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Telomere integrated scoring system of myelodysplastic syndrome

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Abstract

Introduction: Recently, multigene target sequencing is widely performed for the purpose of prognostic prediction and application of targeted therapy. Here, we proposed a new scoring system that encompasses gene variations, telomere length, and Revised International Prognostic Scoring System (IPSS-R) together in Asian myelodysplastic syndrome.

Methods: We developed a new scoring model of these variables: age ≥ 65 years + IPSS-R score + *ASXL1* mutation + *TP53* mutation + Telomere length (<5.37). According to this new scoring system, patients were divided into *four* groups: very good score cutoff (≤3.0), good (3.0–4.5), poor (4.5–7.0), and very poor (>7.0).

Results: The median OS was 170.1, 100.4, 46.0, and 12.0 months for very good, good, poor, and very poor, retrospectively ($p < 0.001$). Meanwhile, according to the conventional IPSS-R scoring system, the median OS was 141.3, 50.2, 93.0, 36.0, and 16.2 months for very low, low, intermediate, high, and very high, retrospectively ($p < 0.001$).

Conclusions: The newly developed model incorporating molecular variations and TL yielded more clear separations of the survival curves. By adding the presence of gene mutation and telomere length to the existing IPSS-R, its predictive ability can be further improved in myelodysplastic syndrome.

Hee Sue Park and Kyongok Im were the co-first authors of this article.

KEYWORDS
myelodysplastic syndrome, Revised International Prognostic Scoring System, targeted capture sequencing, telomere length

1 | INTRODUCTION

Since using classification by French–American British (FAB) based on cell morphology for diagnosis in 1982, the Revised International Prognostic Scoring system (IPSS-R) developed in 2012 has been used as a gold standard scoring system for predicting prognosis in patients with myelodysplastic syndrome (MDS).[1] However, it was raised new scoring model demanded due to the discrepancy between risk grade and clinical courses.[2] Especially, intermediate-risk classified patients were placed in an ambiguous position due to their risk uncertainty and had difficulty determining therapeutic decision.[3] The prognosis of the patients is heterogenous, and reports have been conducted on how the parameters are determined in addition to the IPSS-R score. Benton et al.[4] proposed a new risk value for intermediated risk group. They showed that the inferior survival was significantly associated with the age of 66 years or greater, peripheral blood blasts of 2% or more, and history of red blood cell transfusion.

Molecular genetic alterations have been reported to affect clinical outcomes of patients with myelodysplastic syndrome.[5] Nazha et al.[6] suggested a new scoring model incorporating the mutational data into the IPSS-R in treated patients with myelodysplastic syndrome. They proposed new prognostic factors for survival including age, IPSS-R, *EZH2*, *SF3B1*, and *TP53* mutation. In addition to its role as a prognostic factor, gene mutations were used as diagnostic entities such as *CEBPA*, response to therapy, and minimal residual disease assessment as well as prognostic factors in myeloid malignancy.[7,8] Shorted telomere length reflected the genetic instability.[9] In myelodysplastic syndrome, shorted telomere length has been emerging as a prognostic factor.[10] It was shown shorter than normal control and patients with shorter telomere length showed adverse prognosis on overall survival.[10–12] Shorted telomere length reflected the genetic instability. Decreasing telomere length occurred not only in biological aging but also in clinical fatal condition such as hematologic disorder, cardiovascular disease, fibrotic lung disease, and cancers.[9,13–15] Recently several methods including quantitative polymerase chain reaction (Q-PCR), quantitative fluorescence in situ hybridization (Q-FISH), flow fluorescence in situ hybridization, and single telomere length analysis were used for measuring telomere length.[16,17]

In this study, we assessed gene mutation and telomere length as prognostic role in patient with MDS with integrating this variable into a revised IPSS. And, we aim to find additional factors to be of additive prognostic value in MDS.

