Prognostic impact of *SF3B1* mutation and multilineage dysplasia in myelodysplastic syndromes with ring sideroblasts: a Mayo Clinic study of 170 informative cases

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Abstract

The revised 4th edition of the World Health Organization (WHO4R) classification lists myelodysplastic syndromes with ring sideroblasts (MDS-RS) as a separate entity with single lineage (MDS-RS-SLD) or multilineage (MDS-RS-MLD) dysplasia. The more recent International Consensus Classification (ICC) distinguishes between MDS with *SF3B1* mutation (MDS-SF3B1) and MDS-RS without *SF3B1* mutation; the latter is instead included under the category of MDS not otherwise specified. The current study includes 170 Mayo Clinic patients with WHO4R-defined MDS-RS, including MDS-RS-SLD (N=83) and MDS-RS-MLD (N=87); a subset of 145 patients were also evaluable for the presence of *SF3B1* and other mutations, including 126 with (87%) and 19 (13%) without *SF3B1* mutation. Median overall survival for all 170 patients was 6.6 years with 5- and 10-year survival rates of 59% and 25%, respectively. A significant difference in overall survival was apparent between MDS-RS-MLD and MDS-RS-SLD (*P*<0.01) but not between MDS-RS with and without *SF3B1* mutation (*P*=0.36). Multivariable analysis confirmed the independent prognostic contribution of MLD (hazard ratio=1.8, 95% confidence interval: 1.1-2.8; *P*=0.01) and also identified age (*P*<0.01), transfusion need at diagnosis (*P*<0.01), and abnormal karyotype (*P*<0.01), as additional risk factors; the impact from *SF3B1* or other mutations was not significant. Leukemia-free survival was independently affected by abnormal karyotype (*P*<0.01), *RUNX1* (*P*=0.02) and *IDH1* (*P*=0.01) mutations, but not by MLD or *SF3B1* mutation. Exclusion of patients not meeting ICC-criteria for MDS-*SF3B1* did not change the observations on overall survival. MLD-based, as opposed to *SF3B1* mutation-based, disease classification for MDS-RS might be prognostically more relevant.

Introduction

The entity of refractory anemia with ring sideroblasts (MDS-RS) has been well codified for several decades.[1] According to the 2016/17 World Health Organization (WHO) classification system (revised 4th edition; WHO4R),[2] myelodysplastic syndrome with ring sideroblasts (MDS-RS) was listed as a subcategory of MDS, primarily characterized by the presence of ≥15% ring sideroblasts, in bone marrow (BM) erythroid precursors; additional diagnostic criteria included the absence of ≥5% BM myeloblasts, among nucleated BM cells, or ≥1% peripheral blood (PB) myeloblasts, among PB leukocytes, Auer rods, and diagnostic criteria for MDS with isolated del(5q); of note, the presence of *SF3B1* mutation was used to lower the diagnostically required ring sideroblasts threshold to 5%. The new 2022 International Consensus Classification (ICC-2022) of Myeloid Neoplasms and Acute Leukemias, which represents revision of the WHO4R document, considered *SF3B1* mutation over and above ring sideroblasts in defining a more homogeneous group and have thus replaced the term MDS-RS with MDS-*SF3B1*;[3] diagnostic criteria for the latter required the presence of *SF3B1* mutation (≥variant allele frequency [VAF] 10%), absence of multi-hit *TP53* or *RUNX1* mutation, absence of isolated del(5q), -7/del(7q), abn3q26.2, or complex karyotype, and absence of BM/PB myeloblasts ≥5%/2%, but did not include ring sideroblast percentage. MDS-RS without *SF3B1* mutation has accordingly been relocated to the subcategory of MDS-not otherwise specified (MDS-NOS), regardless

of the percentage of BM ring sideroblasts. MDS-NOS, according to ICC-2022, includes MDS with single lineage (SLD), multilineage (MLD), or no dysplasia.[3] The rationale stated for these changes included the assumption that genetic risk stratification superseded the effect from morphologic distinction between MDS-RS-SLD (Figure 1) and MDS-RS-MLD (Figure 2). The current study examined these assumptions in a retrospective cohort of 170 patients with WHO4R-defined MDS-RS, in the context of presenting features and impact on survival.

