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ORIGINAL ARTICLE

Validation of the revised International Prognostic Scoring System in patients with myelodysplastic syndrome in Japan: results from a prospective multicenter registry

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Abstract The Japanese National Research Group on Idiopathic Bone Marrow Failure Syndromes has been conducting prospective registration, central review, and follow-up study for patients with aplastic anemia and myelodysplastic syndrome (MDS) since 2006. Using this database, we retrospectively analyzed the prognosis of patients with MDS. As of May 2016, 351 cases were registered in this database, 186 of which were eligible for the present study. Kaplan–Meier analysis showed that overall survival (OS) curves of the five risk categories stipulated by the revised international prognostic scoring system (IPSS-R) were reasonably separated. 2-year OS rates for the very low-, low-, intermediate-, high-, and very high-risk categories were 95, 89, 79, 35, and 12%, respectively. In the same categories, incidence of leukemic transformation at 2 years was 0, 10, 8, 56, and 40%, respectively. Multivariate analysis revealed that male sex, low platelet counts, increased blast percentage (>2%), and high-risk karyotype abnormalities were independent risk factors for poor OS. Based on these data, we classified Japanese MDS patients who were classified as intermediate-risk in IPSS-R, into the lower risk MDS category, highlighting the need for careful assessment of treatments within low- and high-risk treatment protocols.

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Introduction

Myelodysplastic syndrome (MDS) is a heterogeneous group of hematopoietic stem cell disorders, characterized by ineffective hematopoiesis and a high propensity to transform into acute myeloid leukemia (AML). The clinical course of MDS is highly variable; the disease may progress into AML quickly, or it may remain in a stable condition for years without any interventions. Disease risk stratification is important for selecting optimal treatments for individual patients. Various systems, including international prognostic scoring system (IPSS) [1, 2], World Health Organization prognostic scoring system [3], Global MD Anderson risk model score for MDS [4], and a gene-only model [5] have been developed for this purpose [6].

IPSS, which was proposed in 1997, remains the most commonly used among the systems. According to the IPSS, patients are categorized into 4 risk groups by calculating risk scores, which are determined by percentages of bone marrow (BM) blasts, karyotypes, and number of cytopenias. The low- and int-1-risk groups in the IPSS are generally regarded as the lower risk MDS, and the int-2- and high-risk groups are regarded as the higher risk MDS. The former groups are often indolent, and the latter groups are prone to progress to AML. If a patient is categorized into the higher risk MDS group, either hematopoietic stem cell transplantation (SCT), intensive chemotherapy, or treatment with hypomethylating agents is proposed depending on age and comorbidities [7]. Revised IPSS (IPSS-R) has recently been developed based on the multivariate analysis of overall survival (OS) and time to AML transformation data for more than 7000 patients with MDS registered worldwide [8]. This system underscores the contribution of chromosomal abnormalities to the prognosis of patients with MDS, and a refined cytogenetic scoring system including 5 risk categories was adapted [9]. In the IPSS-R, patients are stratified into five risk categories (very low, low, intermediate, high, and very high) with significantly different median OS (8.8, 5.3, 3, 1.6, and 0.8 years, respectively) established based on five main features, including cytogenetics, BM blast percentage ($\leq$2, 2–5, 5–10, and $\geq$10%), and depth of cytopenias [Hb ($\geq$10, 8–10, and <8 g/dL), platelet count ($\geq$100, 50–99, and <50 K/μL), and absolute neutrophil count ($\geq$0.8 and <0.8 K/μL)]. The OS curves for the 5 categories have been shown to have clear separation in several recent studies [10–12]. The very low- and low-risk categories in IPSS-R are regarded as lower risk MDS, and the high- and very high-risk categories are higher risk MDS. Patients with the latter categories are candidates for intensive treatments including SCT. However, the optimal treatments for patents in the IPSS-R intermediate-risk category remain ambiguous [8]. In addition, given that IPSS-R was developed from the data for patients who did not receive disease-altering treatments, such as hypomethylating agents, intensive chemotherapy, and SCT, during their MDS phases, the actual prognosis of patients with MDS including those who received such treatments may be different. Furthermore, IPSS-R has not been verified in a large MDS cohort in Japan.

Since 2006, the Japanese National Research Group on Idiopathic Bone Marrow Failure Syndromes has been conducting the prospective registration, central review, and follow-up study for aplastic anemia and MDS. Using the database of this study cohort, we retrospectively analyzed the OS and leukemia-free survival (LFS) of patients with MDS according to IPSS- and IPSS-R-risk categories to verify these systems in the real-world practice in Japan. We also investigated the ratios of patients who received SCT in each risk category.

Methods

Patient database

We used the data from the Prospective Registration, Central Review, and Follow-up Study for Aplastic Anemia and MDS conducted by the Japanese National Research Group on Idiopathic Bone Marrow Failure Syndromes. This prospective registration system collected newly diagnosed cases with aplastic anemia and MDS using the definition of French–American–British (FAB) classification [13] as well as cases with cytopenias with unknown etiologies from 16 institutions in Japan (Supplementary Table S1). After registration, patients with BM blasts <5% were subjected to central review for final diagnosis. At least two hematologists and one pathologist reviewed the peripheral blood and BM smear specimens, BM biopsy and/or clot sections, and chromosomal karyotype data for each case, and diagnosed separately. Morphological diagnoses were essentially based on the criteria of the International Working Group on Morphology of MDS [14, 15]. If the diagnosis was inconsistent,

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the final diagnosis was determined by inspection and discussion in regular inspection meetings held twice a year. We did not take into account the patients' prior history of cytotoxic therapy in the diagnosis. Medical records of registered cases were collected every 6 months. This study was performed in accordance with the Helsinki Declaration. The study protocol was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine and by the ethics committee of each participating institution. Written informed consent was obtained from the participating patients. Cases registered between April 2006 and May 2016 were enrolled in this study.

Statistics

OS was defined as the time from the initial diagnosis to death. LFS was defined as the time until death or the date on which the patient was found with >20% blasts in either peripheral blood or BM. Patients who had been alive at the last follow-up were censored. The Kaplan–Meier method was used for the survival analysis, and comparison between groups was performed using the log-rank test. Cumulative incidences of leukemic transformation were compared using Gray's test [16]. Possible risk factors including age, gender, hemoglobin (Hb), neutrophil counts, platelet counts, BM blast percentage, and chromosomal abnormalities were also analyzed using the Cox proportional hazards model. A multivariate analysis was performed using a stepwise logistic regression model. p values <0.05 were considered to be statistically significant. All the analyses were conducted using EZR, version 1.30 [17].

Results

Patient characteristics

A total of 351 cases were registered to the registry by May 2016. Among these cases, 215 were diagnosed as having MDS as defined by FAB classification in the central review system (Supplementary Table S2). After excluding those who were not registered within 1 year after the institutional diagnosis and those whose follow-up periods were shorter than 4 weeks, 186 MDS cases remained to be analyzed for the study. One hundred and nineteen were male; the median age at diagnosis was 68 and the median follow-up time of survivors was 24 months. According to the WHO classification 2008 [18], this cohort included 30 refractory cytopenia with unilineage dysplasia; 78 refractory cytopenia with multilineage dysplasia; 2 refractory anemia with ring sideroblasts; 2 MDS with isolated deletion 5q; 14 MDS, unclassified; 21 refractory anemia with excess blast-1 (RAEB-1); 16 RAEB-2; 3 MDS with fibrosis; 12 myelodysplastic/myeloproliferative neoplasms (mostly chronic myelomonocytic leukemia); and 8 AML with BM blast <30% patients. The number of cases in the low-, int-1-, int-2-, and high-risk groups according to the IPSS were 37, 94, 44, and 11, respectively. According to the IPSS-R, the number of cases in the very low-, low-, intermediate-, high-, and very high-risk categories was 21, 69, 49, 23, and 24, respectively.

Prognostic significance of age and gender in patients with MDS

Patients were divided into 5 age groups (under 50 years, in their 50s, 60s, 70s, and 80 years or above), and their prognoses were analyzed using the Kaplan–Meier method. Younger age groups tended to show better OS ($p = 0.06$) and LFS ($p = 0.18$) as compared with the elderly groups, but not statistically significant (Supplementary Fig. S1, panels a, b). The cumulative incidences of leukemic transformation were not different between these groups (Supplementary Fig. S1c). When we divided the patients into young (under 60) and elderly (60 or over) groups, those in the former group showed significantly better OS compared with those in the latter group ($p = 0.03$, Supplementary Fig. S1d). However, the LFS and cumulative incidences of leukemic transformation were not different between these groups (Supplementary Fig. S1, panels e, f). Subsequently, we examined the effect of sex on the survival of patients with MDS. Kaplan–Meier analyses showed that the survival curves of OS and LFS were superior in the female patients over the male patients, but the differences were not statistically significant (Supplementary Fig. S2, panels a, b). No significant differences of cumulative incidences of leukemic transformation were observed between genders (Supplementary Fig. S2c).

