IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

WT1 as a Biomarker in Myelodysplastic Neoplasms: Clinical Correlations and Preliminary Data from an Iranian Cohort

Forouzan Bahmani[1], Farhad Zaker[1], Bahram Chahardouli[2], Majid Safa[1], Nasrin Alizadehghandforoush[2], Saeed Mohammadi[2], Mohsen Nikbakht[2], Shahrbano Rostami[2], Davood Babakhani[3], Ghasem Janbabaei[3]

[1]Department of Hematology and Blood Banking, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran
[2]Cell Therapy and Hematopoietic Stem Cell Transplantation Research Center, Research Institute for Oncology, Hematology and Cell Therapy, Tehran University of Medical Sciences, Tehran, Iran
[3]Hematology, Oncology and Stem Cell Transplantation Research Center, Research Institute for Oncology, Hematology and Cell Therapy, Tehran University of Medical Sciences, Tehran, Iran

Corresponding Authors: Shahrbano Rostami, Cell Therapy and Hematopoietic Stem Cell Transplantation Research Center, Research Institute for Oncology, Hematology and Cell Therapy, Tehran University of Medical Sciences, Tehran, Iran
Farhad Zaker; Department of Hematology and Blood Banking, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran
E-mail: Sh-rostami@tums.ac.ir, Zaker.f@iums.ac.ir

Received: 15, Jul, 2025
Accepted: 17, Nov, 2025

ABSTRACT

Background: Although Wilms' tumor 1 (WT1) mRNA overexpression is frequently observed in myelodysplastic neoplasms (MDS), its clinical and molecular significance remains incompletely defined across diverse populations; this study is the first to evaluate WT1 expression in Iranian patients with MDS.

Materials and Methods: WT1 expression was assessed in 58 MDS patients using an ELN-certified quantitative RT-PCR assay. Associations with clinical subtype, hematologic features, cytogenetic profiles, and molecular mutations were analyzed. Survival outcomes were evaluated using Kaplan–Meier and Cox regression analyses.

Results: WT1 overexpression was detected in 79.3% of patients and was significantly associated with advanced subtypes (MDS-EB1/EB2) and higher IPSS-R risk groups. Elevated WT1 levels correlated with an increased bone marrow (BM) blast percentage ($P < 0.01$). Although cytogenetic abnormalities were more frequent in patients with WT1 overexpression, the association did not reach statistical significance. No significant correlations were observed with peripheral blood (PB) cytopenias or mutations in RNA splicing genes. Importantly, WT1 overexpression was associated with shorter overall survival (OS) and progression-free survival (PFS). However, in multivariate analysis, ≥10% BM blasts and an abnormal karyotype remained independent predictors of poor outcome, whereas WT1 overexpression itself was not independently prognostic.

Conclusion: WT1 overexpression in MDS is associated with advanced disease features and poorer survival, though it is not an independent prognostic value. Its measurement may complement existing risk stratification, particularly in resource-limited settings.

Keywords: Myelodysplastic; Wilms' tumor 1 (WT1) proteins; Prognosis; Survival analysis

INTRODUCTION

Myelodysplastic neoplasms (MDS) are a heterogeneous group of acquired hematopoietic stem and progenitor cell (HSPC) disorders. They are characterized by ineffective hematopoiesis, peripheral cytopenias, myeloid dysplasia, genomic instability, and an increased risk of progression to acute myeloid leukemia (AML)[1]. The Wilms tumor 1 (WT1) gene encodes a zinc-finger transcription factor and epigenetic regulator. Physiological WT1 expression is largely restricted to $CD34^{+}CD38^{-}$ bone

DOI: 10.18502/ijhoscr.v20i1.21009

marrow (BM) stem cells, where it helps regulate stemness and cellular quiescence. WT1 is typically absent in lineage-committed progenitors[2,3]. Aberrant WT1 overexpression occurs in several solid tumors, including lung, colon, and pancreatic cancers, as well as in hematologic malignancies such as MDS. High WT1 expression has been linked to increased blast burden, therapeutic resistance, disease progression, relapse risk, and inferior overall survival (OS), underscoring its potential as a clinically relevant biomarker [4-7].

In MDS, WT1 levels rise with disease progression and higher International Prognostic Scoring System (IPSS/IPSS-R) risk categories[4,8]. Serial monitoring of WT1, particularly after chemotherapy or hematopoietic stem cell transplantation (HCT), has been proposed as a surrogate for measurable residual disease (MRD). This approach may enable earlier relapse detection and allow for preemptive therapeutic intervention[9-13]. The development of a standardized, European LeukemiaNet (ELN)-certified quantitative assay now permits precise and reproducible measurement of WT1 mRNA in both peripheral blood (PB) and BM, with validated thresholds distinguishing physiological expression from pathological overexpression [5, 8, 14-16].

Despite advances in morphology-based assessment and molecular diagnostics, risk stratification in MDS remains challenging. The disease's heterogeneity, overlap with non-clonal cytopenias, and limited availability of molecular profiling in routine practice all complicate accurate prognosis[17,18]. In this context, WT1 quantification using a standardized ELN-certified assay offers a reproducible and cost-effective biomarker that may complement current diagnostic tools[4]. However, the prognostic and biological significance of WT1 is not fully defined, and its mechanistic role requires further study.

This study investigates the clinico-molecular relevance of WT1 mRNA expression in MDS by integrating hematologic parameters, cytogenetic abnormalities, and mutations in RNA splicing factors—the most common genetic alterations in MDS. By evaluating WT1 as a marker of disease burden, risk of leukemic transformation, and potential therapeutic stratification, we provide the first systematic analysis of WT1 expression in an Iranian MDS cohort.

MATERIALS AND METHODS

Study design and patient selection

This retrospective observational cohort study was conducted at the Hematology-Oncology and Stem Cell Transplantation Research Center of Shariati Hospital, Tehran, Iran. A total of 58 patients diagnosed with MDS between 2016 and 2024 were included. MDS diagnoses were established according to the 2016 World Health Organization (WHO) classification criteria, based on BM morphology, cytogenetic analysis, and PB parameters. Inclusion criteria were: (1) a confirmed diagnosis of MDS with available baseline WT1 gene expression data; (2) no history of other hematologic malignancies or their treatment; and (3) complete follow-up data regarding disease progression and survival outcomes. Patients with secondary MDS or those exhibiting overlapping myeloproliferative features were excluded to ensure cohort homogeneity.

