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Clinical outcome in chronic myeloid leukemia after allogeneic hematopoietic stem cell transplantation: the experience of the Bone Marrow Transplantation Unit of FUNFARME/ BRAZIL using FISH

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ABSTRACT. Investigation of the efficacy of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in chronic myeloid leukemia patients is essential to predict prognosis and survival. In 20 patients treated at the Bone Marrow Transplantation Unit of São José do Rio Preto (São Paulo, Brazil), we used fluorescence *in situ* hybridization (FISH) to investigate the frequency of cells with BCR/ABL rearrangement at diagnosis and at

distinct intervals after allo-HSCT until complete cytogenetic remission (CCR). We investigated the disease-free survival, overall survival in 3 years and transplant-related mortality rates, too. Bone marrow samples were collected at 1, 2, 3, 4, 6, 12, and 24 months after transplantation and additional intervals as necessary. Success rate of the FISH analyses was 100%. CCR was achieved in 75% of the patients, within on average of 3.9 months; 45% patients showed CCR within 60 days after HSCT. After 3 years of the allo-HSCT, overall survival rate was 60%, disease-free survival was 50% and the transplant-related mortality rate was 40%. The study demonstrated that the BCR-ABL FISH assay is useful for follow-up of chronic myeloid leukemia patients after HSCT and that the clinical outcome parameters in our patient cohort were similar to those described for other bone marrow transplantation units.

Key words: Chronic myelogenous leukemia; Disease-free survival; BCR-ABL fusion; Stem cell transplantation; Cytogenetic remission; Fluorescence *in situ* hybridization

INTRODUCTION

Chronic myeloid leukemia (CML) is a malignant disorder of an early hematopoietic progenitor cell that is genetically characterized by the presence of a BCR/ABL fusion (Haigh and Cuthbert, 2005). In the majority of cases, the fusion arises from a reciprocal translocation between the *ABL* locus on 9q34 and the *BCR* locus on 22q11.2, resulting in the classical Philadelphia (Ph) chromosome. CML is clinically characterized by an initial chronic phase followed by accelerated and blast crisis phases, the latter being frequently resistant to treatment (Aoun et al., 2004).

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the modality of treatment that provides a potential cure for CML, but clinical relapse post-HSCT is an ongoing problem (Soydan et al., 2005). Relapse rates after allo-HSCT vary from 20 to 50%, depending on T-cell depletion of the graft (Posthuma et al., 2004); rates are lower in patients allografted in the chronic phase with unmanipulated cells than when donor marrow cells are T-cell depleted (Olavarria et al., 2003).

The aim of allo-HSCT is to replace autologous hematopoiesis with stem cells harvested from the donor. However, autologous (host) cells may be detected in peripheral blood or bone marrow of some patients after the procedure. The clinical significance of this phenomenon, called mixed chimerism or persistence of residual (host) hematopoiesis, remains controversial. Increased mixed chimerism may be associated with a high risk of relapse or rejection, and a decrease in the number of host cells may indicate a low risk of recurrence. The consequence of the stable presence of a low-level autologous signal is still unclear and results in doubt about the best choice of therapeutic approaches for the patient (Wickenhauser et al., 2002; Turkiewicz et al., 2003).

The major aim of post-transplant monitoring is to detect negative events early, such as disease relapse, graft rejection and graft-versus-host disease, and to start the appropriate therapy. In this context, molecular cytogenetics is an important laboratory tool to monitor post-HSCT outcomes through the detection of positive Ph clones. The fluorescence *in situ* hybridization (FISH) assay is a powerful technique to clarify cytogenetic rearrangements (Vendrame-Goloni

et al., 2003; Fett-Conte et al., 2007). Especially when using a dual-color-dual-fusion probe, FISH can be used to monitor CML patients post-allo-HSCT regularly (Khan et al., 2004). Cytogenetic remission is critical to define engraftment and relapse and is a prerequisite in both myeloablative and non-myeloablative HSCT, in order to assess the graft status and decide future therapeutic strategies (Au et al., 2003; Khan et al., 2004). Therapeutic approaches for patients in relapse after allografting include α-interferon, chemotherapy, a second HSCT, and immunomodulation with donor lymphocyte infusions (Olavarria et al., 2003), as well as tyrosine kinase inhibitors (imatinib and dasatinib, for example) which block progenitor cell proliferation (Fausel, 2006).