Methods

The current study was approved by the Mayo Clinic institutional review board. Study patients were selected from institutional databases based on retrospective review of clinical and laboratory information and confirmation of MDS-RS diagnosis, according to WHO4R criteria.[2] Conventional methods were used for cytogenetic and next-generation sequencing (NGS) studies. Targeted exome sequencing included the following genes: *TET2*, *ASXL1*, *DNMT3A*, *IDH1*, *IDH2*, *TP53*, *SRSF2, SF3B1, SH2B3, NPM1, FLT3, U2AF1, ZRSR2, JAK2, CSF3R, MPL, MFSD11, CEBPA, SETBP1, ZRSR2, RUNX1, IKZF1, CALR, KRAS, NRAS, CBL, PTPN11, STAG2, BCOR*, and *GATA2*, and was performed on diagnostic BM specimens. Wright-Giemsa stain was used to stain PB and BM smears. Prussian blue iron stain was used to detect iron reserves and siderotic granulation in BM smears. The characteristics of dysplasia were classified using established criteria.[4] Dysplasia in more than 10% of cells involving two or three lineages was described as MLD whereas dysplasia in more than 10% of erythroid-lineage cells was characterized as SLD. Erythroblasts with at least five siderotic granules encompassing at least a third of the nuclear circumference were classified as ring sideroblasts. Treatment administered was at the discretion of the treating physician and in concert with standard practice and included erythropoiesis stimulating agents (ESA), often as first-line and several other drugs, often as second- or third-line treatment, including luspatercept (N=41), lenalidomide (N=26), and hypomethylating agents (HMA; N=38). Non-parametric tests were used to compare the distributions of continuous variables, whereas the χ^2 test was used to compare the distributions of nominal variables. The time from diagnosis to death or last follow-up was used to calculate overall survival. Leukemia-free survival was calculated from the time of diagnosis to the date of leukemia transformation, death or the last day of follow-up. For univariate comparisons, the Kaplan–Meier approach was utilized in time-to-event analysis. For univariate and multivariate analysis of overall and leukemia-free survival, the Cox proportional hazard regression model was utilized. *P* value ≤0.05 was consid-

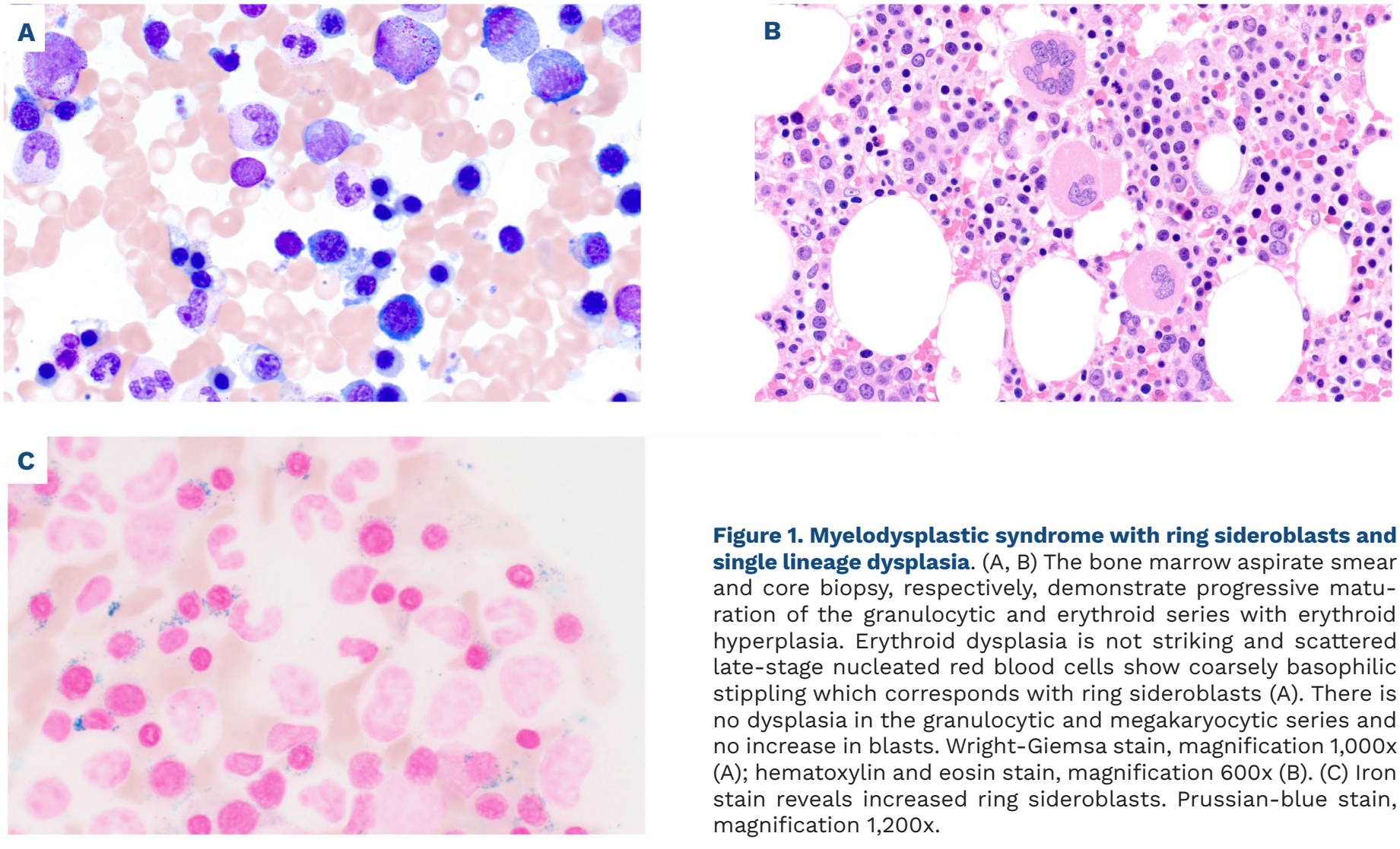

Figure 1. Myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia. (A, B) The bone marrow aspirate smear and core biopsy, respectively, demonstrate progressive maturation of the granulocytic and erythroid series with erythroid hyperplasia. Erythroid dysplasia is not striking and scattered late-stage nucleated red blood cells show coarsely basophilic stippling which corresponds with ring sideroblasts (A). There is no dysplasia in the granulocytic and megakaryocytic series and no increase in blasts. Wright-Giemsa stain, magnification 1,000x (A); hematoxylin and eosin stain, magnification 600x (B). (C) Iron stain reveals increased ring sideroblasts. Prussian-blue stain, magnification 1,200x.

