, only one-half of the patients are cured.[1,2] In Caucasian populations, this type of leukemia is among the most common cytogenetic subtypes of AML, being detected in approximately 12% of adults with primary disease.[2] The prevalence of CBF-AML in México is largely unknown; in a single-center experience in México, we found that the t(8;21)(q22;q22) variant of leukemia is more frequent than in Caucasians,[3] but the prevalence of the inv(16)/t(16;16) variant of AML is not known. We analyze here the prevalence, salient features and therapeutic results of a group of 21 patients with CBF-AML studied and treated in a single institution in Mexico.

MATERIAL AND METHODS

Patients

All consecutive patients with the t(8;21)(q22;q22)) and inv(16)(p13;q22)/t(16;16)(p13;q22) AML studied and treated in the Centro de Hematología y Medicina Interna de Puebla (Puebla, México) were prospectively entered in the study from February 1995 to March 2010.

Diagnosis

Peripheral blood and bone marrow smears stained with May Grümwald Giemsa were studied and the classification was done according to the FAB classification.[4] The immunophenotype of the malignant cells was analyzed by means of flow cytometry.[5,6] Using reverse transcriptase polymerase chain-reaction (RT-PCR), the RUNX1/RUNX1T1 fusion gene (t(8;21)(q22;q22)) was investigated according to Kozu, *et al.,*[7] at diagnosis and along the treatment:[8] One fifth of cDNA was amplified for 40 cycles in 50 µl final volume and analyzed and validated.[7,8] The inv(16)(p13;q22)/t(16;16)(p13;q22) was investigated according to Claxton, *et al.*[9] Molecular biology studies are, in our experience and that of others in developing countries, more reliable than cytogenetics;[3] conventional cytogenetic studies were attempted in all patients, but the final diagnosis relied on the molecular markers.

Treatment

All patients were initially treated with a combined 7 + 3 chemotherapy (CT) course using cytarabine and doxorubicin (cytarabine 100 mg/m^2 in infusion for 7 days and doxorubicin 45 mg/m^2 in bolus for three days); the drugs were delivered as inpatients and the subsequent hypoplasia supported on an outpatient basis.[10] Once the blood cell counts had recovered an additional course of 5 + 2 CT was given, using the same drugs and doses. At this point, per protocol and according to the availability of an HLA-identical sibling donor, patients with no donor were given a course of high-dose cytarabine (2,000 mg/m^2 bid, x 6) or an autologous stem cell transplantation (SCT).[11] Patients with an HLA identical sibling were offered an allogeneic SCT.[12] The analysis of the probability of survival was done according to Kaplan–Meier,[13] for estimates from incomplete observations, a method which censors patients lost to follow-up as alive at the time of the last visit.

RESULTS

Twenty one patients with CBF-AML presented at the Centro de Hematología y Medicina Interna de Puebla from February 1995 to January 2010, 14 with the t(8;21)(q22;q22)) and 7 with the inv (16)(p13;q22)/t(16;16)(p13;q22). Along this period, 365 adult and pediatric patients with acute leukemia presented in this institution: 163 patients with AML

Table 1. Salient features of the patients with CBF-AML, divided into two groups (inv16) and t(8;21). SV: Survival. BMT: Bone marrow transplantation.

	inv(16)	t(8;21)
Number	7	14
Male/female	4/3	8/6
Age, median (range)	33 years (1 - 60)	26 years (7 - 61)
M0	0	1
M2	4	7
M5	0	1
M4	3	3
FLT-3 (+/tested)	1/4	0/3
Biphenotypic	0	2
B cell markers	0	6
T cell markers	0	2
Median overall SV	25 months	Above 165 months
Overall SV	45% at 109 months	62% at 165 months
BMT	5 (3 allogeneic)	8 (5 allogeneic)

(aged 1-83 years) and 202 with acute lymphoblastic leukemia. These data indicate that the CBF-AML represents 13% of all cases of AML in our experience. The median age of the patients was 24 years, (range 1 to 61); there were nine males. The chimerical RUNX1/RUNX1T1 and inv16 genes were identified all patients at diagnosis. According to the French-American-British (FAB) morphological classification of acute leukemia,[2] the morphology was M0 in one case, M2 in 11 cases, M5 in one case and M4 in 6; seven of 14 patients with t(8;21)(q22;q22) had an M2 morphology whereas 3/7 with the inv(16) had an M4 morphology, M4eo in two instances (Table 1). In addition to the myeloid markers identified by flow-cytometry (surface CD13, surface CD33, and cytoplasmic myeloperoxidase),[14,15] some non-myeloid markers were also identified in the blast cells of 8/14 cases of the t(8;21) patients; this information is included in the table.

