Lymphocyte recovery and infused CD34+ cells dose: Effect on the evolution after stem cell autotransplantation

Esperanza Romero Fernández *, Guillermo Montalbán Bravo, Rosario Arrieta Gallastegui, Ana Rodríguez De la Rúa Fernández

Hematology Department, University Hospital La Paz, Paseo de la Castellana 261, C. P. 28046 Madrid, Spain

Abstract

Background and objective: The number of infused CD34+ cells (CD34+i) has been associated with absolute lymphocyte count (ALC) and the outcome undergoing autologous hematopoeitic stem cell transplantation (HSCT) in patients with hematologic malignancies. The study's aim was to analyze the relationship between CD34+i, ALC and prognosis in this patients.

Patients and method: Medical records of 163 patients receiving HSCT between 2005 and 2012 were reviewed. An absolute lymphocyte count (ALC) and the number of days required to reach ALC > 500/μL according to the regression line: days = −0.981 × number of CD34+i + 18.09. We found significant and inversely proportional relationship between the CD34+i and the days required to reach ALC > 500/μL.

Conclusions: We have obtained a predictive model of lymphocyte recovery based on CD34+i dose: Effect on the evolution after stem cell autotransplantation.

Keywords: Immune reconstitution, Stem cell autotransplantation, Infused CD34+ cells, Lymphocyte recovery, Prognosis.

Introduction

Autologous hematopoietic stem cell transplantation (HSCT), consisting of high dose chemotherapy and subsequent infusion of hematopoietic stem cells (CD34+ cells) is a therapeutic procedure used in many hematologic malignancies.

Following HSCT a complex immune reconstitution (IR) is observed. When the infused CD34+ cells (CD34+i) begin to proliferate, an increase in peripheral blood cells is detected. Lymphocytes require more time than granulocytes in order to reestablish their normal function during the early post-transplant period [1].

An absolute lymphocyte count (ALC) > 500/μL at day 15 post-HSCT has been associated with a better prognosis in patients with hematologic malignancies [2–4]. Moreover, the number of CD34+i has been associated with ALC and clinical evolution. Hence, an ALC > 500/μL on day 15 has been proposed as an independent risk factor [3–6].

Our aim was to determine the influence of ALC, at day 15, in predicting post-HSCT outcome in patients with non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL) and multiple myeloma (MM) in terms of progression-free survival (PFS) and overall survival (OS), and to analyze the relationship between the number of CD34+i and the number of days required in order to reach ALC > 500/μL as well as clinical evolution.

Materials and methods

Patients and samples

Medical records of a total of 163 patients with hematologic malignancies receiving autologous-HSCT between January 2005 and March 2012 in our Hospital were reviewed. Patients with incomplete follow-up data and/or absent blood count at day 15 were excluded (n = 7).

Autologous CD34+i during HSCT were obtained after bone marrow mobilization through administration of granulocyte-colony stimulating factor (G-CSF) with or without concomitant chemotherapy. Collection of CD34+ cells were performed using a blood cell separator (CS-3000 Plus, Baxter®). Flow cytometry (FACSscan, Becton Dickinson®) and CD34+ count guides (ISHAGE) were followed.

A retrospective database was created, along with daily peripheral lymphocyte recoutes until an ALC > 500/μL was obtained. Total number of days for endpoint (ALC > 500/μL) along with ALC was noted.

Statistical methods

Patients were distributed into two groups depending on ALC (> or < 500/μL) at day 15 post-HSCT. Correlation between CD34+i and time to ALC > 500/μL was analyzed using Spearman’s correlation coefficient (r) and ANOVA test. OS and PFS were calculated according to the Kaplan–Meier curves and compared by log-rank
test. Multivariate analysis including age, sex, stage, HM, status, ALC and CD34+i/Kg was performed by Cox regression in order to determine Hazard ratio (HR) and Receiver Operating Characteristic curves (ROC curves).

OS and PFS were calculated from infusion day until death/end of follow-up, and disease relapse or progression respectively. Median follow-up of the study was 134 weeks (range: 69–294).

Statistical significance was established with a $p$ value < 0.05. SPSS (version 12/15) was used.