Demographic and clinical data at diagnosis—including age, sex, complete blood counts (CBC), BM blast percentage, cytogenetic profile, and transfusion dependency—were collected from electronic and paper-based medical records. Progression to AML was defined according to WHO criteria as an increase in BM or PB blasts to ≥20%. Risk stratification was performed using the IPSS-R, incorporating BM blast percentage, degree of cytopenias, and cytogenetic abnormalities. Data collection concluded with a final data lock on January 27, 2025. All patients provided written informed consent for genetic testing and the use of their clinical and laboratory data for research purposes. The study protocol was reviewed and approved by the institutional review board (IR.IUMS.REC.1402.195) and was conducted in accordance with ethical guidelines.

Sample collection and WT1 expression analysis

PB and BM aspirate samples were obtained at the time of diagnosis, prior to the initiation of any disease-modifying therapy. WT1 gene expression was quantified using real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR),

following the standardized protocol recommended by the European LeukemiaNet (ELN)[5]. Total RNA was extracted using the TRIzol reagent (Invitrogen) following the manufacturer's instructions. Complementary DNA cDNA synthesis was performed using a reverse transcriptase cDNA synthesis kit (Takara), and WT1 mRNA levels were quantified using the ipsogen WT1 ProfileQuant Kit (Qiagen). WT1 expression was normalized to the ABL1 housekeeping gene and expressed as WT1 copies per 10^4 ABL1 copies. To stratify patients, predefined ELN-recommended thresholds were applied: 250 copies/10^4 ABL in BM and 50 copies/10^4 ABL in PB were used as cutoffs to distinguish between normal and elevated WT1 expression. Patients were categorized into two groups based on these thresholds: those with normal WT1 expression and those with WT1 overexpression.

Comparative analyses were conducted between the two groups with respect to clinical and hematologic parameters, including WHO 2016 MDS subtypes and IPSS-R risk categories. Additionally, differences in progression-free survival (PFS) and OS were evaluated. All molecular analyses were performed in the central molecular hematology laboratory under standardized quality control procedures to ensure data reliability.

Statistical analysis

Data were analyzed using SPSS software (version 27.0) and R software (version 4.4.2). Descriptive statistics were used to summarize baseline characteristics. Continuous variables were expressed as mean ± standard deviation or median (interquartile range), as appropriate, while categorical variables were reported as counts and percentages. Comparisons between the normal WT1 expression and overexpression groups were performed using the independent samples t-test or Mann–Whitney U test for continuous variables, and the Chi-square or Fisher's exact test for categorical variables. Kaplan–Meier survival curves were generated for overall survival (OS) and progression-free survival (PFS), with group differences assessed using the log-rank test. Cumulative incidence (CI) functions were used to evaluate the probability of AML progression and transfusion dependency, accounting for competing risks.

To identify independent prognostic factors among patients with WT1 overexpression, multivariate analysis was performed using Cox proportional hazards regression. Variables with a p-value ≤ 0.05 in univariate analysis were included in the multivariate model. Hazard ratios (HR) with 95% confidence intervals (CI) were calculated, and a p-value < 0.05 was considered statistically significant.

Karyotypic data were unavailable for three patients (5.2%), and IPSS-R scores were missing in 13 patients (22.4%). For all survival analyses and multivariate modeling, cases with missing values were excluded using listwise deletion. No data imputation was applied. The potential impact of missing data was mitigated by consistent trends observed across variables and the use of rigorous statistical methods.

RESULTS

Patient characteristics

A total of 58 patients diagnosed with MDS were included in the study, with a mean age of 58.9 years (range: 35–75 years)(Table 1). The cohort was predominantly male (n = 38, 65.5%), with 20 female patients (34.5%). According to the 2016 WHO classification, the most common subtypes were MDS with excess blasts-2 (MDS-EB2; n = 22, 37.9%) and MDS with excess blasts-1 (MDS-EB1; n = 20, 34.5%). Other identified subtypes included MDS with ring sideroblasts and multilineage dysplasia (MDS-RS-MLD; n = 7, 12.1%), MDS with isolated del(5q) (n = 4, 6.9%), MDS with multilineage dysplasia (MDS-MLD; n = 4, 6.9%), and MDS with single lineage dysplasia (MDS-SLD; n = 1, 1.7%). No patients were classified as having MDS-RS-SLD.

Cytogenetic analysis revealed abnormal karyotypes in 23 patients (39.7%), normal karyotypes in 20 (34.5%), and complex karyotypes in 12 (20.7%). Karyotypic data were unavailable for 3 patients (5.2%). Molecular profiling detected pathogenic variants in genes involved in RNA splicing, including *SF3B1*, *SRSF2*, and *U2AF1*, in 22 patients (37.9%), consistent with established mutational landscapes in MDS. Risk stratification using the IPSS-R showed that most patients fell into the very high (n = 20, 34.5%) or high-risk (n = 15, 25.9%) categories. Intermediate-

and low-risk groups accounted for 13.8% and 3.4% of the cohort, respectively. IPSS-R scores were unavailable for 13 patients (22.4%).

At diagnosis, the median BM blast percentage was 7% (range: 2–19%), and the median BM cellularity was 70% (range: 25–90%). PB findings included a mean WBC count of 4.06×10^9/L (range: 0.68–10.00), a mean hemoglobin level of 8.35 g/dL (range: 4.30–12.70), and a median platelet count of 49×10^9/L (range: 3–270). Transfusion dependency at presentation was common, with 25 patients (43%) requiring packed red blood cell (PC) transfusions and 16 patients (27%) dependent on platelet transfusions.

