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Prognostic impact of immunophenotypic aberrancies of blasts in lower risk myelodysplastic syndrome

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ABSTRACT

Objective/background: Low risk myelodysplastic syndrome (MDS) is a marrow failure state eventually leading to transfusion dependence. Flow cytometry has previously been demonstrated as prognostic tool in MDS, however not thoroughly studied in lower risk MDS. In this study, we assessed whether assessment for immunophenotypic blast aberrancies by flow in low risk MDS patients has a prognostic role in these patients.

Methods: A total of 63 consecutive patients diagnosed with low/intermediate risk MDS were included. We recorded initial flow results, and collected time to transfusion dependence, and AML progression.

Results: On multivariate cox regression analysis, increasing IPSS-R score, an increase in the number of blast aberrancies on flow cytometry, and aberrant expression of CD7 on myeloid blasts increased likelihood of transfusion dependence.

Conclusion:Low risk MDS patients with increasingly aberrant blast phenotypes by flow may be at risk for earlier transfusion dependence.

1. Introduction

The myelodysplastic syndromes (MDS) represent a clonal hematopoietic stem cell disorder with variable clinical presentation and course [1]. Oftentimes, higher risk MDS shares features with Acute Myeloid Leukemia, while lower risk MDS is characterized by eventual development of transfusion dependence, frequently red blood cell dependence. Diagnosis and classification of MDS occurs through a multimodal approach, including the use of flow cytometry.

In addition to aiding diagnosis, flow cytometry has previously been shown to provide prognostic value [2]. In higher risk MDS, studies have shown that aberrant CD7 expression is associated with shorter survival [2]. Wells et al developed a flow cytometry scoring system (FCSS) to aid in the diagnosis and prognostication of MDS [3]. The FCSS has since been validated in predicting outcomes, including risk for transfusion dependence, and risk for progression to AML, independent of other scoring systems for MDS [4,5]. While FCSS provides prognostic information, it has not been adopted into standard workflows at many institutions, including ours.

At our institution, routine flow analysis at time of MDS diagnosis includes the assessment of immunophenotypic (IP) aberrancies in myeloblasts, and we have previously described neoplasia-specific blast aberrancies in this context [6]. Little has been published regarding the relevance of aberrancies in low risk MDS cases. Therefore, in this study, we investigated the prognostic significance of IP aberrancies in low or intermediate risk MDS, namely, whether the presence of aberrances is associated with development of red blood cell transfusion dependence.

2. Methods

This study was performed with Institutional Review Board approval from the Medical College of Wisconsin. We performed a retrospective chart review of patients who received an initial diagnosis of MDS at our institution between January 2010 and December 2017. IPSS-R score was calculated for all identified patients and those with a low or intermediate risk score (IPSS-R <4.5) were included for the study. Patients diagnosed with myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) and chronic myelomonocytic leukemia (CMML) were excluded from the study. Patients who were lost to follow up after initial BM biopsy were also excluded from the study.

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Table 1
Patient Demographics.

Baseline Characteristics	(n=63)
Median Age, yr (range)	66 (31-89)
Gender	
Female (%)	28 (44)
Male (%)	35 (56)
Mean Hgb, g/dL (range)	10.0 (5.2 - 15.3)
Mean Platelet Count, x10^9/L (range)	155.4 (8.0 - 960.0)
Mean ANC, x10^9/L (range)	2.5 (0.1 - 13.8)
Mean BM blasts % (range)	1.9 (0.0 – 9.2)
Cytogenetics risk category	
Very Good/Good (%)	47 (75)
Intermediate (%)	10 (16)
Poor (%)	6 (9)
IPSS-R	
Very Low (%)	15 (24)
Low (%)	29 (46)
Intermediate (%)	19 (30)

Patients were identified as red blood cell transfusion dependent if they had two or more episodes of packed red blood cell (PRBC) transfusions over the course of four weeks. In patients meeting these criteria, date of transfusion dependence was defined as the date of the second PRBC transfusion. Patients who underwent allogeneic hematopoietic stem cell transplantation prior to development of transfusion dependence were censored at the time of transplant.

