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Multiparameter flow cytometry for assessment of minimal residual disease in patients with myelodysplastic syndromes treated with allogeneic stem cell transplantation

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Abstract

Background: Myelodysplastic syndromes (MDSs) are a heterogeneous group of clonal myeloid neoplasms. Allogeneic stem cell transplantation (allo-SCT) remains the curative method for MDS treatment. Little is known about the monitoring of minimal residual disease (MRD) in patients with MDS after allo-SCT. Aim: We aimed to evaluate the significance of leukemia-associated immunophenotypes (LAIPs) identified in acute myeloid leukemia (AML) for MRD monitoring in patients with MDS after allo-SCT. Material and methods: Seven males and 4 females with a median age of 55 years were included. The diagnosis of MDS was established according to 2016 World Health Organization (WHO) criteria. The significance of eight LAIPs in bone marrow samples using multiparameter flow cytometry (MFC) was evaluated for MRD. Results: Eight patients were positive for several LAIPs before allo-SCT. The identified LAIPs included the presence of aberrant lymphoid antigens on myeloblasts and lack of CD33 expression on myeloblasts. All studied MDS patients were negative for LAIPs at Day +30 after the procedure. This was followed by full-donor chimerism in all cases. The Ogata score after allo-SCT decreased in all patients in whom it was indicative for MDS before allo-SCT. Conclusions: MFC could be useful in monitoring MRD in MDS patients after allo-SCT. Further studies in this field are needed.

Keywords:
myelodysplastic syndrome, multiparameter flow cytometry, allogeneic stem cell transplantation, leukemia-associated phenotype, minimal residual disease

Introduction

Myelodysplastic syndromes (MDSs) constitute a heterogeneous group of clonal myeloid neoplasms characterized by the presence of dysplastic cells within the bone marrow (BM) [1, 2]. It results in abnormal hematopoiesis with mono-, bi-, or multilineage cytopenia in peripheral blood (PB) [1]. The diagnosis of MDS requires evaluation of BM aspirate/biopsy, including the assessment of blast proportion and the presence of dysplastic features [3]. Clinical heterogeneity among MDS cases requires the establishment of an individual's risk category, which determines the prognosis and the choice of therapy. The International Prognostic Scoring System (IPSS) and the Revised International Prognostic Scoring System (IPSS-R) are the most widely used tools for a patient's risk assessment. The choice of therapy depends on IPSS-related risk category [3]. First-line treatment for lower-risk patients (low and intermediate-1 IPSS categories) includes erythropoietin, granulocyte-colony-stimulating factor, androgens, transfusions, iron chelation drugs, and supportive care. Patients with 5q deletion usually benefit from lenalidomide [4, 5]. Higher-risk patients (intermediate-2 and high-risk according to IPSS) are usually treated with hypomethylating agents (azacitidine and decitabine), but the only curative therapy for eligible patients is allogeneic stem cell transplantation (allo-SCT) [6].

The development of therapy entails the need to improve existing and find new monitoring methods for patients with MDS. The role of multiparameter flow cytometry (MFC) in the diagnosis, monitoring, and prediction of outcome in patients with acute myeloid leukemia (AML) is well established [7–10]. Immunophenotypic patterns of maturation in hematopoietic lineages are well understood [11, 12]. Minimal residual disease (MRD) is a sensitive method useful in the assessment of pathological cells elusive in cytomorphological evaluation. Assessment of MRD by MFC is based on identifying aberrant clones characterized by specific cell–antigen combinations (immunophenotype) [13]. Several studies investigating the use of MFC in the diagnosis, monitoring, and prediction of outcome in MDS have been conducted [14, 15, 16]. The Ogata score has also been designed as a simple scoring system useful in screening for MDS [17].

Use of MFC in clinical practice for MDS may address technical and interpretational difficulties [18, 19]. There are single reports on MRD monitoring in MDS patients after allo-SCT based on evaluation of

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posttransplant chimerism and mutational status [20, 21]. Data on MFC use for MRD monitoring after allo-SCT in this group of patients are sparse [22, 23].

The aim of the study was to evaluate the utility of leukemia-associated immunophenotypes (LAIPs) identified in AML for MRD monitoring in patients with MDS treated with allo-SCT.

Patients

The preliminary analysis involved 11 patients who underwent allo-SCT at the Department of Hematology and Bone Marrow Transplantation, Silesian Medical University Poland, between March 2017 and July 2018. We evaluated seven males and four females aged between 40 and 66 years (median age: 55 years). All patients signed informed consent before entering the study. The diagnosis of MDS was established according to the revised 2016 World Health Organization (WHO) classification criteria [1]. The disease-risk category was assessed by IPSS-R. Ten patients were treated with one (n = 7), two (n = 3), or three (n = 1) lines of therapy. The therapy was based on corticosteroids and androgens (n = 3), azacitidine (n = 3), and intensive chemotherapy protocols for AML (n = 6). Patients' characteristics are listed in table I.

