Autologous Transplantation in 1st Cr Aml Patients with Purged or Unpurged Bone Marrow: Good Clinical Results in Favorable Cytogenetic Group and in those Infused with a Low Number of Marrow Cells

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Abstract

In a single institution, 31 patients affected with Acute Myeloid Leukaemia (AML) in 1st Complete Remission (CR) received autologous bone marrow transplantation (ABMT). Mafosfamide was employed in a non-randomized fashion to purge marrows, in 15 cases bone marrow cells were purged, while in 16 they were left unpurged. Dose of infused Total Nucleated Cells (TNC) was an important factor for myeloid engraftment (P=0.02). LFS was 58% in purged and 40% in unpurged groups (P=0.26). Patients having a good prognosis cytotype had a LFS of 100% while the group of all other patients had a LFS of 37.5%. Patients receiving a dose of TNC below to median had a LFS of 65% and those receiving a dose of TNC>median had a LFS of 28% (P=0.017). Purging significantly improved LFS in patients “not harbouring good cytogenetic abnormalities” (53% LFS in purged group versus 18% in unpurged group, P=0.05). In conclusion, ABMT is associated with excellent results in “good prognosis cytogenetic”. Purging may improve results in patients belonging to “intermediate cytogenetic group”. A high number of infused TNC produces a fast myeloid engraftment but a poor LFS.

Keywords: Mafosfamide; Acute myeloid leukemia; Autologous bone marrow transplantation

Introduction

In adult patients affected by Acute Myeloid Leukaemia (AML), Autologous Bone Marrow Transplantation (ABMT) offers a clinical advantage over conventional chemotherapy in terms of Disease Free Survival (DFS), although relapse still remains a frequent cause of failure [1-3]. Pharmacological in vitro purging of bone marrow inoculum using mafosfamide or 4-hydroperoxycyclophosphamide (4HC) could reduce relapse risk [4,5], hence this technique has been used extensively by some groups over the last decade [6-8]. Comparative data on the efficacy of in vitro purging in AML obtained in multi-centre retrospective studies [9,10] result in beneficial effect of purging. No randomized study comparing purged and unpurged ABMT is available. A randomized study comparing ABMT using maphosphamide purged marrow versus chemotherapy failed to demonstrate advantages in purged ABMT arm in term of OS or DFS [11]. On the other hand, in retrospective multicenter studies, purging resulted in a reduced rate of treatment failures [9,10] with respect to unpurged ABMT. Furthermore, direct comparisons between purged and unpurged marrow in AML patients, obtained in a single institution, are scarce [12,13]. We report clinical results obtained in our institution using ABMT unpurged or mafosfamide-purged ABMT in the treatment of AML.

Patients and Methods

Thirty-one patients affected by AML in 1st Complete Remission (CR) were treated with ABMT in our Institution from October 1994 to July 2002. Patient selection criteria for ABMT eligibility were: a diagnosis of AML other than type M3, 1st CR; age ≤ 65 years; normal liver, heart and pulmonary functions. Chemotherapy treatment received by patients before bone marrow harvest and ABMT was administered according to GIMEMA AML 8, GIMEMA AML 10 and GIMEMA AML 12 trials. Once in complete remission, all patients received a chemotherapy consolidation course and bone marrow was harvested 6-7 weeks later. Patient characteristics are summarised in Table 1. Although inclusion criteria excluded FAB M3, one patient initially classified as FAB M2 was observed to be affected by AML M3 after transplant, due to the detection of PML/rar alpha transcript in BM cells.

Bone marrow harvest evaluation, processing and purging

Bone marrow was harvested by multiple bilateral aspirations from iliac bone using standard techniques with 10% ACD as anticoagulant [5]. In all cases, bone marrow cells underwent volume reduction and depletion of red cells by means of a Cell Separator (CS3000 Plus – Baxter) before cryopreservation, as reported elsewhere [14]. In 15/31 cases, Bone Marrow cells were treated in vitro with mafosfamide. Purging was offered to patients with a bone marrow harvest containing at least 2x10^6/kg TNC. Use of purging was not randomised and it was limited to some cases due to an irregular availability of mafosfamide. In the case of marrow purging, 15-25% of harvested bone marrow was cryopreserved and unpurged to provide an unpurged back-up inoculum.

Mafosfamide was obtained from ASTA-Medica (Frankfurt, Germany). It was freshly prepared and employed at a final concentration of 50 mcg/ml at 37°C for 30 min in TC199 media (GIBCO); CD34+ cells were determined by cytofluorometry, and CFU-GM by methylcellulose culture stimulated by GM-CSF as already reported [15].

