Brazilian experience using high-dose sequential therapy (HDS) followed by autologous hematopoietic stem cell transplantation (ASCT) for malignant lymphomas

Experiência brasileira utilizando terapia sequencial de alta dose seguido de transplante autólogo de célula-tronco hematopoética para linfomas malignos

Using the overall survival (OS), disease free survival (DFS) and progression free survival (PFS), as well as associated toxicity, the purpose of this work was to evaluate the effectiveness of HDS followed by ASCT as salvage therapy. A retrospective analysis was performed of 106 patients with high grade non-Hodgkin lymphoma receiving HDS followed by ASCT, between 1998 and 2006. Median age was 45 years (Range: 8-65), with 66 (62%) men. Histopathological classification was: 78% DLBCL patients, 12% T and anaplastic and 9% Mantle cell lymphomas; 87% had B cell and 12% T cell lymphomas; 83% were stage III-IV (Ann Arbor Staging), 63% had B symptoms, 32% had bone marrow involvement, 62% bulky disease and 42% high-intermediate or high risk IPI. After HDCY, 9 patients died, 7 from toxicity and 2 from sepsis. Eighty patients underwent ASCT, 47% were in complete remission (CR) and 15% died, all from toxicity. Their OS was 45% over 8 years. During the follow-up, another 35 patients died [4 CR, 1 partial response (PR), 2 relapsed disease (RD) and 28 disease progression (DP)], 11 (31%) had not performed ASCT. OS was 37%; DFS was 49% and PFS 28%. OS by diagnosis was 42% for DLBCL, 40% for T-cell (8 y) and 20% for Mantle Cell (6 y) (P=NS). OS by B symptom patients was 22% vs. 58% (P=0.002) and PFS was 23% vs. 37% (P=0.03). Patients who achieved CR after HDCY (38) had significantly better OS and PFS (38% and 17%) than patients who remained in DP (P<0.0001). Cox Regression demonstrated therapeutic lines before HDCY (Relative risk - RR = 1.41; CI 95%: 1.04-1.90; P= 0.02) and PD both before (RR = 2.70; CI 95%; 1.49-4.91, P<0.001) and after HDCY (RR = 5.38; 95% CI: 2.93-9.87; P<0.0001). Conclusions: Our study suggests HDS is an efficient treatment to improve status and to reduce tumoral burden. Regardless of toxicity-related mortality it is feasible, especially considering the poor prognosis of patients. Rev. Bras. Hematol. Hemoter. 2009;31(Supl. 2):9-14.

Key words: High dose sequential therapy; autologous hematopoietic stem cell transplantation; non-Hodgkin lymphoma; Brazilian experience
Introduction

A dose intensified regimen, such as high dose sequential therapy (HDS) followed by autologous hematopoietic stem cell transplantation (ASCT) is an effective and feasible salvage therapy for resistant and relapsed malignant lymphomas.1,2,3-8 It consists of an intensified debulking phase by sequential administration of high-dose cyclophosphamide (HDCY) followed by high-dose methotrexate (HDMTX), (only for Hodgkin's lymphoma - HL) and high-dose etoposide (HDVP), peripheral blood progenitor cell (PBPC) harvesting, HDS and ASCT.9

Despite proof of its efficacy reported by several investigators for both HL10-12 and non-Hodgkin's lymphoma (NHL),13-15 there are few studies using this strategy in Brazil and few groups around the world evaluated the use of this therapy with over 5-year follow-up periods. Our aim was to evaluate the effectiveness and toxicity of HDS used as salvage therapy for malignant lymphomas, focusing on overall survival (OS), disease free survival (DFS) and progression-free survival (PFS).