OS, LFS, and the incidence of leukemic transformation in IPSS risk groups

In a Kaplan–Meier analysis, the OS curves of the low- and int-1-risk groups by the IPSS were almost completely overlapped, and those of the int-2- and high-risk groups were nearly overlapped (Fig. 1a). The curves of the former two and the latter two were clearly separated. The 2-year OS rates of the low-, int-1-, int-2-, and high-risk groups were 87, 85, 33, and 30%, respectively. If patients were censored when they received allogenic SCT, the OS curve hight of the high-risk group became lower than that in Fig. 1a, but OS curves of the other groups were essentially the same as Fig. 1a (Fig. 1b). The LFS curves of the low- and int-1-risk groups were almost completely overlapped, and these were clearly superior to those of the int-2 and high-risk groups (Fig. 1c). The 2-year LFS of the low-, int-1-, int-2-,

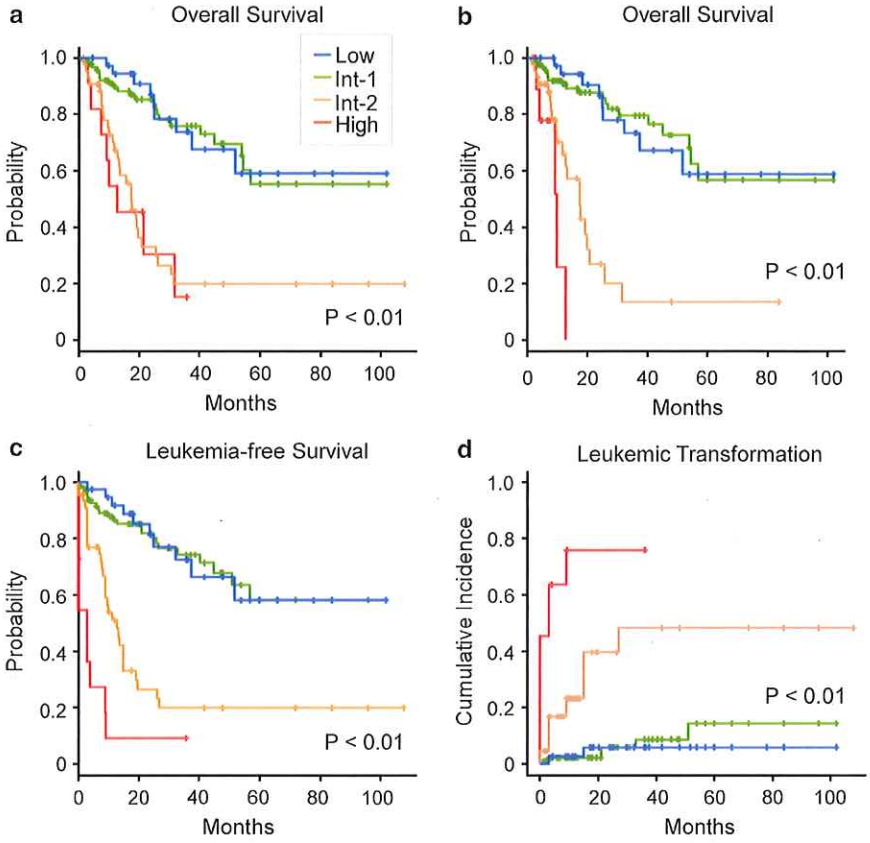

Fig. 1 Survival probabilities and cumulative incidences of leukemic transformation according to the IPSS. **a** Overall survival. **b** Overall survival if patients were censored when they received allogenic SCT. **c** Leukemia-free survival. **d** Cumulative incidences of leukemic transformation

and high-risk groups were 82, 81, 26, and 9%, respectively (Fig. 1c). The cumulative incidence curves of leukemic transformation showed that approximately half of the patients with the int-2- and high-risk groups progressed into AML within 2 years, while less than 20% of those with the low- and int-1-risk groups developed AML during the same duration (Fig. 1d).

OS, LFS, and the incidence of leukemic transformation in IPSS-R risk categories

The OS curves of the 5 risk categories by the IPSS-R were reasonably separated. The 2-year OS rates of the very low-, low-, intermediate-, high-, and very high-risk categories were 95, 89, 79, 35, and 12%, respectively (Fig. 2a). The OS curve of the intermediate-risk category was closer to that of the low-risk category than to that of the high-risk category; the OS curve of the very low-risk category was clearly superior to the curves of the other 4 categories. If patients were censored when they received allogenic SCT, the OS curves of the high- and very high-risk categories further dropped, which were clearly separated from those of the other 3 categories (Fig. 2b). The LFS curves of these 5 categories were also well separated by Kaplan–Meier analysis. However, similar to the OS corves, the LFS curve of the intermediate-risk category was closer to that of the low-risk category rather than that of the high-risk category, and that of the very low-risk category was clearly superior to those of the other 4 categories (Fig. 2c). The 2-year LFS of the very low-, low-, intermediate-, high-, and very high-risk categories were 95, 80, 71, 30, and 11%, respectively. Approximately half of the patients with the high- and very high-risk categories progressed into AML within 2 years, while less than 20% of the other 3 risk categories progressed into AML during the same duration (Fig. 2d). The incidences of leukemic transformation of the very low-, low-, intermediate-, high-, and very high-risk categories at 2 years of diagnosis were 0, 10, 8, 56, and 40%, respectively.

Stratification of the IPSS-defined risk groups by the IPSS-R

To compare the IPSS and IPSS-R in our cohort, we stratified the IPSS-defined risk groups by the IPSS-R. As expected, most of the patients in the IPSS-defined low-risk group were classified into either the very low- or low-risk category, and most of those in the IPSS-defined high-risk group were classified into the high- or very high-risk category in IPSS-R (Fig. 3a). In contrast, the IPSS-defined

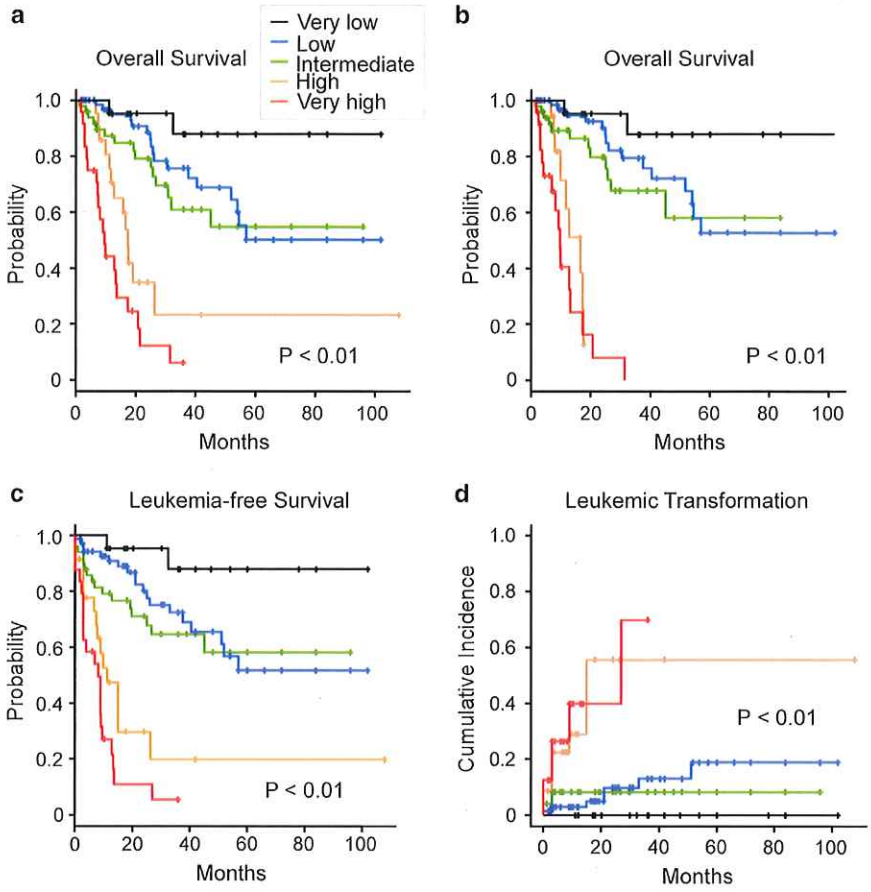

Fig. 2 Survival probabilities and cumulative incidences of leukemic transformation according to the IPSS-R. **a** Overall survival. **b** Overall survival if patients were censored when they received allogenic SCT. **c** Leukemia-free survival. **d** Cumulative incidences of leukemic transformation

