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Cytogenetic Evolution in Myeloid Neoplasms at Relapse after Allogeneic Hematopoietic Cell Transplantation: Association with Previous Chemotherapy and Effect on Survival

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A B S T R A C T

Cytogenetic evolution (CGE) in patients with myeloid neoplasms who relapsed after an allogeneic (allo) hematopoietic cell transplantation (HCT) has been evaluated by only few studies. The effect of the CGE on survival of relapsed allo-HCT recipients is not clear. The effect of previously received chemotherapy to induce CGE in this patient population has not been studied. The aims of our study are to (1) characterize the patterns of cytogenetic change in patients with myeloid neoplasms who relapsed after an allo-HCT, (2) evaluate the effect of CGE on survival, and (3) explore the association of CGE with previous chemotherapy (including the lines of salvage therapy, type of induction, and conditioning therapy). Of 49 patients with a myeloid malignancy (27 acute myeloid leukemia [AML], 19 myelodysplastic syndrome [MDS]/myeloproliferative neoplasm [MPN], and 3 chronic myelogenous leukemia) who relapsed after an allo-HCT, CGE was observed in 25 (51%), whereas 24 patients had unchanged cytogenetic findings at relapse. The CGE group carried more cytogenetic abnormalities at original diagnosis. The most frequent cytogenetic change was the acquisition of 3 or more new chromosomal abnormalities followed by acquisition of unbalanced abnormalities, aneuploidy, and emergence of apparently new clones unrelated to the original clone. The CGE cohort had higher proportion of MDS and MPN and fewer patients with de novo AML. Disease risk assessment category showed a trend to higher frequency of high-risk patients in the CGE group, though the difference was not statistically significant. Time from diagnosis to transplantation and time from transplantation to relapse were not different between the CGE and non-CGE groups. CGE and non-CGE cohorts had similar exposures to salvage therapy and to induction chemotherapy, as well as similar conditioning regimens; thus, no particular type of chemotherapy emerged as a predisposing factor to CGE. CGE was associated with significantly shortened post-transplantation and postrelapse survival when compared with those of the non-CGE group ($P = .004$ and $P < .001$, respectively). Our results underscore the significance of CGE in progression of myeloid malignancies after an allo-HCT.

INTRODUCTION

Cytogenetic characteristics are important prognostic factors in myeloid neoplasms. Disease progression and relapse are commonly accompanied by cytogenetic changes that contribute to the development of a chemotherapy-resistant subclone or emergence of a new clone [1]. Allogeneic (allo) hematopoietic cell transplantation (HCT) has improved the outcome in myeloid neoplasms. However, approximately one-half of the patients experience a relapse [2]. Although cytogenetic evolution (CGE) is well studied in chronic myelogenous leukemia (CML) and acute myeloid leukemia (AML), much less is known about CGE after allo-HCT. Previous studies suggest a higher frequency of clonal evolution in stem cell recipients than in patients treated with conventional chemotherapy [3]. The etiology of this is not entirely clear. Possible contributing factors include the higher dose of mutagenic chemotherapy received in stem cell recipients, the

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genetic instability of the patients selected for allo-HCT, and the presence of an altered bone marrow environment. At present, no studies exist to evaluate the effect of previously received chemotherapy on CGE in the allo-HCT patient population. The effect of the CGE on survival of the stem cell recipients is not clear, as previous limited studies reached no clear conclusions [4,5].

The aims of our study are to characterize cytogenetic changes in patients with myeloid neoplasms who relapsed after allo-HCT and to evaluate the effect of CGE on survival in this patient group. In addition, we evaluate the association between the CGE and the consecutive lines of pretransplantation chemotherapy, including the induction chemotherapy, salvage regimens, and the type of conditioning chemotherapy received.

MATERIALS AND METHODS

Patients

This retrospective case control study was approved by the institutional review board at Mayo Clinic Arizona. All patients with AML, CML, myelodysplastic syndrome (MDS), or myeloproliferative neoplasm (MPN) who had a first allo-HCT at the Mayo Clinic Arizona between March 2004 and January 2015 and who were at least 18 year of age at the time of transplantation were included. The study included only patients who had results of karyotype analysis at diagnosis and at relapse after HCT available. Information about demographic variables, disease and transplantation characteristics, and data of previously received therapy was available from the clinical research database. Disease risk was assigned using a disease risk index for patients undergoing allo-HCT [6]. The disease risk index is a system for risk stratification of heterogenous populations of HCT patients that uses a combination or ternary breakdown for disease type and a binary breakdown for remission status to assign patients into 1 of 4 risk categories that differ statistically and clinically with respect of overall survival and progression-free survival.

Morphologic Evaluation

The classification of the myeloid neoplasms followed the 2008 World Health Organization (WHO) classification of myeloid malignancies [7]. Scheduled bone marrow biopsies were performed at 3, 6, 9, and 12 months after HCT. After 12 months, bone marrow evaluations were performed "as clinically indicated." The definition of relapse for AML was defined as the presence of >5% blasts in the bone marrow. Relapse for CML, MPN, and MDS was defined as recurrent morphological abnormalities compatible with original diagnosis and recurrence of cytogenetic abnormalities if present at diagnosis.