Association of WT1-mRNA expression levels with MDS clinical features and risk stratifications

Among the 58 patients included in the analysis, WT1-mRNA overexpression was identified in 46 individuals (79.3%), while 12 patients (20.7%) exhibited WT1 expression within the established reference range (Table 1). Stratification based on WT1 status revealed significant associations with various clinical and prognostic parameters. Patients with WT1 overexpression were significantly younger than those with normal WT1 expression (mean age: 57.4 vs. 64.6 years; $p = 0.02$). No significant differences were observed with respect to sex distribution between the groups ($p = 0.20$).

Diagnostic classification based on the 2016 WHO criteria differed significantly by WT1 status ($p = 0.007$). WT1 overexpression was predominantly observed in higher-grade MDS subtypes, particularly MDS-EB1 (30.4%) and MDS-EB2 (47.8%). Notably, none of the patients with MDS-EB2 had normal WT1 expression. In contrast, individuals with normal WT1 levels were more frequently diagnosed with lower-grade subtypes (50%), including MDS-SLD, MDS-MLD, and MDS-RS-MLD. Cytogenetic abnormalities were not significantly different between the groups ($p = 0.20$), although normal karyotypes were more common among patients with normal WT1 expression (50% vs. 30%). The frequencies of complex and abnormal karyotypes were comparable across both cohorts. Similarly, the incidence of pathogenic mutations in RNA splicing factor genes (SF3B1, SRSF2, U2AF1) did not significantly differ between groups (33.3% in WT1-normal vs. 39.1% in WT1-overexpressed; $p = 0.70$). Risk stratification using the IPSS-R showed a strong correlation with WT1 expression levels ($p = 0.001$). The intermediate-risk category was most frequent in the WT1-normal group (50%), while the very high-risk category predominated among patients with WT1 overexpression (39.1%). Only 2 patients (16.6%) in the WT1-normal group were classified as very high risk (Figure 1A).

WT1 overexpression was associated with a higher disease burden, as reflected by a significantly elevated median BM blast percentage (8% vs. 5%; $p = 0.03$). However, PB parameters—including WBC count ($p = 0.20$), hemoglobin concentration ($p = 0.60$), and platelet count ($p = 0.90$)—did not significantly differ between groups (Figure 2A). Similarly, transfusion requirements for PC and platelets were not significantly different between WT1 expression groups.

Table 1: Patient and clinical characteristics

Variables	Total (n=58)	Normal WT1 (n=12)	Overexpressed WT1 (n=46)	P value
Age, mean (y)	58.9 (35-75)	64.6 (40-75)	57.4 (35-74)	0.02
Gender				0.2
Female	20 (34.5)	6 (50)	14	
Male	38 (65.5)	6 (50)	32	
WHO 2016 classification				0.007
MDS del5q	4 (6.9)	0	4 (8.7)	
MDS SLD	1 (1.7)	1 (8.3)	0	
MDS MLD	4 (6.9)	2 (16.7)	2 (4.3)	
MDS RS SLD	0	0	0	
MDS RS MLD	7 (12.1)	3 (25)	4 (8.7)	
MDS EB1	20 (34.5)	6 (50)	14 (30.4)	
MDS EB2	22 (37.9)	0	22 (47.8)	
Karyotype				0.2
Normal	20 (34.5)	6 (50)	14 (30)	
Abnormal	23 (39.7)	2 (16.6)	21 (45.65)	
Complex	12 (20.7)	2 (16.6)	10 (21.7)	
Missing	3 (5.2)	2 (16.6)	1 (2.18)	
IPSS-R				0.001
Very low	0	0	0	
Low	2 (3.4)	0	2 (4.3)	
Intermediate	8 (13.8)	6 (50)	2 (4.3)	
High	15 (25.9)	2 (16.6)	13 (28.3)	
Very high	20 (34.5)	2 (16.6)	18 (39.1)	
Missing	13 (22.4)	2 (16.6)	11 (24)	
Splicing factor mutations (SF3B1, SRSF2, U2AF1)	22 (37.9)	4 (33.3)	18 (39.1)	0.7
BM blasts, median (%)	7 (2-19)	5 (3-8)	8 (2-19)	**0.03**
WBC, mean (10^9/L)	4.06 (0.68-10.00)	5.00 (0.68-10.00)	3.78 (0.96-8.80)	0.2
Hemoglobin, mean (g/dl)	8.35 (4.30-12.70)	8.6 (6.6-11.6)	8.3 (4.3-12.7)	0.6
Platelets, median (10^9/L)	49 (3-270)	62 (6-211)	49 (3-270)	0.9
BM cellularity, median (%)	70 (25-90)	50 (25-90)	70 (30-90)	0.3
Transfusion dependency				
Packed red cells	25 (43)	7 (58.3)	18 (39.1)	0.2
Platelet	16 (27)	5 (41.7)	11 (23.9)	0.2

IPSS-R, revised international prognostic scoring system; WBC, white blood cell; BM: bone marrow

