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Quantification of Marrow Hematogones Following Autologous Stem Cell Transplant in Adult Patients with Plasma Cell Myeloma or Diffuse Large B-cell Lymphoma and Correlation with Outcome

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Abstract

In this retrospective study, we quantified the hematogone (normal B-lineage precursor) population by flow cytometric immunophenotyping in post-transplant bone marrow biopsy specimens from adult patients who received an autologous stem cell transplant for either plasma cell myeloma (n=57) or diffuse large B-cell lymphoma (n=73). The majority of patients (80%) had <5% marrow hematogones post-transplant. Extreme (>10%) hematogone percentages were quite rare, seen in only four patients, and were not associated with disease progression. There was a positive association between the post-transplant day and hematogone percentage within the first year after transplant, and a negative association thereafter. Plasma cell myeloma patients with 5% hematogones in any post-transplant flow cytometry study had a worse overall survival as did plasma cell myeloma patients with increased hematogones (as defined by percentile) at 100 days post-transplant. These findings require further study, ideally in a prospective study design.

Keywords

autologous stem cell transplant; autotransplant; hematogones; plasma cell myeloma; multiple myeloma; B-lineage precursors; diffuse large B cell lymphoma

Introduction

Hematogones, or B-lymphocyte precursors, are a normal component of the bone marrow and can be identified by flow cytometry in at least small numbers in most marrow specimens due to their characteristic immunophenotypic pattern [1]. In general, the percentage of marrow cellularity composed of hematogones declines with increasing age, although a broad range has been found at all ages and hematogones may be increased in numerous neoplastic and nonneoplastic conditions [1, 2, 3, 4, 5, 6, 7, 8, 9, 10]. Hematogones may be present in large

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numbers following chemotherapy or bone marrow transplantation and have been associated with outcome in some studies [2, 11, 12, 13, 14, 15, 16].

During our routine clinical practice at a busy transplant center, we noticed sporadic patients with a strikingly large number of marrow hematogones identified by flow cytometry in the post-autologous hematopoietic stem cell transplant setting. To better quantify the frequency and magnitude, and to attempt to identify any factors associated with this phenomenon, we performed a retrospective review of adult patients who received autologous transplant for either plasma cell myeloma or diffuse large B-cell lymphoma.

Materials and Methods

Our Institutional Review Board approved this retrospective study. The study cohort included patients who underwent autologous stem cell transplant (ASCT) for diffuse large B cell lymphoma or plasma cell myeloma between 2004 and 2015, with at least one post-transplant bone marrow flow cytometry study. Patients that received ASCT for plasma cell myeloma were identified through natural language searches of our electronic pathology database. Those transplanted for diffuse large B cell lymphoma were identified via the Blood and Marrow Transplantation Research Database at our institution.

Conditioning regimen for diffuse large B cell lymphoma recipients of ASCT consisted of either BEAM (carmustine 300 mg/m² IV once on day -6, etoposide 100 mg/ m² IV twice daily on days -5 to -2, cytarabine 100 mg/ m² IV twice daily on days -5 to -2, and melphalan 140 mg/ m² IV once on day -1) or Cy/TBI (Cyclophosphamide 60 mg/kg IV on day -7 to -6, and total body irradiation 1320 cGy over days -4 to -1). Autologous stem cell product was administered on day 0 and G-CSF was started on day +5 and continued until neutrophil engraftment (ANC 0.5×10^9 /L for three consecutive days). Peripheral blood stem cells were collected by leukapheresis with a minimal collection goal of 2×10^6 CD34/kg recipient body weight. Conditioning regimen for plasma cell myeloma recipients of ASCT consisted of melphalan 200mg/ m² on day -2 with autologous stem cell product administered on day 0 and G-CSF started on day +5. Peripheral blood stem cells were collected by leukapheresis with a collection goal of 5×10^6 CD34/kg recipient body weight.

The electronic medical records were used to extract clinical information including patient age, gender, medical history, staging, and follow-up. Disease status and course after transplant was determined from summary clinical notes in the electronic medical record. Flow cytometric information was recorded for the immediate pre-transplant marrow (if obtained) and all subsequent post-transplant marrows.

In all cases, flow cytometric immunophenotyping was performed on bone marrow aspirate samples using routine protocols in place at the time the sample was obtained. Samples were processed by a lyse-stain method using an ammonium chloride lysing reagent. The standard number of events collected was either 100,000 or 300,000 depending on the tube. Flow cytometry was performed for the majority of cases by 8-color analysis on the FACSCanto2 flow cytometer [Becton Dickinson (BD, San Jose, CA)] using the following fluorescently labeled antibodies obtained from BD: CD5 PE-Cy7, CD10 APC, CD14 PerCP, CD19 V450,

CD20 APC-H7, CD38 FITC, CD45 V500, CD56 PE-Cy7, CD138 PerCP-Cy5.5, and kappa APC. Lambda FITC, kappa PE, and lambda PE were obtained from Dako (Carpinteria, CA).