Patients and Method

Seventy-seven patients diagnosed with relapsed or refractory HL and 106 patients with high grade NHL were treated with HDS between 1998 and 2006 in three different institutions: University of Campinas, Vera Cruz Hospital and the Boldrini Children's Cancer Center. Data was obtained from the patients' medical records. Eligibility criteria included failure to achieve complete remission (CR) after first-line treatment (non-responsive, NR), relapsed disease even when in CR before mobilization, and adequate psychiatric conditions to participate. All patients or their legal representatives provided written, informed consent before receiving this regimen. Treatment procedures were approved by the Research Ethics Committee of each participating institution, according to the principles of the Helsinki Declaration.

Treatment procedures and definitions

HDS consisted of the sequential administration of HDCY (4 or 7g/m²) and G-CSF (300 µg/day, from day +1 after HDCY), followed by PBPC harvesting when white blood cells increased to > 1.0 x 10⁹/L, with the aim of collecting > 5 x 10⁶ CD34+ cells/kg. G-CSF was stopped after the last day of apheresis. Patients with an insufficient number of CD34+ cells underwent another collection after etoposide. In some cases where peripheral cells could not be harvested, harvesting was made directly from the bone marrow. After PBPC harvesting, methotrexate (8g/m²) plus vincristine (1.4mg/m²) (only in patients with HL) and etoposide (2g/m²) were administered.

Disease status was assessed preferably before HDCY, before ASCT, after ASCT and throughout the long-term follow-up (every three months in the first year, every six months in the second year and annually thereafter).

Data collection and statistical analysis

Analysis was based on data available in February 2009. OS was calculated from HDCY date until the date of death or last follow-up. DFS included only patients who achieved CR and was calculated from the date of CR assessment until the date of relapse, last follow-up or death. PFS included all patients and was calculated from HDCY date until the date of progression, relapse, last follow-up or death due to any cause. Actuarial curves of OS, DFS and PFS were analyzed using the Kaplan-Meier method and compared by the log-rank test. Multivariate predictors of outcome (OS, DFS and PFS) were assessed by Cox regression analysis, using the forward stepwise Wald test (p<0.05).

Results

Hodgkin's Lymphoma

Patients' characteristics

The median age was 23 years (Range: 7-68) at diagnosis, with 46/77 (60%) men. Histopathological diagnosis16 was: 65% nodular sclerosis, 25% mixed cellularity, 6% lymphocyte depleted, 1.5% lymphocyte predominant and 2.5% patients unknown. Stage III-IV17 was 65%, 71% had B symptoms, 14% had bone marrow involvement and 40% had bulky disease (≥ 10 cm). Before HDCY, patients were treated with a median of two therapeutic lines, including conventional chemotherapy and radiotherapy. Three patients were in CR after treatment with conventional therapy for relapsed disease and 54.5% were in disease progression (DP) before mobilization with HCY.

Mobilization, PBPC harvesting and toxicity

CY was administered after a median time of 1.5 years from diagnosis. Thirty patients (39%) received a dose of 4g/m² due to advanced age (> 65 years) or borderline cardiac function, and 47 patients (61%) received 7g/m². The latter group had more patients in DP (57% vs. 50%; p = 0.02) and more patients with B symptoms (80% vs. 57%; p = 0.02). The 4g/m² group had a greater prevalence of previous use of radiotherapy (73% vs. 44%; p = 0.01). There were no significant differences in the other parameters.

The median day of leukapheresis after HDCY was +13 (Range: 8-27), with a median of three sessions (Range: 1-8) and a median number of harvested CD34+ cells of 5.98 x 10⁶ cells/kg (Range: 0.23-45.01 x 10⁶).

Twenty-one patients (27%) died after HDCY. Six died from toxicity, nine from DP, four from sepsis while in DP and insufficient cells were harvested from one; this patient developed myelodisplastic syndrome (MDS) and died from...
sepsis while in CR. Moreover, one case died 16 months after
HDS from refractory congestive heart failure, giving a total of 7/77 (9%) patients with HDS-related mortality. Besides these
patients, three more were not submitted to ASCT, thus 24 patients (31%) did not undergo ASCT.

