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Early assessment of donor $CD34^+$ positive cells chimerism after allogeneic stem cell transplantation in acute myeloid leukemia/myelodysplastic syndrome patients – pilot study

Wczesna ocena chimeryzmu w komórkach $CD34^+$ dodatnich u chorych z ostrą białaczką szpikową/zespołem mielodysplastycznym poddanych allogenicznej transplantacji krwiotwórczych komórek macierzystych – doniesienie wstępne

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Keywords

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Słowa kluczowe

chimeryzm specyficzny liniowo, ostra białaczka szpikowa, zespół mielodysplastyczny, allogeniczna transplantacja krwiotwórczych komórek macierzystych

Konflikt interesów
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Brak konfliktu interesów
None

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Summary

Introduction. In high-risk acute myeloid leukaemia/myelodysplastic syndrome patients, relapse remains the major cause of treatment failure after allogeneic stem cell transplantation (alloSCT). The investigation of lineage-specific chimerism has become an important tool in the management of patients during the post-transplant period.

Aim. Early assessment of lineage specific chimerism in patients who underwent allogeneic hematopoietic stem cell transplantation.

Material and methods. 55 patients with acute myeloid leukemia and myelodysplastic syndrome who underwent alloSCT were included in the study. Flow cytometric analysis and cell sorting was performed in bone marrow collected at day 30 after alloSCT. For the purpose of this study we analyzed sorted immature progenitor cells ($CD34^+CD19^-$) using STR method.

Results. All patients who relapsed presented with lower donor chimerism in $CD34^+$ positive cells in comparison to the group of patients in remission of the underlying disease. Median value of chimerism in $CD34^+$ positive cells in the group of patients who relapsed was 14.5% (range 0-51%), whereas in patients who remained in remission of the underlying disease, chimerism in $CD34^+$ never fell below 97% (median 100%, range 97-100%). All relapses occurred during the first year after alloSCT. Median time to relapse was 107 days (range 28-323).

Conclusions. Early assessment of chimerism in $CD34^+$ cells sorted out of bone marrow is a sensitive technique to detect residual or reoccurring disease after allogeneic SCT. The assessment of donor chimerism in $CD34^+$ cells in day +30 after alloSCT seems to be relevant in post-transplant care of high risk patients.

Streszczenie

Wstęp. W przypadku pacjentów z wysokiego ryzyka ostrą białaczką szpikową/zespołem mielodysplastycznym, poddawanych allogenicznej transplantacji krwiotwórczych komórek macierzystych, wznowa choroby zasadniczej pozostaje najczęstszą przyczyną niepowodzenia. Ocena chimeryzmu specyficznego liniowo jest ważnym narzędziem w monitorowaniu chorych w okresie poprzeszczepowym.

Cel pracy. Celem pracy była wczesna ocena chimeryzmu specyficznego liniowo w grupie chorych poddanych allogenicznej transplantacji krwiotwórczych komórek macierzystych.

Materiał i metody. Do badania włączono 55 chorych ze zdiagnozowaną ostrą białaczką szpikową lub zespołem mielodysplastycznym, którzy poddani zostali allogenicz-

nej transplantacji krwiotwórczych komórek macierzystych. Analizie cytometrycznej oraz sortowaniu poddano próbki szpiku pobrane w dobie +30 po alloSCT. Szczegółowej analizie poddano komórki o fenotypie $CD34^{+}CD19^{-}$, w których oznaczono chimeryzm metodą STR.

Wyniki. W analizowanej grupie chorych, niższy chimeryzm w komórkach $CD34^{+}$ stwierdzono w grupie chorych, którzy doświadczyli wznowy choroby zasadniczej. Chimeryzm w komórkach $CD34^{+}$ w grupie chorych, którzy wznowili, wynosił 14,5% (mediana; zakres 0-51%), podczas gdy w grupie chorych, którzy pozostali w remisji przez okres obserwacji, chimeryzm w komórkach $CD34^{+}$ nie spadł poniżej 97% (mediana 100%, zakres 97-100%). Wszystkie wznowy były obserwowane w ciągu pierwszego roku po alloSCT. Mediana czasu do wznowy wynosiła 107 dni (zakres 28-323).