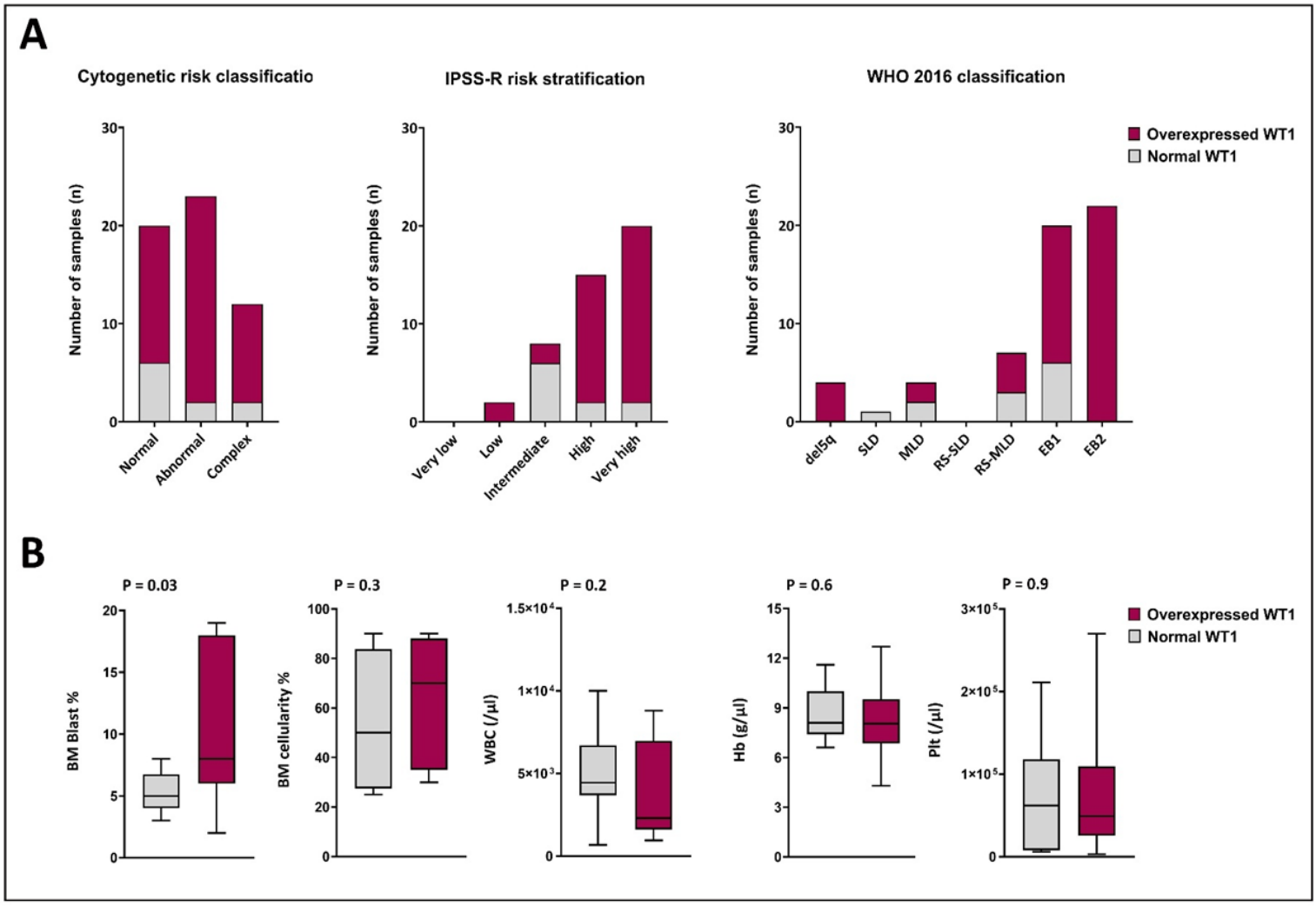

Figure 1. Distribution and hematologic characteristics in MDS patients stratified by WT1-mRNA expression. **(A)** Frequency of WT1 overexpression among the MDS patient cohort. **(B)** Comparison of hematologic parameters between patients with WT1 overexpression and those with normal WT1 levels, including BM blast percentage, BM cellularity, WBC count, Hb concentration, and Plt count. Statistical significance (P-values) for group comparisons is indicated on the corresponding plots. BM, bone marrow; WT1, Wilms' tumor 1; WBC, white blood cell; Hb, hemoglobin; Plt, platelet

Survival analysis

Analysis of OS revealed a significant difference between the two groups. The median OS in the group with WT1 overexpression was 55.5 months (95% confidence interval [CI]: 26.5–78.7). In contrast, the median OS was not reached in the group with normal WT1 expression, with a 95% CI of 32.4 months to not applicable (NA). This difference in OS was statistically significant (P = 0.05), as demonstrated by the Kaplan–Meier survival curve (Figure 2A). These findings indicate that WT1 overexpression is associated with reduced OS in patients with MDS. PFS was also significantly affected by WT1-mRNA expression. The median PFS for patients with WT1 overexpression was 41.3 months (95% CI: 12.1–NA), whereas the median PFS was not reached in the group with normal expression (95% CI: 32.4–NA). This difference was statistically significant (P = 0.03), as illustrated in the Kaplan–Meier plot (Figure 2B). In summary, both OS and PFS were significantly worse in patients with WT1-mRNA overexpression compared to those with normal expression. These results support WT1-mRNA overexpression as a negative prognostic marker in MDS, associated with poorer survival outcomes and earlier disease progression.

Prognostic role of WT1 expression level in AML progression and supportive care needs

The prognostic significance of WT1-mRNA expression in relation to AML progression and transfusion dependency was assessed among patients stratified by WT1 expression status. A significant association was observed between WT1 overexpression and an increased risk of transformation to AML. At 24 months (2 years), the cumulative incidence (CI) of AML progression was 69% in the WT1-overexpressed group, compared to 25% in the group with normal WT1 expression (Figure 2C). This difference was statistically significant (P = 0.007), suggesting that elevated WT1 levels may predict a more aggressive clinical course and a higher likelihood of leukemic transformation in MDS patients.

WT1 overexpression was also associated with a higher CI of platelet transfusion dependency within 2 years, although not over the entire follow-up period (Figure 2C). The 2-year CI of platelet transfusion was 54% in the overexpressed group, compared to 25% in the WT1-normal group. Although this difference was not statistically significant (P = 0.4), the trend suggests a possible association between elevated WT1 expression and increased platelet transfusion needs. Similarly, patients with WT1 overexpression demonstrated higher PC transfusion dependency within 2 years (Figure 2C). The 2-year CI of PC transfusion was 56% in the WT1-overexpressed group, compared to 33% in the normal expression group. This difference also did not reach statistical significance (P = 0.3), but it reflects a consistent pattern of greater transfusion burden among patients with elevated WT1 expression.

In conclusion, WT1-mRNA overexpression was significantly associated with increased AML progression, and although not statistically significant, there was a consistent trend toward greater transfusion dependency within 2 years. These findings support the potential role of WT1 expression as a prognostic biomarker for identifying MDS patients at higher risk for disease progression and transfusion dependence.