We obtained data on toxicity for 71 patients. Sixty-six
patients (93%) presented with hematologic toxicity. Forty patients
experienced gastrointestinal toxicity, nine patients from cardiac
toxicity, six patients from asymptomatic reduction of the ejection
to fraction, one patient from cardiac failure and another one died
with severe heart failure. Seven patients developed acute renal
failure not related to sepsis. Finally, 19 patients had fever of
unknown origin.

**Autografting**

Autografting was performed in 53 patients with a median
of 118 days after HDCY (Range: 62-407). BEAM was used as a
conditioning regimen in 81%. The median times for granulocyte
(>0.5x109/L) and platelet (>20 x 109/L) engraftment were 11 days
(Range: 9-27) and 17 days (Range: 6-88), respectively. Auto-
engraftment related mortality was 6%. OS for transplanted
patients was 46% over five years. Twenty-nine patients died
after ASCT as described in the long-term outcome.

**Long-term outcome**

Of the 77 patients who were submitted to HDCY, 21 died
before ASCT. Another three patients did not undergo ASCT. A
total of 53 patients were submitted to ASCT and until now 45%
are alive. Twenty-nine patients died after ASCT. Another patient
developed MDS and is alive, totaling four patients (5%) who
developed AML/MDS.

A total of 27 patients are alive for a median of 66 months
after HDCY (Range: 3-128). Eighteen patients are in CR for a
median of 70 months after HDCY (Range: 17-128).

OS was 27% with a median time of 18 months (Range: 0.1-
128); DFS was 57% with a median of 45 months (Range: 1.5-125);
PFS was 25% with a median 13 months (Range: 0.1-128).

We analyzed the survival of patients initially in DP (57/77
- 74%) according to their disease status before HDCY. Patients
who achieved CR after HDCY (24/57 - 42%) had a significantly
better OS and PFS (36%-33%) than patients who remained in DP
(10%-17%). We also analyzed the survival of patients according
to HDCY dose, for which no significant difference was found.

Additionally we analyzed survival data based on age, stage
and histopathological and laboratory findings at diagnosis and
found no significant differences.

For the multivariate analysis two variables remained: LDH
(as categorical variable - Hazard ratio - HR = 2.41; 95% CI: 1.04-
5.59; P = 0.04) and DP after HDCY (HR = 3.97; 95% CI: 1.73-9.10;
P = 0.001). In summary, 65% patients died; 46% from DP, 26%
from sepsis, 14% had HDS-related deaths, 6% had ASCT-related
deaths, 6% had AML/MDS and 2% had extensive chronic graft-
versus-host disease (GVHD) due to a reduced intensity conditioning (RIC) allogeneic transplantation.

**Non-Hodgkin Lymphoma**

**Patients’ characteristics**

One hundred and six patients were enrolled with a
median age of 45 years (Range: 8-65), with 66 men. Histopathological classification16 was: 78% diffuse large B-
cell lymphoma (DLBCL), 12% T and anaplastic lymphoma
and 10% mantle cell lymphoma. At diagnosis, 83% were in
stage III-IV, 17 63% had B symptoms, 32% had bone narrow
involvement, 62% bulky disease and 42% had high-
intermediate or high risk IPI 18.

Before HDCY, patients were treated with a median of
one therapeutic line, including conventional chemotherapy
and radiotherapy. Six patients were in CR after their first relapse
after treatment with conventional chemotherapy and 38 were
in partial response (PR). More than half were in DP or
refractory relapsed disease.

**Mobilization, PBPC harvesting and toxicity**

CY was administered after a median time of 10 months
after diagnosis. Forty-two patients received a dose of 4g/
m² and 64 patients received 7g/m². Groups differed statistically according to IPI (66%-4 g/m² vs. 30%-7 g/m²;
 p = 0.006) and in prevalence of altered serum LDH (68% -
4 g/m² vs. 41% - 7 g/m²; p < 0.0001).