This study used FISH to investigate the time to complete cytogenetic remission (CCR) after allo-HSCT in CML patients treated in the FUNFARME Bone Marrow Transplantation Unit from 2002 until 2004. Other clinical outcome parameters investigated were the disease-free survival (DFS), overall survival (OS) and transplant-related mortality (TRM) rates.

PATIENTS, MATERIAL AND METHODS

We studied 20 CML patients, diagnosed according to the World Health Organization criteria. All patients showed a BCR/ABL rearrangement at diagnosis detected by FISH and/or GTG-banding karyotyping in bone marrow cells. The mean frequency of cells carrying the BCR/ABL rearrangement at diagnosis was 84%, varying from 56 to 100%. Bone marrow samples were collected at 1, 2, 3, 4, 6, 12, and 24 months after allogeneic myeloablative HSCT and at other additional intervals according to the medical indication of each case. All patients were previously submitted to the BUCY (busulfan and cyclophosphamide) protocol as conditioning regimen (Thomas and Clift, 1998; Clift et al., 1999).

Bone marrow cells were added to a 24-h non-stimulated culture in RPMI 1640 medium with 20% fetal bovine serum. FISH assays were performed with the dual-color, dual-fusion DNA probe for BCR/ABL (LSI/BCR labeled in SpectrumGreen and LSI/ABL labeled in SpectrumOrange, Vysis/Abbott Molecular). The protocols for hybridization followed those described by Estécio et al. (2002) and manufacturer instructions. At least 800 interphase cells were analyzed per patient in each examination, and data were described according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995). Lymphocytes from healthy individuals were used as control in each assay, in which no *BCR/ABL*-positive cells were identified.

CCR was defined as the absence of BCR/ABL rearrangements evidenced by FISH. DFS was considered as the length of time after HSCT during which no disease was found (no hematological and/or cytogenetic abnormalities). Patients who died were not considered when determining DFS, even if the cause of death was an infection, because the follow-up was interrupted. OS was defined as the percentage of patients who survived for a period of 3 years, and the mean values were calculated using the Kaplan-Meier method.

RESULTS

The ages, time from HSCT to CCR, DFS rate and events observed post-HSCT for each patient are shown in Table 1. The mean of cytogenetic follow-up was 11.8 months. Among the 20 patients, 15 (75%) achieved CCR between one and 19 months after the procedure, with a mean time of 3.9 months. Nine patients (45%) achieved CCR up to 60 days after the HSCT, a time considered indicative of good prognosis. One patient (case 6) achieved CCR only after 19

months, although the frequency of BCR/ABL rearrangement was very low (<0.5%) in the last 14 months before CCR was reached. There was no association between the frequency of cells with BCR/ABL fusion at the diagnosis of CML and the time to achieve CCR.

Table 1. Case, age, time (months) after hematopoietic stem cell transplantation (HSCT) to achieve complete cytogenetic remission (CCR), disease-free survival (DFS) and events observed post-HSCT.

Case	Age (years)	CCR (months)	DFS (months)	Post-transplant events
1	38	0	0	death → (stroke)
2	13	4	31	N
3	36	4	31	death (I)
4	29	2	29	N
5	20	2	5	relapse → 2nd HSCT → death (I)
6	34	19	25	N
7	46	1	28	N
8	25	1	27	N
9	39	2	3	death (I)
10	29	1	12	N
11	37	2	23	N
12	40	2	10	relapse → death (I)
13	37	0	0	relapse → imatinib → bone marrow aplasia → death (I)
14	16	6	11	N
15	27	3	10	N
16	34	0	0	N
17	42	1	11	N
18	44	9	12	N
19	43	0	0	N
20	37	0	0	relapse → death (I)

I = infections; N = no event, patient in follow-up; 0 = did not achieve remission.

The median DFS was 9.8 months and the 3-year DFS rate was 50%. The OS rate was 60% in 3 years. There was no association between period of time to achieve CCR, DFS and OS time (P = 0.001).

In 3 years, the TRM rate was 40% and the relapse rate was 20%. Five patients (25%) did not achieve CCR. Three of them died, one because the disease affected the central nervous system and two due to infections (one after introduction of imatinib mesylate). The other two patients did not present post-HSCT events. Five patients who had achieved CCR died from infections between one and four months after the allo-HSCT.