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Patients were divided in 3 risk groups: favourable, intermediate and adverse. The favourable risk group included patients with t(8;21) (q22;q22) without a del(9q) and inv(16)(p13q22)/t(16;16)(p13;q22). The intermediate risk group included patients with +8,-Y,+6,-12p,del/12p, t(6;9), t(9;22) abn 17p and complex cariotype. The adverse risk group was defined by the presence of one or more of: –5/del(5q), -7/del(7q), inv(3q), abn 11q, 20q,21q, del(9q), t(6;9), t(9;22) abn 17p and complex cariotype.

**Table 1:** Characteristics of Patients and Comparison between Unpurged and Purged Groups.

<table>
<thead>
<tr>
<th>All cases (31 pts)</th>
<th>Unpurged Group (15 pts)</th>
<th>Purged Group (16 pts)</th>
<th>Difference in purged and unpurged (Mann-Whitney test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvested TNC x 10^6/kg</td>
<td>3.9 (1.6-9.4)</td>
<td>3.3 (1.8-7.5)</td>
<td>4.3 (2.0-9.4)</td>
</tr>
<tr>
<td>Harvested CD34+ x 10^6/kg</td>
<td>3.8 (0.3-19.0)</td>
<td>2.3 (0.6-8.0)</td>
<td>4.1 (0.3-19.1)</td>
</tr>
<tr>
<td>Harvested CFU-GM x 10^3/kg</td>
<td>0.94 (0.4-11.2)</td>
<td>0.99 (0.4-11.2)</td>
<td>0.92 (0.5-5.5)</td>
</tr>
<tr>
<td>Infused TNC x 10^6/kg</td>
<td>1.0 (0.5-2.5)</td>
<td>1.1 (0.58-1.9)</td>
<td>0.9 (0.56-2.5)</td>
</tr>
<tr>
<td>Infused CD34+ x 10^6/kg</td>
<td>1.79 (0.4-8.5)</td>
<td>1.2 (0.4-3.7)</td>
<td>2.4 (0.4-8.5)</td>
</tr>
<tr>
<td>Infused CFU-GM x 10^3/kg</td>
<td>0.48 (0.0-4.75)</td>
<td>0.79 (0.2-4.7)</td>
<td>0.04 (0.0-0.8)</td>
</tr>
</tbody>
</table>

**Table 2:** Characteristics of Harvested and Infused Bone Marrow Cells and Comparison Between Unpurged and Purged Groups.

**Eradication regimen and supportive therapy**

High dose chemotherapy administered before autologous BMT was based on high dose Busulphan and Cyclophosphamide (BU-CY), [6]. G-CSF was administered to 7/31 patients, in 4/15 patients of the purged group and in 3/16 of the unpurged group.

**Cytogenetic analysis**

Patients were divided in 3 risk groups: favourable, intermediate and adverse. The favourable risk group included patients with t(8;21) (q22;q22) without a del(9q) and inv(16)(p13q22)/t(16;16)(p13q22). The intermediate risk group included patients with +8,-Y,+6,del/12p, normal cariotype and in addition to all cytogenetic aberration of unknown prognostic significance because of their low frequency in AML. The adverse risk group was defined by the presence of one or more of: –5/del(5q), -7/del(7q), inv(3q), abn 11q, 20q,21q, del(9q), t(6;9), t(9;22) abn 17p and complex cariotype.

**Data studied and statistical analysis**

Neutrophil (N) engraftment was defined as the first day in which N count reached at least the value of 0.5×10^9/l, PLT engraftment was defined as the first day of three consecutive days in which unsupported PLT count reached the value of 20.0×10^9/l. Differences between groups were assessed, for non-normally distributed continuous variables, using Mann-Whitney test, and for categorical variables using Chi test. Correlation was calculated using Spearman rank test. Since all data studied and statistical analysis were assessed, for non-normally distributed continuous variables, using Mann-Whitney test, and for categorical variables using Chi test. Correlation was calculated using Spearman rank test. Since all data studied and statistical analysis were assessed, for non-normally distributed continuous variables, using Mann-Whitney test, and for categorical variables using Chi test. Correlation was calculated using Spearman rank test. Since all data studied and statistical analysis were assessed, for non-normally distributed continuous variables, using Mann-Whitney test, and for categorical variables using Chi test. Correlation was calculated using Spearman rank test. Since all data studied and statistical analysis were assessed, for non-normally distributed continuous variables, using Mann-Whitney test, and for categorical variables using Chi test. Correlation was calculated using Spearman rank test. Since all data studied and statistical analysis were assessed, for non-normally distributed continuous variables, using Mann-Whitney test, and for categorical variables using Chi test. Correlation was calculated using Spearman rank test. Since all data studied and statistical analysis were assessed, for non-normally distributed continuous variables, using Mann-Whitney test, and for categorical variables using Chi test. Correlation was calculated using Spearman rank test. Since all data studied and statistical analysis were assessed, for non-normally distributed continuous variables, using Mann-Whitney test, and for categorical variables using Chi test. Correlation was calculated using Spearman rank test. Since all data studied and statistical analysis were assessed, for non-normally distributed continuous variables, using Mann-Whitney test, and for categorical variables using Chi test. Correlation was calculated using Spearman rank test. Since all data studied and statistical analysis were assessed, for non-normally distributed continuous variables, using Mann-Whitney test, and for categorical variables using Chi test. Correlation was calculated using Spearman rank test. Since all data studied and statistical analysis were assessed, for non-normally distributed continuous variables, using Mann-Whitney test, and for categorical variables using Chi test.