2 | PATIENTS AND METHODS

2.1 | Patients

A total of 137 patients with MDS and related myeloid malignancies were enrolled. These patients were diagnosed according to the World Health Organization 2008 criteria. Bone marrow (BM) samples were collected in Seoul National University Hospital between 2004 and 2014. Eighty-eight males and 49 females were included and a median age of 66 years. Of the 137 patients with MDS, 63 MDS patients with refractory anemia with excess blasts (RAEB), 29 MDS patients with refractory cytopenia with multilineage dysplasia (RCMD), 26 MDS patients with refractory cytopenia with unilineage dysplasia (RCUD), 12 MDS patients with unclassifiable, and seven MDS patients with refractory anemia with ring sideroblasts (RARS) were included. The categorization of patients was performed according to the IPSS-R.[1] This study was approved by the institutional review board of Seoul National University Hospital (1311-091-535, 1604-082-754). All patients provided written informed consent.

2.2 | Cytogenetic studies

Cytogenetic studies using conventional G-banding were performed on BM samples. At least 20 metaphases were analyzed whenever possible. Karyotypes were recorded according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013.[18] Interphase fluorescence in situ hybridization (FISH) analysis was performed on mononuclear cells of BM aspirates to detect frequent abnormalities such as 5/5q-, −7, −7q-, +8, −20/20q-, and +1/1q+ using Vysis LSI EGR1 (5q31), D7S522 (7q31), CEP8, Trisomy 1q (1q25), and D20S108 (20q12) probes (Abott Downers).

2.3 | Telomere length measurement by quantitative fluorescence in situ hybridization

In 113 of 137 patients with MDS, telomere and centromere Q-FISH were performed according to the manufacturer's instruction. This protocol was described in our previous study.[12] Briefly, telomere (Orange) and centromere probe were added on chromosome 2 and the software calculated a telomere/centromere fluorescence intensity ratio, which is as defined telomere length. At least 100

TABLE 1 Clinical characteristics of new score-plus risk category ($n = 113$)

Characteristics	Very good (≤3.0) ($n = 14$)	Good (3.0–4.5) ($n = 31$)	Poor (4.5–7.0) ($n = 36$)	Very poor (>7.0) ($n = 32$)	p-Value
Male, *n* (%)	10 (71.4)	21 (67.7)	21 (58.3)	22 (68.8)	0.740
Age (years)[a]	60.0 (44.5–69.3)	66.0 (56.0–75.0)	66.0 (53.3–73.0)	66.5 (58.8–70.8)	0.348
Survival rate (*n*, %)	10 (71.4)	13 (41.9)	8 (22.2)	2 (6.3)	<0.001
Survival, months	170.1(14.7–287.7)	100.4 (15.6–189.5)	46.0 (10.8–120.4)	12.0 (6.0–37.8)	<0.001
Transformation to AML, *n* (%)	1 (7.1)	2 (6.5)	15 (41.7)	23 (71.9)	<0.001
IPSS-R score[a]	1.5 (1.0–2.3)	3.5 (2.5–4.0)	5.0 (4.0–5.9)	7.5 (6.5–8.5)	<0.001
ASXL1 mutation, *n* (%)	2 (14.3)	2 (6.5)	14 (38.9)	9 (28.1)	0.014
TP53 mutation, *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)	14 (43.8)	<0.001
Telomere length (T/C ratio) <5.37, *n* (%)	0 (0.0)	2 (6.5)	4 (11.1)	9 (28.1)	0.014

Abbreviation: AML, acute myeloid leukemia.

[a]Values presented as the median (interquartile range).

interphase nuclei were scanned for each patient. Samples of peripheral blood were collected from the patient with MDS.

2.4 | Targeted sequencing

We selected 87 genes related to myeloid neoplasm were performed. Genomic DNA (gDNA) was extracted from BM cells, and next-generation sequencing was performed with the Illumina Miseq 2500 platform (Illumina). gDNA shearing, standard library production, and hybridization were performed by Celemics Inc. Variants with low quality and low depth (<10) were excluded. After all of the synonymous variants (SNV) were discarded, single nucleotide variants were filtered out using a population database with an allele frequency of greater 0.5%. The SNVs that are known from previous MDS studies were rescued and we checked mapping error by visual inspection with IGV browser.

