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RESEARCH ARTICLE

RESPONSE TO TREATMENT WITH 5-AZACITIDINE IN MYELODYSPLASTIC SYNDROME IN A SMALL MEXICAN POPULATION

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ABSTRACT

Myelodysplastic syndromes (MDS) are a heterogeneous group of diseases of the bone marrow, characterized by cytopenia and an increased risk for transformation to acute myeloid leukemia (AML). For patients with high-risk MDS, hypomethylating agents have been implemented recently as 5-Azacitidine (5-AZA) in order to improve the overall survival (OS), and additionally the decreased transfusion requirement and the delayed AML progression to AML. At present, 5-AZA is a drug used in our country, so that the analysis of MDS patients treated with this drug in the Centro Médico Nacional "20 de Noviembre", will reveal the clinical benefits and their contribution in the overall survival (OS).

Objective: Learn about the therapeutic response in MDS patients treated with 5-AZA. Secondly, OS and EFS, as well as toxicity will be established.

Patients and Methods: Prospective, experimental, longitudinal, single-center, single-arm study. Patients diagnosed with MDS, treated with 5-AZA at 100 mg/day for 7 days with at least 4 cycles, attended at the Hematology Service of CMN "20 de Noviembre", from 2010 to 2015.

Results: 15 patients with MDS diagnosis, treated with 5-AZA, were assessed. The mean age was 64 years, 60% were women and 40% men. Seven percent (7%) of all patients were diagnosed with refractory anemia with ring sideroblasts; 7% with refractory cytopenia with multilineage dysplasia, 27% with refractory anemia with excess blast 1 (AREB-1), and 60% with refractory anemia with excess blast 2 (AREB-2). The cytogenic risk was good in 67%, intermediate in 7% and highly complex in 27%. Regarding prognostic indices, 27% of the patients were classified by IPSSS as intermediate 1, 53% as intermediate 2, and 20% as high risk. For the IPSS-R, 33% was intermediate risk, 40% high risk, and 27% very high risk. The analysis of the response to treatment was performed by measuring hemoglobin, absolute neutrophils, platelets, number of bone marrow blasts, number of peripheral blood blasts and DHL upon diagnosis, the 4th treatment cycle, and 9th treatment cycle. 33% complete response (CR), 13% partial response (PR), 7% stable disease (SD), and 47% failure were obtained. A relapse was observed in 13% of the patients, and progression to acute myeloid leukemia (AML) in 49%. In the multivariate analysis, the factors having an impact on the response were IPSS score, with p=0.015; IPSS-R with p=0.05. The median overall survival (OS) was 17 months. No patient presented renal or hepatic toxicity. The number of hospitalization days per the risk was a median of 8.5 days for INT1, median of 13 days for INT-2, and a median of 12 days for HIGH (p=0.032). The median number of times the patient had febrile neutropenia according to risk INT1, INT2 and HIGH was 3.5, 6 and 8 times, respectively (p=0.014). The median transfusion requirement was 25 concentrated erythrocytes (CE), and the transfusion requirement according to the degree of response was 12 CE for CR, 10 CE for PR, 16 CE for EE, and 19 CE for failure (p=0.06).

Conclusions: The treatment with 5-AZA has demonstrated to extend OS in patients with MDS diagnosis, especially those with high risk. In our study a 17-months OS was shown, which is comparable to that reported in the literature5. Likewise, it has been associated with a reduced transfusion requirement and, thus, a quality-of-life benefit for MDS patients. Therefore, 5-AZA is considered a treatment of choice in MDS patients not being candidates for intensive chemotherapy in the daily clinical practice.