**Results**

**Characteristics of harvested and infused bone marrow cells**

Features of marrow Total Nucleated Cells (TNC) “harvested” and “infused” are reported in Table 2. Median recovery of TNC, after processing to deplete RBC, was 34% (range 12-69%). No correlation between harvested TNC and infused TNC was found (Rho=0.127; P=0.5), whereas there was a significant correlation between infused TNC and post-processing recovery (Rho=0.418; P=0.03). Due to the
The cytotoxic effect of maphosphamide, median number of infused CFU-GM in “purged” group was significantly lower than in unpurged group (Table 2), (P=0.001). In “Purged” and “unpurged” groups, all other features of “infused” bone marrow cells were comparable (Table 2).

**Engraftment**

All patients reached a Neutrophil count above 0.5×10^9/l in a median of 25 days (range 8-49). Time for Neutrophil engraftment was not different in purged group compared to unpurged patients; 24 days versus 25.5 days (log rank P=0.39) (Figure 1). A statistically significant correlation was found between infused TNC/Kg and day to N=0.5×10^9/l (P=0.002), (Table 3). Furthermore, patients receiving a TNC/Kg dose>median had an engraftment time for N longer than the group receiving a TNC/Kg dose<median; 30 days vs. 16 days, (P=0.02) (Figure 2). There was no significant correlation between time for N. engraftment and either infused CD34+ (P=0.76), infused CFU-GM (P=0.5) or harvested TNC (P=0.51).

All patients reached a PLT count of 20.0×10^9/l and median time for PLT engraftment was 60 days (range 17-270), which was significantly longer in purged transplants than unpurged ones; 85 days vs. 59 days (log rank P=0.041). No statistical correlation was found between times for PLT>20.0×10^9/l and number of infused CD34+ (P=0.75), infused TNC (P=0.7) and infused CFU-GM (P=0.74).

**Toxicity**

No cases of TRM were observed. Purged and unpurged groups did not show significant differences in the incidence of fever (80% vs. 75%, P=0.7). No difference was observed between purged and unpurged groups with regard to the requirement of RBC transfusions (P=0.8) and PLT-apheresis transfusions (P=0.4).

**Relapse and LFS**

In the patients taken as a whole, 16/31 patients relapsed. All relapses were haematological and were diagnosed between 4 and 14 months from transplantation. 15 patients were still in complete remission when mean follow-up for patients was still within response time of 40 months (range 9-96). The Kaplan-Meier estimate of LFS for the entire group of patients is 50.5 % (Figure 3). LFS was 58% in purged group and 40% in unpurged group (log rank: P=0.06) (Figure 4).

**Importance of Infused Tnc for Lfs**

Patients receiving an “Infused TNC<median” (median=1.0×10^8/kg) had a LFS of 65% while those with an “Infused TNC>median” showed a LFS of 28% (P=0.017) (Figure 5). These two groups did not differ in age (P=0.8), diagnosis-transplant interval (P=0.11), number of harvested TNC (P=0.54), FAB M5 frequency (P=0.08), use of purging (P=0.14) and percentage of patients requiring more than 1 cycle to reach CR (P=0.3). Recovery post processing of TNC in the group who received an infused TNC< median was significantly lower than those who received infused TNC>median; 28% vs. 46% (P=0.004).