2.5 | Statistical analysis

The chi-squared test and Fisher's exact test were used to compare categorical variables. Paired *t* test and the Mann–Whitney *U* test were used for continuous variables. Kruskal–Wallis statistics were used to compare the continuous variables of several group. Estimation of overall survival was made using the Kaplan–Meier analysis, and differences among survival curves were analyzed using the log-rank test. Cox proportional hazards regression analysis was used to develop a univariate/multivariate model of prognostic factors by considering the factors that were associated with survival. Statistical analysis was performed using SPSS version 21.0 (SPSS Inc.) and Mathematica (Wolfram). *p*-Values less than 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Mutation profiles and telomere length

A total of 378 candidate mutations in 72 genes were found. One hundred twenty-eight patients of the 137 patients (83.7%) harbored at least on mutation. We selected genes that are detected in patients in more than 5% for conforming as prognostic factors. There were included *ASXL1* (31/137, 22.6%), *U2AF1* (22, 16.1%), *TP53* (18, 13.1%), *TET2* and *RUNX1* (each 15, 10.9%), *SRSF2* and *DNMT3A* (each 13, 9.5%), *EZH2* (8, 5.8%), and *SF3B1*, *STAG2*, *WT1*, *BCOR* (each 7, 5.1%). *TP53* significantly correlated with adverse prognosis by univariant cox analysis ($p < 0.001$), and *ASXL1* with adverse prognosis ($p = 0.011$). The average telomere length (T/C) of MDS was 9.47. Additionally, we assessed the telomere length as prognostic factor. Patients with each telomere length less than 5.37 showed adverse prognosis in MDS ($p = 0.009$). We selected short telomere length (mean T/C ratio < 5.37) as prognostic candidate variable in new scoring model.

3.2 | Development of a new scoring system integrating IPSS-R and molecular genetics

In Cox proportional hazards model using univariate analysis, age, IPSS-R, mutation in *TP53*, *ASXL1*, and telomere length less than 5.37 were statistically related to overall survival. Based on these findings, a new scoring model was developed using the constant value of each prognostic variable: New score = (Age ≥ 65 years) + (IPSS-R score) + (*ASXL1* mutation) + (*TP53* mutation) + (telomere length < 5.37). Each of the variables is scored as 1 when the age is over 65, gene mutations present, and TL is below 5.37.

3.3 | Comparison of new scoring model and IPSS-R

According to this new scoring system, patients were divided into *four* groups: Very good$^{\text{new scoring}}$ (score < 3, $n = 14$), Good$^{\text{new scoring}}$ (3.0–4.5, $n = 31$), Poor$^{\text{new scoring}}$ (4.5–7.0, $n = 36$), and Very poor$^{\text{new scoring}}$ (≥7.0, $n = 29$; Table 1). The age of each group did not show statistical difference between these groups ($p = 0.348$). The median OS was 170.1, 100.4, 46.0, and 12.0 months for the Very good$^{\text{new scoring}}$, Good$^{\text{new scoring}}$, Poor$^{\text{new scoring}}$, and Very poor$^{\text{new scoring}}$ groups, respectively ($p < 0.001$). Meanwhile, according to conventional IPSS-R, the median OS was 141.3, 50.2, 93.0, 36.0, and 16.2 months for the Very low$^{\text{IPSS-R}}$, Low$^{\text{IPSS-R}}$, Intermediate$^{\text{IPSS-R}}$, High$^{\text{IPSS-R}}$, and Very high$^{\text{IPSS-R}}$ groups, respectively ($p < 0.001$). And, the proportion of transformation to acute myeloid leukemia was markedly increased by 6.5% and 41.7% from good$^{\text{new scoring}}$ to poor$^{\text{new scoring}}$ group ($p < 0.001$). The rate of *ASXL1* mutation was higher frequency in poor$^{\text{new scoring}}$ (38.9%) and very poor$^{\text{new scoring}}$ group (28.1%). *TP53* was frequently mutated in the Very poor$^{\text{new scoring}}$ group (43.8%).