It is interesting that, despite being myeloid leukemias as defined by the presence of either the 8:21 or the 16;16 translocations and the characteristic myeloid markers, the blast cells of 57% of patients with the t(8;21) displayed lymphoid markers in addition to the myeloid ones; the lymphoid markers were consonant with B cell lineage in 6 patients and with T cell lineage in two. According to the first and second Latin American consensus for flow cytometric immunophenotyping of hematological malignancies,[14,15] two cases with the t(8;21) displayed enough criteria to classify them as biphenotypic leukemias. Two cases were secondary to a myelodysplasia, whereas the remaining 19 were *de novo* instances of AML.

Patients were observed for periods ranging from 1 to 165 months (median 14). Of the 21 patients, two did not complete the first course of chemotherapy and were lost to follow up. Of the remaining 19 patients, one had a refractory form of the leukemia and died 30 days after the diagnosis; sixteen achieved a complete molecular remission (CR), the CR rate being 84%. Once achieving a CR, seven patients were given autologous (four cases) or allogeneic (three cases) peripheral blood stem cell transplantation. Relapses were observed in 10 of the 16 patients who achieved a CR; the relapsed patients were given chemotherapy only (3 cases), an allogeneic BMT (6 cases) or an autologous BMT (1 case).

Nine patients are alive and leukemia-free 1 - 165 months (median 59) after the diagnosis; all of them in a complete hematological and molecular remission, whereas eight patients died 1- 25 months (median 19) after the diagnosis. Of the five patients who were autografted, two relapsed 2 and 3 months after the graft, one was lost to follow-up, one died as a result of a central nervous bleeding and one remains in a CR 75 months after the transplant. The median probability of overall survival (OS) has not been reached being above 165 months, whereas the 165-month probability of OS and disease-free survival (DFS) was 52%. Figure 1 shows the Kaplan–Meier[14] OS plot of the whole group, whereas figure 2 shows the survival plot for the two groups of patients; despite a tendency for a better outcome of patients with the t(8;21), there were no significant differences in survival of patients with either the t(8;21) or the inv(16)/t(16;16), $p > 0.05$; on the other hand, there were no significant differences in survival between patients allografted or not ($p > 0.05$), probably as a result of the low number of patients included in each group.

DISCUSSION

Acute myelogenous leukemia (AML) represents a group of clonal hematopoietic stem cell disorders in which both failure to differentiate and overproliferation in the stem cell compartment result in accumulation of non-functional cells termed myeloblasts.[2] The evolution of the classification system in AML from morphology to cytogenetic/genetic-based reflects the recognition of the importance of subtype-specific biology. The two major prognostic factors in newly diagnosed AML are currently patient age and chromosome status, and form the basis of important treatment decisions.[16]

The distribution of genetic-based AML subtypes in México is largely unknown. There is infor-

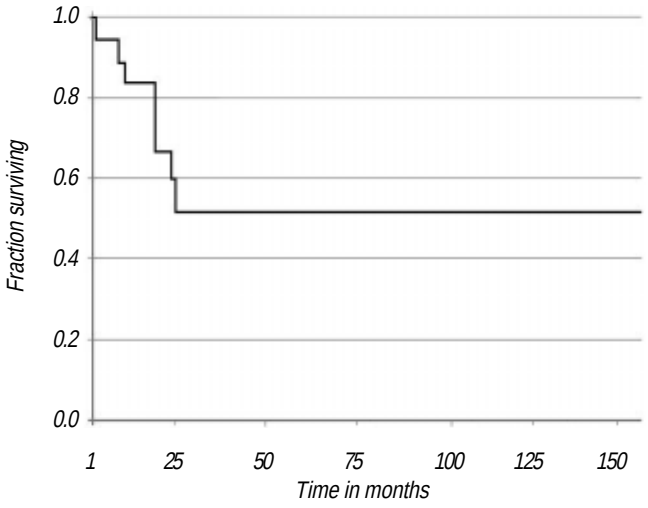

***Figure 1**. Overall and disease-free survival of the 21 patients with core binding factor acute myeloid leukemia treated in the Centro de Hematología y Medicina Interna de Puebla.*

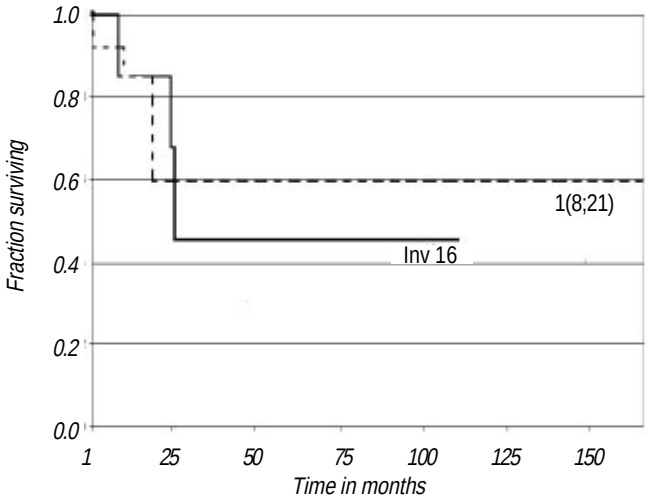

Figure 2. Overall survival in months of the 21 patients with core binding factor acute myeloid leukemia treated in the Centro de Hematología y Medicina Interna de Puebla, divided by the type of genetic alteration inv(16) or t (8;21).