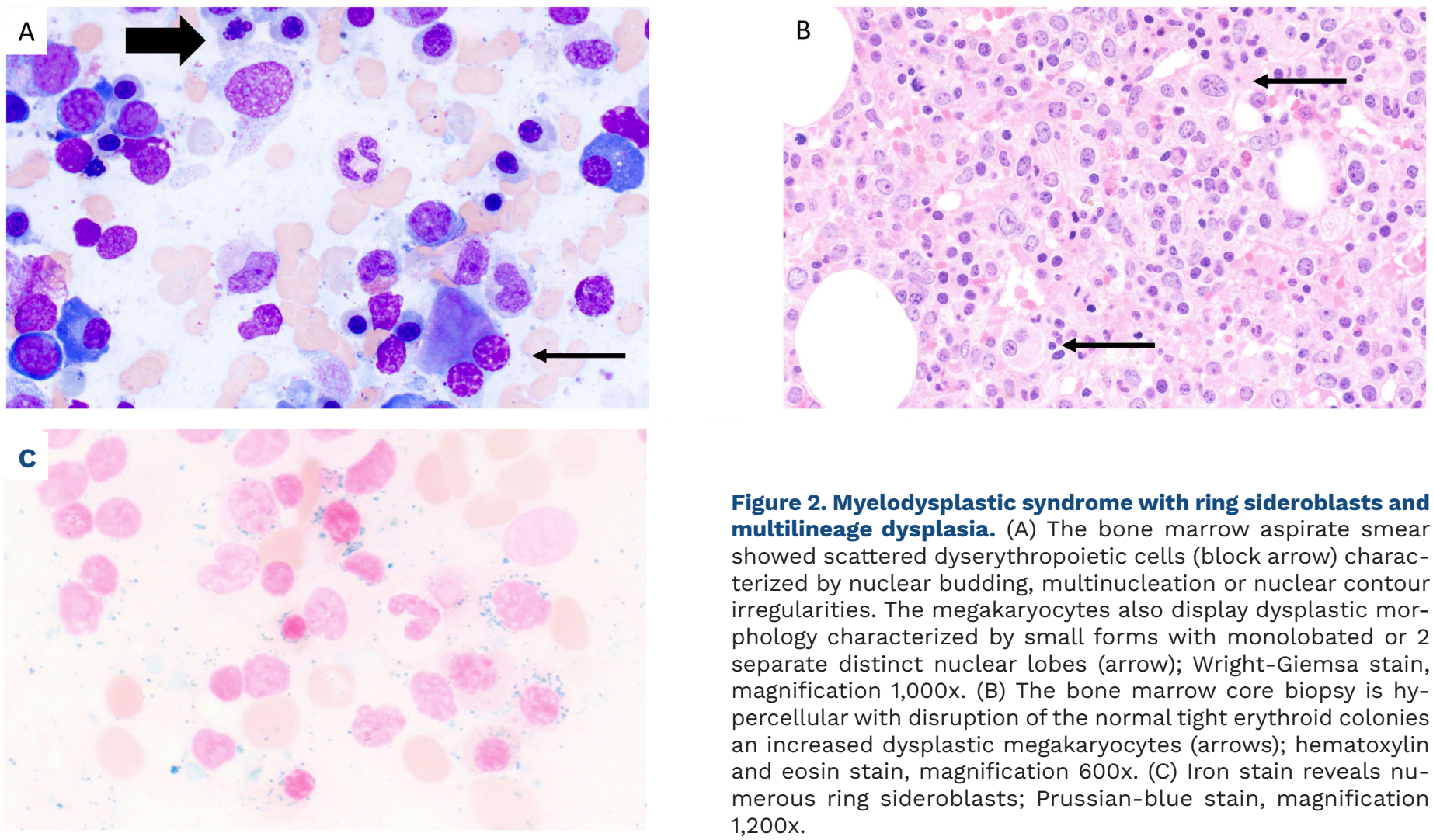

Figure 2. Myelodysplastic syndrome with ring sideroblasts and multilineage dysplasia. (A) The bone marrow aspirate smear showed scattered dyserythropoietic cells (block arrow) characterized by nuclear budding, multinucleation or nuclear contour irregularities. The megakaryocytes also display dysplastic morphology characterized by small forms with monolobated or 2 separate distinct nuclear lobes (arrow); Wright-Giemsa stain, magnification 1,000x. (B) The bone marrow core biopsy is hypercellular with disruption of the normal tight erythroid colonies an increased dysplastic megakaryocytes (arrows); hematoxylin and eosin stain, magnification 600x. (C) Iron stain reveals numerous ring sideroblasts; Prussian-blue stain, magnification 1,200x.

ered significant. JMP Pro 16.0.0 software package, SAS Institute, Cary, NC was utilized for all analyses.