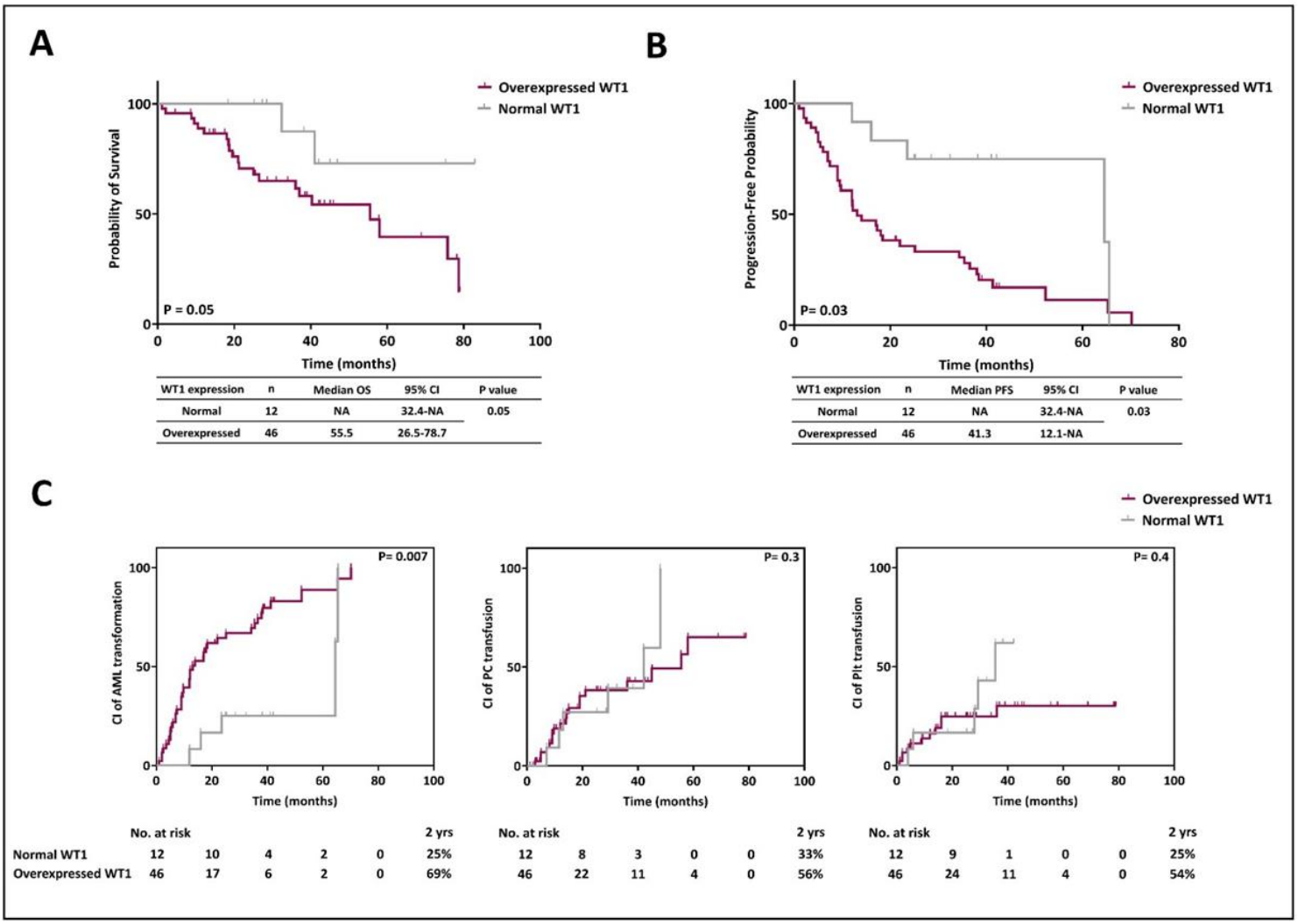

Figure 2. Kaplan–Meier survival and cumulative incidence analyses based on WT1 expression levels in MDS patients. **(A)** OS stratified by WT1 expression status (normal vs. overexpressed); **(B)** PFS according to WT1 expression level; **(C)** CI curves for AML transformation and transfusion dependency (PC and platelets) in patients with normal and overexpressed WT1.P-values indicating statistical significance are shown on the respective plots. OS, overall survival; PFS, progression-free survival; CI, cumulative incidence; AML: acute myeloblastic leukemia; PC: packed red blood cells

Univariate and multivariate analyses of OS in MDS patients with WT1 overexpression

To assess the prognostic impact of clinical, cytogenetic, and molecular factors on OS in MDS patients exhibiting WT1-mRNA overexpression, both univariate and multivariate Cox proportional hazards regression analyses were performed (Table 2). In univariate analysis, several factors were significantly associated with poorer OS. A BM blast percentage ≥10% demonstrated the strongest adverse effect (HR: 9.47; 95% CI: 3.04–29.47; P < 0.001). Cytogenetic abnormalities also conferred a significantly increased mortality risk (HR: 4.73; 95% CI: 1.63–13.74; P < 0.001). Additional unfavorable prognostic indicators included platelet transfusion dependency (HR: 2.76; 95% CI: 1.11–6.83; P = 0.02) and the presence of RNA splicing factor gene mutations (HR: 2.78; 95% CI: 1.05–7.36; P = 0.03). Other variables, such as age ≥60 years, male gender, progression to AML, complex karyotype, isolated del(5q), and PC transfusion dependency, were not significantly associated with OS (Figure 3).

Multivariate analysis confirmed that BM blast count ≥10% remained a strong independent predictor of inferior survival (HR: 11.79; 95% CI: 3.07–45.23; P < 0.001), along with the presence of cytogenetic abnormalities (HR: 5.23; 95% CI: 1.55–17.69; P < 0.001). However, platelet transfusion dependency

(HR: 1.35; P = 0.57) and RNA splicing mutations (HR: 1.63; P = 0.40) lost statistical significance after adjustment for other covariates. These findings emphasize the importance of BM blast burden and cytogenetic profile as independent prognostic markers of OS in WT1-overexpressing MDS patients, while also indicating that the prognostic value of transfusion dependency and molecular alterations may be influenced by coexisting high-risk disease features (Figure 3).