Wnioski. Uzyskane wyniki wskazują, że wczesna ocean chimeryzmu w komórkach $CD34^{+}$ sortowanych ze szpiku kostnego stanowi czułą metodę monitorowania statusu choroby zasadniczej w okresie poprzeszczepowym. Wydaje się, że oznaczanie chimeryzmu dawcy w komórkach $CD34^{+}$ w dobie +30 po alloSCT może stanowić ważny element w opiece potransplantacyjnej chorych, zwłaszcza chorych wysokiego ryzyka.

INTRODUCTION

In high-risk acute myeloid leukemia and myelodysplastic syndrome, relapse remains the major cause of treatment failure after allogeneic stem cell transplantation (alloSCT). Treatment of post-transplant relapse is difficult and most therapeutic approaches including donor lymphocyte infusions and second allogeneic transplantation usually present limited efficacy in the majority of cases. Following hematopoietic stem cell transplantation, monitoring the proportion of donor and recipient hematopoiesis in the patient is an influential tool in directing further treatment decisions (1, 2). The investigation of lineage-specific chimerism has therefore become an important tool for the management of patients during the post-transplant period.

AIM

The aim of the study was early evaluation of donor $CD34^{+}$ positive cells chimerism after allogeneic hematopoietic stem cell transplantation. The study design was approved by the local Bioethical Committee, Medical University of Silesia on June 21, 2011 and on June 30, 2014 (continued). Written informed consent was collected from each patient included into the study. This research was supported by Medical University of Silesia.

MATERIAL AND METHODS

A total of 55 patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) who underwent alloSCT were included in the study (median age 36 years, range: 19-67 years). Only patients with high risk AML and MDS whose disease had been shown to express $CD34^{+}$ by the time of initial diagnosis or at relapse were eligible for the study. Disease categories were as follows: AML (n = 51), MDS and therapy related leukemia (n = 4). All patients had received peripheral blood grafts from sibling (n = 12) or unrelated donors (n = 43) after intensive (n = 23) or reduced-intensity (n = 32) conditioning. The study group consisted of 30 males and 25 females. The data of the study group are summarized in table 1.

Tab. 1. Patients characteristics

Patient and donor characteristics	Number (%)
Acute myeloid leukemia (risk classification according to ELN)	N = 50
Favorable	3
Intermediate I	31
Intermediate II	3
Unfavorable	13
Myelodysplastic syndrome – MDS RAEB2	N = 5
IPSS low	0
IPSS intermediate I	2
IPSS intermediate II	1
IPSS high	2
Female	25
Male	30
Patient age	36 (19-67)
Disease status prior to SCT	N = 50
CR1	33
CR2	7
NR	10
Donor	N = 55
HLA identical family donor	12
HLA identical unrelated donor	29
HLA mismatched unrelated donor	14
Blood group incompatibility	N = 43
Major	24
Minor	10
Bi-directional	9
Donor age	22 (19-49)
Donor-recipient sex	
Female donor to male recipient	19
Other	36
Donor/recipient CMV status	
Positive-negative	12
Positive-positive	35
Negative-negative	8

The major AB0 group mismatch was detected in 24 cases, in 10 – minor, and in 9 donor-recipient pairs major and minor AB0 group incompatibility (bi-directional mismatch) was present. In 12 cases the blood groups between the donor and the recipient showed no difference. Cyclosporine at adjusted doses to concentration in serum and short course of methotrexate were used routinely as an immunosuppressive treatment in all patients. All patients transplanted from unrelated donor received either anti-lymphocyte globulin (ATG) at dose 15 mg/kg on three consecutive days preceding the transplantation or Thymoglobuline at dose 7.5 mg/kg (in 3 cases only).

Flow cytometric analysis and cell sorting was performed in bone marrow collected at day 30 after alloSCT. For the purpose of this study we analyzed immature progenitor cells (CD34⁺CD19-). Lineage specific donor chimerism was assessed using STR method and was calculated following the defined genetic profiles of the donor and the recipient. The methods for DNA isolation, cell sorting and chimerism analysis have been described in detail previously (3).