INTRODUCTION

The myelodysplastic syndromes (MDS) are a group of diseases characterized by ineffective hematopoiesis, cytopenias, and a high rate of transformation to acute leukemia. One third of patients present with a high risk of the disease, with a 60% risk of progression to acute myeloid leukemia (AML), and poor overall survival (OS). The treatment of high-risk MDS is intended to alter the natural course of the disease and extend the OS. The hematological improvement has become the primary point for response

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measurement; the main objective has been the achievement of a complete or partial response (CR or PR) of the disease as requirement for OS extension. Treatment options in this group of patients have evolved significantly during the last decade. Prior to this period, many patients were treated with cytarabine-based therapies after their evolution to AML. This practice has been modified since DNA hypomethylating agents are being used. (Shin et al., 2012) For the use of 5-AZA in MDS, the study titled Cancer and Leukemia Group B (CALGB) 9221 was performed, which included both high and low risk patients, randomizing 191 MDS patients to compare 5-AZA (75 mg/m^2/day subcutaneously for 7 days every 28 days) with the supportive therapy. The response occurred in 60% of the patients in the 5-AZA arm (7% complete response (CR), 16% partial response (PR) and improvement in 37%), in comparison with 5% of improvement in those receiving supportive therapy (P <0.01). The median time of transformation to leukemia or death was 21 months for 5-AZA versus 13 months for supportive therapy (P= 0.007). The transformation to AML occurred as first event in 15% of the patients in the 5-AZA arm, and 38% in supportive therapy arm (p= 0.001). (Silverman et al., 2002) Currently, 5-AZA is a drug used in our country, so that the analysis of MDS patients treated with this drug in this ISSSTE National Medical Center "*20 de Noviembre*" will reveal the clinical benefits and their contribution to overall survival (OS).

DESIGN AND METHODS

Prospective, experimental, longitudinal, single-center, single-arm study, including patients with MDS diagnosis according to WHO 2008 criteria, who were discussed and accepted by the service for the administration of 5-AZA, relative to the following criteria:

- Male and female patients over 18 years of age with MDS diagnosis, treated with 5-AZA at a dose of 100 mg/day for 7 days/month.
- The administration of 5-AZA was performed subcutaneously and in the in-hospital setting during the first 4 cycles of treatment, with posterior re-entry for cytopenia surveillance.
- All patients were matched to selection criteria and the medical record, for which purpose the following variables were recorded: age, sex, WHO classification, karyotype, disease evolution time, initial platelets, initial leukocytes, initial hemoglobin, initial peripheral blood blasts and bone marrow, blasts after 4th and 9th cycle of 5-AZA.
- The assessment of response was performed at the end of the 4th cycle, and patients completing 9 cycles were reassessed.

The statistical analysis was performed using the statistical program IBM SPSS Statistics v22 for Windows. For population characteristics, a descriptive analysis was performed with measures of central tendency and dispersion; absolute measures and percentages according to type of measured variable. The responses were classified in the following categories: CR, PR, SD and failure, assessed according to the obtained hematological response, number of blasts and cytogenetics. Numerical variables were analyzed by means of Student's t test, corroborated by ANOVA table. Toxicity was measured by WHO criteria, as well as the amount of transfusions, number of hospitalizations and infections. For the comparison of two nominal variables, the Chi-square test was used and corroborated with the Pearson test. For OS and event-free survival (EFS), the Kaplan-Meier method was applied, considering statistical significance with a p value < 0.05. Likewise, progression of disease and progression to AML were analyzed.

RESULTS

A population of 15 patients with MDS diagnosis, receiving treatment with 5-AZA, was tested. The demographic characteristics of the population were as follows: mean age of 61 years, with a median age of 64 years (36-80). 60% were women (n=9) and 40% men (n=6). According to the type of MDS, 7% accounted for refractory anemia with ring sideroblasts (n=1), 7% for refractory cytopenia with multilineage dysplasia (n=1), 27% for refractory anemia with excess blast 1 (n=4), and 60% for refractory anemia with excess blast 2 (n=9). The cytogenic risk was good in 67%, represented by patients with normal karyotype (n=10), 7% had an intermediate karyotype (n=1), and 27% had a highly complex karyotype (n=4). With regard to IPSS, 27% of patients were classified as intermediate 1 (n=4), 53% as intermediate 2 (n=8), and 20% as high risk (n=3). For the IPSS-R, 33% were intermediate risk (n=5), 40% high risk (n=6), and 27% very high risk (n=4). (Table 1)

Table 1. Baseline characteristics of patients (n=15)