Only patients who had initial diagnostic studies, including flow, at our institution were included. All flow reports were re-reviewed by a single pathologist for the purpose of this study. Flow cytometry (4- or 8-color) was performed on bone marrow aspirates for the following antigens: CD7, CD11b, CD13, CD14, CD15, CD19, CD20, CD33, CD34, CD36, CD38, CD45, CD56, CD64, CD117, and HLA-DR using a FACS Calibur/Canto. Blasts were identified using cluster analysis (Paint-A-Gate^{TM} software, BD Biosciences, California), after exclusion of all other populations as cohesive, well-delineated clusters, with consistent light scatter and CD45 expression patterns across multiple tubes. Blast IP aberrancies were defined as >1/4 log compared to normal controls. Neoplasia-specific blast IP aberrancies were defined as abnormalities seen exclusively in neoplastic non-acute myeloid diseases, and were adopted from a previous study to include: expression of CD7, CD11b, and CD56; under expression of CD38, CD45, and HLA-DR, and over-expression of CD15 and CD34 [6].

Demographic and disease characteristics were summarized using descriptive statistics and compared between the study cohorts using Wilcox on rank-sum test for continuous and ordinal measures and chi-square tests for categorical outcomes. Patients were followed for transfusion dependence from the time of diagnosis to transplant, death or last follow-up, defined as last complete blood count (CBC). Survival curves were estimated using the Kaplan-Meier method and compared between groups via the log-rank test. Cox regression was used for the multivariable analysis of survival. Analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

3. Results

Between January 2010 and December 2017, we identified 63 patients with a new diagnosis of low or intermediate risk MDS at our institution. The median age of patients was 66 years, and most were male, n=35 (56%). At diagnosis, mean hemoglobin was 10.0 g/dL (range: 5.2-15.3 g/dl), mean platelet count was 155.4 $\times$ 10^9/L (range: 8-960 $\times$ 10^9/L), and mean ANC was 2.5 $\times$ 10^9/L (range: 0.1-13.8 $\times$ 10^9/L). The mean bone marrow blast percentage was 1.9 % (range: 0.0-9.2 %). Overall, 15 (24%) patients were categorized as IPSS-R very low risk, 29 (46%) patients were IPSS-R low risk, and 19 (30%) patients were IPSS-R intermediate risk. Table 1 summarizes demographics. Of note, eight patients initiated therapy with an erythrocyte stimulating agent or hypomethylating agent prior to development of transfusion dependence.

On diagnostic bone marrow biopsy, IP aberrancies on bone marrow blasts were detected in 45 patients (71%). Seven (11%) patients were found to have one IP aberrancy, and 16 (25%) and 22 (35%) patients were found to have 2 and ≥3 IP aberrancies, respectively. The most observed aberrancy was dim CD38 expression in 23 (38.3%) patients, followed by dim HLA-DR, bright CD117, and dim CD33 expression. Furthermore, aberrant CD7 expression was observed in 8 (13.3%) patients. Overall, flow cytometry revealed aberrant expression of CD7, CD11b, CD13, CD15, CD33, CD34, CD38, CD45, CD56, CD64, CD117, CD123, and HLA-DR.

On univariate analysis, several findings were significantly associated with a more rapid time to transfusion dependence. IPSS-R score was predictive of time to transfusion dependence with a higher IPSS-R score predicting a shorter time to transfusion dependence, HR 1.528 (95% CI 1.036 – 2.285, p = 0.0346). Aberrant expression of CD7 on myeloid blasts was predictive of a shorter time from diagnosis to transfusion dependence 2.951 (95% CI 1.083-6.879, p = 0.0194). Finally, an increasing number of aberrancies by flow cytometry was predictive of a more rapid time to transfusion dependence with HR 1.319 (95% CI 1.05-1.65, p = 0.018). Of note, cytogenetic risk, or percentage of blasts at the time of diagnosis alone, or presence of a neoplasia specific aberrancy were not statistically significant predictors of time to transfusion dependence.

On multivariate cox regression analysis, several variables remained significant predictors of transfusion dependence. Increasing IPSS-R score increased the likelihood of transfusion dependence (HR: 1.976, (95% CI 1.130 – 3.454, p = 0.0169)). Moreover, an increase in the number of blast aberrancies on flow cytometry, independent of IPSS-R, was associated with an increase in likelihood of transfusion dependence (HR: 1.453 (95% CI 1.060 – 1.992, p = 0.0203)). Finally, aberrant expression of CD7 on myeloid blasts increased likelihood of transfusion dependence. (HR: 3.727 (95% CI 1.359 – 10.218, p = 0.0106)). Table 2 reports these findings.

Overall, among the 63 patients, median time to transfusion dependence was 48 months. Among patients with three or more aberrancies on blasts, median time to transfusion dependence was significantly shorter at 7.3 months (95% CI 1.3 – 40.5). Conversely, in patients with two or less blast aberrancies, median time to transfusion dependence was over 60 months **(**Fig. 1**).**

4. Discussion

Among lower risk MDS patients, our study demonstrates a potential prognostic role for simply assessing the number of IP aberrancies on blasts by flow cytometry. In patients with MDS with low or intermediate IPSS-R scores, the presence of blasts with three or more aberrancies at diagnosis was associated with more rapid development of transfusion dependence. In the absence of aberrancies, patients in this study rarely

Table 2
Multivariate analysis of variables for transfusion independence.