Table 2: Univariate and multivariate analysis of OS in patients with overexpressed WT1

Variables	HR	95% CI	P value
Univariate			
Age ≥60 years	0.76	0.32-1.85	0.55
Male gender	0.5	0.2-1.24	0.14
BM blasts ≥ 10%	9.47	3.04-29.47	<0.001
AML progression	0.76	0.25-2.34	0.64
Complex karyotype	0.62	0.2-1.9	0.4
del(5q)	1.71	0.49-6.0	0.39
Other CG abnormality	4.73	1.63-13.74	<0.001
PC transfusion dependency	1.94	0.8-4.71	0.14
Plt transfusion dependency	2.76	1.11-6.83	0.02
Splicing factor mutations	2.78	1.05-7.36	0.03
Multivariate			
BM blast count ≥ 10	11.79	3.07-45.23	<0.001
Other CG abnormality	5.23	1.55-17.69	<0.001
Plt transfusion dependency	1.35	0.47-3.87	0.57
Splicing factor mutations	1.63	0.52-5.06	0.4

BM: bone marrow; HR: hazard ratio; CG: cytogenetic abnormality; PC: packed cell; Plt: Platelet

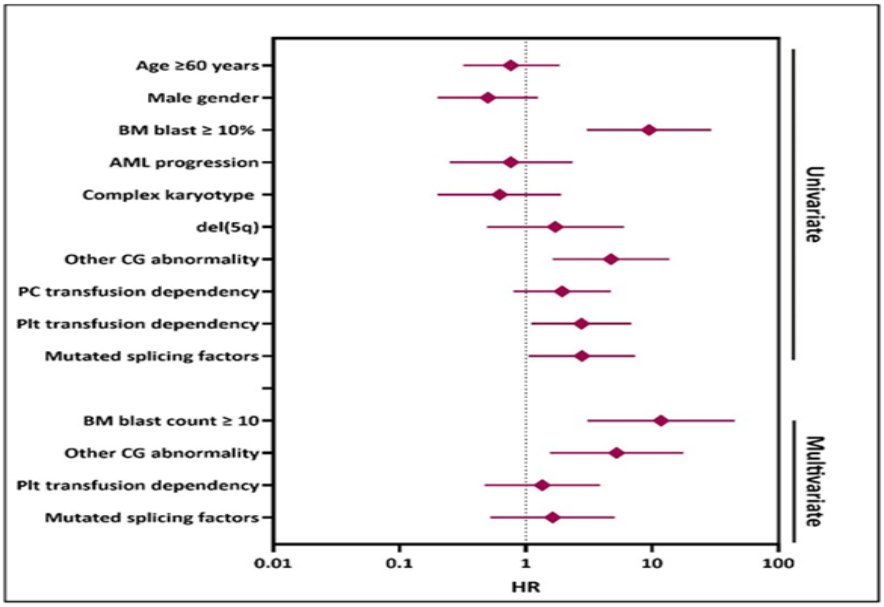

Figure 2. Univariate and multivariate analyses of overall survival in MDS patients with WT1 overexpression.Cox regression analysis identified BM blasts ≥10% and cytogenetic abnormalities as the strongest adverse prognostic factors. In univariate analysis, BM blasts ≥10%, cytogenetic abnormalities, platelet transfusion dependency, and splicing factor mutations were associated with poorer survival. In multivariate analysis, only BM blasts ≥10% (HR: 11.79, P < 0.001) and cytogenetic abnormalities (HR: 5.23, P < 0.001) remained independent predictors of inferior overall survival, whereas platelet transfusion dependency and splicing factor mutations lost significance.

DISCUSSION

In this study, for the first time in an Iranian MDS population, we employed a standardized, ELN-certified quantitative assay to assess WT1-mRNA expression in PB and BM samples and systematically evaluated its clinical, hematologic, and prognostic relevance. Although this represents the first evaluation of WT1 in Iranian MDS patients, previous studies in Iranian AML cohorts—such as the assessment of WT-1, BAALC, and ERG expressions pre- and post-chemotherapy—provide important regional and molecular context, further supporting the potential role of WT1 as a prognostic biomarker[19]. WT1-mRNA overexpression was identified in 79% of the cohort, underscoring its prevalence as a pivotal molecular hallmark within MDS pathophysiology. Crucially, WT1 expression demonstrated a strong correlation with the 2016 WHO classification schema and IPSS-R risk stratification, reinforcing its potential as a robust and clinically actionable biomarker[4, 8, 20-24]. Elevated WT1-mRNA expression portended significantly adverse clinical outcomes, including diminished OS, PFS, and an increased rate of leukemic transformation, thereby substantiating WT1's role as an indicator of clonal dominance and proliferative dysregulation in higher-risk MDS subtypes, notably MDS-EB1 and MDS-EB2.

WT1 operates as a critical transcription factor integral to the self-renewal and differentiation of hematopoietic progenitor cells. Its aberrant upregulation in MDS likely facilitates clonal expansion, suppresses normal hematopoiesis, and contributes to therapeutic resistance [25-27]. Within our cohort, WT1 overexpression was consistently observed in all MDS-EB2 cases, further associating this molecular aberration with high-risk disease phenotypes. A robust positive correlation between WT1 transcript abundance and BM blast percentage was evident, supporting its utility as a dynamic biomarker reflective of disease burden and progression risk. Beyond diagnostic and stratification implications, this association suggests prognostic and predictive potential. Survival analyses demonstrated that patients with heightened WT1 expression exhibited significantly poorer OS and PFS, with a 24-month AML transformation incidence of 69%, compared to 25% in patients maintaining WT1 expression within normal parameters. These data concur with extant literature implicating WT1 in leukemogenic transformation and the molecular evolution of clonal hematopoiesis in myeloid neoplasms [28-31].

Multivariate Cox proportional hazards modeling identified BM blasts ≥10% and cytogenetic abnormalities as independent adverse prognostic determinants of OS in MDS patients with elevated WT1 expression. Conversely, splicing factor mutations and transfusion dependency lost prognostic significance upon multivariate adjustment, suggesting their effects may be confounded by other clinical and genomic variables. These findings posit that WT1 overexpression may serve as an integrative biomarker encapsulating clonal complexity and genomic instability beyond discrete mutational events. Although platelet and PC transfusion dependency did not achieve statistical significance, a discernible trend toward increased transfusion requirements over a 2-year period was observed within the WT1-high subgroup, consistent with progressive marrow failure and escalating transfusion burden. Given WT1's established association with elevated IPSS-R risk scores, increased blast counts, and impaired multilineage hematopoiesis—drivers of cytopenias and transfusion needs—WT1 expression likely functions as a surrogate for disease severity and supportive care intensity.