Parameter	Result
(Median) age in years	64 (36-80)
Sex F/M (n=)	9/6
Type of MDS (n=)	
Refractory anemia with ring sideroblasts	1
Refractory cytopenia with multilineage dysplasia	1
Refractory anemia with excess blast 1 (AREB-1)	4
Refractory anemia with excess blast 2 (AREB-2)	9
Cytogenic risk (n=)	
Good	10
Intermediate	1
Highly complex	4
IPSS (n=)	
Intermediate 1	4
Intermediate 2	8
High	3
IPSS-R (n=)	
Intermediate	5
High	6
Very high	4

Abbreviations: MDS, myelodysplastic syndrome; IPSS, International Prognostic Scoring System; IPSS-R, Revised International Prognostic Scoring System.

The median baseline hemoglobin was 7.3 g/dL (4.5-11-5), the median absolute neutrophils was 1.1 x 10^9/L (0.1-13.4), median platelets was 120 x 10^3/L (6-391), the median of bone marrow blasts at the time of diagnosis was 8% (2-17), and the median of peripheral blood blasts was 0% (0-3); the median DHL was 221 IU/L (133-881). After the 4th treatment cycle with 5-AZA, median hemoglobin was 9 g/dL (7.1-14.8); median neutrophils were 1.6 x 10^9/L (0.1-7.5); median platelets were 105 x 10^3/L (14-553); median bone marrow blasts were 2% (0-25); median peripheral blood blasts were 0% (0-18); median DHL was 167 IU/L (107-578). The control karyotype was normal in 80% and abnormal in 20%. (Table 2).

Only 73% of the patients completed 9 cycles of treatment; from the remaining patients, 3 progressed to acute myeloid leukemia, and there was one death for infection, with the

following response: median hemoglobin 8.3 g/dL (2.5-12.6); median neutrophils 1.3 x 109/L (0.2-0.5); median platelets 131 x 103/L (12-464); median bone marrow blasts 1% (0-85); median peripheral blood blasts, 0% (0-28); median DHL was 175 IU/L (120-267). The response assessment at the 4th cycle was CR 33% (N=5), PR 13% (N=2), SD 7% (N=1) and failure 47% (N=7). The karyotype was normalized in 13% of the patients. There was a relapse in 13% (N=2) of the patients who achieved any response, whether CR or PR; 49% (N=7) progressed to AML, of which 33% (N=5) were in failure, and 13% in stable disease.

Table 2. Results

Parameter	Baseline	4th cycle	*P*
Hb g/dL (median)	7.3 (4.5-11.5)	9 (7.1- 14.8)	0.6
Neutrophils x 10^9/L (median)	1.1 (0.1-13.4)	1.6 (0.1- 7.5)	0.3
Platelets x 10^3/L (median)	120 (6-391)	105 (14- 553)	0.7
BMO % (median)	8 (2- 17)	2 (0- 25)	0.4
BSP % (median)	0 (0-3)	0 (0- 18)	0.6
DHL IU/L (median)	221 (103-881)	167 (107- 578)	0.08
Karyotype % (Normal/abnormal)	67/33	80/20	0.2

Abbreviations: Hb, hemoglobin; BMO, bone marrow blasts; BSP, peripheral blood blasts; DHL, lactate dehydrogenase.

At the 4th cycle, the erythroid hematological response behaved as follows: median hemoglobin was 13.4 g/dL in CR, 10 g/dL in PR, 9.3 g/dL in SD, and 8.3 in failure (p=0.049). The neutrophil response, with a median of 1.9x 109/L in CR, 0.89 x 109/L in PR, 0.60 x 109/L in SD, and 3.7 x 109/L in those who had treatment failure (p=0.39). The median platelet response was 160 x 103/L in CR, 128 x 103/L in PR, 97 x 103/L in SD, and 88 x 103/L in failure (p=0.30) (Table 3).