Results

Phenotypic and genotypic comparisons between myelodysplastic syndrome with ring sideroblasts multilineage and myelodysplastic syndrome with ring sideroblasts single lineage

Online Supplementary Table S1 outlines the presenting clinical and laboratory features, along with postdiagnosis events, in 170 Mayo Clinic patients with WHO4R-defined MDS-RS (median age 73 years; range, 41-94; males 67%): 87 (51%) were classified as MDS-RS-MLD (median age 74 years; range, 47-94; males 71%) and 83 (49%) as MDS-RS-SLD (median age 72; range, 41-89; males 63%); there was no significant difference between the two morphologic variants in age and sex distribution. Patients with MDS-RS-MLD, compared to those with MDS-RS-SLD, were more likely to display lower leukocyte count (median $4.4x10^9$/L *vs.* $5.6x10^9$/L; $P<0.01$), lower absolute lymphocyte count (ALC; median $1.2x10^9$/L *vs.* $1.6x10^9$/L; $P<0.01$), lower absolute neutrophil count (ANC; median $2.6x10^9$/L *vs.* $3x10^9$/L; $P=0.06$), and lower platelet count (median $202x10^9$/L *vs.* $275x10^9$/L). Patients with MDS-RS-MLD were also more likely to display neutropenia (ANC $<1x10^9$/L in 10% *vs.* 2%; $P=0.02$); lymphopenia (ALC $<1.2x10^9$/L 47% *vs.* 31%; $P=0.04$), and thrombocytopenia (platelets $<100x10^9$/L in 18% *vs.* 4%; $P<0.01$); a non-significant association for MDS-RS-MLD was also apparent for a lower hemoglobin level ($P=0.19$) and a higher incidence of transfusion need at diagnosis ($P=0.19$). Although not at a significant level, MDS-RS-SLD was associated with higher percentage of BM ring sideroblasts ($P=0.11$; *Online Supplementary Table S1*). BM and PB blast percentages were similar between the two morphologic cohorts; BM median 1 (range, 0-4) and PB median 0 (range, 0-1) (*Online Supplementary Table S1*). Cytogenetic information was available in 166 patients of whom 121 (73%) displayed normal karyotype, nine (5%) loss of Y chromosome only, eight (5%) sole trisomy 8, seven (4%) sole del(20q), five (3%) complex karyotype, three (2%) -7/7q- abnormality, and 13 (8%) other abnormalities. The frequency of abnormal karyotype other than LOY was 31% in MDS-RS-MLD *versus* 12% in MDS-RS-SLD ($P<0.01$); as depicted in *Online Supplementary Table S1*, the significant difference in abnormal karyotype distribution was mostly attributed to complex/-7/7q- abnormalities seen in eight (10%) of the 84 evaluable patients with MDS-RS-MLD and none of the 82 evaluable patients with MDS-RS-SLD ($P<0.01$). NGS information was available in 145 patients; mutational frequencies were 87% for *SF3B1*, 25% for *TET2*, 19% for *DNMT3A*, 11% for *ASXL1*, 5% for *SRSF2*, 5% for *TP53*, and 2% each for *IDH1* and *CSF3R*; as depicted in *Online Supplementary Table S1* and unlike the case with karyotype, there were no significant differences between

the two morphologic groups of MDS-RS in regard to the distribution of these mutations.

Phenotypic and genotypic comparisons between *SF3B1*-mutated and -unmutated myelodysplastic syndrome with ring sideroblasts

NGS information, including *SF3B1* mutational status, was available in 145 of the 170 study patients. *Online Supplementary Table S2* outlines presenting features stratified by the presence or absence of *SF3B1* mutation, including 126 (87%) with and 19 (13%) without the mutation. *SF3B1* mutation was more likely to be associated with higher leukocyte (P<0.01), neutrophil (P<0.01), lymphocyte (P=0.03), monocyte (P=0.02), and platelet (P<0.01) counts and less likely to be associated with neutropenia (P=0.02), lymphopenia (P<0.01), or thrombocytopenia (P<0.01). Unlike the case with SLD *versus* MLD morphologic variants of MDS-RS, differences in karyotype distribution between *SF3B1*-mutated and -unmutated MDS-RS cases were not as pronounced while NGS-derived mutation distribution revealed significant clustering of wild-type *SF3B1* with *SRSF2* (26% *vs.* 2% mutational frequency in *SF3B1*-mutated cases; P<0.01), *TP53* (16% *vs.* 3%; P=0.04), *RUNX1* (10% *vs.* 25; P<0.01), *IDH1* (16% *vs.* 0%; P<0.01), and *U2AF1* (10% *vs.* 0%; P=0.01) mutations (*Online Supplementary Table S2*). In addition, borderline significance was apparent for higher prevalence of normal karyotype in *SF3B1*-mutated cases (79% *vs.* 58%) and that of complex karyotype in patients with wild-type SF3B1 (16% *vs.* 2%; *Online Supplementary Table S2*).