RESULTS

Clinical data: All but one patient engrafted. The infused allograft contained a median of 4.56 $\times$ 10^6CD34⁺ cells/kg (range 1.7-11.91) and 22.31 $\times$ 10^8CD3⁺ cells/kg (range 7.8-33.87). Hematological recovery was as follows: neutrophils recovered to > 500/μL at a median of 13 (range 12-28) days post-transplant. All patients received IV antibiotics to treat febrile neutropenia. The median time to a platelet count > 50,000/μL was 15 days (range 11-21) post-transplant with 7 patients never dropping their platelet count below 20,000/μL. Patients were not given prophylactically hematopoietic growth factors to enhance engraftment.

The diagnosis of GVHD was based on physical examination and laboratory tests. Viral, allergic, drug-related causes of symptoms were ruled out. Acute GVHD was graded according to the modified Seattle Glucksberg criteria. In 39 patients acute GVHD symptoms developed till day +30, most of the patients presented grade I. Skin involvement was noted most often. In 4 patients severe GVHD symptoms (grade III) were seen, with gastrointestinal tract and liver involvement. Topical steroids were applied in 36 patients and systemic steroid therapy was needed in 24 cases (the maximum dose of methylprednisolone was 2 mg/kg of body weight/day).

Donor lineage-specific chimerism assessment at day +30 after alloSCT: All patients underwent assessment at day +30 after SCT. The median follow-up for all patients was 23 months (range 2-47 months). The analysis of chimerism in the cell subsets of interest was done in most patients. Some of the measurements were not possible, owing to technical reasons, including poor quality of clinical samples and low cell count.

The clinical data of the patients who relapsed are summarized in table 2.

All of the patients who relapsed were high risk patients, mainly due to their cytogenetic and/or molecular profile, two patients with intermediate risk AML according to ELN classification, one with chemotherapy related AML (treated previously with high dose chemotherapy with subsequent autologous stem cell transplantation for relapsed Hodgkin lymphoma). All but two of these patients underwent myeloablative conditioning before stem cell transplantation from HLA-identical unrelated donors (except 3 cases of HLA-identical sibling donors). All but one were transplanted with active disease, so prompt reduction of cyclosporine was applied. The symptoms of acute graft-versus-host disease were observed in three patients (skin involvement, stage II) in the subgroup of patients who relapsed.

Tab. 2. Assessment of post-transplant donor chimerism at day +30 after alloSCT in AML/MDS patients who relapsed

Pt no.	Diagnosis, risk factors	Conditioning regimen	Time of survival since relapse (days)	Time to relapse (days)	Assessment of BM blasts at day +30 after alloSCT (%)	MRD at day +30 after alloSCT	Donor chimerism in unsorted BM cells (%)	Donor lineage-specific chimerism (%)						
								B cells	T cells	CD34⁺ cells	NK cells	NK/T cells	Granulocytes	Monocytes
1	AML NOS	MAC	38	323	< 1	negative	87	100	92	–	100	95	100	100
2	AML, Flt3-ITD(+)	MAC	100	28	2	positive	100	94	96	36	100	100	100	98
3	AML M4, del7, inv(3)	MAC	33	107	1	positive	87		100	13	71	–	98	–
4	AML, Flt3(+)	MAC	220	55	1	positive	100	22	59	100	98	37	100	100
5	MDS RAEB2, complex karyotype	RIC	unknown	57	3	positive	84	100	99	51	–	–	99	97
6	AML, del7	RIC	360	161	1	positive	100	100	73	16	–	–	87	90
7	AML, del7	MAC	312	215	2	positive	99	100	100	0	–	–	92	34
8	Chemotherapy-related AML	MAC	153	293	0	positive	100	100	99	3	99	92	100	98
9	MDS RAEB2	RIC	45	69	1	N/A	56	100	35	8	80	96	99	100

MRD N/A – minimal residual disease not available

All patients who relapsed presented with lower donor chimerism in CD34+ positive cells in comparison to the group of patients in remission of the underlying disease. The results of lineage-specific chimerism assessments in the group of patients who remained in remission of the underlying disease during the time of observation are presented in table 3.