mation about an increased prevalence of the t(15;17)(q22;q21) AML in México,[17-21] but there is no large data concerning the t(8;21)(q22;q22)[3] or the inv16 AML variants. In this study, we have found that the CBF-AML variants represent 13% of all cases of AML in our institution, whereas in Caucasian populations, this type of leukemia is among the most common cytogenetic subtypes of AML, being detected in the same proportion (13%) of adults with primary disease[2] and 30 to 40% of cytogenetically abnormal cases of AML-M2.[22] There are other alterations that may represent cooperative events in CBF-AML leukemogenesis; these include mutations in the KIT, FLT3, JAK2 and RAS genes, haploinsufficiency of the putative tumor suppressor genes TLE1 and TLE4 in t(8;21)-positive patients with del(9q), MN1 overexpression in inv(16) patients, and epigenetic and posttranscriptional silencing of CEBPA.[2] In this group of patients we investigated the FLT3 mutations in seven cases and found them only in one patient with the inv16 (Table 1).

The (8;21)(q22;q22) translocation has been associated with the morphological M2 AML subtype of the French-American-British classification;[2] in our experience, 7/14 patients were found to display this morphological subtype. It was also noteworthy that 8/14 patients displayed lymphoid surface and/or cytoplasmic markers, which in two cases were consonant with the diagnosis of biphenotypic leukemias according to the first and second Latin American consensus for flow cytometric immunophenotyping of hematological malignancies;[12,13] the expression of lymphoid markers in cases of t(8;21)(q22;q22) AML has been previously described.[23]

Because both t(8:21) and inv(16) disrupt core binding factor in AML, these cytogenetic groups are often treated similarly.[24] The t(8;21) AML usually occurs in *de novo* AML and generally predicts a good response to chemotherapy, with a high remission rate and long survival, particularly following high-dose cytosine arabinoside intensification therapy.[1,25] In our experience, despite the fact that relapses were observed in 62% (10/16) of patients achieving a CR, the median probability of overall survival (OS) has not been reached being above 165 months, whereas the 165-month probability of OS and DFS was 52%, probably because some patients could be rescued with either chemotherapy or bone marrow transplantation. These survival figures are better than those found in México for all types of AML (18% OS at 77 months),[26] but worse than those recorded in our same institution for patients with promyelocytic leukaemia (85% OS at 133 months) (Figure 3).[21] These data confirm that for most, both t(8:21) and inv(16) are regarded as a favourable prognostic factor, within individuals with AML. Since this study was conducted during 16 years, most treatment and supportive measures could have changed during the period; this has to be taken into account when analyzing the data.

In summary, in this single institution experience in México, we have found that the t(8;21)(q22;q22) and inv(16) variants of AML have a similar prevalence as compared with Caucasian populations, that

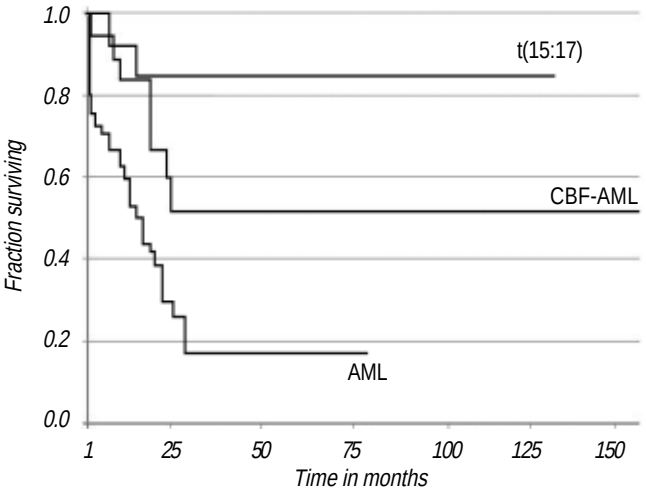

Figure 3*. Overall survival in months of Mexican patients with promyelocytic leukemia [t(15;17), reference number 21], core binding factor acute myeloid leukemia (CBF-AML) (these data) and a cohort of AML Mexican patients (reference number 26).*

the co-expression of lymphoid markers in the blast cells was frequent in the t(8;21)(q22;q22) variant and that these two CBF-AML were associated with a relatively good long-term prognosis. Further studies are needed to describe with more detail the precise biological features of these types of leukemia.

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