Impact of multilineage or *SF3B1* mutation on overall and leukemia-free survival in revised 4th edition of the World Health Organization-defined myelodysplastic syndrome with ring sideroblasts

At a median follow-up of 5.2 years (range, 0.1-12.6) for living patients, 104 (61%) deaths, eight (5%) leukemic transformations, and seven (5%) allogeneic hematopoietic stem cell transplantations (AHSCT) were documented (*Online Supplementary Table S1*). There were significantly more deaths among patients with MDS-RS-MLD *versus* MDS-RS-SLD (72% *vs.* 49%; P<0.01; *Online Supplementary Table S1*) while this was not the case during comparison of *SF3B1*-mutated *versus* wild-type cases (63% *vs.* 47%; P=0.2; *Online Supplementary Table S2*). Median overall survival for all 170 patients was 6.6 years with 5- and 10-year survival rates of 59% and 25%, respectively (Figure 3A); the corresponding figures for MDS-RS-SLD were 7 years, 67%, and 31%; for MDS-RS-MLD, 5.5 years, 52%, and 17% (Figure 3B); for MDS-RS with *SF3B1* mutation 6.8 years, 64%, and 26%; and MDS-RS with wild-type *SF3B1* 9.7 years, 52% and 0% (Figure 3C). Figure 3B reveals a significant difference in overall survival between MDS-RS-MLD and MDS-RS-SLD (hazard ratio [HR]=1.7, 95% confidence interval [CI]: 1.14-2.5; P<0.01) while such was not the case when comparing MDS-RS with and without *SF3B1* mutation (P=0.36; Figure 3C).

The difference in overall survival between MDS-RS-MLD and MDS-RS-SLD was confirmed by multivariable analysis that included, individually, *SF3B1* mutational status, age, ANC <1x10^9/L, ALC <1.2x10^9/L, platelets <100x10^9/L, red cell transfusion need at diagnosis, abnormal karyotype, and *TP53* mutation; significance was also sustained (HR=1.8, 95% CI: 1.1-2.8; P=0.01) in an all-inclusive multivariable analysis that included SF3B1 mutational status, karyotype, transfusion need at diagnosis, age, ANC, ALC, and platelet count, with the later four entered as continuous variables; additional independent risk factors in the latter analysis included age (P<0.01), transfusion need at diagnosis (P<0.01), and abnormal karyotype (P<0.01) and the results were not influenced by the addition of mutation information in the multivariable model: *SF3B1* (P=0.48); *TP53* (P=0.22); *SRSF2* (P=0.97); *IDH1* (P=0.93); *RUNX1* (P=0.47); or *U2AF1* (P=0.22).

There was borderline significance for a shorter leukemia-free survival in patients with MDS-RS-MLD *versus* MDS-RS-SLD (HR=3.3, 95% CI: 0.7-16.2; P=0.15) while a significant difference was apparent when comparing cases with wild-type *versus* mutated *SF3B1* (HR=8.2, 95% CI: 1.1-58.7; P=0.03). However, the latter significance was lost during multivariable analysis that included other mutations that clustered with *SF3B1* mutation including *RUNX1* (HR=53.7), *IDH1* (HR=54.9), and *TP53* (HR=22.4); no other mutation or clinical variable (e.g., age, transfusion need, neutrophil, lymphocyte, or platelet count) displayed additional prognostic significance for leukemia-free survival. Furthermore, *RUNX1* (P=0.02) and *IDH1* (P=0.01), but not *TP53* (P=0.35) mutations retained their significance for shortened leukemia-free survival when karyotype was added to the multivariable model with abnormal karyotype showing additional prognostic contribution (P<0.01).

Re-analysis of survival impact after exclusion of patients not meeting International Consensus Classification-2022 criteria for myelodysplastic syndromes with *SF3B1* mutation

In order to address the confounding effect of revised criteria for diagnosis of MDS-SF3B1,[3] we repeated the above outlined analyses after limiting the study population to patients with available NGS information (N=145) and excluding those with complex (N=5) or -7/7q- (N=3) cytogenetic abnormalities, multi-hit *TP53* (N=2) or *RUNX1* (N=5) mutations, and *SF3B1* VAF ≥10% (N=3). After these adjustments, 130 patients were evaluable for further analysis with 63 MDS-RS-MLD and 67 MDS-RS-SLD cases; and 115 with and 15 without *SF3B1* mutation. In univariate analysis, overall survival was similar between patients with and without *SF3B1* mutation (P=0.92) but significantly worse in those with MLD compared to SLD (P=0.04). Multivariable analysis for overall survival demonstrated the significant difference