3.4 | Shifting of intermediate group to other group by new scoring system

Compared with IPSS-R, all patients of intermediate-risk group$^{\text{IPSS-R}}$ moved into good risk group$^{\text{new scoring}}$ of the new scoring model (Table 2). Furthermore, 34.6% (9/26) of intermediate $^{\text{IPSS-R}}$ risk group moved to poor risk group$^{\text{new scoring}}$ by the new scoring model. All of these nine patients moved to poor risk group$^{\text{new scoring}}$, 33.3% (3/9) had gene mutations (at least one mutation in *TP53*, *ASXL1*), and 88.9% (8/9) were over 65 years old. We compared overall survival according to the shifting of Intermediate group$^{\text{IPSS-R}}$. The patients shifting good$^{\text{new scoring}}$ showed better prognosis compared with those shifting poor$^{\text{new scoring}}$ group ($p = 0.085$).

3.5 | Overall survival analysis

The new scoring model showed a higher hazard ratio than the IPSS-R. The hazard ratio of Poor$^{\text{new scoring}}$ for Good$^{\text{new scoring}}$ was statistically larger than that of Intermediate$^{\text{IPSS-R}}$ for Low$^{\text{IPSS-R}}$ in overall survival and acute myeloid leukemia transformation (Table 3). In the IPSS-R, the survival graph was reversed for the Low$^{\text{IPSS-R}}$ group and the Intermediate$^{\text{IPSS-R}}$ group (Figure 1). New scoring system showed higher C-index (0.838) than IPSS-R (0.769) for adverse prognosis (acute leukemia transformation or decreased). The frequency of gene mutations was different between the new scoring model and the IPSS-R. In the new scoring model, the number of mutations increased significantly in poor$^{\text{new scoring}}$ (38.9%) and very poor$^{\text{new scoring}}$ (65.6%), respectively. Similar patterns were observed in IPSS-R, but IPSS-R showed no differences in the number of mutations between the low and intermediate-risk group. Additionally, mutations in *ASXL1*, *RUNX1*, *SFSR2*, *SF3B1*, *STAG2*, and *TP53* were observed more frequently in the Poor$^{\text{new scoring}}$ and Very poor$^{\text{new scoring}}$ groups.

4 | DISCUSSION

In our previous studies, we reported that somatic mutations appeared at a very high frequency in patients with MDS.[19] Telomere length was shorter than normal control group and shorted telomere length was related with poor survival rate.[12] Based on the existing reports, we aimed to develop a new scoring model for predicting the prognosis of patients with MDS.

The profiles of the detected genes were similar to other previous reports.[20] The genes detected in more than 5% were *ASXL1*, *U2AF1*, *TP53*, *RUNX1*, *TET2*, *DNMT3A*, *SRSF2*, *EZH2*, *SF3B1*, *BCOR*, *STAG2*, and *WT1*. Among these genes, *ASXL1* and *TP53* were associated with adverse prognoses. The frequency of these genes was further different in the new scoring model than existing in this study. *DNMT3A* and *TET2* showed similar frequencies regardless of the risk group in the existing IPSS-R and in the new model, whereas *ASXL1* was high in the poor risk group in the new scoring model, respectively. *DNMT3A*, *ASXL1*, and *TET2* were known to be common mutations with aging.[6,21] Unlike *DNMT3A* and *TET2*, the frequency of *ASXL1* gene mutation should be considered to be more weighted when the myeloid neoplasm was present. Actually, *DNMT3A* or *TET2* loss of function has been reported the altered methylation patterns in pluripotency genes but cancer rarely develops in mice.[22,23] In the case of *TP53* mutation, it seems to be strongly associated with adverse prognosis in both scoring models as previously known. *RUNX1*, *SRSF2*, and *SF3B1* also had similar patterns, but they did not have statistical significance in new model despite relatively sufficient patient numbers. Thus, it seems that the new scoring model could be able to determine the weight of gene more clearly, which makes the new model better at predicting outcomes. Especially, it was confirmed the effects of gene mutations on the intermediate-risk group$^{\text{IPSS-R}}$. The moving of prognostic group in intermediate-risk group$^{\text{IPSS-R}}$ was determined by these gene mutations. Recently, NGS has been changed the diagnostic workflow in clinical fields as routine inspection.[24,25] In particular, it is very helpful when the number of genes involved in disease is large. It may detect candidate genes that cause the disease or target of drug.[26] However, in the clinical field, this test is time consuming and costly. Therefore, it may be more useful to proceed with the examination of some clinically critical genes first and to establish a faster treatment plan accordingly.