Flow cytometry data (raw fcs files) was reanalyzed using Kaluza analysis software version 1.2 (Beckman Coulter Life Sciences, Indianapolis, IN) by study authors (VS and/or EC) in all cases with 8-color flow cytometry with the reanalyzed data percentages used for subsequent study analysis. A subset of the cases, 81/631 (13%), had flow cytometry performed by 4-color analysis and for these cases, data was extracted from the pathology report.

In the retrospective analysis, for patients transplanted for plasma cell myeloma, hematogones, mature B cells, and total lymphocytes were identified in a tube containing CD19, CD20, CD38, CD45, CD56, CD138, and cytoplasmic kappa and lambda. Hematogones were identified as CD19 positive cells with expression of CD38, a spectrum of CD20 expression and no to minimal dim expression of cytoplasmic light chains and were calculated as a percentage of total cells. For the patients transplanted for diffuse large B cell lymphoma, hematogones, mature B cells, and total lymphocytes were identified in a tube containing CD5, CD10, CD14, CD19, CD20, CD45, and surface kappa and lambda. Hematogones were identified as CD19 positive cells with co-expression of CD10, a spectrum of CD20 expression, and no to minimal dim expression of surface light chains and were calculated as a percentage of total cells. Representative flow cytometry plots showing hematogone identification are shown in Figure 1, including a representative image from a Wright-Giemsa stained marrow aspirate showing the typical hematogone morphology. In a subset of flow cytometry studies (34/631, 5%, all in patients transplanted for plasma cell myeloma), a hematogone percentage was obtained by both of the above methods, with a similar hematogone percentage obtained between the two methods in all but one flow cytometry study..

SPSS version 22 was used for statistical analysis. The independent samples Mann-Whitney U Test was used to compare continuous data and a Fisher's exact test was used to compare categorical data. Correlations were tested using Spearman's rho test. Survival analysis was performed using Kaplan-Meier curves with log rank statistics.

Results

Plasma cell myeloma versus diffuse large B-cell lymphoma patients

Our study cohort included 130 adult patients who received autologous stem cell transplant for plasma cell myeloma (n=57) or diffuse large B-cell lymphoma (n=73). Patient features are compared in Table 1. Each patient had between one and 13 post-transplant flow cytometry studies, ranging from 21 days to 15 years post-transplant. Six (11%) of the plasma cell myeloma patients and one (1%) of the diffuse large B cell lymphoma patients had a second autologous stem cell transplant. Additionally, two (4%) of the plasma cell myeloma patients and six (8%) of the diffuse large B cell lymphoma patients had a subsequent allogeneic stem cell transplant. Data from flow cytometry studies following subsequent transplant were included in this study. There was a significant difference in the distribution of post-transplant hematogone percentages (Figure 2), polyclonal B-cell

percentages, and lymphocyte percentages between patients transplanted for plasma cell myeloma and patients transplanted for diffuse large B-cell lymphoma.

Hematogone % peaks approximately 1 year after transplant and correlates with mature B cell %

The majority (80%) of patients had <5% marrow hematogones post-transplant, irrespective of the post-transplant day (Figure 3A). Sixty-five percent (24/37) of flow cytometry studies with 5% hematogones occurred within the first year of autologous transplant. Of the remaining 13 flow cytometry studies with 5% hematogones, six were after second transplant (either second autologous or subsequent allogeneic transplant).

By Spearman's rho test, there was no significant correlation between the hematogone percentage and post-transplant day, either for all patients ($p=0.780$) or for the subsets of plasma cell myeloma patients ($p=0.467$) or diffuse large B-cell lymphoma patients ($p=0.836$). However, there was a statistically significant positive association between hematogone percentage and post-transplant day ($r_s = +0.241$, $p<0.001$) when analysis was restricted to cases within a year of first autologous transplant and excluding the three patients who received a second transplant within a year, which remained when plasma cell myeloma patients and diffuse large B cell lymphoma patients were evaluated separately ($p=0.005$ and $p=0.001$, respectively). In contrast, there was a statistically significant *negative* association between the hematogone percentage and the post-transplant day ($r_s = -0.244$, $p<0.006$), when evaluation was restricted to cases after a year of first autologous transplant and excluding patients with a second transplant (at any time), which remained when plasma cell myeloma patients and diffuse large B cell lymphoma patients were evaluated separately ($p=0.016$ and 0.014). Data is presented in Figure 3B.