**Table 3: Importance For Time For N > 0.5 X 10^9/L of The Following Variables: Infused Tnc, Harvested Tnc, Infused Cd34+ and Infused Cfu-Gm**

<table>
<thead>
<tr>
<th></th>
<th>INFUSED TNC</th>
<th>HARVESTED TNC</th>
<th>INFUSED CD34+</th>
<th>INFUSED CFU-GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL CASES</td>
<td>Spearman: P=0.002</td>
<td>Log rank: P=0.02</td>
<td>Spearman: P=0.51</td>
<td>Log rank: P=0.17</td>
</tr>
<tr>
<td></td>
<td>Regression analysis:</td>
<td></td>
<td>Spearman: P=0.76</td>
<td>Log rank: P=0.35</td>
</tr>
<tr>
<td></td>
<td>Exp (coef): 2.964</td>
<td>95% lower CI: 1.160</td>
<td>95% upper CI: 7.448</td>
<td>95% lower CI: 1.180</td>
</tr>
<tr>
<td></td>
<td>P=0.02</td>
<td></td>
<td>P=0.02</td>
<td></td>
</tr>
<tr>
<td>UNPURGED GROUP</td>
<td>Spearman: P=0.18</td>
<td>Logrank: P=0.2</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>PURGED GROUP</td>
<td>Spearman: P=0.001</td>
<td>Logrank: P=0.0001</td>
<td>Not evaluated</td>
<td>Not evaluated</td>
</tr>
</tbody>
</table>

Relationship between Time for N > 0.5 x 10^9/l and the considered variable was studied using: Spearman Rank correlation test; Log rank test after categorisation of the considered variable in two groups (< median and > median); Univariate regression analysis was also used to allow for evaluating the Infused TNC as a continuous variable in a time dependent analysis.
As opposed to what was found for an "infused TNC", patients with a number of "harvested TNC>median" (median=3.75×10^8/Kg) had a LFS not different in respect to patients with a TNC<median at harvest, 63% vs. 40% (P=0.20).

### Cytogenetic defined groups and LFS

A favourable cytogenetic abnormality was found at diagnosis in 35% of patients in which cytogenetic was available (7/20 cases), this group had a LFS of 100% (Figure 6).

Group of patients with intermediate prognosis cytogenetic abnormalities or normal cytogenetic represented 60% of patients in which cytogenetic was available, in this group LFS was 50% (Figure 6).

Bad prognosis cytogenetic was present in only one patient while cariotype at diagnosis was not available in 35.4% of patients and this subgroup had a LFS of 27% (Figure 6).

Distribution of cytogenetic subgroups was different, although not significantly, in the two strata of patients that received a TNC>median and TNC<median. In the group that received Infused TNC<median, good cytogenetic abnormalities were present at diagnosis in 46.1% (6/13) while in the group that received infused TNC>median, good prognosis cytogenetic patients were 14.2 % (1/7) (Chi square p=0.15).

Effect of purging (Figure 7) was studied in the strata of patients "not having at diagnosis good prognosis cytogenetic abnormalities" or in which "cytogenetic was not done".

In this strata, composed of large part of patients having intermediate cytogenetic, LFS was 53% in purged group (n=13) versus a LFS of 18% in unpurged marrow group (n=11), (P=0.05). All patients with good prognosis cytogenetic (n=7) were in continuous complete remission either after purged or unpurged marrow.

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**Figure 3:** Leukaemia Free Survival. LFS after ABMT in all cases is 50%.

**Figure 4:** Leukaemia Free Survival. LFS in purged patients and unpurged patients. LFS was 58% in purged group and 40% in unpurged group.

**Figure 5:** Leukaemia Free Survival. LFS in patients receiving TNC < median in respect to patients receiving TNC > median (p=0.017).

**Figure 6:** LFS in patients identified by cytogenetic abnormalities present at diagnosis: All patients harbouring good prognosis cytogenetic are alive and in CR, LFS is 50% in intermediate cytogenetic group and 29% in patients having cytogenetic not done at diagnosis.

**Figure 7:** Effect of Maphosphamide purging on LFS in cytogenetic defined subgroups. LFS is significantly shorter in patients "not harbouring at diagnosis Good cytogenetic alterations" and receiving unpurged marrow (p=0.05).
Discussion

After unpurged bone marrow transplantation in AML patients, myeloid and platelet engraftment were delayed [9,10,16-18]. Such lengthy marrow aplasia is a clinically significant relevant problem, resulting in high transfusion requirements and prolonged hospitalisation. It is also believed to contribute to a discrete rate of TRM. Using purged marrow, myeloid engraftment time may be further delayed, and a period of 28-45 days for N>0.5×10^9/l was reported in this context [7-10,16,19]. However comparison between purged and unpurged marrow obtained in a controlled study of AML patients was not available, while data using purged and unpurged ABMT generated in a single institution were scarce [12,13]. In our study, we observed that purging can prolong the period of PLT engraftment but not that of myeloid engraftment. Although the low number of patients limits the statistical power of our study, nevertheless no difference in myeloid engraftment time was also reported when comparing purged and unpurged marrow in acute leukaemia patients in two recently published large multi-centre studies [9,20]. The absence, of any evidence of a prolonged myeloid aplasia in purged bone marrow transplantation together with good clinical tolerability may further encourage the study of purging in acute leukaemia patients as a means of decreasing the relapse rate.