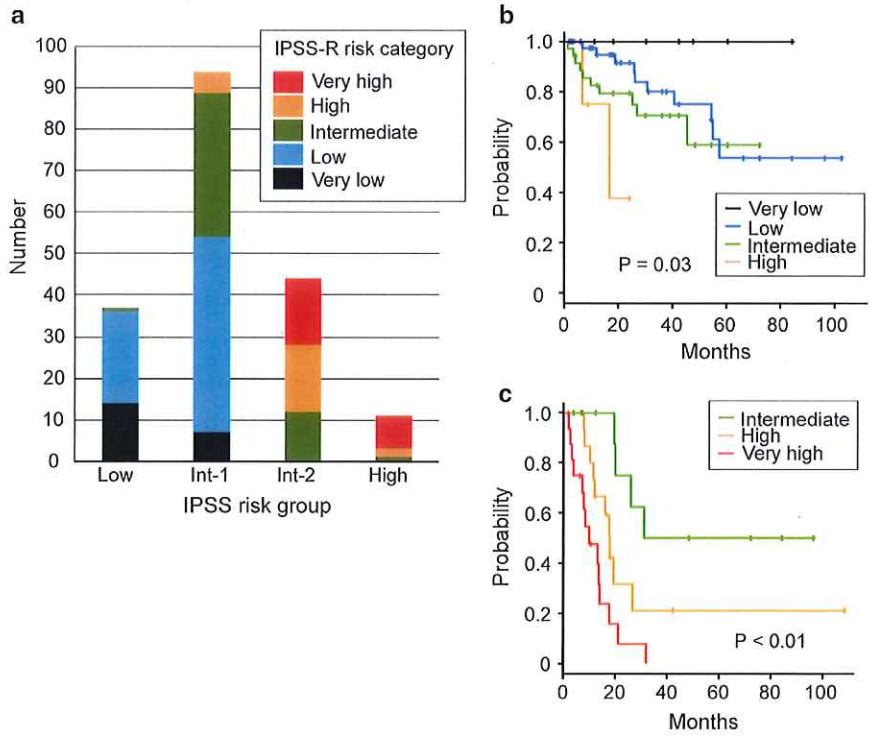

Fig. 3 Stratification of the IPSS-defined risk groups into five IPSS-R-defined risk categories. **a** Numbers of cases in each IPSS-defined risk group are shown in a stacked bar chart. **b** Overall survival of each IPSS-R category among the IPSS-defined int-1-risk group. **c** Overall survival of each IPSS-R category among the IPSS-defined int-2-risk group

int-1- and int-2-risk groups could be further separated by IPSS-R into 4 and 3 risk categories, respectively. The majority of patients in the IPSS-defined int-1-group were classified into the low- or intermediate-risk category in IPSS-R; however, a certain number of patients in this group were classified into the very low- and high-risk categories (Fig. 3a). OS curves of these 4 categories were reasonably separated (Fig. 3b); it is noteworthy that no death was recorded among the patients in the very low-risk category. Patients with the IPSS-defined int-2-risk group were divided into nearly equal proportions of 3 risk categories, from intermediate to very high (Fig. 3a). The OS curves of these categories were also well separated (Fig. 3c).

Risk factors for OS

In the IPSS-R, chromosomal karyotype, BM blast percentage, Hb, neutrophil counts, and platelet counts were categorized and incorporated into the calculation. We divided the patients with MDS into 5 groups according to karyotype categories of the IPSS-R [9] and assessed their OS by the Kaplan–Meier method. The OS curves of patients with the very good-, good-, or intermediate-risk karyotypes were nearly overlapped, and these were clearly superior to those with the poor- and very poor-risk karyotypes (Fig. 4a). We then divided the patients into 4 groups by BM blast percentages: ≤2%, 2–5%, 5–10%, and >10%. The OS curve of patients with BM blasts ≤2% was evidently superior to those of the other 3 groups (Fig. 4b). Therefore, we set the cutoff of blast percentage to be 2%. Then, we assessed the risk factors for OS by univariate analysis (Table 1). The cut-off values of Hb (8.0 g/dL), neutrophil counts (800/μL), and platelet counts (100,000/μL) were took from the IPSS-R. Age (cutoff 60 years) and gender were also included as confounders in the model. Age ≥60 years, Hb <8 g/dL, platelet counts <100,000/μL, BM blast >2%, and the high- and very high-karyotype risks were significantly associated with poor OS, whereas gender and neutrophil counts <800/μL were not. In a multivariate analysis, male sex, low platelet counts (<100,000/μL), increased blast percentage (>2%), and the high-risk karyotype abnormalities (the high or very high-risk groups) were shown to be independent risk factors for poor OS. The highest hazard ratio of >10 was observed in the high-risk karyotype abnormalities (Table 1).

Ratios of patients receiving SCT

We evaluated the ratios of patients who received SCT among those ≤65 years old, because patients >65 years are generally regarded not to be eligible for SCT. In the IPSS, less than 20% of those with the low- and int-1-risk groups received SCT, while more than 60% of those with the int-2- and high-risk groups received SCT during their follow-up periods (Fig. 5a). When the patients were divided according to IPSS-R categories, a third of those in the intermediate-risk category, three-fourths of those in the high-risk category, and nearly half of those in the very high-risk category underwent SCT during the follow-up period, while very few in the very low- and low-risk categories underwent SCT (Fig. 5b).

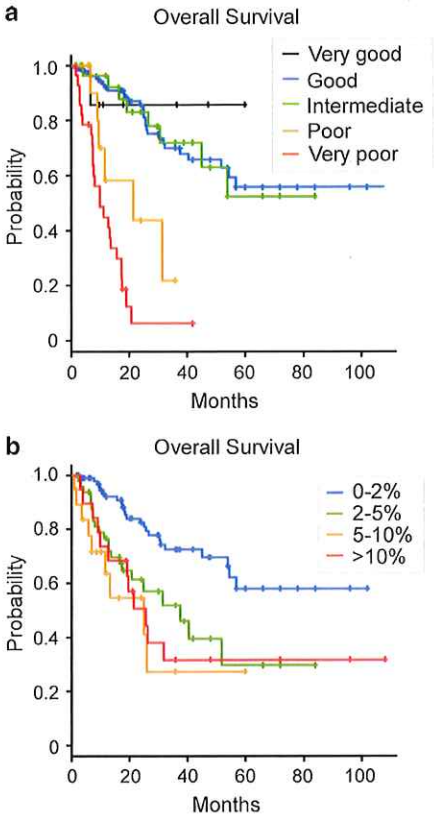

Fig. 4 Survival probabilities according to **a** karyotype risks by IPSS-R and **b** bone marrow blast percentage

Discussion

In this study, we analyzed the OS and LFS of patients with MDS in Japan. When we divided our patients according to IPSS, the OS curves for the low- and int-1-risk groups almost completely overlapped, and those for the int-2- and high-risk groups nearly overlapped; the former two were clearly superior to the latter two (Fig. 1). These results are consistent with our understanding that, in the IPSS, the low- and int-1-risk groups are regarded as the lower risk MDS and the int-2- and high-risk groups are the higher risk MDS. When we divided the patients according to the IPSS-R, the OS curves of risk categories were well separated,

Table 1 Risk factors of OS

Categories	Criteria	*n*	Median OS (months)	Univariate analysis	Multivariate analysis	
				p	Hazard ratio (95% confidence intervals)	*p*
Age	<60 years	48	NR	**0.009**	1	**0.004**
	≥60 years	138	45		2.58 (1.36–4.88)	
Gender	Female	67	NR	0.294	1	**0.017**
	Male	119	52		1.96 (1.13–3.43)	
Hemoglobin	≥8 g/dL	126	NR	**0.003**		
	<8 g/dL	60	26			
Neutrophil counts	≥800	139	54	0.094		
	<800	47	26			
Platelet counts	≥100,000	82	NR	0.051	1	**0.041**
	<100,000	104	45		1.69 (1.02–2.81)	
BM blast percentages	≤2%	99	NR	**<0.001**	1	**0.031**
	>2%	79	26		1.79 (1.06–3.05)	
Karyotype risks (IPSS-R)	Very low, low, or intermediate	146	NR	**<0.001**	1	**<0.001**
	Poor or very poor	40	12		10.35 (5.66–18.95)	

p values <0.05 are shown in bold

OS overall survival; *NR* not reached; *BM* bone marrow; *IPSS-R* revised-international prognostic scoring system

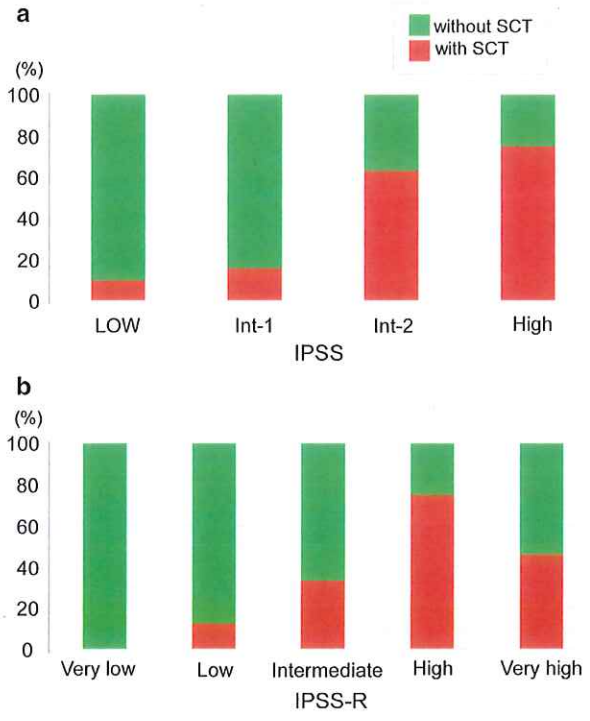

Fig. 5 Ratios of patients receiving allogenic stem cell transplantation among those ≤65 years according to the **a** IPSS and **b** IPSS-R

and their estimated survivals were comparable with those by the original IPSS-R and its validation studies that followed [8, 10–12]. However, the OS and LFS curves for the low- and intermediate-risk categories were relatively close, and those for the high- and very high-risk categories were also close; furthermore, the curve of the very low-risk category was clearly superior to those of the other 4 categories (Fig. 2, panels a, b). Thus, the IPSS-R seems to be quite useful into identifying the very low-risk patients with extremely favorable prognosis, who could not be stratified by conventional IPSS.