The choice of conditioning regimen was dependent on patient's age, performance status, and comorbidities. Transplantation details are included in table II.

Table I. Patient and disease characteristics

Number of analyzed patients (%)	11 (100)
Gender	
Male (%)	7(64)
Female (%)	4 (36)
Median age at diagnosis, years (range)	55 (39–65)
Median age at allo-SCT, years (range)	55 (40–66)
Diagnosis, number (%)	
MDS-SLD	1 (9)
MDS-MLD	2 (18)
MDS-EB-1	1 (9)
MDS-EB-2	7 (64)
Disease risk category, number (%)	
IPSS-R	
Very low	1 (9)
Low	1 (9)
Intermediate	4 (36)
High	3 (27)
Very high	2 (18)
Disease status at allo-SCT, number (%)	
MDS-SLD	1 (9)
MDS-MLD	4 (36)
MDS-EB-1	2 (18)
MDS-EB-2	4 (36)
Transfusion dependency, number (%)	10 (90)
Cytopenia before allo-SCT, number (%)	9 (81)
1-lineage	2 (18)
2-lineage	5 (45)
3-lineage	2 (18)
Blast percentage in PB before transplantation, median (range)	0 (0–7)
Blast percentage in BM before transplantation, median (range)	5.5 (0–16)

MDS-SLD – myelodysplastic syndrome with single lineage dysplasia; MDS-MLD – myelodysplastic syndrome with multilineage dysplasia; MDS-EB – myelodysplastic syndrome with excess; IPSS-R – Revised International Prognostic Scoring Systeml; allo-SCT – allogeneic stem cell transplantation; PB – peripheral blood; BM – bone marrow

Table II. Transplantation procedure details

Number (%)	11 (100)
Type of donor	
Matched related, n (%)	1 (9)
Matched unrelated, n (%)	7 (64)
Mismatched unrelated, n (%)	3 (27)
Source of hematopoietic stem cells	
Peripheral blood, n (%)	11 (100)
Conditioning regimen	
Myeloablative conditioning, n (%)	6 (54)
BuCy, n (%)	5 (45)
BuFlu, n (%)	1 (9)
Reduced intensity conditioning, n (%)	5 (45)
TreoFlu, n (%)	5 (45)
GvHD prophylaxis	
CsA + Mtx, n (%)	10 (91)
CsA + MM, n (%)	1 (9)

BuCy – busulfan plus cyclophosphamide; BuFlu – busulfan plus fludarabine; TreoFlu – treosulfan plus fludarabine; GvHD – graft-versus-host disease; CsA + Mtx – cyclosporine plus methotrexate; CsA + MM – cyclosporine plus mycophenolate mofetil

Material and methods

MRD was evaluated by MFC using BM samples collected in ethylenediaminetetraacetic acid (EDTA) tubes after obtaining informed consent from 11 patients with MDS [at two time points – before and at Day +30 after allo-HSCT (n = 11)]. Staining with monoclonal antibodies (moAb) was made after erythrocyte lysis procedure. The evaluation or MRD was done immediately after moAb staining. The analysis was performed on eight-color flow cytometer FACS Canto II with FACS Diva program (Becton-Dickinson, USA). The analysis was performed according to European LeukemiaNet recommendations on minimum 500,000 CD45+ cells [24]. All moAbs and isotype-matched negative control were produced by Becton-Dickinson (San Jose, USA). The percentage of CD34+ myeloblasts, B-cell precursors among CD34+ cells, and lymphocytes/

myeloblasts CD45+ ratio, as well as granulocytes-to-lymphocytes side scatter (SSC) peak ratio, was assessed to evaluate the Ogata score for each patient before allo-HSCT [25].

The antigens aberrantly expressed on myeloblasts (CD2, CD7, CD56, CD13+CD33-) were identified within CD45^{dim} SSC$^{low/int}$ CD34+ and CD45^{dim} SSC$^{low/int}$ CD34-CD117+cell compartments [18]. The expression of aberrant antigens on myeloid progenitors >30% was considered as a positive result. The myeloblast gating strategy is shown in figure 1.