Table 3. Hematological response at 4th cycle

Measurement	CR	PR	SD	Failure	*p*
Hb g/dL (median)	13.4	10	9.3	8.3	0.049
Neutrophils x 10^9/L (median)	1.9	0.89	0.60	3.7	0.39
Platelets x 10^3/L (median)	160	128	97	88	0.30

Abbreviations: Hb, hemoglobin; BMO, bone marrow blasts; BSP, peripheral blood blasts; DHL, lactate dehydrogenase.

At 9th cycle, median hemoglobin was 12 g/dL in CR, 8 g/dL in PR, 7 g/dL in SD, and 3 g/dL in those having treatment failure (p=0.001). Median neutrophils were 2.5 x 109/L in CR, 1.3 x 109/L in PR, 1.8 x 109/L in SD, and 1.0 x 109/L in those having treatment failure (p=0.9). Median platelets were 129 x 103/L in CR, 60 x 103/L in PR, 32 x 103/L in SD, and 12 x 103/L in failure (p=0.01) (Table 4).

Table 4. Hematological response at 9th cycle

Measurement	CR	PR	SD	Failure	*p*
Hb g/dL (median)	12	8	7	3	0.001
Neutrophils x 10^9/L (median)	2.5	1.3	1.8	1	0.9
Platelets x 10^3/L (median)	129	60	32	12	0.01

Abbreviations: Hb, hemoglobin; BMO, bone marrow blasts; BSP, peripheral blood blasts; DHL, lactate dehydrogenase.

In accordance with the treatment response, the median transfusion was 12 concentrated erythrocytes for those who obtained CR, 10 for PR, 16 for SD and 19 for those who were in failure, with p=0.06. On the other hand, this transfusion requirement was assessed according to the risk established at the diagnosis: a IPSS INT1 had a median transfusion requirement of 12 packs of red cells; INT 2, median of 18.5 packs of red cells; and high IPSS with a median transfusion of 34 packs of red cells (p=0.14) (Table 5). The number of times that the patient was in the febrile neutropenia scheme according to the risk was a median of 3.5 times for INT1, median of 6 times for INT2; high median of 8 times in febrile neutropenia (p=0.014), and regarding hospitalization days according to risk: median of 8.5 days for INT1, median of 13 days for INT2, and high median of 12 hospitalization days (p= 0.032) (Table 6). None of the patients presented renal toxicity, with a median creatinine of 0.6, nor hepatic toxicity (Table 7).

Table 5. Transfusion requirement

Parameter		Median transfusion of CE	
IPSS (n=)			
	Intermediate 1	12	
	Intermediate 2	18.5	*p*= 0.14
	High	34	
Response (n=)			
	CR	10	
	PR	12	
	SD	16	*p*= 0.06
	Failure	19	

Table 6. Febrile neutropenia and hospitalization days

Parameter		Number of times in febrile neutropenia		Number of hospitalization days	
Response (n=)			IC 95%		IC 95%
	CR	4		4	
	PR	6	*p*=0.5	8	*p*=0.2
	SD	8		8.5	
	Failure	8		12	
IPSS (n=)			IC 95%		IC 95%
	Intermediate 1	3.5		8.5	
	Intermediate 2	6	*p*= 0.014	13	*p*=
	High	8		12	0.032

Table 7. Renal and hepatic toxicity

Parameter	Baseline	4th cycle	*p*
Creatinine	0.6 (0.3-1.5)	0.5 (0.3- 1.6)	0.30
AST/ALT	32/38 (26-46/28-52)	28/32 (26-40/26-50)	0.26

The time to AML progression was 7.5 months, the median event-free survival was 12 months (Figure 1), the median OS was 17 months; the median diagnosis at the time of death was 11.2 months (7-28). (Figure 2)