In addition, ethnic differences will be considered in developing a new scoring model. Nazha et al. included *TP53*, *EZH2*, and *SF3B1* in new molecular scoring model. In our study, the *SF3B1* and *EZH2* mutation was detected in 5% of total patients and there was no statistical significance. It might be due to low frequency. In *SF3B1* gene, this is due to the low frequency of myelodysplastic syndrome, refractory anemia with ring sideroblasts (MDS, RARS), and refractory anemia with ring sideroblasts with thrombocytosis (RARS-T) in the Asian group. The frequency of RARS and RARS-T was reported 12% and 2% in the Europe chronic myeloid disorders working group of international cancer genome consortium report, and *SF3B1* mutation was strongly associated only with this subtype. On the contrary, according to the Japanese MDS working group, the incidence of RARS

TABLE 2 Clinical characters of patient with interchange prognostic group from Intermediate$^{IPSS\text{-}R}$ to other groups$^{new\ scoring}$

N	Age	Sex	MDS subtype	New scoring group	IPSS-R	Telomere length (T/C)	Mutation			Diagnosis date	Treatment	Survival
							ASXL1	TP53	EZH2			
1	18	M	RCMD	Good	4.5	18.82	No	No	No	2013-03-05	Vidaza (2013.12–2014.2) → alloPBSCT (2014.04)	Die
2	27	M	RCUD	Good	4.5	5.59	No	No	No	2008-06-13	Dacogen (2008.10–2009.2)	Die
3	30	M	RCUD	Good	3.5	9.64	No	No	No	2006-08-16	alloPBSCT (2007.5)	Survival
4	36	F	RAEB	Good	4.0	9.31	No	No	No	2008-07-28	Dacogen (2008.6–2008.12) → alloPBSCT (2009.1)	Die
5	32	M	RCMD	Good	4.5	14.02	No	No	No	2011-08-08	Dacogen (2011.2–6) → alloPBSCT (2011.10)	Survival
6	69	M	RCMD	Good	3.5	19.04	No	No	No	2009-04-14	Supportive care	Die
7	47	M	RAEB	Good	4.5	11.21	No	No	No	2011-07-28	Dacogen(2011.5–7) → alloPBSCT (2011.9)	Survival
8	61	M	RCUD	Good	4.0	8.64	No	No	No	2009-05-21	alloPBSCT (2009.5)	Die
9	59	F	RCMD	Good	4.0	7.98	No	No	No	2013-10-17	Supportive care	Survival
10	57	M	RCMD	Good	4.0	5.40	No	No	No	2011-07-26	Dacogen (2011.08–2014.09)	Survival
11	61	F	RAEB	Good	4.0	6.62	No	No	No	2005-06-27	Vidaza (2006.09)	Die
12	50	F	RCUD	Good	3.5	9.65	No	No	No	2011-10-24	Dacogen (2011.11) →alloPBSCT (2013.1)	Survival
13	56	F	RCMD	Good	4.0	9.95	No	No	No	2007-10-10	Supportive care	Survival
14	50	F	RARS	Good	3.5	7.69	No	No	No	2010-06-07	Supportive care	Survival
15	38	F	RAEB	Poor	4.0	6.82	Yes	No	No	2008-08-19	Vidaza (2008.9–12) →alloPBSCT(2009.03)	Die
16	75	F	RAEB	Good	3.5	12.05	No	No	No	2013-08-22	Supportive care	Survival
17	75	M	RCMD	Poor	4.0	16.40	No	No	No	2012-04-19	Supportive care	Die
18	74	F	RCMD	Poor	4.0	13.68	No	No	No	2009-09-28	Vidaza (2010.11)	Survival
19	75	F	RCUD	Good	3.5	16.22	No	No	No	2011-04-25	Supportive care	Survival
20	72	F	RCMD	Poor	4.0	12.02	No	No	No	2012-02-08	Dacogen (2012.2–5)	Die
21	56	M	RCUD	Good	3.5	4.36	No	No	No	2006-09-13	alloPBSCT (2007.1)	Die
22	66	F	RAEB	Poor	4.0	6.26	No	No	No	2007-09-10	Vidaza (2006.9–2007.9)	Die
23	73	M	RCUD	Poor	4.5	8.85	No	No	No	2011-10-10	Vidaza (2011.6–9)	Survival
24	81	M	RAEB	Poor	4.5	6.73	No	No	No	2012-05-09	Supportive care	Survival
25	72	M	RCMD	Poor	4.0	21.47	Yes	No	Yes	2012-10-04	Vidaza (2013.3–7)	Die
26	73	M	RCMD	Poor	3.5	11.75	Yes	No	Yes	2011-05-20	Supportive care	Die