The MRD analysis was conducted by evaluation of expression of selected aberrant lymphoid antigens (CD2, CD7, CD22, and CD56) on CD34+ myeloblasts and lack of expression of CD33 on CD13+CD34+ myeloblasts, which were found in AML [9, 24, 25]. All the analyzed aberrant immunophenotypes, i.e., LAIPs, are highly specific for myeloblast identification: their expression in healthy or regenerative BM is <0.1% [25]. MRD results >0.1% (>1/1,000 total BM cells) are considered positive [9]. Detected immunophenotypes used for MRD evaluation are shown in table III.

The gating strategy was based on identifying CD34+ cells within CD45dim SSC$^{low/int}$ and then distinguishing the percentage of CD13+CD7+, CD13+CD33-, CD13+CD22+, CD33+CD22+, CD13+CD56+, CD33+CD56+, CD13+CD2+, and CD33+CD2+ in this population [9]. BM and PB smears were prepared for cytomorphological evaluation by the May-Grünwald-Giemsa staining and counted for 200 nuclear cells. The evaluation of chimerism was based on deoxyribonucleic acid (DNA) analysis by polymerase chain reaction (PCR) by the short tandem repeat method.

Table III. Immunophenotypes used for MRD evaluation

Immunophenotypes
CD13+CD33-CD34+
CD22+CD13+CD34+
CD7+CD13+CD34+
CD22+CD33+CD34+
CD13+CD56+CD34+
CD33+CD56+CD34+
CD2+CD33+CD34+
CD2+CD13+CD34+

Results

Aberrant immunophenotypes on BM myeloblasts were identified in eight cases. The final three patients did not reveal any LAIPs. The immunophenotypes were characterized by the expression of lymphoid

