Leukemia Research 38 (2014) 334–339

Contents lists available at ScienceDirect

Leukemia Research

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

The prevalence and prognostic value of concomitant eosinophilia in chronic graft-versus-host disease after allogeneic stem cell transplantation

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ARTICLE INFO

Article history:
Received 19 September 2013
Received in revised form
21 November 2013
Accepted 7 December 2013
Available online 24 December 2013

Keywords:
Allogeneic stem cell transplantation
Chronic graft-versus-host disease
Eosinophilia
Glucocorticoids
Myeloablative
Clinical outcome

ABSTRACT

The prognostic significance of eosinophilia after myeloablative allogeneic stem cell transplantation (ASCT) remains to be established. Patients, whom developed chronic graft-versus-host disease (cGVHD) after ASCT, were included ($n = 142$). Eosinophil count was analyzed at cGVHD onset. We observed no significant association between EO and the grade of cGVHD, thrombocytopenia, nor extensive skin involvement. Importantly, we observed no significant association between cGVHD with concomitant eosinophilia and long-term clinical outcomes, and subgroup analyses revealed a considerable confounding effect of ongoing steroid treatment. In conclusion, we advocate that prognostic conclusions regarding cGVHD with concomitant eosinophilia after ASCT should be interpreted with caution.

1. Introduction

Chronic graft-versus-host disease (cGVHD) is a common and often severe condition affecting long-term survivors of allogeneic stem cell transplantation (ASCT). Although the condition has been subjected to extensive research, the pathogenesis behind the syndrome remains to be fully established. cGVHD is caused by alloreactive T cells transferred into the recipient along with hematopoietic stem cells and autoreactive T cells developed after ASCT [1]. New theories suggest that impaired thymic function with defective immune reconstitution may contribute to cGVHD [2,3] by a defective peripheral immune regulation, cytokine dysregulation [4,5] and a succeeding imbalance in the so-called T helper cell 1/2-cytokine secretion pathways [1,6–8]. In this context, cGVHD has been associated with an activation of the T helper cell 2 (Th2) pathway where certain Th2 cytokines, e.g. interleukin (IL)-5, also act as potent eosinophilopoietic factors [7]. Hence, it has been hypothesized that eosinophilia ($\geq 0.5 \times 10^9$/L, EO), which may be present at the time of cGVHD diagnosis, can act as a surrogate marker of Th2 pathway-activation, which again has been proposed to be associated with a more favorable prognosis among cGVHD patients [9].

The eosinophilic granulocyte differentiates from myeloid precursor cells in the bone marrow. The development is controlled by *globin transcription factor 1*, while proliferation and differentiation are controlled by the cytokines IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor, which are all produced by Th2 lymphocytes [10]. In healthy adults the level of eosinophils in peripheral blood does not exceed 0.5×10^9/L [11]. Several studies have focused on the prognostic impact of EO on transplant outcome [9,12–17], however only two studies have analyzed patients with cGVHD and *concomitant* EO [9,12]. In a retrospective analysis of both myeloablative and non-myeloablative ASCT recipients with a median follow-up of 31 months, Kim et al. observed significantly improved overall survival (OS) and lowered non-relapse mortality (NRM) in patients exhibiting peripheral blood EO at the time of cGVHD debut [9]. Ahmad et al., having performed a similar study on non-myeloablative ASCT recipients with a median follow-up of 58 months, observed that EO was associated with a high positive predictive value for cGVHD and that thrombocytopenia (TP) at cGVHD diagnosis was associated with EO. Ahmad et al. could not demonstrate any significant associations between cGVHD and

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http://dx.doi.org/10.1016/j.leukres.2013.12.009

EO regarding OS and NRM [12]. Accordingly, the prognostic significance of EO after ASCT and the relationship between cGVHD and concomitant EO remain to be established.

The primary objective of this study was to identify pre-transplant factors associated with development of cGVHD with concomitant EO. Secondly, we aimed to determine the prevalence and clinical impact (OS, NRM and relapse incidence) of cGVHD with concomitant EO in a large population of patients who have undergone myeloablative ASCT with a long follow-up.

2. Patients and methods

2.1. Patients

From January 1, 1999, to December 31, 2009, a total of 372 adult patients (>15 years) with hematological disease received myeloablative ASCT at the Blood and Marrow Transplant Clinic in Copenhagen, Denmark. Patients who subsequently received a second transplant or additional donor leukocyte infusion ($n = 88$) were excluded from this study. Patients who did not develop cGVHD ($n = 133$) and patients who did not have eosinophils measured within 7 days before cGVHD onset ($n = 9$) were excluded as well. Ultimately, 142 patients were included in the analysis all of whom developed cGVHD after having received unmanipulated grafts.

