REGULAR ARTICLE

blood advances

U2AF1 and EZH2 mutations are associated with nonimmune hemolytic anemia in myelodysplastic syndromes

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Key Points

- Hemolysis in MDSs is nonimmune, relates to inferior survival trends in low-risk disease, and lower response to erythroid-stimulating agents.
- U2AF1 and EZH2 MT were more common with hemolysis, suggesting synergy between alleles encoding splicing factors and RBC membrane components.

Hemolysis is a well-recognized but poorly characterized phenomenon in a subset of patients with myelodysplastic syndromes (MDS). Its pathobiological basis seems to underpin a nonimmune etiology whose clinical significance has not been adequately characterized. Hemolysis in MDS is often attributed to either ineffective intramedullary erythropoiesis or acquired hemoglobinopathies and red blood cell (RBC) membrane defects. These heterogeneous processes have not been associated with specific genetic subsets of the disease. We aimed to describe the prevalence of hemolysis among patients with MDS, their baseline characteristics, molecular features, and resulting impact on outcomes. We considered baseline serum haptoglobin <10 mg/dL a surrogate marker for intravascular hemolysis. Among 519 patients, 10% had hemolysis. The baseline characteristics were similar among both groups. Only 13% of patients with hemolysis were Coombs-positive, suggesting that hemolysis in MDS is largely not immune-mediated. Inferior survival trends were observed among lower-risk patients with MDS undergoing hemolysis. Decreased response rates to erythropoiesis-stimulating agents (ESA) and higher responses to hypomethylating agents (HMA) were also observed in the hemolysis group. *U2AF1* and *EZH2* hotspot mutations were more prevalent among those undergoing hemolysis ($P < .05$). *U2AF1* mutations were observed in 30% of patients with hemolysis and occurred almost exclusively at the S34 hotspot. Somatic mutations encoding splicing factors may affect erythrocyte membrane components, biochemical properties, and RBC metabolic function, which underpin the development of atypical clones from erythroid precursors in MDS presenting with hemolysis. Future studies will explore the contribution of altered splicing to the development of acquired hemoglobinopathies.

Introduction

Myelodysplastic syndromes (MDS) are clonal disorders of hematopoiesis characterized by abnormal maturation of progenitor cells resulting in bone marrow failure and clinical complications arising from peripheral blood cytopenias.[1] This group of highly heterogeneous disease subsets is further marked by different clinical phenotypes, behavior, and outcomes ultimately driven by perpetuation and amplification of aggressive clones with a proclivity to transform into acute leukemia.

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Data are available on request from the corresponding author, Rami Komrokji (rami.komrokji@moffitt.org).

Hemolysis is a well-recognized but poorly characterized phenomenon seen in a subset of patients with MDS and other hematologic malignancies. For instance, autoimmune hemolytic anemia (AIHA), immune thrombocytopenia, and other autoimmune cytopenias (aplastic anemia, pure red cell aplasia, autoimmune neutropenia) have been more commonly observed concurrently with lymphoproliferative neoplasms, that is, chronic lymphocytic leukemia and lymphomas, with a reported incidence of up to 10% in the former and even higher incidence in specific subsets of the latter such as with marginal zone lymphoma and angioimmunoblastic T-cell lymphoma.[2-5] Conversely, the relative incidence of autoimmune cytopenias in MDS and other myeloid disorders remains scarce, with AIHA being reported in only up to 1% of cases, whereas immune thrombocytopenia and other cytopenias appear relegated to case reports.[6]

The mechanisms driving hemolysis can be broadly divided into autoimmune and nonimmune etiologies. The former is usually caused by loss of peripheral tolerance to endogenous erythroid antigens by self-reactive B cells (such as in warm AIHA in which typically immunoglobulin G antibodies are directed against the Rh complex or glyclophorin antigens leading to extravascular lysis), which itself may be secondary to viral infections resulting from molecular mimicry, connective tissue disorders, and drugs, among others.[7,8] In contrast, nonimmune etiologies comprise a diverse group of processes that include hemoglobinopathies (thalassemia, sickle cell disease), enzymopathies (G6PD, PK deficiency), red blood cell (RBC) membrane defects (hereditary spherocytosis, elliptocytosis), paroxysmal nocturnal hemoglobinuria (PNH), and mechanical/osmotic insults. Coombs/direct antibody testing (DAT) is used in such instances to distinguish autoimmune from nonimmune causes with profound sensitivity, but it may be negative in up to 5% of cases.[8]