In this study, we found that the IPSS-R can be utilized for restratification of each of the IPSS-defined risk groups (Fig. 3a). Indeed, the IPSS-R allowed reclassification of the IPSS-defined int-1-risk group, which included the largest number of MDS patients, into various prognostic subgroups, from the very low- to high-risk categories. Among patients in the IPSS-defined int-1-risk group, no death was observed for those classified into the IPSS-R-defined very low-risk category during the observation period, while the median OS for those classified into the high-risk category was only 1.4 years (Fig. 3b). Thus, patients with IPSS-defined int-1 risk group could be stratified more precisely by IPSS-R.

One of the main questions in the current study was whether Japanese patients with MDS in the intermediate-risk category in the IPSS-R should be treated as the lower risk or higher risk MDS. A German study group investigated the OS and incidence of leukemic transformation of 1313 patients with MDS including those who received

disease-altering therapies [10]. In this study, the OS curve and cumulative incidence of leukemic transformation of patients in the intermediate-risk category by IPSS-R were clearly inferior to that of the low-risk category, rather close to that of the high-risk category. Recently, a similar study from The Netherlands was published [19]. In this study, 222 patients with MDS by the definition of WHO classification from a single institute were analyzed; the median OS of the intermediate-risk category was 22 months, which was closer to that of the high-risk category (19 months) than that of the low-risk category (49 months). These studies suggest that the intermediate-risk category in IPSS-R is a group to consider aggressive treatments for the higher risk MDS including SCT. In contrast, a report from a Serbian study group showed different results [12]. They analyzed 173 patients with MDS using the definition of FAB classification excluding those who received disease-altering therapies. Their results showed that the OS curve of the intermediate-risk category was closer to that of the low-risk category rather than that of the high-risk category (the median OS of the low-, intermediate-, and high-risk categories were 35, 22, and 7 months, respectively). Another study from an Italian group validated the IPSS-R using 380 patients with MDS including those who were treated with lenaridomide, azacitidine, and cytotoxic agents [20]. In their cohort, the survival curves for the high- and very high-risk categories were nearly overlapped, and the curve for the intermediate-risk category was located in between those of the low- and high-risk categories [20]. Thus, ethnic differences may exist in the prognosis of patients in this disease category. In our study cohort, in contrast to the aforementioned German and Dutch studies, the OS curves for the low- and intermediate-risk categories were relatively close, and those for the high- and very high-risk categories were also close; the former were clearly superior to the latter (Fig. 2, panels a, b). The cumulative incidences of leukemic transformation were low in the former categories, less than 20% in 24 months, whereas those of the latter categories were clearly high (Fig. 2d). These results support the idea that Japanese patients with MDS with the IPSS-R intermediate-risk category should be regarded as the lower risk MDS together with those in the low- and very low-risk categories. However, among patients ≤65 years, 33% of those in the intermediate-risk category received SCT, whereas only less than 12.5% of those in the low-risk category received it. Taken together, as suggested in the original paper of IPSS-R [8], we suggest to classify Japanese patients with MDS with intermediate-risk category in IPSS-R into the lower risk MDS regarding their potential therapeutic management, but simultaneously, we suggest to carefully assess optimal treatments of these patients within low- and high-risk treatment protocols.

This study has several study limitations. First, the number of enrolled patients is relatively small. Second, the registration period was relatively long; thus, the treatment strategies, which may have affect their prognosis, was not consistent during the enrolment period. Third, because a considerable proportion of patients were referred to local hospitals or clinics after diagnosis, the median follow-up period (24 months) was relatively short. Fourth, given that informed consent was required prior to enrolment patients into this study, those with very poor performance status were likely to be excluded. Fifth, only relatively large institutes participated in this study, which may have caused selection bias to relatively young patients. A large proportion of elderly patients were probably managed by physicians in much smaller local hospitals or clinics. According to a study by Chihara et al., the incidence of MDS in Japan sharply increases with age, particularly in those over age 70 years, and the median age of MDS diagnosis in Japan is 76 [21], which is much higher than that in our cohort (69 years). To approach these problems, it is necessary to continue the research systematically under the cooperation of all over Japan.

The results of this study was based on a database of a multicenter Japanese MDS and aplastic anemia patient registry, and the diagnosis of each case was strictly made by a central review system including morphological inspection at the time of registration. Although there are several study limitations as mentioned above, we believe that this study provides valuable basic data of the real-world prognosis of patients with MDS in Japan.

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Compliance with ethical standards

Conflict of interest Dr. Kawabata reports Grants from the Ministry of Health, Labor and Welfare, Japan, during the conduct of the

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RESEARCH ARTICLE

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A nationwide survey of hypoplastic myelodysplastic syndrome (a multicenter retrospective study)

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Abstract

Hypoplastic myelodysplastic syndrome (hMDS) is a distinct entity with bone marrow (BM) hypocellularity and the risk of death from BM failure (BMF). To elucidate the characteristics of hMDS, the data of 129 patients diagnosed between April 2003 and March 2012 were collected from 20 institutions and the central review team of the National Research Group on Idiopathic Bone Marrow Failure Syndromes, and compared with 115 non-hMDS patients. More RA and fewer CMMoL and RAEB-t in French-American-British (FAB) and more RCUD and MDS-U and fewer RCMD in World Health Organization (WHO) classifications were found in hMDS than non-hMDS with significant differences. The overall survival (OS) and AML progression-free survival (AML-PFS) of hMDS were higher than those of non-hMDS, especially in patients at age ≥50 and of lower risk in Revised International Prognostic Scoring System (IPSS-R). In competing risks analysis, hMDS exhibited decreased risk of AML-progression in lower IPSS or IPSS-R risk patients, and higher risk of death from BMF in patients at age ≥50. Poor performance status (PS ≥2) and high karyotype risks in IPSS-R (high and very high) were significant risk factors of death and AML-progression in Cox proportional hazards analysis.

1 | INTRODUCTION

Hypoplastic MDS (hMDS) is a distinct disease entity of myelodysplastic syndrome (MDS) characterized by bone marrow (BM) hypocellularity and dysplasia.[1] The BM cellularity <30% had been the criterion for hMDS in the early literature.[1,2] However, based on the evidence that BM cellularity decreases with aging,[3] the stratified criteria of BM cellularity <30% for patients younger than age 60 and <20% for patients older than (or equal to) age 60 have been proposed for the definition of hMDS.[4,5] More than 50% of hMDS patients were refractory anemia (RA) in FAB classification,[6] but other background information, such as past medical histories, family histories, and smoking habits, has hardly been discussed.

The hMDS was originally reported to be of more favorable prognosis with less frequent chromosomal abnormalities than non-hMDS,[1] while some other reports exhibited similar prognostic outcomes of hMDS compared with non-hMDS,[2] so the prognosis of hMDS is still controversial. A study by Huang TC et al. showed that hMDS has more favorable prognosis than non-hMDS, especially in lower risk groups (low and intermediate-1 in the International Prognostic Scoring System (IPSS)), and that hMDS also has lower risk to progress to acute leukemia.[6] Since Revised IPSS (IPSS-R) was made available and proved to predict the clinical outcomes of MDS better than IPSS,[7] it is desirable to investigate the outcomes of hMDS according to IPSS-R as well.

The standard therapy for hMDS remains unknown. Because of severe pancytopenia, the patients with hMDS may be at higher risk of death from BM failure (BMF) rather than the risk of progression to acute myeloid leukemia (AML), and therefore, the adequacy of applying the intensive therapies to hMDS is still controversial.[8,9] A report from Czech included 9 hMDS patients to whom cyclosporine A (CsA) was administered, and 8 of them (80%) responded well to CsA alone or in combination with other agents such as erythropoietin (EPO),[10] while the hMDS patients responded poorly to CsA in another study.[11] The patients in these studies did not progress to acute leukemia after immunosuppressive therapy (IST), but there is a report on a patient with hMDS who transformed to AML after the administration of CsA,[12] so it remains to be clarified to what extent IST is indicated for hMDS. Some studies on successful allogeneic hematopoietic stem cell transplantation (HSCT) included patients with hMDS,[13–15] but their ages were much younger than the median age of hMDS patients, which varied from 46 to 69.[1,2,5,16,17] Therefore, accumulation of data on the outcomes of HSCT for hMDS including the elderly patients is desired.

The purpose of this study is to elucidate the patient background, clinical characteristics, treatment response, and prognosis of patients with hMDS by conducting a nationwide multicenter survey, and retrospectively analyze the survival and risk factors of these patients. In particular, this study is focused on the prognosis of subpopulations according to the age and risk groups of hMDS.