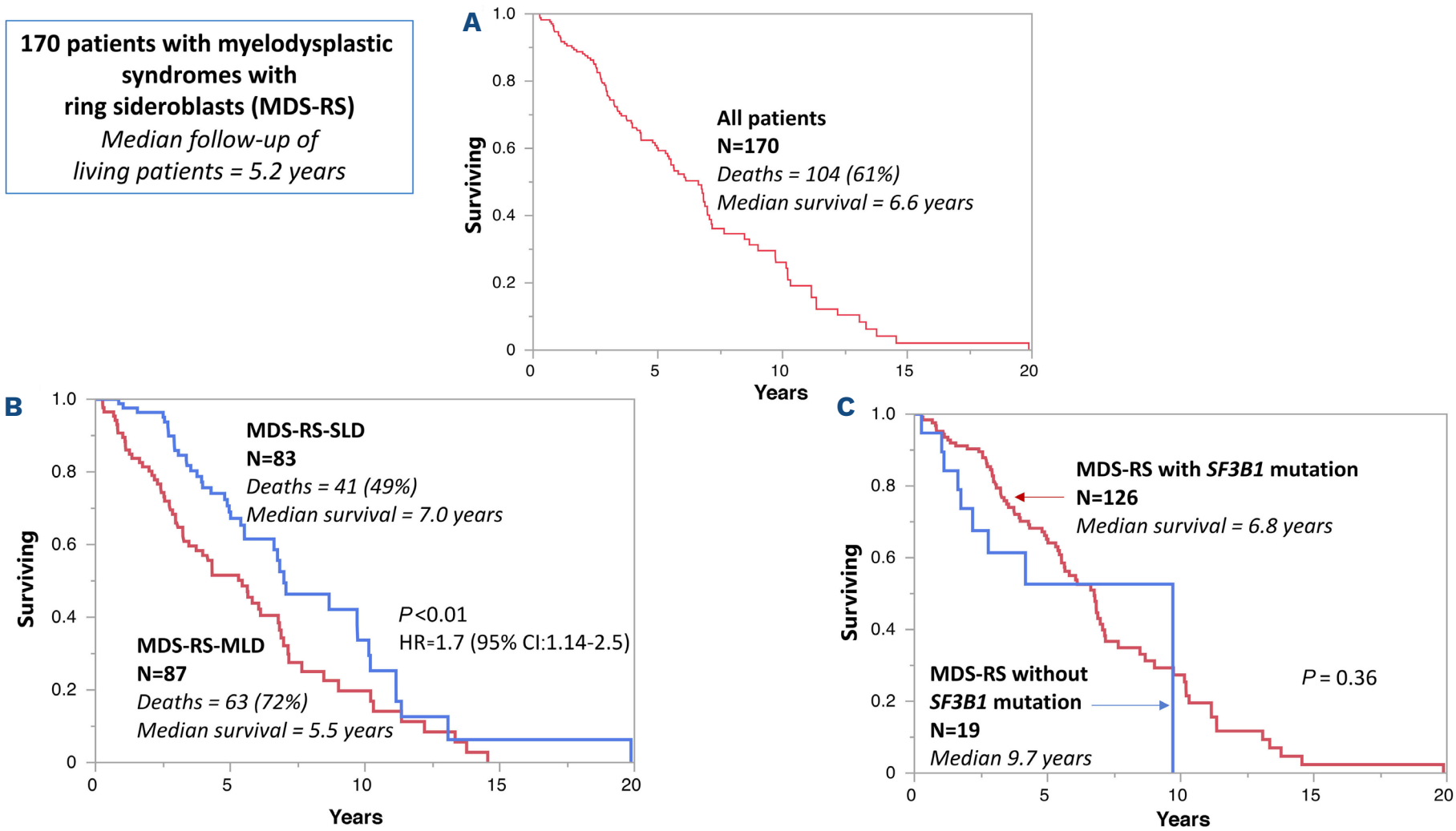

Figure 3. Overall survival among 170 patients with myelodysplastic syndrome with ring sideroblasts. (A) All patients; (B) all 170 patients stratified by single lineage *versus* multilineage dysplasia; and (C) a subset of 145 patients with information on *SF3B1* mutation available.

in overall survival between MDS-RS-MLD and MDS-RS-SLD was independent of age, karyotype, and transfusion-need at diagnosis, with all four variables remaining significant: P=0.02, P<0.01, P<0.01, and P<0.01, respectively. A similar analysis for leukemia-free survival was handicapped by the small number of informative cases that included only three incidents of leukemic transformation with one of the three harboring *TP53* mutation (P=0.08).

Impact of multilineage dysplasia or *SF3B1* mutation on treatment response

Accurate treatment information was available for luspatercept (N=41), revlimid (N=26), and hypomethylating agents (N=38). These drugs were often used after failure of prior therapy with ESA, which was historically mentioned in 121 patients, including 64 (74%) with MDS-RS-MLD and 57 (69%) with MDS-RS-SLD (P=0.48). Overall anemia response rates were 17% (7/41 patients) for luspatercept, 19% (5/26 patients) for revlimid, and 45% (17/38 patients) for HMA; the corresponding response rates in patients with MDS-RS-MLD *versus* MDS-RS-SLD were 16% (5/31 patients) *versus* 20% (2/10 patients) for luspatercept (P=0.78), 25% (4/16 patients) *versus* 10% (1/10 patients) for revlimid (P=0.33), and 46% (12/26 patients) *versus* 42% (5/12 patients) for HMA (P=0.8). A similar analysis comparing *SF3B1*-mutated *versus* wild-type patients showed luspatercept response in 13% (4/31 patients) *versus* 17% (1/6 patients; P=0.8), revlimid response in 26% (5/19 patients) *versus* 0% (0/3 patients; P=0.2), and HMA response in 32% (8/25 patients) *versus* 50% (3/6 patients; P=0.4), respectively. Similar data on treatment response to ESA were not available.