The clinical significance of hemolysis in MDS has not been adequately characterized, but its pathobiological basis seems to underpin a nonimmune etiology. Nonimmune hemolysis in MDS is often attributed to either ineffective intramedullary erythropoiesis or acquired hemoglobinopathies and RBC membrane defects, with the latter having been identified only in rare instances. These vastly heterogeneous processes mediating RBC lysis in MDS may be seen in association with specific genetic subsets of disease, and their nature may be directly consequential to the compromised end product arising from aberrant gene expression and the resulting cascade of downstream effects. In this study, we report the prevalence of hemolysis among patients with MDS, their clinical characteristics, and molecular features of the predominant disease clones at baseline, as well as the resulting impact on outcomes.

Methods

Patient selection and inclusion

We retrospectively identified and analyzed all patients presenting to the H. Lee Moffitt Cancer Center with a diagnosis of MDS who had serum haptoglobin measured at time of diagnosis or referral. Laboratory values and prognostic scores were determined at the time of diagnosis or before treatment. Haptoglobin was included in our new MDS patient panel as routine testing at the time of diagnosis or first referral. Assumption of hemolytic anemia (HA) was made based on a very stringent haptoglobin cutoff of <10 mg/dL to ensure very high sensitivity and specificity at the time of patient selection.[9] This threshold was arbitrarily decided upon by the authors by referencing 2 historic validation studies looking into low plasma haptoglobin as an accurate marker of hemolysis.[9,10] We subsequently compared the baseline characteristics, response to treatment, and overall survival (OS) in those with hemolysis (haptoglobin < 10 mg/dL) with those in the nonhemolysis group. We then explored the mutational landscape of MDS among patients with hemolysis compared with those in the nonhemolysis group. This study was approved by the institutional review board of the H. Lee Moffitt Cancer Center and Research Institute and conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Mutational profiling

All patients underwent molecular profiling by next-generation sequencing performed on DNA extracted from peripheral blood or bone marrow mononuclear cells. The next-generation sequencing panels targeted up to 406 genes. A minimum variant allele frequency of 5% was used to define single nucleotide variants, and a cutoff of 10% was used for insertions or deletions. The reported mutations were subsequently evaluated by the investigators and referenced in the Catalogue of Somatic Mutations in Cancer (COSMIC) database to ensure pathogenicity.

Definitions of response and survival

Response to erythroid-stimulating agent (ESA) and hypomethylating agent (HMA) therapies was defined according to the International Working Group 2006 response criteria for hematologic improvement in MDSs. OS was measured from the time of diagnosis. Leukemia-free interval (LFI) was defined as the time from the diagnosis to the development of acute myeloid leukemia.

Statistical methods

Categorical variables were compared using Fisher exact and χ^2 tests, and quantitative data were compared using the Mann-Whitney *U* test and Wilcoxon rank-sum tests. The Kaplan-Meier method was used to estimate OS, and the log-rank test was used to compare OS between groups in univariable analyses. Patients sequenced on targeted panels that did not include a specific gene of interest were excluded from all analyses evaluating that gene. Statistical analyses were performed using IBM SPSS statistics version 27.

Results

Baseline characteristics of the cohort

Among 519 patients with known serum haptoglobin at first referral or diagnosis, 54 (10%) had serum haptoglobin levels <10 mg/dL (the hemolysis group). Other laboratory markers associated with hemolysis such as total bilirubin (1.4 vs 0.7, $P < .005$) and elevated lactate dehydrogenase ($P < .005$) were significantly increased in the hemolysis group as expected. The baseline characteristics were similar among the 2 cohorts, except for a slightly younger age (68 vs 71 years, $P = .02$) and lower platelet counts (86 000 vs 135 000, $P = .002$) among those in the hemolysis group. However, no differences in Coombs positivity rates were observed. Only 13% of patients in the hemolysis group and 9% of those with no evidence of hemolysis had a positive Coombs test ($P = .3$), suggesting that hemolysis in MDS is not primarily immune- mediated