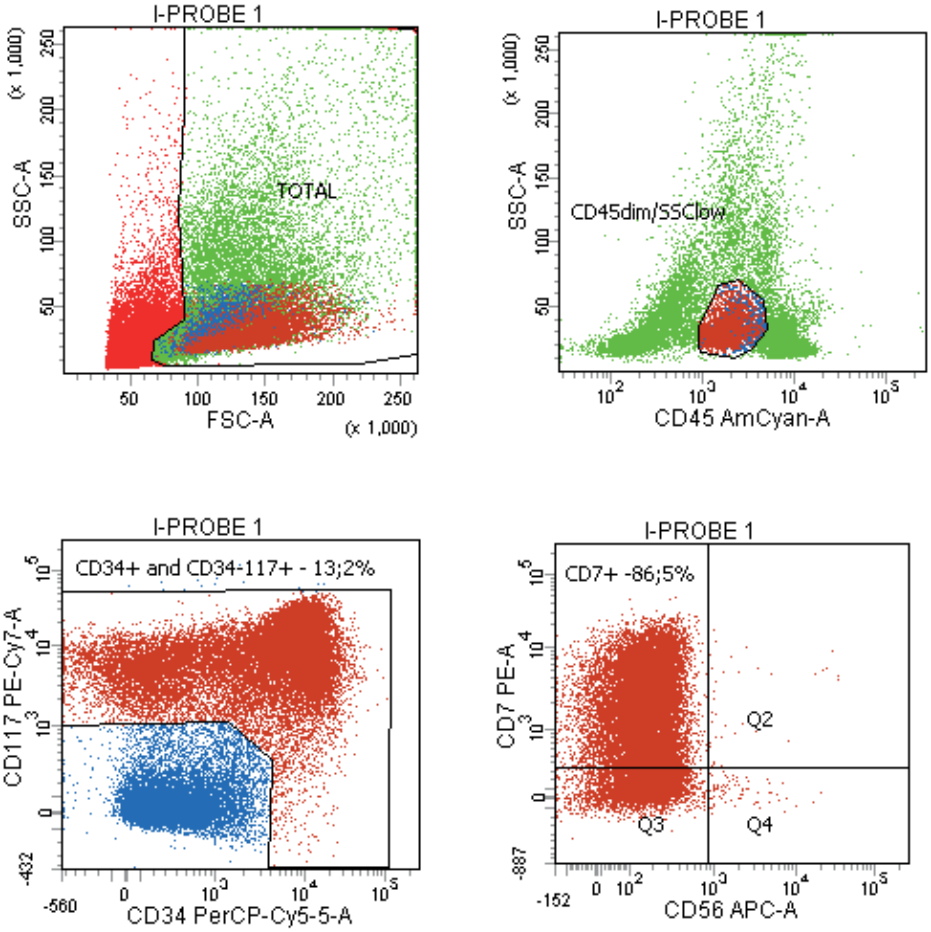

Fig. 1. Gating strategy of MFC aberrancies in myeloid progenitor cells

lineage-specific antigens, viz., CD7 (n = 3) and CD2 (n = 1), or the presence of CD13+CD33 coexpression (n = 6). Eight patients were MRD-positive before allo-SCT (median of MRD was 0.226; range: 0.12–4.79). All of them were MRD-negative at Day +30 after allo-SCT (median: 0.005; range: 0.001–0.079), which corresponds with full-donor chimerism at the second time point. The Ogata score was calculated for all patients before and at Day +30 after allo-SCT. This score was designed as a simple scoring system useful in screening for MDS. It evaluates the percentage of myeloblasts within all marrow cells, percentage of B-precursors within the CD34+ cell subset, ratio of CD45+ lymphocytes to CD45+ myeloblasts, and granulocyte-to-lymphocyte SSC ratio. Results ≥2 indicate a diagnosis of MDS [17]. All studied cases indicated as MDS by the score before allo-SCT showed decreased value at Day +30 after transplantation. The study results are shown in table IV.

Discussion

Myeloablative allo-SCT procedure is applicable for patients without severe comorbidities. Thus, the development of reduced-intensity conditioning allo-SCT (RIC allo-SCT), which is characterized by less toxicity, is a more suitable option for patients with MDS who are usually in advanced age [26]. Relapse after allo-SCT is considered to be the major cause of death in this group of patients. This indicates the importance of MRD monitoring in posttransplant care, which helps to predict the relapse and decide on further therapeutic approaches [22, 27]. Single studies showing the utility of mutational status monitoring of *Wilms' Tumor 1* gene (*WT*1) after allo-SCT in patients with MDS have been conducted [28, 29]. These data are limited and the method is not widely used. Another study on a large population of patients with MDS has revealed that almost 80% of

Table IV. Study results

Patient number	Sex / age (years)	Disease status at allo-SCT	Detected immunophenotypes	MRD/ 100 total BM before allo-SCT (positive >0.1)	MRD/ 100 BM at Day +30 after allo-SCT (positive >0.1)	Ogata score before allo-SCT	Ogata score at Day +30 after allo-SCT	Donor chimerism at Day +30 after allo-SCT (%)
1	M / 44	MDS-EB-2	CD13+CD33-CD34+ CD13+CD56+CD34+ CD33+CD56+CD34+	0.209 (+) 0.204 (+) 0.185 (+)	0.048 (–) 0.001 (–) 0.001 (–)	3	2	100
2	F / 46	MDS-MLD	No immunophenotype detected	–	–	2	1	100
3	M / 55	MDS-MLD	CD7+CD13+CD34+ CD13+CD33-CD34+	0.461 (+) 0.198 (+)	0.015 (–) 0.085 (–)	3	0	100
4	F / 57	MDS-EB-2	CD13+CD33-CD34+ CD13+CD56+CD34+	4.79 (+) 0.51 (+)	0.002 (–) 0.003 (–)	3	2	100
5	F / 60	MDS-MLD	No immunophenotype detected	–	–	3	0	100
6	M / 44	MDS-MLD	No immunophenotype detected	–	–	1	1	99
7	M / 64	MDS-EB-2	CD13+CD33-CD34+ CD22+CD13+CD34+	0.45 (+) 0.183 (+)	0.016 (–) 0.005 (–)	3	0	99
8	M / 40	MDS-EB-2	CD7+CD13+CD34+ CD22+CD13+CD34+ CD22+CD33+CD34+	2.62 (+) 0.992 (+) 0.942 (+)	0.003 (–) 0.005 (–) 0.008 (–)	3	1	99
9	M / 57	MDS-EB-1	CD7+CD13+CD34+ CD13+CD33-CD34+	0.645 (+) 0.19 (+)	0.079 (–) 0.023 (–)	2	1	100
10	M / 53	MDS-SLD	CD13+CD33-CD34+ CD22+CD13+CD34+	0.243 (+) 0.204 (+)	0.001 (–) 0.001 (–)	0	0	100
11	F / 66	MDS-SLD	CD22+CD13+CD34+ CD22+CD33+CD34+ CD2+CD13+CD34+ CD2+CD33+CD34+	0.112 (+) 0.37 (+) 0.12 (+) 0.15 (+)	0.008 (–) 0.006 (–) 0.005 (–) 0.006 (–)	1	2	100

MDS-SLD – myelodysplastic syndrome with single lineage dysplasia; MDS-MLD – myelodysplastic syndrome with multilineage dysplasia; MDS-EB – myelodysplastic syndrome with excess; allo-SCT – allogeneic stem cell transplantation; MRD – minimal residual disease

patients have mutation in at least one of the 111 analyzed genes [30]. These data are promising; however, analysis of such a large number of genes in clinical practice raises difficulties. Monitoring of MRD with cytogenetics is also challenging since only 50% of patients reveal cytogenetic abnormalities [31]. MFC seems to give the opportunity to overcome these limitations. This precise method allows obtaining quantitative results in a relatively short time at lower costs [32]. The role of MFC in MRD monitoring in acute leukemias is well established, and the evaluation of immunophenotype is possible in > 90% patients [33]. The most widely used MFC approach of MRD evaluation is based on the identification of LAIPs. This method allows for the identification of expression of abnormal antigens on blasts, which are not detected or extremely rare (<0.1%) in healthy or regenerative BM.