For both the plasma cell myeloma and the diffuse large B-cell lymphoma patients, a higher hematogone percentage correlated with a higher polytypic B cell percentage ($r_s = +0.371$, $p<0.001$ and $r_s = +0.138$, $p=0.046$, respectively). For the plasma cell myeloma patients, but not the B-cell lymphoma patients, a higher hematogone percentage correlated with a lower total lymphocyte percentage ($r_s = -0.238$, $p<0.001$).

Increased marrow hematogones persist short-term and may be associated with progressive disease

The hematogone percentages from three post-transplant time points were evaluated (approximately 1 month, 100 days, and 6 months post-transplant, Table 2) for the combined diffuse large B cell lymphoma and plasma cell myeloma cohorts. For each time point, a hematogone percentage at or above the 75th percentile was defined as "increased hematogones". There was a significant association between increased hematogones at one month and 100 days ($p=0.004$), and between increased hematogones at 100 days and at 6 months ($p<0.001$); there was a borderline significant association between increased hematogones at 1 month and at 6 months ($p=0.056$). For the plasma cell myeloma patients, there was a borderline significant association between increased hematogones at 1 month (1%) and progressive disease at any timepoint ($p=0.046$). Eight of 15 (53%) of patients with

increased hematogones at 1 month had disease progression while only 7/32 (22%) of patients with <1% hematogones at 1 month had disease progression.

There was a difference in age distribution between the patients with and without increased hematogones at 100 days [median age at transplant 52 years (range 34–74) versus median 59 years (range 20–73), respectively, $p=0.014$] and at 6 months [median 53 years (range 33–67) versus median 59 years (range 44–74), respectively, $p=0.011$]. There was no difference in age distribution at diagnosis or transplant between the patients with and without increased hematogones at 1 month.

There was no significant association between patients with $\geq 5\%$ hematogones (at any time) and age at diagnosis, age at transplant, gender, underlying hematopoietic neoplasm (diffuse large B cell lymphoma or plasma cell myeloma), or pre-transplant hematogone percentage. When flow cytometry studies following a second transplant were excluded, there was no statistically significant association between any post-transplant study with $\geq 5\%$ hematogones and a second transplant (either autologous or allogeneic, $p=0.204$).

An increase in marrow hematogones is associated with worse overall survival in patients transplanted for plasma cell myeloma

When all patients with known follow-up (107/130, 82%) were evaluated, patients transplanted for plasma cell myeloma had a better overall survival than patients transplanted for diffuse large B-cell lymphoma ($p=0.005$), and there was no difference in overall survival based on $\geq 5\%$ or $< 5\%$ hematogones in any post-transplant study. In contrast, those transplanted for plasma cell myeloma with $\geq 5\%$ hematogones in any post-transplant study had a worse overall survival ($p=0.011$) and borderline significantly worse progression-free survival ($p=0.061$). Excluding increased hematogones after a second transplant, there was a significantly worse overall survival ($p=0.008$) and progression-free survival ($p=0.030$) for the plasma cell myeloma patients with $\geq 5\%$ hematogones in any post-transplant study before a second transplant (Figure 4A and 4B). Patients transplanted for plasma cell myeloma with increased hematogones ($>3\%$) at 100 days had a significantly worse overall survival ($p=0.004$) (Figure 4C) while those with increased hematogones ($\geq 1\%$) at 1 month after transplant had a borderline worse overall survival ($p=0.059$) and significantly worse progression-free survival ($p=0.005$) (Figure 4D). There was no difference in overall survival based on the hematogone percentage (increased hematogones at 1 month, 100 days, or 6 months or $\geq 5\%$ in any post-transplant study) for the patients transplanted for lymphoma. Plasma cell myeloma patients with progressive disease had a worse overall survival ($p=0.032$).

Extreme marrow hematogone percentages are rare

Extreme hematogone percentages ($>10\%$) were quite rare, with only four cases in our series, occurring in four different patients. A female patient who received an autologous transplant at age 45 for diffuse large B cell lymphoma did not have an extreme hematogone percentage following autologous transplant (maximum hematogone percentage of 3%) but did have 13% hematogones 1 year following subsequent allogeneic transplant for relapsed disease. The patient was alive at last follow-up (1759 days post-autologous transplant/1098 days after