Table 1. Baseline characteristics according to presence or absence of hemolysis

		Hemolysis, n = 54 (%)	No hemolysis, n = 465 (%)	*P* value
Age, y	**Mean**	**68**	**71**	**.02**
Gender	Male	40 (74)	316 (68)	.97
Race	White	50 (93)	437 (95)	.22
WHO 2016	MDS-SLD	2 (4)	35 (8)	.16
	MDS-MLD	22 (41)	120 (26)	
	MDS-SLD-RS	2 (4)	35 (8)	
	MDS-MLD-RS	3 (6)	53 (11)	
	EB1	12 (22)	103 (22)	
	EB2	13 (24)	104 (22)	
	U	0	15 (3)	
R-IPSS	Very low	6 (11)	54 (12)	.4
	Low	17 (32)	151 (33)	
	Int	8 (15)	74 (16)	
	High	14 (26)	72 (16)	
	Very high	9 (17)	107 (23)	
CBC (mean)	Hgb	9.4	9.6	.47
	WBC	3.7	3.8	.66
	ANC	1.7	1.9	.44
	Platelets	**86**	**135**	**.002**
Myeloblasts	Mean	5.4	5.7	.7
LGL clone	Yes	13 (24)	79 (17)	.4
PNH clone	Yes	2 (4)	12 (3)	.7
Acute myeloid leukemia transformation		11 (20)	93 (20)	.95
Immature retic	**Mean %**	**22**	**16**	**<.005**
Retic	**Mean %**	**3.5**	**2.3**	**.02**
LDH (elevated)	**Yes**	**45 (83)**	**164 (35)**	**<.005**
DAT poly (Coombs)	Positive	7 (13)	43 (9)	.3
DAT IgG	Positive	4 (7)	33 (7)	.97
T bilirubin	**Mean**	**1.4**	**0.7**	**<.005**
Baseline EPO	Mean	106.5	173	.26

ANC, absolute neutrophil count; CBC, complete blood count; EPO, erythropoietin; Hgb, hemoglobin; IgG, immunoglobulin G; LDH, lactate dehydrogenase; LGL, large granular lymphocyte; MLD, multilineage dysplasia; R-IPSS, Revised International Prognostic Scoring System; RS, ring sideroblast; SLD, single lineage dysplasia; WBC, white blood cell; WHO, World Health Organization.

(table 1). The presence of small PNH clones was similar between both groups (4% vs 3%, $P = .7$).

Examination of a small number of peripheral smears from the subset of patients with HA-MDS showed significant poikiloanisocytosis.

Covariates for OS

The median OS (mOS) for the entire patient cohort was 38.5 months (95% confidence interval [CI], 28.9-48.1 months) (Figure 1). The mOS was 30.8 months (95% CI, 7.5-54.1 months) for those patients in the hemolysis group vs 38.5 months (95% CI, 27.8-49.2 months) among those with no evidence of hemolysis ($P = .98$). The median duration of follow-up was 25 months (95% CI, 22-27 months).

There was a trend toward inferior survival among patients with hemolysis in the lower and intermediate R-IPSS risk subgroups. In the very low/low-risk R-IPSS subgroup, the mOS was 44 months for those patients with hemolysis compared with 89 months for those with no hemolysis ($P = .2$). Conversely, in the intermediate risk subgroup, the mOS was 19 months for those with evidence of hemolysis vs 34 months for those with no hemolysis ($P = .7$). The median LFI for those patients with hemolysis was 60.3 months (95% CI, 16.3-104.3 months) (Figure 2) vs not reached ($P = .870$).

Response to ESA and HMA therapy

A total of 19 patients (35%) in the hemolysis group and 130 patients (87.2%) in the nonhemolysis group received ESAs. Mean EPO levels at baseline (before starting treatment) were 106.5 mU/mL for the hemolysis group and 173 mU/mL for the nonhemolysis group ($P = .261$). The hematological improvement rate on ESA therapy was 16% (3 of 19 patients) in the hemolysis group compared with 30% (39 of 130 patients) in the nonhemolysis group ($P = .2$).

The hematological improvement rate at best response with HMAs was 70% (22 of 31) for those with hemolysis compared with 45% (127 of 280) in the nonhemolysis group ($P = .05$).