2 | METHODS

The medical institutions participating in the National Research Group on Idiopathic Bone Marrow Failure Syndromes and its central review team were contacted, and the nationwide survey data were collected from the institutions that participated in this study. The protocol of this study was approved by the Research Ethics Committee of the Graduate School of Medicine and Faculty of Medicine, The University of Tokyo, and by the ethics committee of each participating institution. The criteria for the hypocellularity of hMDS are the BM cellularity <30% for patients at age <60, and <20% for age ≥60 in BM biopsy specimens[4,5]; for patients diagnosed without BM biopsy, the same criteria were applied to the clot samples of the BM aspirates. The hMDS cases previously untreated and newly diagnosed by BM biopsy and/or aspiration between April 2003 and March 2012 according to the French-American-British (FAB) classification were enrolled in this study, and their medical records and data were studied throughout the same period. The data collected for analysis included the patients'

clinical characteristics such as age at diagnosis, sex, past medical history of malignancies and/or hematological diseases, family history of malignancies and/or hematological diseases, performance status (PS), complete blood counts (CBC), blasts in peripheral blood (PB) and BM, cellularity of BM, chromosome abnormalities, FAB and World Health Organization (WHO) classifications, risk groups in IPSS and IPSS-R, treatments, the dates of initial diagnosis, progression to AML, and death or the last follow-up. The data of the hMDS patients were compared with those of non-hMDS patients in terms of clinical characteristics, survival, risk factors, causes of death, and responses to treatments. The clinical characteristics of hMDS patients and non-hMDS patients were analyzed using *t*-test for parametric variables and BM blasts, and Fisher's exact test for categorical data.

Overall survival (OS) was defined as the time from the initial diagnosis to death; patients who had been alive at the last follow-up were censored. The AML progression-free survival (AML-PFS) was defined as the survival time until the date on which the patient was found with leukemia either by CBC or BM analysis. The Kaplan-Meier method was used for the survival analysis, and log-rank test was used to compare the survival distributions of hMDS and non-hMDS. The risk factors were also analyzed using the Cox proportional hazards model. In the multivariate analysis of Cox proportional hazards model, variables were optimized using Akaike's information criterion (AIC).[18] Although discussed and defined pathologically, there have been no proposed threshold values to diagnose BMF. For the purpose of this study, death from BMF was defined as the death with cytopenia of at least two lineages (the actual causes of deaths shall be given below). The risks of progression to AML and death from BMF were analyzed by competing risks analysis.

The original definition of hypocellularity for hMDS was given only by BM biopsy, so the clinical study of hMDS only with the histology-proven patients would be ideal. However, less than 50% of the hMDS patients were diagnosed by BM biopsy, so the clot-diagnosed hMDS were included in this study, and a subset analysis of only the histology-proven MDS patients (i.e., the MDS patients diagnosed by BM biopsy) was studied as well. Furthermore, in order to investigate whether the subset of histology-proven patients represents the characteristics of the entire population, two-sample Kolmogorov-Smirnov test was applied to the subpopulations of histology-proven patients and the other patients.[19]

The MDS patients were classified according to their initial treatments, and the OS was studied for each treatment from the days on which the treatment started (patients with best supportive care (BSC) and no treatment were observed from the day on which they were diagnosed).

Throughout this study, all of the statistical analyses were performed using R version 3.2.1.

3 | RESULTS

Thirty-four of the 54 institutions responded to the preliminary survey (63.0%); 27 institutions agreed to participating in the study, 4 institutions had no hMDS cases, and 3 institutions withdrew from the study. The data of 129 patients with hMDS were collected from 20

TABLE 1 Patient characteristics at initial diagnosis

Variables	hMDS (*N* = 129)	non-hMDS (*N* = 115)	*P*-value
Age, years			.22
Median (range)	65 (16–89)	66 (19–88)	
Sex			.061
Male (%)	75 (58)	81 (70)	
Female (%)	53 (41)	34 (30)	
Unknown (%)	1 (0.78)	0 (0)	
Past illness (%)[b]	41 (32)	32 (28)	.74
Family history (%)[b]	20 (16)	38 (33)	<.001[a]
Smoking (%)	32 (25)	58 (50)	<.001[a]
Hemoglobin, g/dL			.32
Median (range)	9.2 (4.9–14)	8.9 (4.4–15)	
Platelet, $\times 10^4/\mu L$			.0079[a]
Median (range)	7.0 (0.60-44)	8.5 (0.50–87)	
Neutrophil, $\times 10^3/\mu L$			.0019[a]
Median (range)	1.2 (0.042-9.9)	1.3 (0.11–36)	
PB blast, %			.016[a]
Median (range)	0 (0–19)	0 (0–16)	
BM blast, %			.069
Median (range)	1.8 (0–28)	3.0 (0–25)	
FAB classification (%)			.021[a]
RA	84 (65)	55 (48)	
RARS	3 (2.3)	6 (5.2)	
RAEB	32 (25)	27 (23)	
RAEB-t	2 (1.6)	6 (5.2)	
CMMoL	2 (1.6)	14 (12)	
Unknown/others	6 (4.7)	7 (6.1)	
WHO classification			<.001[a]
RCUD	40 (31)	8 (7.0)	
RARS	2 (1.6)	2 (1.7)	
RCMD	33 (26)	55 (48)	
RAEB-1	22 (17)	16 (14)	
RAEB-2	12 (9.3)	17 (15)	
MDS-U	11 (8.5)	3 (2.6)	
5q-	1 (0.78)	0 (0)	
Unknown/others	8 (6.2)	14 (12)	
IPSS			.31
Low (%)	19 (15)	18 (16)	
Intermediate-1 (%)	63 (49)	55 (48)	
Intermediate-2 (%)	33 (26)	27 (23)	
High (%)	7 (5.4)	13 (11)	
Unknown (%)	7 (5.4)	2 (1.7)	
IPSS-R			.17
Very low (%)	10 (7.8)	6 (5.2)	
Low (%)	36 (28)	35 (30)	
Intermediate (%)	33 (26)	28 (24)	
High (%)	22 (17)	18 (16)	
Very high (%)	18 (14)	26 (23)	
Unknown (%)	10 (7.8)	2 (1.7)	

[a]Statistically significant.
[b]Malignancies and/or hematological diseases.
BM, bone marrow; FAB, French-American-British; IPSS, International Prognostic Scoring System; IPSS-R, Revised IPSS; PB, peripheral blood; WHO, World Health Organization.

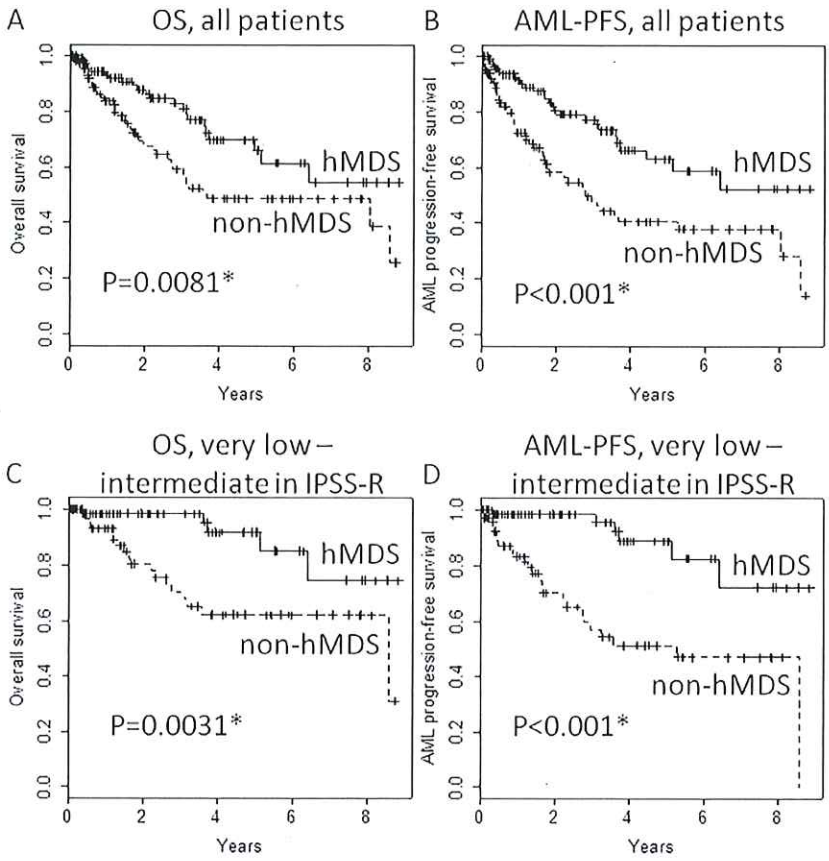

FIGURE 1 OS and AML-PFS, hMDS versus non-hMDS. (A) Overall survival (OS). hMDS patients exhibited significantly higher rates of OS than non-hMDS patients. hMDS: hypoplastic myelodysplastic syndrome (MDS). (B) AML progression-free survival (AML-PFS). hMDS patients exhibited significantly higher rates of AML-PFS than non-hMDS patients. AML: acute myeloid leukemia. (C) OS, lower risk groups (very low, low and intermediate) in IPSS-R. (D) AML-PFS, lower risk groups in IPSS-R. The hMDS of lower risk groups exhibited significantly higher rates of OS and AML-PFS than non-hMDS patients. IPSS-R: Revised International Prognostic Scoring System. *: statistically significant

institutions and from the central review team of the National Research Group on Idiopathic Bone Marrow Failure Syndromes by September 7, 2013. These data were compared with 115 non-hMDS cases of The University of Tokyo Hospital (Supporting Information Table S1). Excluding 2 institutions whose total numbers of MDS patients were unknown, the percentage of hMDS patients was 6.4% (125/1957).