Cytogenetic Analysis

Giemsa-banded (G-banded) chromosome analysis was performed on bone marrow samples according to conventional methods. When available, at least 20 metaphases were analyzed. Karyotypes of G-banded chromosomes were described according to the 2009 International System of Human Cytogenetic Nomenclature [8]. *Abnormal clones* were defined as 2 or more cells with the same structural abnormality, or the same extra chromosomes, or the presence of 3 or more cells with loss of the same chromosome. Fluorescence in situ hybridization (FISH) procedures were performed on cell suspensions prepared from fresh bone marrow aspirate pellets using standard FISH techniques. FISH was performed by codenaturation on a HYBrite instrument (Vysis/Abbott, Abbott Park, IL) at a denaturation temperature of 72°C for 2 minutes for freshly dropped cells, followed by overnight hybridization at 37°C. At least 100 nuclei were examined for each probe whenever possible. Images were captured on a Leica DM5000B microscope (Leica Microsystems, Buffalo Grove, IL).

Evaluation of Cytogenetic Change

Cytogenetic findings were categorized following the 2008 WHO classification of myeloid malignancies. Patterns of cytogenetic change were categorized as (1) *CGE*, defined as acquisition of new cytogenetic alterations; (2) unchanged cytogenetic results; or (3) *clonal cytogenetic regression*, defined as loss of previously detectable cytogenetic alterations. Additional cytogenetic changes were characterized as balanced rearrangements, unbalanced aberrations, emergence of a new clone, or *complex alterations*, defined as the acquisition of at least 3 new chromosomal alterations.

Molecular Studies

Molecular evaluation was performed at original diagnosis following the guidelines of WHO Classification of Myeloid Neoplasms and included NPM1, FLT3 internal tandem duplication (ITD), and FLT3 D835 mutation in patients with normal karyotype acute myeloid leukemia, JAK2 V617F mutation in patients with MPN, KIT mutation in AML with t(8;21), and BCR ABL p190 and p210 in CML. FLT3 ITD and FLT3 D835 and NPM1 mutation analysis was performed by DNA PCR by Roche Molecular Systems (Pleasanton, CA), with fragment analysis by capillary gel electrophoresis. KIT mutation was performed by Sanger sequencing of DNA. JAK2 V617F mutation was evaluated by quantitative PCR. BCR/ABL p190 and p210 analysis was performed by a quantitative RT-PCR using GeneXpert (Cepheid, Sunnyvale, CA). Post-transplantation chimerism analysis was performed on genomic DNA extracted from either unsorted bone marrow aspirate cells or from cell-sorted CD3-positive and CD33-positive fraction of peripheral blood using multiplex PCR.

Statistical Analysis

Patient demographics and clinical variables were compared between patients who developed CGE and those who had unchanged cytogenetic findings (non-CGE). The comparison between 2 groups was performed by the Wilcoxon rank-sum test for continuous variables and by the chi-square test for categorical variables. All tests were 2-sided, and a P value < .05 was considered statistically significant. Post-transplantation survival and postrelapse survival were estimated by the method of Kaplan-Meier and compared by log-rank test. Patients were considered censored at the date last known alive if death was not documented.

RESULTS

Clinical and Transplantation Characteristics

Of 316 patients undergoing allo-HCT for a myeloid malignancy at Mayo Clinic Arizona from 2004 to 2014, 56 patients relapsed after HCT; 49 cases met inclusion criteria. CGE was observed in 25 (51%) (CGE group), whereas 24 (48%) demonstrated unchanged cytogenetic results between primary diagnosis and relapse. Demographic, clinical, treatment, and transplantation characteristics are summarized in Table 1. The average age, 49.4 years, was similar in CGE and non-CGE cohorts. The male/female ratio was 48%/52% and 33%/67% in the CGE and non-CGE cohorts, respectively, indicating a female predominance if the non-CGE cohort. The median time from original diagnosis to transplantation was similar in the CGE and non-CGE group (206 days). The median time from HCT to relapse was similar in both groups (112 days versus 110 days, $P = .50$). The MDS and MPN disease spectrum represented 48% in the CGE cohort and represented 29% of non-CGE cohort ($P = .43$). Cohort risk comparison revealed baseline high-risk disease in 64.0% of the CGE cohort versus 45.8% in the non-CGE cohort respectively, ($P = .20$) using a disease risk index for patients undergoing allo stem cell transplantation [6]. Criteria of therapy-related myeloid neoplasm were met by 4 patients (8.1%). Cytogenetic findings of these 4 patients were characteristic of therapy-related myeloid neoplasms and included 2 with chromosome 7 deletion, 1 with chromosome 5 deletion, and 1 with a t(11;19) MLL gene rearrangement. Two of these patients developed CGE (1 del(7) and 1 MLL rearranged) and the other 2 did not develop CGE.

Cytogenetic and Molecular Characteristics at Original Diagnosis

Cytogenetic findings at original diagnosis and at relapse are summarized in Table 2. Cytogenetic findings were more frequently abnormal at baseline diagnosis in the CGE cohort (72%), as opposed to 41% of the non-CGE group (Figure 1). Complex karyotypes (defined as 3 or more cytogenetic abnormalities) were present in 32% of the CGE group and in 16% of the non-CGE group, respectively. Monosomal karyotypes (defined as -5/5q- or -7/7q- present and less than 3 cytogenetic abnormalities) were not common, with only 2 patients in CGE and 1 patient in no-CGE group.

Molecular mutation results at original diagnosis included the following: in the CGE group, of 7 patients with normal cytogenetic findings at diagnosis, 3 had combined FLT3