TABLE 3 Overall survival and acute myeloid leukemia transformation by revised IPSS and new scoring models

	Overall survival			AML transformation		
Risk factors	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Revised IPSS			<0.001			<0.001
Very low vs. Low	2.724	0.930–7.976	0.068	1.860	0.193–17.937	0.591
Very low vs. intermediate	1.913	0.647–5.656	0.241	0.954	0.086–10.556	0.970
Very low vs. high	3.637	1.268–10.49	0.016	9.715	1.272–74.226	0.028
Very low vs. very high	4.629	1.622–13.215	0.004	19.368	2.577–145.550	0.004
Low vs. intermediate	0.732	0.386–1.385	0.337	0.626	0.104–3.758	0.608
New scoring						
Very good vs. Good	2.360	0.798–6.979	0.121	1.326	0.119–14.761	0.819
Very good vs. Poor	3.758	1.313–10.753	0.14	10.644	1.389–81.565	0.023
Very good vs. Very poor	7.225	2.517–20.739	<0.001	33.896	4.425–259.628	0.001
Good vs. Poor	1.685	0.929–3.057	0.086	8.816	2.007–38.719	0.004

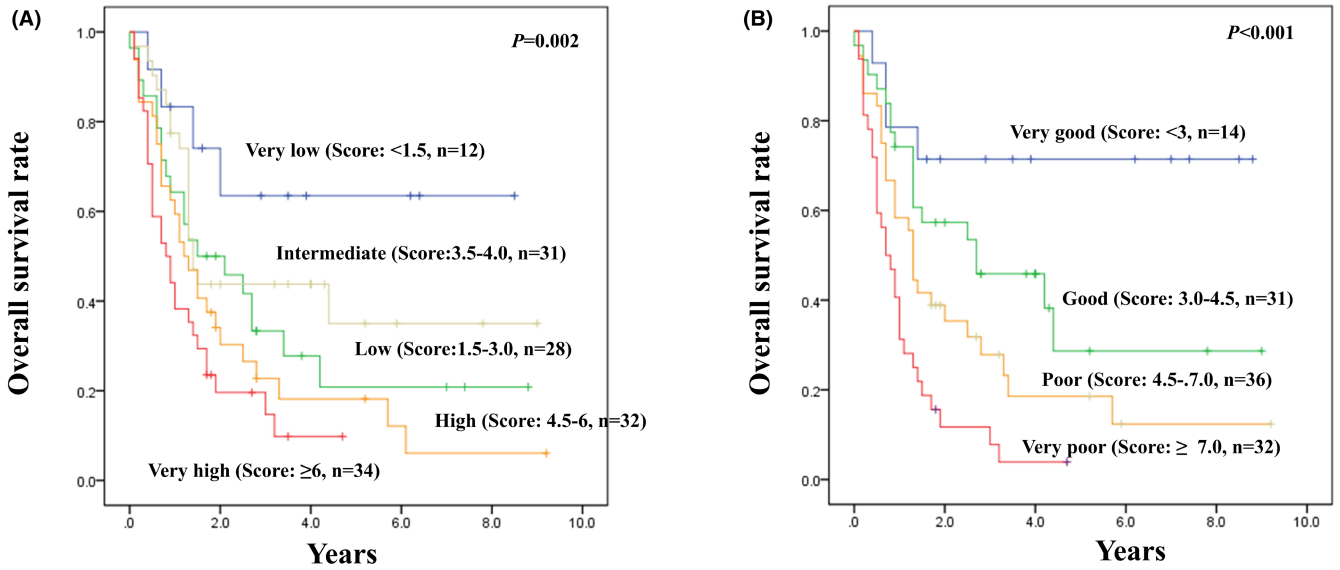

FIGURE 1 Overall survival rate by Kaplan–Meier estimated survival curve according to scoring system (A) IPSS-R scoring (B) New scoring system in Korean MDS

was 12.6% in Caucasian, while in Japanese group, it was 4%, which was about one-third lower.[27] Therefore, differentiated prognostic models for each ethnic group should be applied.