identification of the extreme hematogone percentage). A male patient who received autologous transplant for plasma cell myeloma at age 48 achieved partial remission after transplant; his extreme hematogone value was 12% in a sample obtained 100 days after transplant. He subsequently had progressive disease (228 days after transplant) and died at 325 days post-transplant (224 days after the extreme hematogone value). The third extreme hematogone percentage occurred in a woman who received an autologous transplant for plasma cell myeloma at age 65 and a second autologous transplant for progressive disease occurring about 4 years after the initial transplant. This patient had 31% hematogones in a study obtained 6 months after second autologous transplant. She achieved very good partial remission after both autologous transplants and was alive in complete remission at last follow-up (2146 days after initial transplant/187 days after extreme hematogone value). The final extreme hematogone percentage occurred in a woman transplanted at age 48 for plasma cell myeloma who achieved and remained in complete remission post-transplant (alive at last follow-up 737 days post-transplant, 555 days after extreme hematogone value). The patient had 15% hematogones 6 months after autologous transplant. All four patients had at least one marrow flow cytometry study subsequent to the extreme hematogone value, with all subsequent flow cytometry studies showing no or minimal neoplastic cells (a single case had less than 1% clonal plasma cells). Subsequent studies in three patients showed a decrease in hematogone percentage while the single subsequent flow cytometry study from the patient with 31% hematogones showed persistence of the hematogones hyperplasia with a similar hematogone percentage.

Discussion

In our study, plasma cell myeloma patients with increased hematogones post-transplant had a worse overall survival and progression-free survival. There was no association between increased post-transplant hematogones and overall survival for patients transplanted for diffuse large B cell lymphoma. In contrast, most prior studies evaluating the association between post-transplant marrow hematogone percentage and transplantation outcome in the setting of allogenic stem cell transplant [15, 17], including umbilical cord blood transplant [18, 19, 20, 21], have reported an association between a higher proportion of hematogones and improved survival rate after transplantation. The association of increased hematogones with lower transplant-related mortality in allogenic transplant may be due to a lower incidence of severe acute GVHD and extensive chronic GVHD [21]. This hypothesized “protective” association would not apply in the post-autologous transplant setting, but does not entirely explain the negative association between hematogones and survival in our plasma cell myeloma cohort. The worse survival seen in plasma cell myeloma patients with increased hematogones may be related to disease progression. There was a worse progression-free survival for plasma cell myeloma patients with increased hematogones at 1 month. We found an association between increased hematogones at 1 month and progressive disease and plasma cell myeloma patients with progressive disease had a worse overall survival. One hypothesis is that neoplastic plasma cells may persist/regress as more immature cells with immunophenotypic overlap with hematogones to then re-emerge as progressive disease. However, the large time-span between the increased hematogones (1 month post-transplant) and progression of disease (ranging from months to several years

later) and the lack of association of increased hematogones at 100 days or 6 months with progressive disease, makes this explanation less likely.

In our cohort of adult patients, the hematogone percentage peaked at approximately one year after transplant and then declined, with a significant positive association between post-transplant day and hematogone percentage within the first year post-transplant and a negative association after the first post-transplant year. While the associations are statistically significant, the correlation coefficients are weak (+0.241 within the first year and -0.244 after the first post-transplant year), indicating that post-transplant day is not a robust predictor of hematogone percentage. Most of the post-transplant studies with 5% hematogones occurred within a year of transplant. Previous work on hematogones following autologous transplant [13] found that CD10 positive mononuclear cells in the bone marrow peaked at 3 months post-transplant; that study included both children and adults and excluded patients who had not achieved morphologic remission. In our study, a higher hematogone percentage correlated with a higher polytypic B cell percentage, suggesting that, in general, the normal B-cell precursor maturation pathway was complete leading to restitution of the marrow mature B cell population.

Hematogones can be identified by morphology or by flow cytometric immunophenotyping [2]. Both methods have variability due to differences in staining method and observer interpretation (morphology) and technical considerations, immunophenotypic panel for identification, and gating strategies (flow cytometry). Quantitative information regarding hematogones identified by flow cytometry is particularly difficult to compare between institutions and care needs to be taken in interpreting data between laboratories. In our study, we used an erythrocyte-lysis method and total events as our denominator. Even within our single institution study, the multi-color flow cytometry panels and gating strategies to identify hematogones differed between the patients transplanted for plasma cell myeloma and diffuse large B-cell lymphoma. In the subset of cases in which both flow cytometry panels/gating strategies were utilized for the same sample, the hematogone percentage was similar in absolute value for most cases but the difference relative to hematogone percentage varied substantially in many of the cases (data not shown). The differences in hematogone enumeration between the plasma cell myeloma and diffuse large B cell lymphoma cases likely contributes to the differences in hematogone distribution between the two patient populations, in addition to the differences in immediate pre-transplant treatment regimens and conditioning.