Description	Point Estimate	Lower 95% Wald Confidence Limit	Upper 95% Wald Confidence Limit	p-value	Overall p-value
IPSS-R	1.976	1.130	3.454	0.0169	0.0169
Blast % at diagnosis	1.000	0.998	1.002	0.9291	0.9291
Number of aberrancies	1.453	1.060	1.992	0.0203	0.0203
Aberrant CD7 expression	3.727	1.359	10.218	0.0106	0.0106

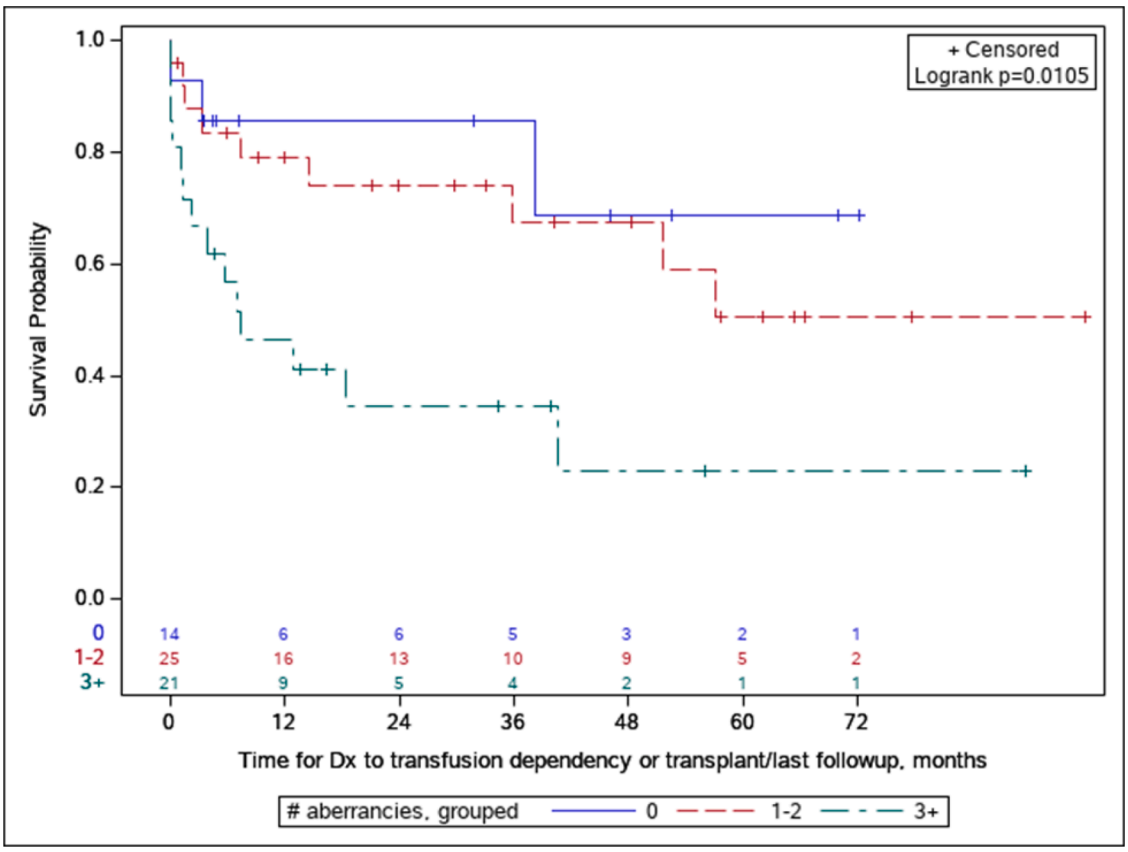

Fig. 1. Time to transfusion dependency by number of aberrancies.

developed transfusion dependence. Furthermore, like previous studies, aberrant CD7 expression was associated with poorer outcomes; this also indicated an earlier time to transfusion dependence.

As more centers routinely perform flow analysis during initial assessment for MDS, stratifying blasts into high (3+) IP aberrances or low IP aberrancies, is a simple assessment that may inform clinicians into how carefully to monitor their LR-MDS patients. Prospective studies are required to confirm these results, and future studies could also examine the relationship between high IP blast aberrancy burden and pathologic mutations present with MDS.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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