Table 1 provides the demographic and clinical characteristics of patients at their initial diagnoses. The numbers of hMDS patients with family histories of malignancies and/or hematological diseases and with smoking habits were significantly fewer than those of non-hMDS patients ($P < .001$ and $P < .001$, respectively). Patients with hMDS exhibited significantly lower platelet, neutrophil and blast counts in PB than non-hMDS patients ($P = .0079$, $P = .0019$, and $P = .016$, respectively). Also, statistically significant differences between hMDS and non-hMDS patients were found in both FAB and WHO classifications: in particular, the percentage of RCUD was higher in hMDS than in non-hMDS (31% versus 7.0%), whereas that of RCMD was lower in hMDS than in non-hMDS (26% versus 48%), and more MDS-U patients were found in hMDS than in non-hMDS (8.5% versus 2.6%), whereas fewer patients with RAEB-t and CMMoL were found in hMDS than in non-hMDS (1.6% versus 5.2%, and 1.6% versus 12%, respectively). The differences between hMDS and non-hMDS in other characteristics such as past medical histories were not statistically significant.

The OS and the AML-PFS of hMDS patients were evaluated by Kaplan-Meier method, and analyzed further by dividing the hMDS patients into two groups according to the age, IPSS, or IPSS-R (Supporting Information Figure S1). The 5-year OS of hMDS patients was 66% (95% confidence interval (C. I.) = 54 to 81%) (Supporting Information Figure S1A), whereas their 5-year AML-PFS was 63% (95% C. I. = 51 to 78%) (Supporting Information Figure S1B). Patients at age <50 showed significantly higher 5-year AML-PFS than patients at age ≥50 (94% versus 57% ($P = .032$)) (Supporting Information Figure S1F), and their 5-year OS exhibited similar results as well (Supporting Information Figure S1E). According to the IPSS, 5-year OS and AML-PFS were significantly higher in low and intermediate-1 risk groups than in intermediate-2 and high risk groups (81% versus 37% ($P = .0052$), and 80% versus 30% ($P < .001$), respectively) (Supporting Information

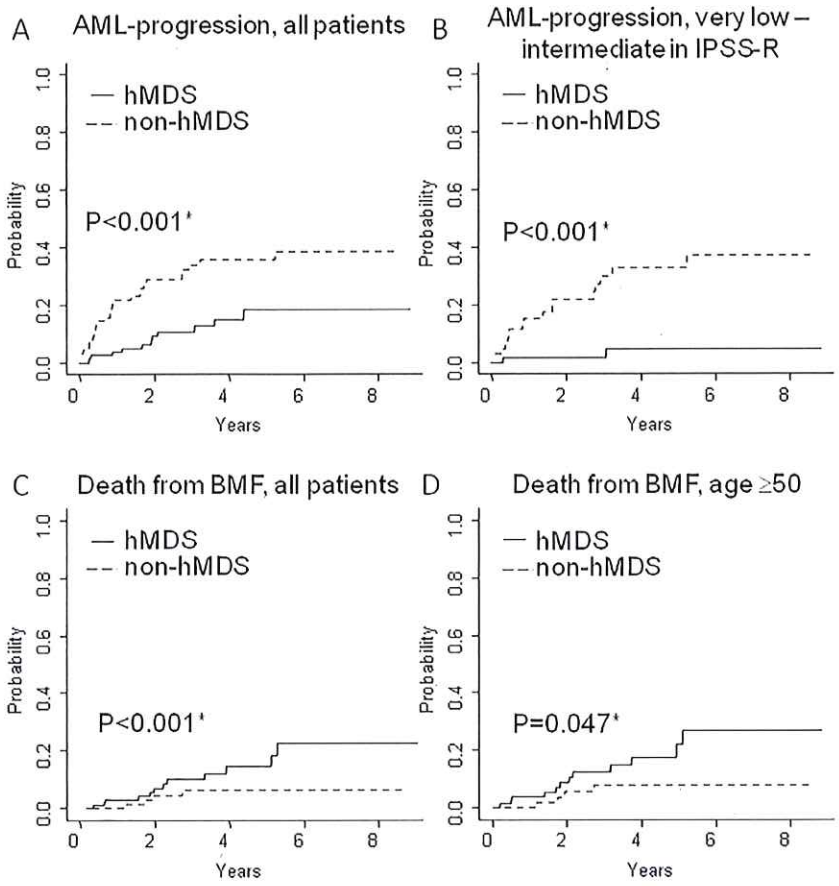

FIGURE 2 The risks of progression to AML and of death from bone marrow failure (BMF) by competing risks analysis. (A) AML-progression, all patients. AML: acute myeloid leukemia. (B) AML-progression, very low, low and intermediate risk groups in IPSS-R. The hMDS patients exhibited significantly lower risk of AML-progression than non-hMDS patients, especially in lower risk groups of IPSS-R. IPSS-R: Revised International Prognostic Scoring System. (C) Death from bone marrow failure (BMF), all patients. (D) Death from BMF, age ≥50. The hMDS patients exhibited higher risk of death from BMF than non-hMDS, especially at age ≥50. The death from BMF is defined as the death caused by cytopenia of at least two lineages. *: statistically significant

Figure S1I,J); likewise, the 5-year OS and AML-PFS in very low, low and intermediate risk groups in IPSS-R were significantly higher than in high and very high risk groups (92% versus 11% ($P < .001$), and 89% versus 11% ($P < .001$), respectively) (Supporting Information Figure S1M,N).

Then, OS and AML-PFS of hMDS were compared with those of non-hMDS (Figure 1, Supporting Information Figure S2). The difference in the OS between hMDS and non-hMDS was statistically significant ($P = .0081$), and their 5-year rates of OS were 66% and 49%, respectively (Figure 1A). The difference in the AML-PFS between them was also statistically significant ($P < .001$), with their 5-year rates being 63% and 41%, respectively (Figure 1B). Based on the findings in Supporting Information Figure S1, hMDS and non-hMDS patients were divided into two groups by IPSS, IPSS-R and age (Supporting Information Figure S1C,D,G,H,K,L). The OS and AML-PFS of patients in low and intermediate-1 risk groups of IPSS were analyzed separately from those in intermediate-2 and high risk groups (Supporting Information Figure S2). For low and intermediate-1, 5-year OS and AML-PFS of hMDS patients exhibited significantly higher rates than those of non-hMDS patients (81% versus 57%) ($P = .034$), and 80% versus 49% ($P = .0027$), respectively) (Supporting Information Figure S2A,B). In intermediate-2 and high risk groups of IPSS, hMDS patients failed to show better OS but did show significantly superior AML-PFS compared to those of non-hMDS patients (37% versus 33% ($P = .11$), and 30% versus 23% ($P = .024$), respectively) (Supporting Information Figure S2C,D). Likewise, OS and AML-PFS of patients in very low, low and intermediate risk groups of IPSS-R were analyzed separately from high and very high risk groups. The 5-year OS and AML-PFS of hMDS patients in very low, low and intermediate risk groups were significantly higher than those of non-hMDS patients (92% versus 62% ($P = .0031$), and 89% versus 51% ($P < .001$), respectively) (Figure 1C,D), whereas the OS and AML-PFS in high and very high risk groups were as low in hMDS as in non-hMDS (11% versus 13% ($P = .50$), and 11% versus 25% ($P = .40$), respectively) (Supporting Information Figure S2E, F). Therefore, the higher survival rates of hMDS than those of non-hMDS were attributed to the favorable outcomes of lower-risk hMDS.

TABLE 2 Cox proportional hazards analysis, hMDS patients ($N = 129$)

Univariate hMDS	Overall survival			AML progression-free survival		
	Hazard ratio	95% C. I.	*P*-value	Hazard ratio	95% C. I.	*P*-value
Sex	3.4	1.2–10	.025[a]	4.3	1.5–12	.0075[a]
Age	1.0	0.99-1.0	.25	1.0	1.0–1.1	.12
Past illness[b]	3.0	1.3–6.7	.010[a]	2.5	1.2–5.3	.018[a]
Family history[b]	1.6	0.58-4.3	.38	1.3	0.48-3.4	.63
Smoking	2.4	0.89-6.4	.083	3.2	1.3–8.1	.015[a]
Hemoglobin	0.88	0.74-1.1	.16	0.91	0.77-1.1	.23
Platelet count	0.90	0.81-0.99	.026[a]	0.94	0.88-1.0	.092
Neutrophil count	1.0	1.0–1.0	.034[a]	1.0	1.0–1.0	.053
PB blast	0.96	0.78-1.2	.68	0.95	0.78-1.2	.63
BM blast	1.1	1.0–1.1	.055	1.1	1.0–1.2	<.001[a]
Performance status	7.1	2.5–20	<.001[a]	5.3	2.1–14	<.001[a]
Karyotype risks in IPSS-R	3.3	1.2–8.8	.019[a]	3.8	1.6–9.0	.0030[a]
Multivariate hMDS	**Overall survival**			**AML progression-free survival**		
	Hazard ratio	**95% C. I.**	***P*-value**	**Hazard ratio**	**95% C. I.**	***P*-value**
Sex	6.3	1.3–30	.020[a]	5.4	1.2–24	.11
Platelet count	0.87	0.75-1.0	.062			
BM blast				1.1	1.0–1.2	.0033[a]
Performance status	4.1	1.3–13	.016[a]	4.5	1.7–12	.0029[a]
Karyotype risks in IPSS-R	3.5	0.99-12	.051	5.2	1.7–15	.001[a]

C.I.: confidence interval.
[a]Statistically significant. Sex: 1 for male, 0 for female.
[b]Past illness/family history of malignancy/hematological disease.
PB, peripheral blood; BM, bone marrow. Performance status (PS): 1 for score ≥2, 0 for score ≤1. Karyotype risks in IPSS-R, 1 for poor and very poor risk groups, 0 for very good, good and intermediate risk groups; IPSS-R, Revised International Prognostic Scoring System.