Shorter TLs were reported in MDS than in healthy individuals, suggesting TL as an adverse prognostic factor in MDS.[10] Müezzinler et al.[28] reported telomere length of leukocyte shorted by 24.7 bp per year using sighted linear regression. In addition, due to heterogeneity of MDS, it remains controversial whether telomere length plays a role as a prognostic factor. But, in the present study, we investigated whether TL has additive predictive value in a new scoring model. It was confirmed that a short telomere length of less than 5.37 is an adverse prognostic marker independently of age. To the best of our knowledge, this is the first report of a new scoring model that combines gene mutations and telomere length with the existing IPSS-R.

This study has some limitations. First, we did not include enough patients for statistical analysis to draw new prognostic system and did not verify this new model with a larger number of patients. Nevertheless, even with a small number, it was possible to show the effect of shorter telomeres and genetic aberration as prognostic markers. And, we showed a new prognostic model is more powerful than the existing IPSS-R for predicting adverse prognoses. Therefore, if it is validated, this new scoring system will be useful in clinical practice. Second, the method for measuring telomere lengths diverse such as terminal restriction fragment analysis, Q-PCR including Q-FISH we used,[29] and method standardization has been not established yet. Considering the convenience and reproducibility of these methods that can be used in clinical laboratories, it should be extensive verification of telomere length measurement method.

In conclusion, by adding gene mutations specific to Asian MDS and TL to the existing IPSS-R, we were able to more clearly predict patient prognosis, especially in regard to intermediate-risk patients. When considering the long turnaround time of next-generation sequencing, gene testing for prognostic mutations may be more useful for establishing a treatment plan quickly.

AUTHOR CONTRIBUTIONS

H.S.P. and K.I. carried out the experiment, analyzed the data, and wrote the study. D.-Y.S. and S.-S.Y contributed to the interpretation of clinical data. S.K provided critical feedback and helped shape the research. S.W.K. helped supervise the project. D.S.L. designed the study and supervised this project.

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CONFLICT OF INTEREST

There are no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are not publicly available.