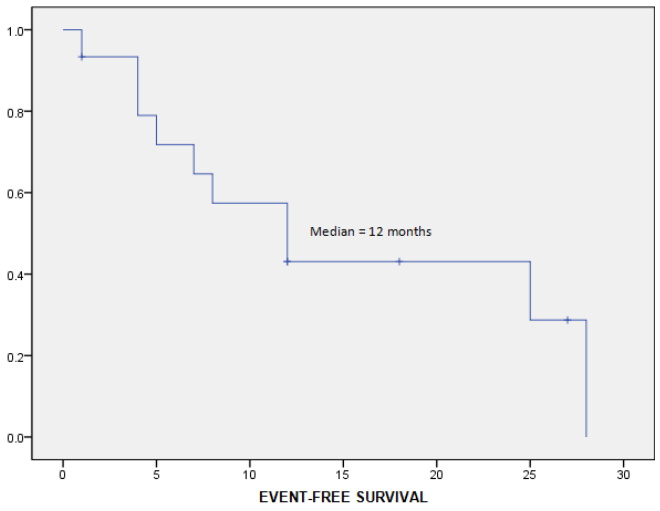

Figure 1. Event-free survival

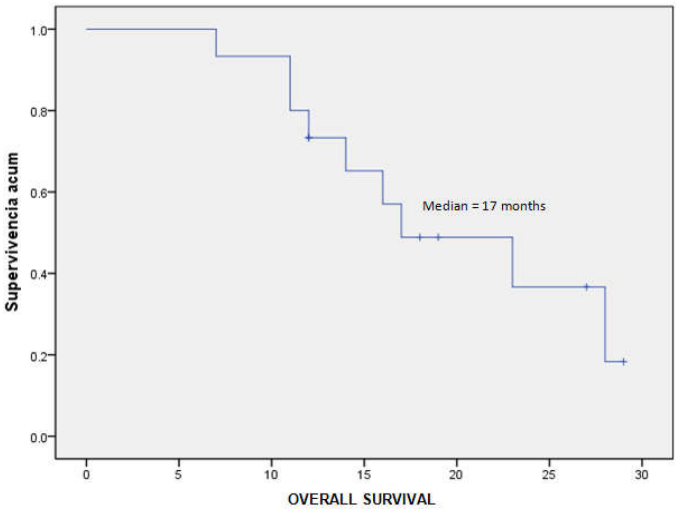

Figura 2. Overall Survival

DISCUSSION

The results obtained in this analysis demonstrate that 5-AZA is a treatment offering the possibility of improving MDS conditions, in those achieving a degree of response. The rate of incidence of MDS varies from 4.1 to 4.6 cases per 100 000 persons per year during 2003 and 2008, according to analyses conducted in the US. Existing data in the literature suggest that MDS is a disease predominantly occurring in the elderly. Approximately 86% of the patients are over 60 years of age at the time of diagnosis (median, 76 years), and only 6% of the cases are diagnosed before 50 years of age.[5] In our analysis, the study population ranged from 36 to 80 years, with a median age at diagnosis of 64 years. Regarding the sex, the literature reports a higher rate of incidence mayor in men than in women; however, in this study the prevailing population were women with 60% and men with 40%. Upon conducting the analysis with related age and sex with the response, no significant data was obtained (p=0.70 and 0.41, respectively).
Previous studies have identified several prognostic factors, including age, sex, transfusion dependency, type of MDS, percentage of bone marrow blasts, number of cytopenias, and cytogenetics. The last three parameters represent determining factors in the prognostics of MDS patients according to models such as IPSS and IPSS-R. (Xiaomei Ma, 2012) This could be confirmed in the population of this study since, by comparing the response - whether complete or partial - with the IPSS and IPSS-R score, a statistical significance with p=0.015 and p= 0.05, respectively, was obtained. The same did not happen with the type of MDS and cytogenic risk (p= 0.45 and 0.52, respectively). According to cytopenia, the result obtained from baseline measurements, was that hemoglobin, neutrophil and platelet values do not have an impact on the response with p=0.8.