Results

Patient characteristics are detailed in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>n=163</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>95</td>
<td>58.3</td>
</tr>
<tr>
<td>Female</td>
<td>68</td>
<td>41.7</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>18-68</td>
<td></td>
</tr>
<tr>
<td>Hematologic malignancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHL</td>
<td>86</td>
<td>52.7</td>
</tr>
<tr>
<td>DLBCL</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>FL</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>NHL-T</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>MCL</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>45</td>
<td>27.6</td>
</tr>
<tr>
<td>IgG</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Light chain</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Non secretor</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>HL</td>
<td>32</td>
<td>19.7</td>
</tr>
<tr>
<td>NE</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Stage at diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>44</td>
<td>27</td>
</tr>
<tr>
<td>III</td>
<td>60</td>
<td>37</td>
</tr>
<tr>
<td>IV</td>
<td>59</td>
<td>36</td>
</tr>
<tr>
<td>Status disease pre-HCST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR1</td>
<td>79</td>
<td>48.4</td>
</tr>
<tr>
<td>CR2</td>
<td>32</td>
<td>19.6</td>
</tr>
<tr>
<td>PR</td>
<td>37</td>
<td>22.5</td>
</tr>
<tr>
<td>PROG</td>
<td>15</td>
<td>9.5</td>
</tr>
<tr>
<td>Conditioning, NHL/HL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEAC</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>BEAM</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Conditioning, MM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melphalan</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>CD34+i/Kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.99 $\times 10^6$/Kg</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.54–26.23</td>
<td></td>
</tr>
<tr>
<td>Days to ALC &gt; 500/μl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3–30</td>
<td></td>
</tr>
<tr>
<td>ALC (μl) at day 15 post-HCST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 500</td>
<td>97</td>
<td>59.5</td>
</tr>
<tr>
<td>&lt; 500</td>
<td>66</td>
<td>40.5</td>
</tr>
</tbody>
</table>

NHL indicates, non Hodgkin lymphoma; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MM, multiple myeloma; HL, Hodgkin lymphoma; NE, nodular sclerosis; MC, mixed cellularity; CR1, first complete remission; CR2, second complete remission; PR, partial remission; PROG, progression; BEAC, Carmustine plus Etoposide plus Citarabine and Melphalan; ALC, absolute lymphocyte count.

Patients with ALC > 500/μl, n=94 (59.5%) had a higher PFS and OS compared with those with ALC below the limit, n=64 (40.5%): PFS 67 months (CI95%: 60.5–72.5) vs. 23 months (CI95%: 15.1–30.8), $p<0.001$ and OS of 283.5 weeks (CI95%: 262.7–304.4) vs. 211.8 weeks (CI95%: 171.3–252.3), $p<0.001$ (Fig. 1). We found a significant effect of disease state in OS, with a lower OS in patients with partial remission (PR) pre-HCST compared to first complete remission (CR1) with HR=4.28 (IC=1.39–13.2), $p=0.011$ in patients with NHL, HL and MM.

Median CD34+i was 2.99 $\times 10^6$/Kg (range: 0.54–26.23) and the median of days required for an ALC$\geq$500/μl was 14 (range: 3–30). We found a significant and inversely proportional relationship between the number of CD34+i and the days required to reach ALC$\geq$500/μl in patients with NHL ($r$=-0.625; $p<0.001$), HL ($r$=-0.801; $p<0.001$) and MM ($r$=-0.662; $p<0.001$) according to regression line (Fig. 2): $y=-0.981 \times$ number of CD34+i+18.09.

Moreover, CD34+i$\geq$2.0 $\times 10^6$/Kg directly and significantly related to an ALC$>500/μl$ on day 15: RR=7.77; $p<0.001$ (IC95%:5.55–90.25) and post-HCST survival so that ALC$>500/μl$, n=97(59.5%) was associated with better PFS and OS, compared to patients with lower ALC, n=66 (40.5%): PFS 67 months (CI95%: 62–73) vs. 23 months (CI95%: 15–31) $p<0.001$ and OS 82 months (CI95%: 78–85) vs. 55 months (CI95%: 44–65) $p<0.001$.

Univariate analysis showed a statistically significant association between ALC at day 15 and prognosis, with higher OS and PFS in patients attaining ALC $\geq$500/μl directly and significantly related to an ALC$>500/μl$ on day 15: RR=7.77; $p<0.001$ (IC95%:5.55–90.25) and post-HCST survival so that ALC$>500/μl$, n=97(59.5%) was associated with better PFS and OS, compared to patients with lower ALC, n=66 (40.5%): PFS 67 months (CI95%: 62–73) vs. 23 months (CI95%: 15–31) $p<0.001$ and OS 82 months (CI95%: 78–85) vs. 55 months (CI95%: 44–65) $p<0.001$.