Discussion

The presence of ring sideroblasts in low-risk MDS has traditionally been considered as a marker of favorable prognosis in terms of both overall and leukemia-free survival, despite progressive anemia and red cell transfusion need in the majority of cases.[4-6] In a recently published natural history study of 138 patients with low-risk MDS with ≥5% ring sideroblasts, 65% of patients became red cell transfusion-dependent in their first 5 years of study enrollment with 42% deaths and 14% leukemic progressions reported during the same period.[6] In our own previously published series of 76 patients with WHO4R-defined MDS-RS and followed for a median of 33 months, median overall survival was 46 months and leukemic transformation rate 3%.[5] In the current study, we have expanded our WHO4R-defined MDS-RS patient population to 170 cases and the follow-up period to 5.2 years, in living patients; median overall survival was 6.6 years with 5-year and

10-year survival rates of 59% and 25%, respectively (Figure 3A); the incidence of leukemic transformation was 5%.

Most cases of MDS-RS are associated with an *SF3B1* mutation,[7] which is now considered a characteristic feature of the disease in whose presence diagnosis of MDS-RS can be made with BM ring sideroblast percentage >5% rather than the ≥15% threshold otherwise required.[3] WHO4R classification also considered the number of lineages exhibiting morphologic dysplasia, in order to subclassify MDS-RS into two morphologic subcategories: MDS-RS-MLD and MDS-RS-SLD.[2] It is to be recalled that MDS-RS-SLD was previously recognized as refractory anemia with ringed sideroblasts (RARS) and MDS-RS-MLD as refractory cytopenia with multi-lineage dysplasia (RCMD), according to the 2008 WHO classification system (4th edition).[8] Microscopically, MDS-RS-SLD exhibits increased BM erythroid precursors associated with erythroid-lineage dysplasia, without associated dysplasia in granulocytes or megakaryocytes (i.e., <10% dysplastic forms; Figure 1). MDS-RS-MLD also exhibits erythroid-lineage dysplasia but also ≥10% dysplastic forms in granulocyte (e.g., nuclear hypolobulation) or megakaryocyte (e.g., micro-megakaryocytes, nuclear hypolobulation) lineages (Figure 2).[2]

ICC-2022 considered *SF3B1* mutation over and above ring-sideroblasts in defining a more homogeneous group of low-risk MDS and have thus replaced the term MDS-RS with MDS-*SF3B1*.[3] MDS-RS cases without *SF3B1* mutations are now included in the ICC category of MDS-NOS, which also includes the subcategories of MDS-NOS with unilineage, multilineage, or no dysplasia, regardless of the percentage of BM ring sideroblasts.[3] By contrast, the proposed 5th edition of the WHO classification (WHO5) considers the distinction between SLD and MLD optional.[9] The rationale stated for the ICC-2022 changes included the assumption that genetic risk stratification superseded the effect from morphologic distinction between MDS-RS-SLD and MDS-RS-MLD.[4] In support of this assumption, Malcovati *et al*. found similar survival between *SF3B1*-mutated MDS patients with SLD *versus* MLD (P=0.4) while the same group of patients stratified by BM blast percentage at 5% resulted in significantly different survival (P<0.01).[10] In the same study, the authors showed significantly longer survival for *SF3B1*-mutated *versus* unmutated cases in a spectrum of MDS subcategories, including RARS and RCMD-RS, with the exception of those with excess blasts.[10] In contrast to the aforementioned study by Malcovati *et al.*, we have, in the past, repeatedly failed to demonstrate an independent prognostic effect from *SF3B1* mutations in the context of WHO4R-defined MDS-RS. In the first (published in 2012) of several related work in MDS-RS, we examined the phenotypic and prognostic relevance of BM ring sideroblast percentage in an otherwise loosely-defined MDS-RS (MDS without excess blasts and RS% ≥1%);[11] we reported direct correlation of ring sideroblast percentage with age, platelet count, transfusion need and *SF3B1* mutational frequency and inverse correlation with hemoglobin level, multilineage dysplasia and high-risk karyotype; more importantly, ring sideroblast percentage did not affect overall or leukemia-free survival. In a similar work published the same year (2012), we examined the prognostic interaction between *SF3B1* mutation, morphology, and karyotype in MDS patients with ≥15% ring sideroblasts, regardless of BM blast content;[12] in the particular study, *SF3B1* mutations did not display MLD-independent prognostic value, which was otherwise suggested in univariate analysis. Similarly, in a 2018-published study of 76 patients with MDS-RS,[5] including 57 with MDS-RS-SLD and 19 with MDS-RS-MLD, we reported higher frequency of *SF3B*1 and *DNMT3A* mutations in the latter with no difference in overall survival, which was otherwise adversely affected by the presence of *ASXL1* or absence of *SF3B1* mutation. In a more recent communication,[13] we reported similar survival in *SF3B1*-mutated MDS and *SF3B1*-mutated MDS/MPN, which otherwise shared similar mutational landscape, with the exception of higher frequency of *JAK2* mutations in the latter.