For both age <50 and age ≥50, the survival rates of hMDS tended to be higher than those of non-hMDS (Supporting Information Figure S2G-J), especially with statistically significant differences in OS and AML-PFS for age ≥50 (61% versus 42% ($P = .011$), and 57% versus 34% ($P < .001$), respectively) (Supporting Information Figure S2I,J).

The risks of progression to AML and death from BMF were investigated by competing risks analysis (Figure 2, Supporting Information Figure S3). The difference in the risk of AML-progression between hMDS and non-hMDS was statistically significant ($P < .001$), with the 5-year cumulative incidence of 18% and 36%, respectively (Figure 2A). Therefore, hMDS patients face lower risk to progress to AML than non-hMDS patients. Dividing the patients into two groups by IPSS, IPSS-R or age revealed that the hMDS patients have significantly lower risk of AML-progression than non-hMDS patients also in the subpopulations of low and intermediate-1 risk groups in IPSS (5-year cumulative incidence = 5.0% versus 31% ($P < .001$)) (Supporting Information Figure S3A), very low, low and intermediate risk groups in IPSS-R (5-year cumulative incidence = 4.5% versus 33% ($P < .001$)) (Figure 2B), and age ≥50 (5-year cumulative incidence = 21% versus 39% ($P = .0015$)) (Supporting Information Figure S3E). As given above, the criterion of death caused by cytopenia of at least two lineages was used for the death from BMF (the actual causes of deaths for hMDS patients are given in Supporting Information Table S2). Applying this criterion to the analysis revealed that the 5-year cumulative incidence of hMDS patients to die from BMF was significantly higher than that of non-hMDS patients (18% versus 6.3% ($P < .001$)), implying that hMDS patients face higher risk of death from BMF than non-hMDS patients (Figure 2C). It was revealed further that hMDS patients face higher risk of death from BMF than non-hMDS patients at age ≥50 (5-year cumulative incidence = 22% versus 8.0% ($P = .047$)) (Figure 2D).

The univariate and multivariate Cox proportional hazards models were used to analyze which characteristics of patients served as the risk factors to affect the rates of OS and AML-PFS (Table 2, Supporting Information S4). Among the statistically significant risk factors of survivals for hMDS ($N = 129$) in the univariate proportional hazards analysis, sex (male = 1, female = 0) and PS remained significant risk factors of OS in multivariate analysis, whereas BM blast, PS and karyotype risks in IPSS-R (poor and very poor risk groups) remained significant risk factors of AML-PFS in multivariate analysis, respectively (Table 2).

Similar results were exhibited for all patients ($N = 244$) and non-hMDS patients ($N = 115$) as well (Supporting Information Table S4).

The subset analysis of histology-proven patients is given in Supporting Information Figure S4. The rates of OS and AML-PFS for this subpopulation exhibited similar results as those for the entire population including patients who were diagnosed by BM aspiration alone; 5-year OS and AML-PFS for histology-proven hMDS were 69% (95% C.I. = 52 to 94%) and 68% (95% C.I. = 51 to 91%), respectively (Supporting Information Figure S4A,B). The hMDS patients exhibited trends for higher rates of survival for both OS and AML-PFS than the non-hMDS patients, especially the latter with statistical significance (5-year OS = 69% versus 40% ($P = .10$), and 5-year AML-PFS = 68% versus 36% ($P = .011$), respectively) (Supporting Information Figure S4C,D). Competing risks analysis for histology-proven patients also exhibited similar results as those for all patients; 5-year cumulative incidences of AML-progression for hMDS and non-hMDS were 14% versus 38% ($P = .0040$), and 5-year cumulative incidences of death from BMF for hMDS and non-hMDS were 24% versus 9.7% ($P = .34$), respectively (Supporting Information Figure S4E,F). Therefore, it was confirmed that hMDS patients face lower risk of AML-progression and higher risk of death from BMF in this subset analysis as well.

Applying two-sample Kolmogorov-Smirnov test to the subpopulations of histology-proven patients and the other patients confirmed that all of the background continuous variables for histology-proven patients, except for neutrophil count, follow the same distributions as those of the other patients (Supporting Information Table S3). Therefore, the data of all patients, including the data of those who were diagnosed by BM aspiration alone, can be interpreted to represent the clinical characteristics of hMDS patients, even though the importance of diagnosing by BM biopsy cannot be overemphasized.

The OS of patients according to their initial treatments were also analyzed (Supporting Information Figure S5), but the OS between hMDS and non-hMDS did not exhibit statistically significant differences in any treatment, at least in part due to the limited sample sizes. Twenty-seven percent of hMDS patients received no treatments throughout the entire clinical courses, 17% were administered vitamins as their first treatment, and 10% underwent hematopoietic stem cell transplantation (HSCT) as their initial treatments (Supporting Information Table S5).

4 | DISCUSSION

There have been a few reports of hMDS exhibiting poorer prognosis than aplastic anemia (AA) and better prognosis than non-hMDS, but they dealt with a limited number of hMDS patients, and a study with a larger sample size was desirable.[6,17] Since IPSS-R has already been acknowledged worldwide, the prognosis of hMDS needs to be discussed according to IPSS-R.[7] This study is the first multicenter study with the data of >100 hMDS patients on hMDS that dealt with IPSS-R. The fact that in neither OS nor AML-PFS did the hMDS patients differ significantly from non-hMDS patients in higher risk groups of IPSS-R may reflect some advantages of IPSS-R to predict high risk patients compared with IPSS because in int-2 and high risk groups of IPSS, AML-PFS was significantly prolonged in hMDS patients. Furthermore, the very low–intermediate risk groups of hMDS exhibited higher OS and AML-PFS than those of non-hMDS patients with statistically significant differences, implying that treatments for hMDS patients in very low–intermediate risk groups of IPSS-R should be considered separately from those for non-hMDS in the same risk groups.

It is often difficult to distinguish hMDS from AA, because both the clinical courses and pathological findings of hMDS may overlap with those of AA, while treatments and prognosis of these two entities may differ.[20–22] Although there have been several reports to propose the criteria for distinction, such as CD34-positive BM cell analysis and measurement of tumor necrosis factor receptors,[21,23] the morphological study of the BM still remains the standard means to diagnose hMDS and distinguishing it from AA. The diagnosis of hMDS for the current study is based on the morphological dysplasia, but there remains the need for the methods of diagnosis with more objective criteria.[24]

In addition to the finding in Table 1 that more than half of the hMDS patients were classified as RA in FAB classification, which coincides with the previously reported literature,[6] it was exhibited in this study that there were significant differences between hMDS and non-hMDS in WHO classification. On the other hand, the marrow blast percentages of hMDS and non-hMDS were not significantly different, which yielded similar fractions of RAEB-1/2; therefore, the significant differences in WHO classification were mainly due to the higher percentage of RCUD and the lower percentage of RCMD. A recent study suggests that RCUD exhibits higher rates of OS than RCMD,[25] but the OS of hMDS in the current study did not exhibit statistically significant difference between RCUD and RCMD (data and graph not shown).

High hazard ratios of PS and karyotype risks for both OS and AML-PFS of hMDS patients in Cox proportional hazards analysis coincide with the previously published literature,[26,27] but it was shown further in univariate Cox proportional hazards analysis that other factors such as the male gender, past illnesses and smoking habits can also be the risk factors of death and AML-progression for hMDS.

Competing risks analysis revealed that hMDS is less likely to progress to AML than non-hMDS, as Huang et al. exhibited.[6] However, the anticipation that hMDS may have higher risk of death from BMF had never been confirmed before. Applying a criterion of death from BMF for the purpose of this study confirmed that hMDS has higher risk of death from BMF. Therefore, there may be some hMDS patients for whom myelo-suppressing therapies are not indicated.

The cytogenetic abnormalities of hMDS in this study were summarized into the karyotype risk groups of IPSS-R. According to Koh Y et al., the difference between the AML-PFS of hMDS patients with cytogenetic abnormalities and of those without were not significant.[17] IPSS-R classifies some chromosomal abnormalities into the same risk group as the normal karyotype, and there exist some hMDS patients with chromosomal abnormalities that have more favorable outcomes than those without. Therefore, survival analyses by IPSS-R exhibited earlier may give more adequate assessments for the prognoses of hMDS.

Abnormalities in fluorescent in situ hybridization (FISH) were not dealt with in this study. It has been reported that AA positive of trisomy 1q by FISH progressed to acute leukemia frequently, whereas no single karyotype or FISH abnormality in hMDS predicted leukemic progression.[17] Therefore, it is likely that FISH analysis for the data of hMDS patients in this study would not have yielded significant outcomes.

Another recent report suggests that hMDS acquires fewer somatic mutations than hyperplastic MDS, and exhibits genomic differences in driver clones when compared with hyperplastic MDS,[28] so a combined analysis of genetic data and clinical outcomes of hMDS in comparison with those of non-hMDS is also desired.

