Leukemia Research 38 (2014) 551–556

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Role of minimal residual disease and chimerism after reduced-intensity and myeloablative allo-transplantation in acute myeloid leukemia and high-risk myelodysplastic syndrome

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ARTICLE INFO

Article history:
Received 10 August 2013
Received in revised form 21 January 2014
Accepted 1 February 2014
Available online 10 February 2014

Keywords:
Minimal residual disease
Chimerism
Allogeneic stem cell transplantation
Acute myeloid leukemia
Myelodysplastic syndrome
Relapse

ABSTRACT

We evaluated the impact of detection of minimal residual disease by flow cytometry (FCMRD) and CD3 chimerism in relapse in a cohort of 87 patients with acute myeloid leukemia or myelodysplastic syndrome undergoing stem cell transplantation. Patients with a positive FCMRD at day +100 after transplantation showed higher relapse rates and worse overall survival. In multivariate analysis, a positive FCMRD after transplantation was a significant predictor of relapse. Mixed chimerism showed a trend to statistical signification. We conclude that FCMRD at day 100 after SCT is the best predictor of relapse after SCT in patients with aggressive myeloid malignancies.

1. Introduction

Allogeneic stem cell transplantation is the only curative therapeutic approach in myelodysplastic syndromes (MDS) and high-risk acute myeloid leukemia (AML) [1]. The advances in supportive care during the post-transplant period have decreased procedure-related mortality, leaving relapse as the main cause of transplantation failure. Some factors related to relapse, such as cytogenetics [2] or molecular abnormalities [3], have been identified. Additionally, the intensity of the conditioning regimen may also influence relapse risk [4]. Recent studies have highlighted the negative impact of a positive minimal residual disease in flow cytometry assays (FCMRD) before conditioning [5,6] and during the early post-transplant period [7,8] to predict relapse, suggesting that those cases with positive FCMRD could benefit from additional therapeutic interventions.

Another factor that has been related to relapse is chimerism. Monitoring of chimerism during the post-transplant period is critical in the context of reduced-intensity conditioning regimens or cord blood transplantation [9]. Whereas chimerism in whole bone marrow or CD34 populations is indicative of persistence of recipient hematopoiesis, chimerism analysis of the CD3 compartment may reflect the immunological relationship between host and graft as it is associated with different probabilities of acute graft versus host disease (GVHD), rejection or relapse [10]. For instance, complete CD3 chimerism is related to acute GVHD [11] and mixed CD3 chimerism is related with relapse in acute myeloid leukemia [12].

In this study, we have attempted to evaluate the impact of both CD3 chimerism and FCMRD in relapse in a population of patients with MDS/AML who received an allogeneic stem cell transplantation. The analysis has been restricted to patients who reach complete remission at day +100 post-transplant to exclude early relapses or deaths, in which therapeutic interventions are limited due to the high risk of acute GVHD.

2. Patients and methods

Local ethic committees approved this observational study, and all patients signed informed consent prior to transplantation.

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http://dx.doi.org/10.1016/j.leukres.2014.02.001
0145-2126/

2.1. Patients

From 2005 to 2011, 124 patients with an AML or MDS diagnosis according to 2008 WHO criteria [13] received an allogeneic stem cell transplantation from related or unrelated donors in one of the two participant hospitals (Hospital Universitario de Salamanca, Hospital Universitario Central de Asturias, Spain). Demographical and clinical data from these patients, including disease-related variables, transplantation procedure and follow up were collected and analyzed. Treatment response was defined according to International working groups [14,15]. Cytogenetic risk was classified following the United Kingdom Medical Research Council [16]. Patients with morphological relapse at day +100 post-transplant were excluded from the analysis.

Reduced-intensity conditioning was defined according EBMT criteria [17] and included fludarabine plus busulphan (8–10 mg/kg orally or the equivalent intravenous formulation). Different schemes of GVHD prophylaxis were used, including cyclosporine plus methotrexate (83 patients) or mycophenolate mophetil (8 patients), tacrolimus plus rapamicyn (20 patients), cyclosporine alone (6 patients), mycophenolate mophetil alone (5 patients) or antithymocyte globulin alone (1 patient). Data from one patient was not available. Among the 91 patients receiving a cyclosporine-based combination regimen, 16 received antithymocyte globulin as a third immunosuppressor. Diagnosis and clinical staging of acute and chronic GVHD and relapse were performed according established criteria [14,18–20].

2.2. Chimerism analysis

Whole blood was fractionated using a Ficoll gradient. CD3-positive cells were separated using an immunomagnetic procedure (CD3 MicroBeads, Miltenyi Biotec). Presence of chimerism was determined in DNA obtained in granulocyte and CD3 populations by analysis of polymorphic short tandem repeat markers (Powerplex 16, Promega). Percentage of donor chimerism was computed according to Thiede et al. [21] and classified as mixed (5–94% recipient cells) or full chimerism (more than 95% donor cells). Samples were analyzed at day +100 post-transplant, then every 3 months or when clinically indicated because of relapse or graft failure.