In addition to method variability, variability in the definition of increased hematogones complicates correlation of post-transplant hematogone percentage with clinical features/follow-up. In this study, we used 5% as a cut-off based on the literature and our institutional experience. Additionally, we calculated a percentile based percentage at each of three time-points based on our cohort of flow cytometry studies. The optimal method of hematogone quantification and threshold determination by flow cytometry is not entirely clear. However, in the absence of a standard method of processing and interpretation across flow cytometry laboratories, it is reasonable to suggest an institution/study establish their own cut-off for increased hematogones, or verify one reported in the literature.

Our study of hematogones after autologous bone marrow transplant in adult patients in the era of modern multi-color flow cytometry provides new information to the field that suggests areas of future research. We found that the hematogone percentage peaked at one year following autologous transplant and, in contrast to other reports, we did *not* find a positive association between hematogone percentage and survival. To the contrary, we found a negative association between hematogone percentage 5% and overall survival in patients transplanted for plasma cell myeloma. Further studies are needed to fully understand this finding, including whether this is a correlative or causative association. Finally, we found that “extreme” hematogone values (< 10%) were actually quite rare in our post-autologous transplant population and tended to occur following a second transplant.

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Figure 1.

Representative flow cytometry plots illustrating hematogone identification in post-transplant study from plasma cell myeloma patient (A) and post-transplant study from diffuse large B-cell lymphoma patient (B). The “Mature B cells” gate is color-gated blue in (A) while the “hematogones” gate is color-gated pink in (B); the remaining plots show banded coloring. (C) Representative marrow aspirate morphology from patient with numerous hematogones (Wright Giemsa stained slide, 100X objective).

Figure 2.
Boxplots for post-transplant hematogone distributions based on transplant indication.

Figure 3.

Figure 3A: Hematogone percentage versus post-transplant day, separated for diffuse large B cell lymphoma and plasma cell myeloma patients.

Figure 3B: Hematogone percentage versus post-transplant day for all patients, with separation of cases within and after the first year (365 days) post-transplant.

Figure 4.

(A) Overall survival curves for all plasma cell myeloma patients with (green line) and without (blue line) increased hematogones in studies following first autologous transplant. (B) Progression-free survival curves for all plasma cell myeloma patients with (green line) and without (blue line) increased hematogones in studies following first autologous transplant. (C) Overall survival curves for plasma cell myeloma patients with flow cytometry study at 100 days with (green line) and without (blue line) increased hematogones. (D) Progression-free survival curves for plasma cell myeloma patients with flow cytometry study at 1 month with (green line) and without (blue line) increase hematogones.

Table 1

Details of Patients Transplanted for Plasma cell myeloma or Diffuse large B-cell lymphoma

	Plasma cell myeloma n=57	Diffuse large B-cell lymphoma n=73	p value
Age at diagnosis, years, mean (range)	58 (33–74)	53 (19–73)	0.006
Age at transplant, years, mean (range)	59 (34–74)	56 (20–74)	0.201
Gender, Male:Female	28:29	42:31	NS
Bone marrow involvement at diagnosis	N/A	24 (33%)	
Pre-transplant ^A hematogone percentage, median (range)	0.1 (0–2)	0.1 (0–4)	NS
Day of pre-transplant ^A flow cytometry study, median (range)	28 (10–111)	40 (14–75)	<0.001
Number of post-transplant studies, median (range)	5 (1–13)	2 (1–10)	<0.001
Second auto-transplant	6/57 (11%)	1/73 (1%)	0.043
Subsequent allo-transplant	2/57 (4%)	6/73 (8%)	0.233
Status at last follow-up, alive/deceased/unknown	42/5/10	39/21/13	0.006^B
Post-transplant lymphocyte percentage, median (range)	12 (1–59)	10 (0–98)	0.043
Post-transplant polytypic B cell percentage, median (range)	1 (0–8)	0 (0–7.5)	<0.001
Post-transplant hematogone percentage, median (range)	1 (0–31)	0.4 (0–13)	<0.001

^ARelative to first transplant if multiple transplants;^Bp-value calculating excluding patients with unknown follow-up.

Bold denotes statistical significance.

Table 2

Distribution of hematogone percentages at one month, 100 days, and 6 months post-transplant

	One month	100 days	6 months
Number of patients	84	98	72
Days post-transplant, median (range)	28 (21–31)	99 (80–115)	181 (160–200)
Hematogone percentage, median (range)	0.4 (0–8.0)	1.1 (0–12.0)	1.0 (0–15.0)
Percentiles			
25 th	0.1	0.3	0.3
50 th	0.4	1.1	1.0
75 th	1.0	3.0	2.2