The aberrancies may manifest as expression of different-lineage-specific antigens, asynchronous expression, and overexpression or loss of lineage-specific antigens [9, 34, 35]. Immunophenotypes identified in our study reveal the presence of CD2 and CD7, which are typical for T-lineage and are aberrantly expressed in cases with myelodysplasia or AML [19, 35, 36]. Data indicate that expression of CD7 on myeloblasts is frequently seen in cases of AML with Fms-like tyrosine kinase-3 internal tandem duplication (FLT3/ITD) and – through it – is associated with worse outcome [37]. The prognostic significance of CD7 expression on myeloblasts in MDS has also been evaluated; however, the results are divergent [38, 39]. Another study has shown that increase in CD7 and decrease in CD15 on CD34+ myeloblasts are seen in more advanced stages of the disease [40]. In our observation, CD7 is found in patients with different disease stages – MDS-MLD, MDS-EB-1, and MDS-EB-2. We have also found two immunophenotypes with expression of CD56, which is the aberrant feature in CD34+ cells. This aberrancy is also identified in cases of myelodysplasia [19]. Expression of this antigen on blasts is supposed to be associated with worse response to growth factors in lower-risk MDS [41]. CD56 is an antigen used for LAIP evaluation in patients with AML and seems to be associated with poor prognosis in certain AML subgroups [42, 43]. The most frequently seen immunophenotype identified in our analysis includes CD13+CD33- antigen combination, which can be found in progenitors, neutrophils, and monocytes in MDS and has been identified in LAIPs in AML [18, 19, 25, 44].

Several studies have assessed the impact of MRD status on the outcome in patients with acute leukemias treated with allo-SCT. It has been shown that patients transplanted for acute lymphocytic leukemia (ALL) who had positive MRD at transplant had higher relapse rate and shorter progression-free survival [45]. Higher incidence of relapse and death after allo-SCT is also seen in MRD-positive patients transplanted in complete remission of AML [46]. Available data suggest that patients with MDS and positive MRD at the time of transplantation are characterized by worse posttransplant outcome; however, it differs depending on the type of applied conditioning regimen [22]. All of our patients with identified immunophenotypes were MRD positive at the time of allo-SCT; one of them died because of acute graft versus host disease (aGvHD) and one because of chronic graft versus host disease (cGvHD). One patient relapsed early after allo-SCT; however, none of the identified immunophenotypes before transplantation was detected at the relapse (data not shown). Limited data of monitoring MRD after allo-SCT in patients with MDS suggest that the outcome of patients is better when the MRD value is lower at Day +100 after allo-SCT [23]. All of our patients with identified immunophenotypes had negative MRD at Day +30 after allo-SCT, which corresponded with 100% chimerism detected in most of cases.

We also evaluated the Ogata score before and at Day +30 after allo-SCT. Several studies analyzed the score's utility as a diagnostic tool for MDS; however, they demonstrated its limitation [17, 19, 47]. There are no data of application of this score after allo-SCT. In eight of our cases, the results obtained before allo-SCT indicated MDS diagnosis (the value was ≥2). All of these eight results decreased when evaluated at Day +30 after allo-SCT. This may be associated with eradication of the clonal population of MDS in the BM, which seems to be confirmed by full-donor chimerism in these cases.

Conclusions

In our opinion, MFC may be a useful tool for evaluation of LAIPs and monitoring of MRD in patients with MDS qualified to allo-SCT. We would like to emphasize that this study is a pilot project in this field. The obtained results suggest the need for further observation and assessment of MRD at subsequent time points after allo-SCT in larger populations. Further studies on standardization of MFC application in MDS are needed.

Authors' contributions

GH, JDS, KC, KWP – design of the study. AWK, PZ, KW, DK, IGW, AK – data collection. JDS, KWP – data analysis. KWP, JDS – review of the literature. KWP, GH – writing manuscript. All authors – final approval.

Conflict of interest

The authors have no competing interest.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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