2.3. Minimal residual disease monitoring by flow cytometry

Flow cytometry analysis was performed on bone marrow aspirates obtained at diagnosis, before conditioning and on day +100 of follow up. Erythrocyte-lysed whole BM samples were studied using quadruple staining with different fluorochrome-conjugated (FITC, PE, PerCP or PE-Cy5, APC) antibody combinations, as previously described [22]. Briefly, at least 50,000 cells were acquired at diagnosis using a FACSCalibur or a FACSCanto-II flow cytometer (Becton Dickinson Biosciences, San Jose, CA). Using a six-dimensional space formed by the two light scatter parameters – forward (FSC) and sideward light scatter (SSC) – and the four antibody-associated fluorescence emissions, antigenic expression on neoplastic cells was systematically analyzed at diagnosis to identify phenotypic aberrancies that could be used to define patient specific panels for detection of residual neoplastic cells in the BM once patients achieved morphological complete remission. In order to evaluate minimal residual disease, at least 500,000 cells were studied in each tube. Abnormal patterns of antigen expression on specific cell lineages at different stages of maturation were also considered neoplastic disease, as defined by Walter et al. [5]. In our hands, the sensitivity of this technique is at least 10^{-4}. Detection of disease at any level was considered positive. The Paint-a-Gate Pro (Becton Dickinson Biosciences, San Jose, CA) and Infinicyt (Cytognos, Santa Marta, Salamanca) software programs were used for further data analysis.

2.4. Statistical analysis

Data are presented as mean ± standard deviation or as median (range). Univariate comparisons were done using the chi-square test (categorical variables), Fisher's exact test (for contingency tables with frequencies below 5) or *T*-test (for continuous variables). Variables with a *p* value in the comparison lower than 0.1 were introduced in logistic regression models. This multivariate analysis was performed using a backward elimination technique, and odds ratios (OR) and their 95% confidence intervals were computed. Goodness-of-fit was measured using the Hosmer–Lemeshow statistic, and was good in all the cases ($p > 0.05$). In the multivariate analysis, a *p* value lower than 0.05 was considered significant.

Events were analyzed from day +100 after transplantation. For event free survival, the event analyzed was relapse or death, whatever occurred first. Overall survival was calculated from transplantation until death from any cause. Transplantation-related mortality was defined as death due to causes unrelated to the underlying disease. Cumulative incidence of relapse was analyzed using the Nelson–Aalen estimator and the general bootstrap algorithm for curve comparisons [23]. Overall and relapse-free survival were analyzed using the Kaplan–Meier method and the log-rank test. Statistical analysis was performed using SPSS (v 20.0) software (SPSS Inc. Chicago, IL).

Table 1
General characteristics of the study population.

Number of study patients (%)	87 (100)
Median age, y (range)	54 (17–69)
Median follow up after transplantation, months (range)	26 (4–146)
Gender [male (%)/female (%)]	50 (57)/37 (43)
Primary diagnosis, number (%)	
Acute myeloid leukemia	49 (56)
Myelodysplastic syndrome	38 (44)
Status at transplantation (%)	
1st and 2nd complete remission	68 (78)
Partial remission	6 (7)
Relapse	1 (1)
Refractory	4 (5)
Up-front	8 (9)
HLA (%)	
Matched related	65 (75)
Matched unrelated	18 (20)
Mismatched unrelated	4 (5)
Source (%)	
Peripheral blood	81 (94)
Bone marrow	3 (3)
Umbilical cord blood	3 (3)
Conditioning (%)	
Myeloablative	14 (16)
Reduced intensity	73 (84)
CD34 dose ($\times 10^6$/kg)	5.3 ± 2.1

3. Results

3.1. Patients

A total of 124 consecutive patients were studied. 37 patients were excluded from the analysis due to early relapse (14 patients), death (4 patients) or incomplete data (19 patients). The remaining 87 patients were included in the study. Cytogenetics at diagnosis was available in 73 of 87 patients and was classified into intermediate group in 56 patients (76%) favorable in 3 patients (4%) and adverse in 14 (19%). Characteristics of the study sample are detailed in Table 1.

Fourteen patients received myeloablative conditioning, whereas 73 received a reduced intensity regimen. As expected, patients receiving myeloablative conditioning were significantly younger than those in the reduced-intensity conditioning group. There were no differences between myeloablative and reduced-intensity conditioning groups regarding status of disease at transplantation, HLA matching or infused CD34 cells. Eleven patients (79%) in the myeloablative conditioning group and 2 (3%) in the reduced-intensity conditioning group received prophylaxis with antithymocyte globulin ($p = 0.06$). Acute and chronic GVHD were less frequent after myeloablative conditioning. All these data are shown in Table 2.

3.2. CD3 chimerism

Univariate analysis of factors associated with CD3 chimerism at day +100 is detailed in Table 3. In univariate comparisons, patients with mixed CD3 chimerism were older ($p = 0.04$), more frequently male (OR 2.2 [0.9–5.4], $p = 0.07$) and there was an increased proportion of donor–recipient sex mismatch (OR 2.3 [9.9–5.8], $p = 0.07$). Presence of acute GVHD was related to a lower rate of mixed chimerism (OR 0.4 [0.1–0.9]). In multivariate analysis, variables related to mixed chimerism were acute GVHD, older age, male gender and donor–recipient sex mismatch (Table 4).

3.3. Minimal residual disease

Factors associated to positive FCMRD at day +100 post-transplant were positive FCMRD before conditioning (OR 14.3