Mutational landscape and spectrum of mutations

Table 2 summarizes the mutational landscape between hemolysis and nonhemolysis groups. *U2AF1*and *EZH2* hotspot mutations were significantly more common among those undergoing hemolysis ($P < .05$). Of the 16 patients harboring *U2AF1* mutations in the hemolysis group, 94% (15 patients) harbored a hotspot mutation at serine 34 (S34), whereas only 40% of those in the nonhemolysis cohort harbored the same hotspot mutation (22 of 55 pts), suggesting that the association between *U2AF1* and hemolysis is variant specific ($P = .0001$).

Discussion

Hemolysis in MDS is a poorly characterized phenomenon attributed to ineffective erythropoiesis and, in rare instances, autoimmunity or acquired hemoglobinopathies. Only occasionally have autoimmune cytopenias been reported in conjunction with myeloid malignancies, and the association is less known compared with lymphoproliferative disorders.[11]

Haptoglobin as a clinical variable plays a critical role as a surrogate of hemolysis. The rationale behind its use as a laboratory marker of RBC lysis comes from observational studies that showed that haptoglobin becomes depleted in the presence of higher concentrations of free hemoglobin.[12-14] In myelodysplasia, reduction in serum haptoglobin concentration has mostly been attributed to ineffective erythropoiesis resulting in intramedullary hemolysis.[15,16] In our study, the proportion of individuals presenting with anemia and who had assessment of haptoglobin levels amounted to roughly 20% of the entire MDS cohort.

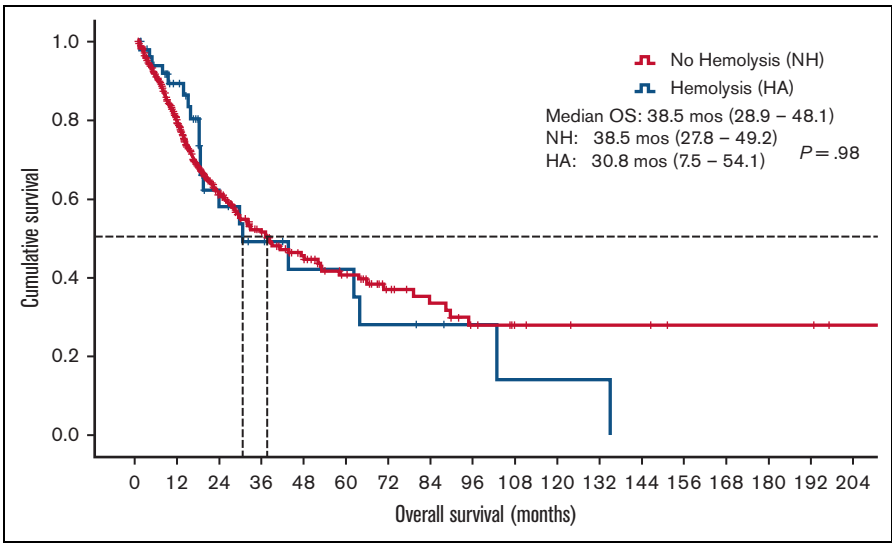

Figure 1. Estimated OS based on clinical phenotype (hemolysis vs no hemolysis).

AIHA has been reported in approximately 0.5% to 1% of patients with MDS, in which most cases seem to occur in those with low-risk disease and appear to be related to production of warm autoantibodies.[6,11,17] Rarely, red cell enzyme abnormalities expressed by aberrant clones result in clinically relevant hemolysis, a phenomenon that has been described in MDS as Coombs-negative HA.[18]

U2AF1 and *EZH2* mutations have been associated with poor outcomes among patients with myelodysplasia.[18-22] In this respect, a meta-analysis investigating the prognostic significance of *U2AF1* mutations in patients with MDS across 10 different studies showed a pooled hazard ratio (HR) for OS of 1.60 (95% CI, 1.33-1.92, $P < .001$). Along similar lines, one of the most comprehensive analyses of the mutational landscape of MDS done to date revealed a significant risk of leukemic transformation for those harboring *U2AF1* (adjusted HR, 1.28; 95% CI, 1.01-1.61) and *EZH2* mutations (adjusted HR, 1.31; 95% CI, 0.98-1.75).[18] The less favorable survival trends seen in those patients with hemolysis in our study can likely be attributed to the nature of the ancestral clones driving the disease phenotype.