Previous studies have corroborated the association of WT1 expression with FAB classification, WHO subtypes, and IPSS/IPSS-R risk stratifications [14, 32]. Our data further validate these correlations, establishing WT1 quantification via a standardized, ELN-certified assay as a practical, reproducible biomarker reflecting disease burden and clonal heterogeneity in MDS. This is especially relevant considering current diagnostic and prognostic limitations in MDS patient management, where WT1 measurement constitutes a valuable adjunct for risk stratification.

Differentiating MDS from non-clonal cytopenias such as aplastic anemia, megaloblastic anemia, and age-related cytopenias remains diagnostically challenging, particularly in cases exhibiting subtle or equivocal dysplastic features. Although morphological evaluation remains the diagnostic cornerstone, its sensitivity is inherently limited[33-36]. Furthermore,

detection of common MDS-associated mutations—including *SF3B1*, *U2AF1*, and *SRSF2*—is not definitive, as these mutations can also occur in elderly individuals with clonal hematopoiesis, thereby complicating the differential diagnosis (37-39). While the IPSS-M model enhances prognostic accuracy through incorporation of molecular data, its clinical applicability is constrained by technical complexity, high cost, and limited accessibility, particularly in resource-restricted settings. In contrast, WT1 quantification offers a widely available, cost-effective, and reproducible assay with straightforward implementation, representing an accessible tool for MDS diagnosis and risk stratification.

This study has several limitations that warrant consideration. The relatively modest sample size and single-center design may limit the generalizability of our findings across broader and more diverse populations. Additionally, the observational nature of the study precludes definitive conclusions regarding causality between WT1 expression and clinical outcomes. Importantly, prospective validation of our results in larger, multi-center cohorts is needed to confirm the prognostic utility of WT1-mRNA expression and to further elucidate its role within diverse genetic and clinical contexts of MDS.

CONCLUSION

WT1-mRNA overexpression is closely associated with advanced disease features and inferior survival in patients with MDS. Its correlation with blast burden and cytogenetic abnormalities highlights its value as a surrogate marker of disease aggressiveness. Measurement of WT1 using a standardized ELN-certified assay provides a reproducible and cost-effective tool to complement existing risk stratification. These findings support the clinical utility of WT1 as a prognostic biomarker, while further studies are needed to clarify its biological role and therapeutic relevance.

ACKNOWLEDGEMENTS

The authors sincerely appreciate the invaluable contributions of their colleagues at the Hematology-Oncology and Stem Cell Transplantation Research Center, Shariati Hospital. They also extend their gratitude to the physicians for their dedication in patient care, sample collection, and data gathering, which were essential to this study.

Ethics approval and consent to participate

Written informed consent was obtained from all participants for genetic analysis and the use of their clinical and laboratory data for research purposes. The study protocol was approved by the institutional review board (IR.IUMS.REC.1402.195) and conducted in accordance with institutional ethical guidelines.

Data availability

The datasets supporting the findings of this study are accessible from the corresponding author upon reasonable request and in compliance with institutional and ethical guidelines.

Competing interests

The authors declare that they have no competing interests.

Funding support

Not applicable.

Consent for publication

Written informed consent for publication was obtained from all participants included in the study.

REFERENCES

1. Tefferi A, Vardiman JW. Myelodysplastic syndromes. New Engl J Med. 2009;361(19):1872-85.
2. Oji Y, Nakamori S, Fujikawa M, et al. Overexpression of the Wilms' tumor gene WT1 in pancreatic ductal adenocarcinoma. Cancer Sci. 2004;95(7):583-7.
3. Rampal R, Figueroa ME. Wilms tumor 1 mutations in the pathogenesis of acute myeloid leukemia. Haematologica. 2016;101(6):672-9.
4. Rautenberg C, Germing U, Pechtel S, et al. Prognostic impact of peripheral blood WT1-mRNA expression in patients with MDS. Blood Cancer J. 2019;9(11):86.
5. Cilloni D, Renneville A, Hermitte F, et al. Real-time quantitative polymerase chain reaction detection of minimal residual disease by standardized WT1 assay to enhance risk stratification in acute myeloid leukemia: a European LeukemiaNet study. J Clin Oncol. 2009;27(31):5195-201.
6. Bergmann L, Miething C, Maurer U, et al. High levels of Wilms' tumor gene (wt1) mRNA in acute myeloid