All patients were released home at a median of 28 days after alloSCT (range 26-45 days) presenting good clinical condition. The hematological status was analyzed at day +30 after alloSCT. All patients presented hematological remission (less than 5% of blast cells in bone marrow aspiration), but nine out of ten patients in the group of patients who relapsed presented positive minimal residual disease (MRD) status at day +30 after alloSCT. Donor chimerism in unsorted bone marrow cells was measured in all cases and the median value was 99%, range 56-100% (in the group of patients who relapsed) and 99.5%, range 84-100% in those patients who remained in remission. Interesting data were revealed upon investigation of chimerism in CD34+ positive cells (CD34+CD19-) – the median value was found to be 14,5% (range 0-51%) and correlated with unfavourable clinical outcome. In one case (patient no. 4) chimerism in CD34+ cells was 100%, but it can be explained by the fact that blast cells were CD19 positive. It is seen also in low chimerism in B cells (22%). In patients who remained in remission of the underlying disease, chimerism in CD34+ never fell below 97% (median 100%, range 97-100%). All relapses occurred during the first year after alloSCT. Median time to relapse was 107 days (range 28-323). One patient (no. 6) experienced extramedullary relapse (skin lesions confirmed on skin biopsy) six months after alloSCT. One patient (no. 7) underwent two more transplantations from HLA-matched unrelated donor (third transplantation was from another donor) and died eventually due to severe graft versus host disease. In one case DLI (donor lymphocyte infusion) was performed together with concomitant therapy with demethylating agent, which provided transient positive effect (no. 8).

DISCUSSION

Chimerism analysis is routinely performed after alloSCT to monitor engraftment. Achieving complete hematopoietic chimerism after allogeneic transplantation is one of the main factors that contribute to the successful outcome. The issues of chimerism analysis in selected cell subsets (lineage-specific chimerism) is still under investigation. Nowadays, monoclonal antibodies are widely used to detect specific surface antigens. This precise evaluation enables to confirm the donor-origin hematopoiesis. When mixed chimerism is found it may provide valuable information about further immunotherapy, such as donor lymphocyte infusion or immunosuppression therapy adjustment (1, 2). Moreover, chimerism analysis provides data about imminent relapse or risk of GVHD development. The most important factor is to investigate chimerism changes over time. There are no strict recommendations about the timing of chimerism assessment (2). Generally, serial and quantitative analysis of chimerism by STR-PCR allows the identification of patients at the highest risk for relapse. However, the time interval between onset of mixed chimerism and relapse can be very short and nowadays, we would rather like to have early marker of high risk patients rather than multiply follow up. Analysis of donor chimerism in cell subpopulations increases the sensitivity form 1-5% up to 0.1-0.001% and can therefore be regarded a surrogate marker for MRD. Applying lineage-specific chimerism and MRD analysis as a standard management of post-transplant care provides an accurate monitoring of engraftment and surveillance of remission status. The only problem with that approach is the labor and cost effectiveness. That prompted us to look for a sensitive and early marker that would distinguish patients who are at high risk of relapse.

The most important factor in lineage-specific chimerism evaluation is to determine which cells would provide the most valuable data. In general, it is advised to assess chimerism in at least a few different cell lines. In this study we decided to focus on sorting the following cell lines: B cells (CD19+CD38-/+), T cells (CD3+ or CD7+CD56-), monocytes (CD14+), granulocytes, monocytes, NK cells and NK/T cells, and immature progenitor cells from myeloid line (CD34+CD19-).