U2AF1 (U2 Small Nuclear RNA Auxiliary Factor 1) is a splicing modulator gene that encodes a small subunit crucial in RNA splicing through spliceosome assembly by recognizing and binding to AG nucleotides at the 3′ splice site.[23,24] Heterozygous mutations have been reported in about 11% of patients with myelodysplasia and are more prevalent in MDS without increased ring sideroblasts.[25,26] *U2AF1* somatic mutations exclusively involve 2 amino acid positions (S34 or Q157) within the zinc finger motifs flanking the U2AF-homology motif domain.[25] Experimental *U2AF1* SF34 knock-in murine models have shown that hotspot mutations lead to differentiation defects in hematopoietic stem cells with subsequent decrease in common myeloid progenitors and

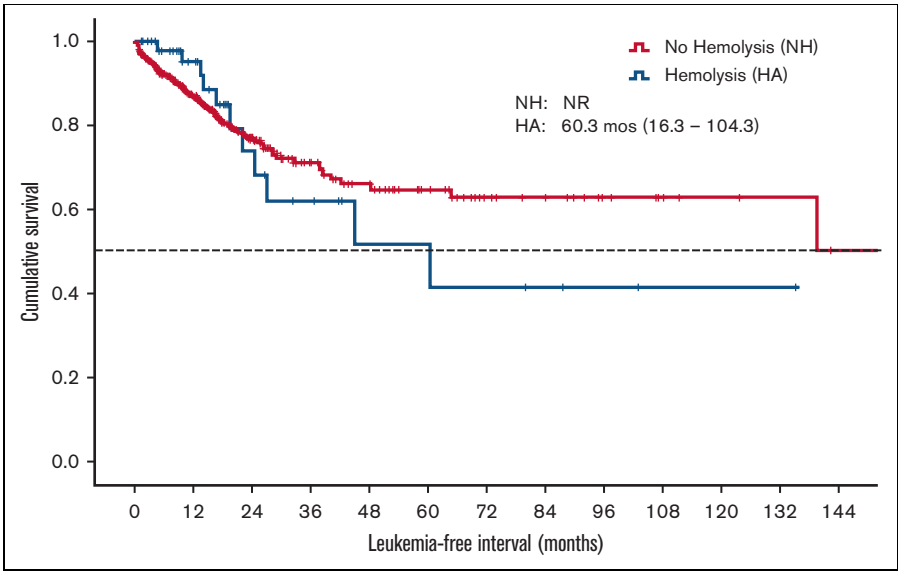

Figure 2. Estimated LFI based on clinical phenotype (hemolysis vs no hemolysis). NR, not reported.

Table 2. Mutational landscape according to disease phenotype

Mutation	Hemolysis % (n = 53)	No hemolysis % (n = 462)	*P* value
Cytokine receptor/tyrosine kinase			
FLT3-ITD	2	0.6	.11
FLT3-TKD	2	0.2	.20
KIT	0	1	.45
JAK2	2	2	.9
MPL	0	0.2	.9
RNA splicing			
U2AF1	**30**	**12**	**<.005**
SF3B1	**7**	**19**	**.03**
SRSF2	6	13	.14
ZRSR2	6	4	.5
Chromatin modification			
EZH2	**13**	**5**	**.008**
ASXL1	32	21	.08
PHF6	0	2	.4
Others			
NPM1	2	0.4	.2
SETBP1	4	3	.8
RAS signaling			
NRAS	4	2	.5
CBL	2	2	.9
DNA methylation			
IDH1	0	2	.3
DNMT3A	17	13	.36
IDH2	2	3	.64
TET2	25	22	.7
Transcription			
ETV6	6	3	.5
RUNX1	13	12	.75
Checkpoint cycle			
TP53	17	23	.3

megakaryocyte-erythrocyte lineage-restricted progenitors, leading to ineffective hematopoiesis and marrow failure manifested as severe leukopenia with neutrophil hypersegmentation and erythroid dysplasia.[26] More importantly, the link with *U2AF1* and erythropoiesis was further explored in a series of assays by Zhang et al, who demonstrated that *U2AF1* plays a critical role in erythropoiesis through regulation of alternative splicing, which was further highlighted by the fact that it was significantly expressed in the progenitor burst-forming-unit and colony-forming-unit stages of erythroid maturation.[27]