leukemias are associated with a worse long-term outcome. Blood. 1997;90(3):1217-25.
7. Hou HA, Huang TC, Lin LI, et al. WT1 mutation in 470 adult patients with acute myeloid leukemia: stability during disease evolution and implication of its incorporation into a survival scoring system. Blood. 2010;115(25):5222-31.
8. Nagasaki J, Aoyama Y, Hino M, et al. Wilms tumor 1 (WT1) mRNA expression level at diagnosis is a significant prognostic marker in elderly patients with myelodysplastic syndrome. Acta Haematol. 2017;137(1):32-9.
9. Santaliestra M, Garrido A, Carricondo M, et al. Bone marrow WT1 levels in patients with myeloid neoplasms treated with 5-azacytidine: Identification of responding patients. Eur J Haematol. 2019;103(3):208-14.
10. Nomdedéu JF, Esquirol A, Carricondo M, et al. Bone marrow WT1 levels in allogeneic hematopoietic stem cell transplantation for acute myelogenous leukemia and myelodysplasia: clinically relevant time points and 100 copies threshold value. Biol Blood Marrow Transplant. 2018;24(1):55-63.
11. Casalegno-Garduño R, Schmitt A, Spitschak A, et al. Immune responses to WT1 in patients with AML or MDS after chemotherapy and allogeneic stem cell transplantation. Int J Cancer. 2016;138(7):1792-801.
12. Ino K, Fuji S, Tajima K, et al. Clinical utility of Wilms' tumor 1 monitoring in patients with myeloid malignancy and prior allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2017;23(10):1780-7.
13. Bahmani F, Shayanmanesh M, Safari M, et al. Bone marrow microenvironment in myelodysplastic neoplasms: insights into pathogenesis, biomarkers, and therapeutic targets. Cancer Cell Int. 2025;25(1):175.
14. Cilloni D, Gottardi E, Messa F, et al. Significant correlation between the degree of WT1 expression and the International Prognostic Scoring System Score in patients with myelodysplastic syndromes. J Clin Oncol. 2003;21(10):1988-95.
15. Tamura H, Dan K, Yokose N, et al. Prognostic significance of WT1 mRNA and anti-WT1 antibody levels in peripheral blood in patients with myelodysplastic syndromes. Leuk Res. 2010;34(8):986-90.
16. Ueda Y, Mizutani C, Nannya Y, et al. Clinical evaluation of WT1 mRNA expression levels in peripheral blood and bone marrow in patients with myelodysplastic syndromes. Leuk Lymphoma. 2013;54(7):1450-8.
17. DeZern AE, Greenberg PL. The trajectory of prognostication and risk stratification for patients with myelodysplastic syndromes. Blood. 2023;142(26):2258-67.
18. Garcia-Manero G. Myelodysplastic syndromes: 2023 update on diagnosis, risk-stratification, and management. Am J Hematol. 2023;98(8):1307-25.
19. Mehralizadeh H, Aliparasti MR, Talebi M, et al. WT-1, BAALC, and ERG expressions in Iranian patients with acute myeloid leukemia pre-and post-chemotherapy. Adv Pharm Bull. 2021;11(1):197-203.
20. Gallo D, Nicoli P, Calabrese C, et al. The Wilms' tumor (WT 1) gene expression correlates with the International Prognostic Scoring System (IPSS) score in patients with myelofibrosis and it is a marker of response to therapy. Cancer Med. 2016;5(7):1650-3.
21. Israyelyan A, Goldstein L, Tsai W, et al. Real-time assessment of relapse risk based on the WT1 marker in acute leukemia and myelodysplastic syndrome patients after hematopoietic cell transplantation. Bone Marrow Transplant. 2015;50(1):26-33.
22. Rautenberg C, Lauseker M, Kaivers J, et al. Prognostic impact of pretransplant measurable residual disease assessed by peripheral blood WT1-mRNA expression in patients with AML and MDS. Eur J Haematol. 2021;107(2):283-92.
23. Giudice V, Gorrese M, Vitolo R, et al. WT1 expression levels combined with flow cytometry blast counts for risk stratification of acute myeloid leukemia and myelodysplastic syndromes. Biomedicines. 2021;9(4):387.
24. Huang QS, Wang JZ, Qin YZ, et al. Overexpression of WT1 and PRAME predicts poor outcomes of patients with myelodysplastic syndromes with thrombocytopenia. Blood Adv. 2019;3(21):3406-18.
25. Yang L, Han Y, Saurez Saiz F, et al. A tumor suppressor and oncogene: the WT1 story. Leukemia. 2007;21(5):868-76.
26. Svensson E, Vidovic K, Lassen C, et al. Deregulation of the Wilms' tumour gene 1 protein (WT1) by BCR/ABL1 mediates resistance to imatinib in human leukaemia cells. Leukemia. 2007;21(12):2485-94.
27. Zhou B, Jin X, Jin W, et al. WT1 facilitates the self-renewal of leukemia-initiating cells through the upregulation of BCL2L2: WT1-BCL2L2 axis as a new acute myeloid leukemia therapy target. J Transl Med. 2020;18(1):254.
28. Rostami S, Kazemi A, Chahardouli B, et al. The Prognostic Impact of WT1 Expression Levels, Mutations, and SNP rs16754 in AML Patients: A Retrospective Cohort Study. J Adv Med Biomed Res. 2021;29(133):109-17.
29. Lyu Y, Lou J, Yang Y, et al. Dysfunction of the WT1-MEG3 signaling promotes AML leukemogenesis via p53-dependent and-independent pathways. Leukemia. 2017;31(12):2543-51.

30. Sugiyama H. Wilms Tumor Gene WT1 as a: Tumor Marker for Leukemic Blast Cells and Its Role in Leukemogenesis. Methods Mol Med. 2002:68:223-37.
31. Potluri S, Assi SA, Chin PS, et al. Isoform-specific and signaling-dependent propagation of acute myeloid leukemia by Wilms tumor 1. Cell Rep. 2021;35(3):109010.
32. Kobayashi S, Ueda Y, Kurokawa M, et al. WT-1 Expression Level In BM Is The Great Prognostic Marker In Three Of Classification IPSS, WPSS, and Latest Revised IPSS (IPSS-R). Blood. 2013;122(21):2795.
33. Heuser M, Thol F, Ganser A. Clonal Hematopoiesis of Indeterminate Potential. Dtsch Arztebl Int. 2016;113(18):317-22.
34. Parmentier S, Schetelig J, Lorenz K, et al. Assessment of dysplastic hematopoiesis: lessons from healthy bone marrow donors. Haematologica. 2012;97(5):723-730..
35. Senent L, Arenillas L, Luño E, et al. Reproducibility of the World Health Organization 2008 criteria for myelodysplastic syndromes. Haematologica. 2013;98(4):568-575.
36. Valent P, Orazi A, Steensma DP, et al. Proposed minimal diagnostic criteria for myelodysplastic syndromes (MDS) and potential pre-MDS conditions. Oncotarget. 2017;8(43):73483-73500.
37. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126(1):9-16.
38. Ariste O, de la Grange P, Veitia RA. Recurrent missense variants in clonal hematopoiesis-related genes present in the general population. Clin Genet. 2023;103(2):247-51.
39. Pritzl SL, Badar T, Ferrer A, et al. U2AF1 Mutant Clonal Hematopoiesis Is Infrequent, Context Dependent, and Commonly Co-Occurs with BCOR Mutations. Blood. 2022;140(Supplement 1):11408-9.