Some authors proved that measurements performed in cell line that was the origin of the clonal (malignant) disorder (leukemia lineage specific chimerism) are the most important clinically (4, 5). An interesting issue has been raised by Lange et al. (6), who concluded that post-transplant monitoring of WT1 expression in peripheral blood and CD34+ donor chimerism in bone marrow seems to be good predictors of early relapse not only in AML patients, but also in patients suffering from myelodysplastic syndromes who underwent hematopoietic cell transplantation with reduced-intensity conditioning. It is worth mentioning, that CD34+ positive blastic cell phenotypes are found only in around

Tab. 3. Assessment of post-transplant donor chimerism at day +30 after alloSCT in patients who remained in remission during the follow-up period

Number of patients (n = 46)	Donor lineage-specific chimerism (%)						
	Lymphocytes B (n = 44)	Lymphocytes T (n = 45)	CD34+ (n = 42)	NK cells (n = 26)	NK/T cells (n = 21)	Granulocytes (n = 45)	Monocytes (n = 41)
Median (%)	100	99	100	100	98.5	100	100
Range (%)	81-100	63-100	97-100	97-100	96-100	97-100	98-100

80% of AML patients (7), so anti-CD34$^+$ antibody is not a perfect marker of relapse. It is advisable then to adjust the assessment of specific cell lines to particular patient looking closely at the leukemic cell phenotype detected at the time of diagnosis. It is also worth noting that expression of CD34$^+$ may change in the course of the disease. In our material CD34$^+$ positivity in blast cells was found in 89% of patients at the time of diagnosis (in 14 cases the data was unavailable since the patients were treated in another centers and the initial data were not forwarded). We showed in our material that chimerism analysis in cell subpopulations may serve as a surrogate marker for MRD, especially low chimerism in CD34$^+$ positive cells may act as a prognostic marker of imminent relapse. Similar data were shown by Thiede et al. (8) by analyzing peripheral blood CD34$^+$ positive cells. The only obstacle to use widely this method is the need for at least 50 ml of blood since this populations is found at a very low percentage in peripheral blood. On the other hand, it is less invasive and quicker method in comparison to bone marrow aspiration. Bornhäuser et al. (7) evaluated the measurements of chimerism in CD34$^+$ cells in peripheral blood in 90 AML/MDS patients. They concluded that cumulative incidence of relapse significantly increased in cases with decreasing or incomplete CD34$^+$ donor chimerism. They also found that the interval between a decrease of CD34$^+$ chimerism of less than 80% and hematologic relapse was 61 days (range 0-567).

CONCLUSIONS

Currently, fluorescence-based PCR amplification of short tandem repeats is the gold standard for analyzing post-transplant chimerism and has been used in most studies. Lineage specific chimerism allows for precise monitoring of patients after alloSCT. Assessment of donor chimerism in CD34$^+$ positive cells may be worth applying in selected AML/MDS patients with high risk of relapse. In our study the analysis of CD34$^+$ donor chimerism allowed the prediction of relapse in most cases. But still, the experience of our study confirms once again that recurrence of AML is difficult to prevent by preemptive strategies in the subgroup of patients with low CD34$^+$ donor chimerism even if detected as early as in day +30 after alloSCT. Strategies to triger graft-versus-leukemia reaction like tapering of immunosuppression or DLI do not provide long-term efficacy in most cases. In these patients, new approaches including immunomodulatory therapies should be tested in prospective trials.

In our opinion, the most sensitive and specific methods of post-transplant follow up in MDS/AML patients are MRD and chimerism of CD34$^+$ cells assessment. Both methods present similar level of sensitivity. Since MRD phenotype is not always available, we regard these two tests as complementary ones. Moreover, for reliable evaluation of donor chimerism in CD34$^+$ cells, the phenotype of blasts cells at the time of diagnosis should be known.

In summary, the results of this study show that early assessment of chimerism in CD34$^+$ cells sorted out of bone marrow is a sensitive technique to detect residual or reoccurring disease after allogeneic SCT. It allows the prediction of relapse in most cases presenting with CD34$^+$ disease. We proved that donor chimerism in CD34$^+$ cells is much more sensitive and specific than donor chimerism in unsorted bone marrow. Since this method is time and cost-consuming, it needs further optimization before applying in clinical practice. Monitoring of CD34$^+$ donor chimerism might be very informative in cases when no MRD phenotype or other molecular marker of disease is available. But still, assessment of donor chimerism in CD34$^+$ cells in day +30 after alloSCT may be helpful in post-transplant care of high risk patients. These data are preliminary but indicate a very interesting direction for further investigation.

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