The median leukapheresis day after the HDCY was
+13 (Range: 3-83), with a median of two sessions (Range: 1-
7) and a median number of harvested CD34+ cells of 6.74 x
10⁹ cells/kg (Range: 1.29-44.01 x10⁹).

Eighteen patients (17%) died after HDCY. As well as
these patients, 8 did not undergo ASCT, totaling 26 patients
(24.5%) who were not submitted to ASCT.

We were able to recover toxicity data for 102 (96%)
patients. All patients presented hematologic toxicity. Forty-
seven patients experienced gastrointestinal toxicity, ten
cardiac toxicity, six asymptomatic reduction of the ejection
to fraction, two heart failures, one severe pleural effusion and
one died due to severe congestive heart failure. Four patients
presented acute renal failure that was not due to
sepsis. Finally, 27 (25.5%) patients presented fever of
unknown origin.

**Autografting**

ASCT was performed in 80 patients after a median of
123 days (Range: 45-1710) from HDCY. BEAM was used as a
conditioning regimen in 87. The median time for
grafluence engraftment (neutrophil count > 0.5x10⁹/L) was
11 days (Range: 6-29) and 16 days (Range: 5-70) for platelet
engraftment (platelet count >20 x 10⁹/L). Auto-engraftment
related to mortality was 14%. Twenty-six patients did not
autograft.

Only five patients who were not submitted to ASCT
are alive: 1 DP, 2 CR and 2 PR. OS for patients submitted to
ASCT was 45% over eight years.
**Long-term outcome**

Twenty-six patients did not undergo ASCT: 18 died prior to the procedure and eight patients were not transplanted for other reasons, with six of them still alive. Another 37 patients died during follow-up, 11 due to ASCT toxicity, 12 due to DP, 13 from sepsis and one from chronic GVHD after being submitted to a RIC allogeneic transplantation. One patient developed MDS and remains alive.

Until the closing date of this analysis, 49/106 patients are alive, with a median of 68 months. OS was 41% with a median of 30 months (Range: 0.2-124), DFS was 49% with a median of 36 months (Range: 1.5-118) and PFS was 31% with a median of 16 months (Range: 0.2-124).

We observed better OS (60 and 40%) and PFS (27 and 24%) for patients without B symptoms at diagnosis ($p<0.03$ and $p=0.02$, respectively). Patients who achieved CR after HDCY (38) had significantly better OS and PFS (44%-27%) compared to patients who remained in DP (24 patients with 0% and 0%).

OS, PFS and DFS were not affected by the CY dose. We also analyzed the outcome of patients based on age, stage, histopathological findings at diagnosis and IPI and found no significant differences.

From the variables included in univariate analysis, DP before (HR = 2.56; CI 95%: 1.42-4.62; $p=0.02$) and after (HR = 5.52; CI 95%: 3.01-10.1; $p<0.001$) HDCY were associated with worse OS.

On multivariate analysis, the results were: presence of B symptoms, LDH, and DP or relapse before HDCY for OS, whereas for PFS, B symptoms and DP or relapse before HDCY remained.

Overall, mortality was 57/106 (54%); causes were 42% DP, 12% related to HDCY, 19% related to ASCT, 23% infections, 2% from chronic GVHD after being submitted to a RIC allogeneic transplantation and 2% from an unknown cause. In addition, one patient developed MDS and remains alive.

**Discussion**

The intention of analyzing a Brazilian cohort has several implications because the frequency of some important poor prognostic factors, such as B symptoms and bulky disease, are higher in our patients compared to series from the Northern Hemisphere. In an Italian study evaluating HDS in 102 patients with refractory or recurrent HL, 42% had B symptoms and 29% had bulky disease. In another study evaluating the use of HDS and ASCT in 494 Spanish patients with refractory or recurrent HL, 40.5% had B symptoms and 33% had bulky disease. These numbers contrast with the 71% of patients with B symptoms and the 40% of patients with bulky disease seen in our patients with HL. The same is true with NHL patients.