When the IPSS was proposed for MDS, it was estimated that the patients in the low-risk category had a median survival of 5.7 years, and a median time of progression to AML of 9.4 years. Both the median OS and the time of progression to AML, decrease progressively with the risk increase, respectively, 3.5 and 3.3 years for IPSS Intermediate 1; 1.2 and 1.1 years for IPSS Intermediate 2; and 0.4 and 0.2 years for high-risk patients. The rate of progression to AML in our sample was 49%; comparing it with the degree of response obtained it was concluded that those progressing to AML remain in stable disease or have treatment failure, and the OS was 17 months, ranging from 11.2 to 28 months, which is comparable to the literature. (Santini, 2009) The response to 5-AZA may occur even when the MDS clone persists, indicating that the treatment with hypomethylating agents may modulate the sensitivity of hematopoietic progenitors in this disease. In a series of cases evaluating this response, the patients who remained with an abnormal karyotype despite the treatment accounted for 27%. The eradication of las cytogenetic anomalies is possible with the 5-AZA treatment. However, there patients requiring 36 to 40 months to achieve the full eradication, indicating the benefit of the maintenance therapy. (Silverman, 2009) In our study, the karyotype normalization in all patients was 13%. The survival advantages with 5-AZA are apparently independent from age, percentage of bone marrow blasts, and karyotype. Obtain a hematological improvement (acquiring transfusion independence and an increased platelet count), even in absence of partial or complete remission, is associated with a better survival rate. (Adés, 2014) In this report, the hematological improvement determined by median hemoglobin and platelets at the 9th cycle, relative to the type of obtained response, had statistical significance with p=0.001 and p=0.01, respectively. On the other hand, despite large advances in therapies aimed at MDS, this therapy with 5-AZA does not replace the only treatment that finally provides healing, such as bone marrow transplantation.

The dose approved in the US and in Europe is 75 mg/m^2/day, administered subcutaneously on days 1 to 7 of 28-day cycles, until the progression of the disease or unacceptable toxicity. There are records evaluating the effectiveness and tolerability of different schemes according to the days of administration (García-Delgado et al., 2014), but not the dose. Innovatively, to avoid wasting resources, in this study a dose of 100 mg/day was standardized, obtaining results not differing from those reported by other groups with the above mentioned usual doses. With regard to toxicity, for which, beyond the assessment of renal and hepatic adverse effects, the rate of hospitalizations and frequency of infections were analyzed, measured by the number of times that the patient had febrile neutropenia and transfusion requirement, statistical significance of 0.014 was obtained for the number of times in febrile neutropenia, and 0.032 for hospitalization days according to IPSS. This did not happen according to the degree of response, but with an evident increase of times in febrile neutropenia and hospitalization days according to the degree of response of p=0.5 and p=0.2, respectively.

With the chronic nature of this disease, the benefit of treatment is aimed at controlling the transfusion requirement and thus improving quality of life. Given that the transfusion dependence is a prognostic factor for survival and progression to AML, and in association to other known poor prognostic factors, with these results still getting worse. The treatment modalities reducing the need of transfusion in low-risk patients, may increase their survival. This is a justifiable reason for the use of 5-AZA in myelodysplastic syndromes rated as minor risk. (Santini, 2009) The decreased transfusion requirement in our study was measured by the number of concentrated erythrocytes transfused according to the type of obtained response, evidencing that those who achieved a response had a lesser amount of transfusions, with p=0.06.

Conclusion

The use of 5-azacitidine in patients with myelodysplastic syndrome has demonstrated efficacy in previous clinical studies, evaluated by the normalization of hematopoiesis, decreased transfusion requirement, increase in disease-free survival and overall survival; thus allowing a decrease of expenses for frequent and prolonged hospitalizations, a lower use of antibiotics for associated infections, and a lower amount of input used to satisfy transfusion requirements. The above is of great benefit to the patient by allowing him/her to return, as far as possible, to usual activities, and reintegrate into employment, with subsequent earnings, in addition to an increased overall survival, facing a catastrophic disease. This study demonstrated that 5-AZA is a treatment useful in patients with MDS in the group and profile of patients studied in this CMN. 5-AZA improves the medulla function, reducing and delaying significantly the transformation to AML. It improves quality of life in terms of a lower rate of transfusion requirement. For that reason, 5-AZA shall be considered a treatment of choice in patients with MDS meeting the criteria required for its use. The Hematology Service is favored with a clear and objective learning of a drug aimed at a disease without standard treatment, allowing to disseminate knowledge for being the first study at institutional level. Upon knowing the results of this study in our institution, it is possible to reproduce the benefit in other hospital centers.

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