In leukaemia patients undergoing syngeneic Bone Marrow Transplantation, a high marrow cell dose has been shown to favourably affect Transplant Related Mortality (TRM) and relapse rate and hence increased the probability of this procedure being successful [21]. After autologous BMT in patients affected with AML, a low dose of Total Nucleated Cells (TNC) predict a high Transplant Related Mortality (TRM) [16,22], while a high CPU-GM content has been associated with improved Leukaemia Free Survival (LFS) [23]. Various authors have studied the role of infused marrow cell dose on engraftment after ABMT for AML. Demirer et al. [16] reported that dose of harvested TNC is important for engraftment as well as for TRM. Abdallah et al. found in patients undergoing purged ABMT [19] a significant correlation between infused TNC dose and time of engraftment. Other authors after unpurged transplant [18,24], did not found that marrow cell dose was an important factor for engraftment. These discrepancies regarding the role of marrow cell dose on engraftment could be explained by differences in status of the disease at transplant, the amount and type of chemotherapy previously administered [25], the techniques of bone marrow processing, and the use of TBI as eradication treatment [18] with respect to purging. In our study we observed that purging can prolong the period of PLT engraftment but not that of myeloid engraftment. Although the low number of patients limits the statistical power of our study, nevertheless no difference in myeloid engraftment time was also reported when comparing purged and unpurged marrow in acute leukaemia patients in two recently published large multi-centre studies [9,20]. The absence, of any evidence of a prolonged myeloid aplasia in purged bone marrow transplantation together with good clinical tolerability may further encourage the study of purging in acute leukaemia patients as a means of decreasing the relapse rate.

Besides these effects on the speed of engraftment, in our study, we observed that TNC dose is important for LFS. In fact the group of patients receiving a TNC dose below median had a better LFS. The more obvious explanation is that TNC dose and neoplastic contamination of inoculum are proportional, and thus an increase in TNC dose could be associated with a higher probability of neoplastic contamination and, in turn, will lower LFS. Conversely, a reduced TNC dose implies a reduction in neoplastic contamination of bone marrow inoculum and thus a reduction in relapse risk.

An alternative possible explanation for the high LFS of patients having a low infused TNC is that these patients have a higher sensitivity to chemotherapy both in leukaemia cells and in normal myeloid precursors. In fact, high sensitivity to chemotherapy could lead to a lower minimal residual disease and therefore to a better LFS while higher sensitivity of normal non-leukemic marrow cells lead to higher percentage of differentiated cells in marrow at CR, and hence to a lower recovery after cell processing and also a lower number of "infused TNC". An association between chemo-resistance of normal non-leukemic marrow cells in AML and of leukemic cells persisting in complete remission could, indeed, exist and this may be based on an "activated functional status of microenvironment cells" in some patients. This hypothesis has been put forward recently by our group [26] on the basis of experimental work in marrow cell of AML patients in complete remission. In these experiments, we found that a chemo-sensitivity of normal non-leukemic cells in complete remission is heterogeneous and that this heterogeneity correlated with LFS [27].

In this regard, it should be recalled that there has been a recent report on the existence of a group of AML patients having a low ability of CD34+ cells mobilization, low level of minimal residual disease in P.B., long length of aplasia after chemotherapy and good DFS [28,29]. The bone marrow features in this group of patients were not described. The group of patients with a low infused TNC and a good prognosis after ABMT may indeed belong to this recently identified group of AML patients.

In our study, clinical usefulness of purging is less evident in the stratum of "good risk cytogenetic"; these patients had excellent clinical results using unpurged marrow so that their low relapse risk was not further be lowered by purging. Excellent results, with OS ranging from 70% to 80% have been, indeed, reported in AML belonging to "good cytogenetic group" using unpurged ASCT [30-35]. However, after purging, we observed in the group of patients "not harbouring good prognosis cytogenetic abnormalities", a better LFS was present, compared to the group that received unpurged marrow.

Conclusion

It was observed that ABMT is associated with excellent results in "good prognosis cytogenetic". Purging may improve prognosis in patients belonging to "intermediate cytogenetic group". The number of "infused" TNC determines the rate of myeloid engraftment and also the rate of LFS i.e. the higher the number, the faster the engraftment but the poorer the LFS.

References

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