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REFERENCES

1. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. *Blood*. 2012;120(12):2454-2465.
2. Zeidan AM, Sekeres MA, Wang XF, et al. Comparing the prognostic value of risk stratifying models for patients with lower-risk myelodysplastic syndromes: is one model better? *Am J Hematol*. 2015;90(11):1036-1040.
3. Alzahrani M, Power M, Abou Mourad Y, et al. Improving revised international prognostic scoring system pre-allogeneic stem cell transplantation does not translate into better post-transplantation outcomes for patients with myelodysplastic syndromes: a single-center experience. *Biol Blood Marrow Transplant*. 2018;24(6):1209-1215.
4. Benton CB, Khan M, Sallman D, et al. Prognosis of patients with intermediate risk IPSS-R myelodysplastic syndrome indicates variable outcomes and need for models beyond IPSS-R. *Am J Hematol*. 2018;93(10):1245-1253.
5. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013;122(22):3616-3627.
6. Nazha A, Narkhede M, Radivoyevitch T, et al. Incorporation of molecular data into the revised international prognostic scoring system in treated patients with myelodysplastic syndromes. *Leukemia*. 2016;30(11):2214-2220.
7. Pastore F, Kling D, Hoster E, et al. Long-term follow-up of cytogenetically normal CEBPA-mutated AML. *J Hematol Oncol*. 2014;7(1):1-8.
8. Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular minimal residual disease in acute myeloid leukemia. *N Engl J Med*. 2018;378(13):1189-1199.
9. Zhang X, Zhao Q, Zhu W, et al. The Association of Telomere Length in peripheral blood cells with cancer risk: a systematic review and meta-analysis of prospective StudiesMeta-analysis of telomere length and cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2017;26(9):1381-1390.
10. Williams J, Heppel NH, Britt-Compton B, et al. Telomere length is an independent prognostic marker in MDS but not in de novo AML. *Br J Haematol*. 2017;178(2):240-249.
11. Poloni A, Serrani F, Berardinelli E, et al. Telomere length, c-myc and mad-1 expression could represent prognosis markers of myelodysplastic syndrome. *Leuk Res*. 2013;37(11):1538-1544.
12. Park HS, Choi J, See C-J, et al. Dysregulation of telomere lengths and telomerase activity in myelodysplastic syndrome. *Ann Lab Med*. 2017;37(3):195-203.
13. Hammadah M, Al Mheid I, Wilmot K, et al. Telomere shortening, regenerative capacity, and cardiovascular outcomes. *Circ Res*. 2017;120(7):1130-1138.
14. Lansdorp PM. Maintenance of telomere length in AML. *Blood Adv*. 2017;1(25):2467-2472.
15. Steenstrup T, Kark JD, Verhulst S, et al. Telomeres and the natural lifespan limit in humans. *Aging (Albany NY)*. 2017;9(4):1130-1142.
16. Barkovskaya MS, Bogomolov AG, Knauer NY, Rubtsov NB, Kozlov VA. Development of software and modification of Q-FISH protocol for estimation of individual telomere length in immunopathology. *J Bioinform Comput Biol*. 2017;15(2):1650041.
17. Hills M, Lücke K, Chavez EA, Eaves CJ, Lansdorp PM. Probing the mitotic history and developmental stage of hematopoietic cells using single telomere length analysis (STELA). *Blood*. 2009;113(23):5765-5775.
18. Shaffer LG, McGowan-Jordan J, Schmid M. *ISCN 2013: An International System for Human Cytogenetic Nomenclature (2013)*. Karger Medical and Scientific Publishers; 2013.
19. Hwang SM, Kim SY, Kim JA, et al. Short telomere length and its correlation with gene mutations in myelodysplastic syndrome. *J Hematol Oncol*. 2016;9(1):1-13.
20. Xu F, Wu L-Y, He Q, et al. Exploration of the role of gene mutations in myelodysplastic syndromes through a sequencing design involving a small number of target genes. *Sci Rep*. 2017;7(1):1-10.
21. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26):2488-2498.
22. Jeong M, Sun D, Luo M, et al. Large conserved domains of low DNA methylation maintained by Dnmt3a. *Nat Genet*. 2014;46(1):17-23.
23. Koh KP, Yabuuchi A, Rao S, et al. Tet1 and Tet2 regulate 5-hydroxymethylcytosine production and cell lineage specification in mouse embryonic stem cells. *Cell Stem Cell*. 2011;8(2):200-213.
24. Pagin A, Devos A, Figeac M, et al. Applicability and efficiency of NGS in routine diagnosis: in-depth performance analysis of a complete workflow for CFTR mutation analysis. *PLoS ONE*. 2016;11(2):e0149426.
25. Luthra R, Patel KP, Reddy NG, et al. Next-generation sequencing-based multigene mutational screening for acute myeloid leukemia using MiSeq: applicability for diagnostics and disease monitoring. *Haematologica*. 2014;99(3):465-473.
26. Mitsui T, Narumi S, Inokuchi M, et al. Comprehensive next-generation sequencing analyses of hypoparathyroidism: identification of novel GCM2 mutations. *J Clin Endocrinol Metabol*. 2014;99(11):E2421-E2428.

27. Miyazaki Y, Tuechler H, Sanz G, et al. Differing clinical features between Japanese and Caucasian patients with myelodysplastic syndromes: analysis from the international working Group for Prognosis of MDS. *Leuk Res*. 2018;73:51-57.
28. Müezzinler A, Zaineddin AK, Brenner H. A systematic review of leukocyte telomere length and age in adults. *Ageing Res Rev*. 2013;12(2):509-519.
29. Aubert G, Hills M, Lansdorp PM. Telomere length measurement—caveats and a critical assessment of the available technologies and tools. *Mutat Res*. 2012;730(1-2):59-67.

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