The current manuscript includes the largest (N=170) single institutional cohort of WHO4R-defined MDS-RS with the objective to clarify the phenotype and genotype correlates of multilineage dysplasia and its impact on long-term survival, in the context of *SF3B1* mutation and karyotype. The key observation from the current study was in regard to the independent prognostic relevance of MLD to overall survival in WHO4R-defined MDS-RS. In our contemporary study population, we were able to confirm the adverse survival impact of MLD in the context of other risk factors, including age, transfusion need at time of diagnosis, abnormal karyotype with or without inclusion of complex/-7/7q- abnormalities, previously recognized high-risk mutations, lymphopenia, neutropenia, and thrombocytopenia. By contrast, we were not able to demonstrate prognostic contribution from *SF3B1* mutation, regardless of whether or not more recent criteria[3] for its diagnosis were applied. This was despite the fact that wild-type *SF3B1* was associated with adverse disease features, including high-risk mutations, thrombocytopenia, lymphopenia, and neutropenia (*Online Supplementary Table S2*). Of note, MLD was also associated with some of these adverse features and, more importantly, with adverse karyotype (*Online Supplementary Table S1*), but still showed an independent adverse effect on overall survival. In addition to MLD, our study highlights the prominent prognostic contribution from abnormal karyotype, both in terms of overall and leukemia-free survival, an effect that was mostly attributed to complex karyotype and -7/7q- abnormalities. The latter observation is in line with the ICC-2022 criteria for diagnosis of MDS-*SF3B1*, which requires exclusion of cases with the specific cytogenetic abnormalities.[3] However, in the current study, abnormal karyotype other than complex karyotype or

-7/7q- abnormalities remained prognostically significant for overall survival, independent of other risk factors. On the contrary, we were not able to demonstrate prognostic relevance for *SF3B1* or other mutations, in regard to overall survival. The current study also found *RUNX1*, *IDH1*, and *TP53* mutations to show prognostic relevance in regard to leukemia-free survival, again in line with ICC-2022 diagnostic criteria for MDS-*SF3B1*, which requires exclusion of cases with multihit *TP53* and *RUNX1* mutations.[2] The latter were also reported by others to be associated with leukemic progression in low-risk MDS.[14]

Recent developments in the treatment of MDS-RS include the introduction of new drugs, such as luspatercept.[15] In the original phase III study of luspatercept *versus* placebo in transfusion-dependent patients with very low/low/intermediate-risk MDS-RS who were either refractory or unlikely to respond to treatment with ESA,[15] red blood cell transfusion-free period of at least 4 months was documented in 28% of study patients during weeks 1 through 48. In the particular study, response to luspatercept was not influenced by *SF3B1* VAF or the total number of baseline somatic mutations.[15] Similar observations regarding the lack of mutation impact on luspatercept treatment response in MDS-RS has been made by others.[16] In a more recent phase III study of luspatercept *versus* epoetin α in transfusion-dependent and ESA-naïve patients with very low/low/intermediate risk MDS (regardless of ring sideroblast percentage), response to luspatercept was more likely in the presence of *SF3B1* mutation but whether the same can be said in the context of MDS-RS is uncertain.[17] In our own retrospective experience on the use of luspatercept in MDS-RS, neither *SF3B1* nor other mutations appeared to effect treatment response.[18] Taken together, it is reasonable to question the value of *SF3B1* or other mutations in predicting treatment response to currently approved drugs in MDS-RS.

Based on the observations from the current study, it is reasonable to conclude that MLD remains a powerful morphologic marker of aggressive disease in WHO4R-defined MDS-RS and is characterized peripherally by trilineage cytopenias and prognostically by shortened survival. These observations are in line with previous reports on the subject matter[19,20] but are now confirmed in a contemporary patient population that accounted for confounding influence from karyotype and mutations, including *SF3B1*. The current study also underscored the limited value of the *SF3B1* mutation as a prognostic marker, in the context of WHO4R-defined MDS-RS, even after adjustments made to comply with criteria used in ICC-2022 for the diagnosis of MDS-*SF3B1*.[3] These findings are consistent with those previously published by us[12] as well others.[21] Taken together, these observations support the retention of MLD as a disease classifier in WHO4R-defined MDS-RS and suggest additional studies to clarify the role of *SF3B1* mutation in a similar capacity, especially considering its promiscuity across the spectrum of myeloid neoplasms, with or without ring sideroblasts.[21,22]

Disclosures

No conflicts of interest to disclose

Contributions

AT designed the study, performed analyses and wrote the paper. FF and MA collected data. AM, MP, AA, MAE, KHB, CCH, WJH, AP, MRL and NG contributed patients. RK reviewed cytogenetic studies. DAA, AO, RH and KR provided pathological expertise. All authors reviewed and approved the final draft.

Data-sharing statement

Data will be shared by email request addressed to the corresponding author.

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