2.2. Definitions

Engraftment was defined as consecutive achievement of sustaining absolute neutrophil counts above 0.5×10^9/L for 2 days and peripheral platelet counts exceeded 20×10^9/L for at least 3 consecutive days without transfusion. Acute graft-versus-host disease (aGVHD) was defined as characteristic symptoms appearing before day +100 post-ASCT, diagnosis and grading were performed according to the revised Glucksberg criteria [18]. Disease risk was defined according to the EBMT risk score and employed retrospectively [19]. 'Donor–recipient sex combination' grouping was performed according to the donor T cell contribution to GVHD [20]. cGVHD, defined as characteristic symptoms appearing before or after day +100, was diagnosed and graded according to the Seattle criteria which separate the condition into a 'limited' and 'extensive' degree [21]. The revised Seattle criteria were employed retrospectively [1]. Confirmation of diagnosis by biopsy was obtained only when found relevant for treatment.

In order to assess the severity of cGVHD, three risk factors for cGVHD survival [22,23] were assessed at time of cGVHD diagnosis: progressive type onset (PTO) (aGVHD progressing directly to cGVHD), extensive skin involvement (ESI) (>50% of body surface area), and thrombocytopenia (TP) ($<100 \times 10^9$/L). The modes of cGVHD presentation were characterized according to the time relation to aGVHD as: (i) 'overlap' (features of both cGVHD and aGVHD present simultaneously), (ii) 'quiescent' (cGVHD occurring after complete resolution of aGVHD), and (iii) 'de novo' (cGVHD appearance without preceding aGVHD).

EO was defined as an absolute eosinophil count of $\geq 0.5 \times 10^9$/L in peripheral blood and analyzed at the time of cGVHD diagnosis. In case of missing eosinophil values at the date of cGVHD onset, we used the eosinophil value from the nearest date within 7 days prior to cGVHD onset. If no such value was available, patients were excluded from further analyses ($n = 9$). If multiple eosinophil analyses were available at the date of cGVHD onset, an average of these was used.

2.3. Transplant procedure

The transplantation procedures and post-transplantation management were performed in accordance with international directives [24]. Conditioning regimens predominantly consisted of high dosage cyclophosphamide (total dose 120 mg/kg) or etoposide (total dose 60 mg/kg) combined with either total body irradiation (12 Gy) or busulfan (total dose 16 mg/kg). Cyclophosphamide, given unaccompanied or with low dosage total body irradiation (2 Gy), were the standard treatment for severe aplastic anemia. In general, mismatched transplant recipients received additional immunosuppressive treatment with anti-thymocyte globulin (total dose 7.5 mg/kg). Post-transplant granulocyte colony-stimulating factor and immunoglobulin were not routinely administered, unless rendered necessary in the presence of neutropenia or hypogammaglobulinemia.

2.4. GVHD prophylaxis

The vast majority (98%) of the cohort received GVHD prophylactic regimens consisting of cyclosporin (CYA), either administered alone (4%) or with methotrexate (94%). A minority received tacrolimus combined with methotrexate (2%). Three months after transplantation, without regard to donor–recipient relation, CYA taper was initiated, unless ongoing GVHD necessitated continued immunosuppressive treatment. According to standard practice, CYA tapering was to be discontinued six months after transplantation.

2.5. Management of GVHD

First-line treatment for aGVHD was i.v. methylprednisolone 2 mg/kg/day in combination with a calcineurin inhibitor. Steroid taper was initiated after 7–14 days if a response to therapy was obtained. Second line treatment of steroid refractory aGVHD involved infliximab, mycophenolate mofetil, or sirolimus. Extensive cGVHD occurring after immunosuppressive taper was treated with prednisone 1 mg/kg/day for at least two weeks, whereupon dosage was decreased in accordance with treatment response. Patients developing cGVHD in spite of ongoing CYA treatment were started on glucocorticoid treatment in addition to the immunosuppressive treatment. Second line treatment of steroid refractory cGVHD included tacrolimus, mycophenolate mofetil, sirolimus, rituximab, or extracorporeal photopheresis.

2.6. Statistics

Patient characteristics of EO-positive (EO+) and EO-negative (EO−) patients were compared using chi-square tests, apart from 'CD34+/kg in graft', which was displayed by medians and compared with a Wilcoxon rank test. Time of cGVHD onset was the time origin for all event time analyses. The following outcomes were analyzed; time to death from any cause (OS), time to relapse-unrelated death (NRM), and time to relapse or relapse-related death (relapse). OS was analyzed with the Kaplan–Meier method, log-rank test, and Cox regression. Relapse and NRM were analyzed in a competing risks frameworks using the Aalen–Johansen method, log-rank test, cause-specific Cox regression for effects on the cause-specific hazard scale, and finally Gray test and Fine-Gray regression for effects on the cumulative incidence scale. In order to adjust for confounding the regression models included the EBMT risk score, in which patients belonged to one of three groups: 0–2, 3–4, 5–6 according to pre-transplant risk, PTO and 'ongoing steroid treatment'. For all endpoints, patients were right-censored at the end of follow-up if they were event-free and alive. Sensitivity analyses were performed in which patients with ongoing steroid treatment at the date of cGVHD onset were excluded. All analyses were performed with "R" software packages (R Core Team, 2012, R Foundation for