DISCUSSION

The success of allo-HSCT depends to a great extent on the cytogenetic follow-up of the patient, which influences the therapeutic management. Patients who show the BCR/ABL rearrangement in progressively higher frequencies after the procedure are at greater risk of relapse and are candidates for additional and more aggressive therapies. However, patients with stable or decreasing frequencies seem to have immunological mechanisms suppressing the malignant clone, or perhaps this clone may lose its proliferative capacity resulting in a lower risk of relapse (Gopcsa et al., 2003).

The FISH technique is especially valuable for investigating patients after HSCT, because it is more sensitive and can be performed faster than classical cytogenetics. The dual-

color interphase FISH has higher sensitivity in detecting *BCR/ABL* fusion gene, but interphase-dual-color and dual-fusion FISH is a more reliable, sensitive and practicable test for monitoring minimal residual disease and CCR after transplantation (Liu Q et al., 2005; Qian et al., 2006). This was the test used here. Dynamic detection of *BCR/ABL* fusion gene level by FISH may predict disease changes and guide individual therapy (Qian et al., 2006). Other techniques such as reverse transcriptase-polymerase chain reaction, real time quantitative polymerase chain reaction and microarray are also sensitive (Yokota et al., 2002).

Most of the patients (75%) treated in the FUNFARME BMT unit during the selected period achieved CCR, 45% of whom within the first two months after HSCT. These results are similar to reported CCR rates in developing countries such as China and Greece, which varied from 56 to 95.9% (Vinogradova et al., 2002; Liu et al., 2004a,b; Liu DH et al., 2005; Qin et al., 2006).

Patients with CCR within two months have a better prognosis (Imparato et al., 1999; Kebriaei et al., 2004). However, four patients in our study who had good prognosis died: two of them relapsed, were submitted to another therapeutic modality (second HSCT and therapy with imatinib mesylate) and died due to infections; two others did not relapse but had fatal infections. In other countries, the CCR rates are similar. For example, Vinogradova et al. (2002) found 56% in Russian patients.

In Brazil, the data on CCR post-allo-HSCT are rare. The only data that we found were of Chauffaille et al. (2001). They observed a 83% CCR rate one year after HSCT using FISH, but they used a double-color and single-fusion probe. Although all of these 12 patients studied showed the fusion, the cut off level was established as 10%.

Patients in relapse after allo-HSCT have few therapeutic options. A second allo-HSCT may be a good therapeutic alternative for selected patients with relapsed leukemia after the first HSCT. Yang et al. (2004) related a relapse rate at 2 years after the second allo-HSCT of 30%. The use of imatinib may also have a significant effect (Fujisawa et al., 2003; Gopcsa et al., 2003). Soydan et al. (2005) concluded that imatinib treatment for molecular relapse after HSCT has an acceptable adverse event profile and provides a molecular remission rate of over 60%, but the response is short and 25% of the patients relapse again soon after drug cessation.

We found an OS rate of 60% in 3 years. It was not too good but did not differ greatly from other reported findings. In the USA, Simon et al. (2006) found a rate of 86% in 3 years.

The TRM rates described in the literature for a period of approximately 2 years after HSCT are variable. In Chinese patients, the mortality rate reported by Liu et al. (2004b) was 16.7%. The rate experienced by Liubimova et al. (2004) was 14%. De Souza et al. (2005) reported a rate of 45% and Valcarcel et al. (2005) published a rate of 30%.

The TRM rate in this study was 40%. In the majority of these cases (87.5%), death occurred due to infections unrelated to relapse of disease in 43% of the patients, and Burroughs and Storb (2005) described a mortality rate unrelated to relapse in 15 to 55%. Infections are complications considered relatively common after HSCT and are described in 38.6 to 59.6% of patients (Petzer and Gunsilius, 2003; Liubimova et al., 2004; Yang et al., 2004; Burroughs and Storb, 2005; De Souza et al., 2005; Valcarcel et al., 2005; Simon et al., 2006).

CONCLUSION

The dual-color, dual-fusion BCR/ABL FISH assay is useful in identifying cytogenetic remission after allogeneic HSCT in CML. The results of cytogenetic remission, disease-free

survival rate and rate of transplant-related mortality in our study are in agreement with those published by other authors from different HSCT units.

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