Among *U2AF1* mutations, S34 variants seem to have better outcomes relative to those harboring mutations in codons Q157 or R156, particularly among lower-risk individuals.[22] This may also be related to co-mutational patterns not captured in our analysis. Teffari et al, as an example, demonstrated a higher incidence of *ASXL1* mutations coinciding with *U2AF1* Q157 variants, but not with S34.[28] *ASXL1* mutations have long been implicated with worse outcomes in multiple studies.[18,19,29]

Mutations in genes encoding splicing factors are known to be mutually exclusive (meaning *SF3B1* and *U2AF1* mutations are unlikely to coexist).[22,30,31] Despite both genes belonging to the same functional class and having similar phylogeny and biological functions, because of differential splice site recognition, *U2AF1* likely affects different downstream targets relative to *SF3B1*, resulting in distinct lineage-specific splicing alterations and clinical phenotypes.[22]

EZH2 (Enhancer of Zeste homolog 2), a histone methyltransferase, acts as a critical epigenetic regulator involved in stem cell self-renewal, maintenance, and cell differentiation through histone H3 methylation and recruitment of DNA methyltransferases to regulate development and lineage commitment in embryonic and adult stem cells.[32] Heterozygous mutations have been reported in about 4% to 6% of patients with MDS and has been associated with worse outcomes.[18,33] In the context of hematopoiesis, *EZH2* knock-in murine models have shown that enhanced expression causes increased number of repopulating hematopoietic stem cells, which results in a myeloproliferative phenotype that features leukocytosis, myeloid expansion, and splenomegaly.[32]

We hypothesize that *U2AF1* splicing mutations may lead to red cell enzymopathy or alteration of RBC membrane leading to hemolysis. Co-mutational patterns unequivocally may play a crucial role in further defining distinctive clinical phenotypes among spliceosome mutations, which, in our case, were defined by presence or absence of Coombs-negative hemolysis.

Characterization of RBCs isolated from patients with MDS with evidence of Coombs-negative hemolysis suggests they may have different metabolic properties that make them prone to destruction. In this regard, Van der Weyden et al were the first to report a case of MDS in which dysplastic RBCs had an adenosine deaminase (ADA) expression that was elevated eightfold relative to the ADA activity of RBCs of patients with hemolysis from AIHA or hereditary spherocytosis.[34] The metabolic features of the patient's RBCs had remarkable similarity to those from patients with a congenital hyperactive ADA hemolytic disorder, which presents with mild hemolysis because of an intracorpuscular defect associated with intraerythroid ADA overexpression and decreased adenosine triphosphate production.[34] The increased intracellular ADA was deemed to be unrelated to structural chromosomal aberrations given that its locus is on chromosome 20 and the patient had a 5q deletion.[34]

Morphologically, structural abnormalities have been described in rare instances in patients with myelodysplasia, such as acquired elliptocytosis in which the cardinal pathophysiological mechanism alludes to decreased expression of protein 4.1 (encoded by the *EPB41* gene), a major structural component of the RBC membrane responsible for regulating erythrocyte deformability and mechanical stability through actin-spectrin interactions.[35,36] Approximately 75% of all reported cases seem to denote an association between acquired elliptocytosis and del(20q), with no knowledge as to why this observation remains so rare despite del(20q) being a commonly reported cytogenetic abnormality.[35-38] In our study, examination of a small number of peripheral smears from the subset of patients with HA-MDS showed

significant poikiloanisocytosis, which seems to corroborate this hypothesis.