The higher prevalence of such variables in our population indicates that they are probably expected to have poorer results when compared to other populations in developed countries.

Another possible factor contributing to a worse outcome in our patients is a high prevalence of Epstein-Barr virus-associated (EBV-associated) HL, although we did not collect this information. Despite the controversy surrounding the possible influence of EBV on the outcome of HL, a few studies suggest that OS is worse when EBV is present in adult patients. While the prevalence of EBV in developed countries is around 30%, one study performed at our institution showed a prevalence of 64%. This difference may be due to low socioeconomic status (as we are a governmental institution which provides care for low income patients) and not due to ethnic differences between our population and those from developed countries. A lower income leads to a delay in diagnosis.

The higher prevalence of worse prognostic factors in our population is reflected by the poor OS and PFS of HL patients (27% and 25%) when compared to other studies, with OS and PFS ranging from 50%-65%. However, this was not observed for NHL patients, with OS of 41% and a PFS of 31%, similar to those observed by other authors, where OS ranged from 40%-45%.

This difference in response between HL and NHL patients has not been previously reported. Although the greater prevalence of high-risk patients can justify the worse survival observed in our HL patients, it should have also impacted survival of NHL patients. This finding, in a population of similar social-demographic conditions, with high-risk, advanced disease treated under the same protocol and within the same institution shows the need of a better understanding of the differences between the response of HL and NHL to HDS.

These data should point to a review of HDCY use in HL, usually a more benign disease, where patients could benefit from less intensive regimens. The lower number of studies evaluating HDS in HL than in NHL only reinforces the need for such a review.

However, the use of different doses, and sometimes drugs, between our study and others should be highlighted as a factor which could justify the differences mentioned above.

We did not expect the lack of benefit of using a higher dose regimen in both HL and NHL. We employed at our institution a 7g/m$^2$ dose of HDCY and a less intense one of 4g/m$^2$ based on findings of the Italian group. This was developed based on the Norton-Simon hypothesis that we should seek a treatment regimen that employs the highest possible dosing, over the minimum period of time, with acceptable toxicity. By showing no extra benefit, neither in OS nor in response rates, of the higher dose regimen, our study suggests that its benefit was exceeded by its toxicity, resulting in a similar survival rate, when compared to a less dose-intense regimen.
Even though the 7 g/m² regimen did not improve survival, the role of a high-dose debulking regimen is highlighted by the observation that patients previously in DP, who responded to HDCY and achieved CR, had a better overall survival. This not only shows the ability of HDCY to overcome primary chemo-resistance in a significant proportion of refractory patients, but also its importance in assessing malignant lymphoma chemo-sensitivity, since there was no benefit in submitting patients to HDS and ASCT when they did not respond to HDCY.

Nevertheless, this high-dose debulking regimen imposed a high toxicity burden, with HDCY-related mortality of 6.6% for NHL and 7.8% for HL, which is slightly worse than the 5% rate observed elsewhere, but still acceptable.

One point that should be noted is the incidence of secondary hematological malignancy, especially in HL patients. We know today that death due to secondary cancers is now the most common cause of mortality among long-term survivors of HL. The incidence of secondary hematological malignancy for HL in our study was 5.2%. This rate appears to be similar to those observed in other studies, and although credited to the cytotoxic effects of alkylating agents, the role of cytogenetic instability related to the disease and ASCT has yet to be determined, especially when we consider the significantly lower incidence of this complication in patients with NHL in our study (0.9%) treated under the same regimen.

Our study has some limitations typical of retrospective studies. However, we can conclude that despite the significant number of toxicity-related deaths, our data suggest that this regimen is feasible, especially for chemo-sensitive patients. The development of secondary neoplasias is a special concern in this setting, particularly for HL patients.

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