Univariate analysis showed a statistically significant association between ALC at day 15 and prognosis, with higher OS and PFS in patients attaining ALC $>500/μl$. On the other hand, patients with CD34+i$\geq$2.0 $\times 10^6$/Kg showed a superior outcome, independent of the lymphocyte count and a protective effect on progression with an increase in PFS but no statistically significant relationship between CD34+i and OS. As expected, previous disease state

![Fig. 1](image-url)
seemed to have prognostic influence, with patients experiencing disease progression presenting with a worse OS and PFS than those in CR1 pre-HSCT. No significant differences on survival between patients in CR2 and those in PR were observed. In our experience, no significant correlation was found between the number of lines of therapy pre-HSCT and CD34 cell counts or lymphocyte recovery.

Multivariate analysis for ALC, CD34+i, disease state, age and sex showed that not reaching an ALC > 500 at day 15 post-HSCT was an adverse factor for PFS with HR = 7.72 (CI95%: 4.05–14.7), \(p < 0.001\) and for OS with HR = 9.7 (CI95%: 3.1–30.34), \(p < 0.001\). In this series, we additionally observed that infusing \(\geq 4.38 \text{ CD34}^+ \times 10^9/\text{Kg}\) was associated with better survival in all groups, \(p = 0.015\) and HR = 0.27 (CI95%: 0.094–0.774). Additionally, disease progression was found to be a statistically significant risk factor when compared to patients on CR1 pre-HSCT with HR = 4.28 (CI95%: 1.39–13.2), \(p = 0.011\) but no significant differences were detected between patients with PR or CR2 with respect to those achieving CR1 with HR = 1.59 (CI95%: 0.53–4.75), \(p = 0.400\) and HR = 1.03 (CI95%: 0.30–3.52), \(p = 0.100\), respectively. No statistically significant differences could be found between age or sex and ALC at day 15 with HR = 1.01 (CI95%: 0.99–1.04), \(p = 0.200\) and HR = 1.76 (CI95%: 0.76–4.05), \(p = 0.180\), respectively.

**Discussion**

In accordance with the results, we have been able to determine the existence of several factors with an impact on outcome after autologous HSCT. Additional variables have been reported [5–7] but detailed description of every single prognostic factor apparently involved in post-transplant evolution was beyond the scope of this study.

In accord with other authors [3–7], patients in our study with localized disease at diagnosis showed better outcome compared to those whose disease was in an advanced stage. Furthermore, patients in CR1 or CR2 pre-HSCT showed better survival rates than those who presented with disease progression. Nevertheless, in contrast with other authors [6,7], there did not seem to be any significant difference in prognosis between patients in PR and those who achieved CR2 pre-HSCT.

Porrata et al. were the first to describe prognostic significance of early ALC in patients with HM undergoing HSCT [2–5] and we confirmed that. Some authors have attributed this improvement in survival to a decrease in infection susceptibility in the subgroup of patients who managed to attain an early lymphocytic reconstitution (ELR) after HSCT [1,2]. Even though we did not specifically analyze infection risk in our patients, we proposed that such a relation can be responsible for this observation.

Developing a means to predict ELR (to express as ALC) after HSCT could be of great use during transplantation. In order to try to prove this point, we obtained a predictive model capable of estimating total days for ELR according to CD34+i at day 0. Late ALC (after day 15) can probably be associated with a greater incidence of infections due to impaired immunity and, hence, a worse prognosis [6–8]. Consequently, pre-transplant knowledge of lymphocyte reconstitution dynamics should be able to define which subgroup of patients could benefit from early antibiotic therapy, and which optimal CD34+i count should be reached in order to guaranty ELR. In this sense, we propose early ALC as a predictive prognostic marker. Additionally, we found a positive correlation between CD34+i and dynamics of lymphocyte recovery, especially in patients with HL.

We have obtained a regression line as a predictive model of lymphocyte recovery, not previously described, based on CD34+i and it could be used as a prognosis tool in this patients. Nonetheless, future prospective studies analyzing lymphocyte subpopulations are needed to achieve a better understanding of IR. There are still many questions that remained unanswered.

**Role of the funding source**

The work was not supported for any funding source.

**Authors’ contributions**

Contribution: E.R.F and R.A.G designed and performed research, analyzed and interpreted data. E.R.F wrote the paper and performed statistical analysis. G.M.B wrote and oversaw the translation into English. A.R.R.F made a general supervision of the paper.

**Acknowledgments**

The authors thank the participating of Elia Rodríguez, Irina Kikinadze and Andrew Doyle.

**References**


[5] Porrata LF, Inwards DJ, Ansell SM, Micallef IN, Johnston PB. Early lymphocyte recovery predicts superior survival after autologous stem cell transplantation in