As for other forms of acquired hemoglobinopathies in MDS, there have been rare reports of somatic mutations involving *ATRX*, a chromatin remodeling factor linked to chromosome X, resulting in decreased alpha globin expression and an alpha thalassemia phenotype.[39] Less common are somatic mutations resulting in clonal deletion of the alpha globin cluster on the short arm of chromosome 16.[39] Both events result in a clinical entity that has been described as acquired alpha thalassemia/myelodysplastic syndrome (ATMDS).[40] In comparison, reports of acquired beta-thalassemia (BTMDS) remain scarce, with at least one instance attributing its phenotype to HBB haploinsufficiency resulting from loss of chromosome 11p in aberrant clones.[40]

Differences in survival trends between hemolysis and nonhemolysis cohorts could be explained by examining the molecular landscape driving clonal hematopoiesis in both phenotypic subsets of the disease. Patients with hemolysis were more likely to harbor *U2AF1* and *EZH2* mutations, relative to the *SF3B1* mutations more commonly seen in the nonhemolysis cohort.

Regarding responses seen to HMA, our findings seem to contrast with what has been reported in the literature. In this respect, a study by Nazha et al exploring potential genomic biomarkers predictive of HMA resistance among patients with MDS revealed higher rates of treatment resistance among those harboring *EZH2* mutations ($P = .04$).[29] In contrast, the presence of *SF3B1* or *U2AF1* mutations did not seem to predict treatment response in other studies.[20,41] The discordance between our observations and other clinical reports could be explained by the differential effect in treatment susceptibilities caused by distinctive co-mutational patterns accompanying one of the canonical spliceosome mutations (*SF3B1* or *U2AF1*).

There were several limitations to our study. First, the small proportion of patients identified as having hemolysis (54 among 516 patients with MDS) may have compromised our ability to detect significant differences with respect to other measurements such as response to therapy, OS, and LFI. Second, lack of archived peripheral blood smears and lack of serial haptoglobin measurements were also noteworthy. Finally, we did not capture additional co-mutation patterns involving synchronic canonical mutations of potential interest nor identified whether the size of the dominant clones alluded to ancestral or subclonal ontogenies.

To our knowledge, this represents the first major study attempting to illustrate a correlation between nonimmune hemolysis in myelodysplastic syndromes and molecular data. Consequently, our analysis serves to highlight that somatic mutations involving 3 canonical genes affecting chromatin remodeling and spliceosome assembly in MDSs (*EZH2*, *U2AF1*, and *SF3B1*) appear to affect the biology and clinical phenotype of myelodysplasia in distinctive ways through complex interactions among combinations of genetic and epigenetic insults.

Conclusion

Nonimmune hemolysis is a clinicopathological phenomenon that has been described in MDS. Our data show that ~10% of patients with MDS develop clinical features of hemolysis. Inferior survival trends were observed among lower-risk patients with MDS with hemolysis. Furthermore, there was a lower trend for responses to ESA and higher trend for responses to HMA in the hemolytic cohort. *U2AF1* mutations were observed in 30% of patients with hemolysis and occurred almost exclusively at the S34 hotspot. Our data suggest that nonimmune hemolysis is associated with allele-specific somatic mutations. It appears that synergism between mutant alleles encoding splicing factors and erythrocyte membrane components, among other interactions, may account for structural abnormalities, change in biochemical properties, and metabolic RBC dysfunction, which underpin the aberrant development of atypical clones from erythroid precursors in MDS presenting phenotypically with hemolysis. Future studies will explore the contribution of altered splicing to these acquired hemoglobinopathies.

Authorship

Contribution: R.K. and L.E.A. contributed to conception and design; R.K. and N.A.-A. contributed to administrative support; R.K., L.E.A., D.S., E.P., and N.A.-A. contributed to provision of study materials or patients; N.A.-A., R.K., and L.E.A. collected and assembled data; R.K., L.E.A., and N.A.-A. interpreted and analyzed data; and all authors contributed to manuscript writing, gave the final approval of manuscript, and completed the STROBE reporting checklist.

Conflict-of-interest disclosure: R.K. reports honoraria from BMS, Novartis, Incyte, Jazz Pharmaceuticals, AbbVie, Agios, Acceleron, and Geron, and speaker's bureau with BMS, Jazz, and Agios. D.S. reports research funding from Celgene and Jazz Pharmaceuticals; and consultancy agreements with Agios, Bristol-Myers Squibb, Celyad Oncology, Incyte, Intellia Therapeutics, Kite Pharma, Novartis, and Syndax. D.R. reports board membership with NanoString. E.P. reports honoraria from Novartis and research funding from Incyte, Kura, and BMS. The remaining authors declare no competing financial interests.

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