Comparison of OS between hMDS and non-hMDS according to the initial treatments did not exhibit statistically significant differences between hMDS and non-hMDS because of the limited sample size and also due to the fact that treatments for hMDS were selected at clinicians' own discretions. Therefore, treatment choices according to the characters and factors of the patients, as well as their adequacies, need to be investigated with even a larger size of population.

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CONFLICT OF INTERESTS

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AUTHOR CONTRIBUTIONS

T. Kobayashi analyzed the data and wrote the manuscript; Y. Nannya designed the entire study and helped write the final version of the manuscript; K. Oritani, A. Tomita, M. Kobune, H. Kawabata, M. Shindo, Y. Yonemura, N. Hanaoka, D. Hasegawa, N. Fujishima, N. Fujii, Y. Morita, A. Matsuda, A. Fujieda, H. Suzuki, Y. Terada, and K. Sato collected the data; H. Kawabata offered the data base of the central review team of the National Research Group on Idiopathic Bone Marrow Failure Syndromes; M. Kurokawa supervised the entire study and wrote the manuscript; and all authors reviewed and approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

Clinical utility of next-generation sequencing for inherited bone marrow failure syndromes

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Purpose: Precise genetic diagnosis of inherited bone marrow failure syndromes (IBMFS), a heterogeneous group of genetic disorders, is challenging but essential for precise clinical decision making.

Methods: We analyzed 121 IBMFS patients using a targeted sequencing covering 184 associated genes and 250 IBMFS patients using whole-exome sequencing (WES).

Results: We achieved successful genetic diagnoses for 53 of 121 patients (44%) using targeted sequencing and for 68 of 250 patients (27%) using WES. In the majority of cases (targeted sequencing: 45/53, 85%; WES: 63/68, 93%), the detected variants were concordant with, and therefore supported, the clinical diagnoses. However, in the remaining 13 cases (8 patients by target sequencing and 5 patients by WES), the clinical diagnoses were incompatible with the detected variants.

Conclusion: Our approach utilizing targeted sequencing and WES achieved satisfactory diagnostic rates and supported the efficacy of massive parallel sequencing as a diagnostic tool for IBMFS.

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Key Words: Fanconi anemia; inherited bone marrow failure; next-generation sequencing; target sequencing; whole-exome sequencing

INTRODUCTION

Inherited bone marrow failure syndromes (IBMFS) are part of a heterogeneous disease category involving a family history in which at least one hematopoietic cell lineage is decreased in the bone marrow. IBMFS consist of more than 25 defined disease entities, including Fanconi anemia (FA), Diamond-Blackfan anemia (DBA), and dyskeratosis congenita (DC).[1] Certain IBMFS have been associated with an increased risk of secondary malignancies. The diagnosis is based on hematological and physical findings with the aid of several disease-specific diagnostic tests such as the chromosomal breakage test for FA and molecular diagnosis with conventional Sanger sequencing for a very limited number of the causative genes. With recent advances in clinical molecular studies that revealed a considerable amount of pathognomonic molecular lesions in IBMFS,[2–4] the role of genetic tests has become more important in the diagnosis of these diseases. However, because clinical and laboratory findings can overlap among different IBMFS, the selection

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of targeted genes for genetic diagnosis is difficult. Therefore, a comprehensive genetic diagnostic approach is warranted for patients with a suspicious diagnosis of IBMFS due to the increased number of genes to be analyzed.

Next-generation sequencing (NGS) encompasses a broad range of techniques that enable the simultaneous sequencing of a massive amount of nucleic acid molecules.[5] These complementary approaches include targeted gene sequencing, whole-exome sequencing (WES), and whole-genome sequencing (WGS), each with distinct advantages and disadvantages. Compared with WES/WGS, targeted gene sequencing is a relatively inexpensive approach for the identification of pathogenic mutations in more than 100 genes and has relatively higher sequence coverage. NGS has been used for research studies regarding certain IBMFS, but its clinical utility is limited. Only two studies used targeted gene sequencing with NGS technology to diagnose patients with distinct IBMFS.[6,7] The majority of these patients had previously undergone extensive genetic testing, all of which yielded negative results. Targeted gene sequencing efficiently identified causative mutations in both studies, which supported the utilization of NGS for genetic screening of patients with IBMFS.

In this study, we developed a targeted gene sequencing platform with 184 genes that were specifically designed for the diagnosis of IBMFS. This platform was used as a first-line diagnostic test for 121 patients clinically diagnosed with IBMFS. In addition, we performed diagnostic WES for 250 patients with IBMFS who could not be genetically diagnosed using conventional genetic tests.

MATERIALS AND METHODS

Patients for targeted sequencing

A total of 121 consecutive patients who were clinically diagnosed with IBMFS at Nagoya University Hospital and other nationwide institutions were included in this study. None of the patients had previously undergone genetic tests. Clinical diagnoses included DBA (n = 26), DC (n = 13), FA (n = 22), Shwachman-Diamond syndrome (SDS, n = 6), severe congenital neutropenia (SCN, n = 7), other anemia (n = 21), other neutropenia (n = 3), other thrombocytopenia (n = 13), and other bone marrow failure (BMF) (cytopenia with ≥2 lineages; n = 10) (**Table 1**, **Supplementary Table S1** online and **Supplementary Figure S1** online).

Whole-exome sequencing of patients

In 2011, we established a government-supported nationwide program, the Research on Measures for Intractable Diseases Project of the Ministry of Health, Labor, and Welfare, for rare inherited blood disorders. As of December 2013, a total of 733 patients were registered with the program (**Supplementary Table S1** online) and had clinical diagnoses including FA (n = 117), DBA (n = 110), congenital hemolytic anemia (HA; n = 261), DC (n = 62), congenital dyserythropoietic anemia (CDA; n = 21), congenital sideroblastic anemia (CSA; n = 34), congenital amegakaryocytic thrombocytopenia (CAMT; n = 10), hereditary hemophagocytic lymphohistiocytosis (HLH; n = 65), SCN (n = 47), and unclassified IBMFS (n = 6). Conventional genotyping such as Sanger sequencing had confirmed the clinical diagnoses of 262 patients; however, no causative candidate germline variants were identified (n = 267) or no genotyping was performed (n = 204) for the remaining 471 patients. Of these 471 patients, 250 patients with FA (n = 73), DBA (n = 61), HA (n = 44), DC (n = 29), CDA (n = 12), CSA (n = 9), CAMT (n = 7), HLH (n = 6), SCN (n = 3), and unclassified IBMFS (n = 6) were enrolled in the present study (**Table 2**, **Supplementary Tables S2 and S3** online). Most of these patients (182/250, 73%) underwent various genetic tests with negative results before WES analysis, whereas the remaining 68 (27%) patients were not evaluated with genetic tests other than WES.

Sample preparation and next-generation sequencing

Written informed consent was obtained from patients or their legal guardians. This study was approved by the ethics committees of Nagoya University Graduate School of Medicine and Graduate School of Medicine, Kyoto University.

Genomic DNA was extracted from peripheral blood, bone marrow, and Epstein-Barr virus–transformed lymphoblastoid cell lines (EBV-LCL) using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). For targeted sequencing, the target region of the genomic DNA was enriched using SureSelect custom bait (Agilent Technologies, Santa Clara, CA) covering the exons and 10 bases surrounding the exons of 184 genes (**Supplementary Table S4** online). For WES, genomic DNA was captured using SureSelect Human All Exon 50M, V4, or V5 Kits (Agilent Technologies). Captured genomic DNA was analyzed by massively parallel sequencing using a HiSeq 2000 or 2500 (Illumina, San Diego, CA) next-generation sequencer with a 100×2 paired-end option.

Detection of causative variants

We detected germline variants using our established pipeline (Genomon-exome, http://genomon.hgc.jp/exome/). In brief, sequence reads were aligned to the hg19 reference genome using the Burrows-Wheeler Aligner; variants were detected using our in-house variant caller. Variant allele frequency (VAF) >0.2 (20%) was used as the cut-off value.

Following the guidelines published by the American College of Medical Genetics and Genomics,[8] we removed common single-nucleotide polymorphisms (SNPs) showing minor allele frequency values of more than 1% in (i) the ESP6500 exome variant server (the National Heart, Lung, and Blood Institute Exome Sequencing Project, Seattle, WA; http://evs.gs.washington.edu/EVS/, as of April 2014); (ii) the 1000 genomes project;[9] or (iii) our in-house SNP database.[10] These variants were considered the causative variants that were previously reported to be pathogenic (category 1) or were otherwise highly expected to cause the associated disorders (e.g., nonsense, frameshift, and splice site variants) (category 2). Other variants of unknown significance such as missense variants without further evidence of pathogenicity were treated as

Table 1 Summary of clinical and genetic diagnosis by target sequencing

Categories of clinical diagnosis	*N*	Patients with genetic diagnosis	Clinical and genetic diagnosis		Identified gene mutations (*n*)
			Matched	Unmatched	
FA	22	15 (68%)	15	0	*FANCA* (11), *FANCF* (1), *FANCG* (3)
DBA	26	12 (46%)	11	1	*RPL5* (3), *RPS17* (3), *RPS19* (4), *RPS24* (1), *SPTB* (1)
CHA	8	4 (50%)	4	0	*PIEZO1* (2), *SPTB* (2)
DC	13	5 (38%)	4	1	*DKC1* (2), *SBDS* (1), *TINF2* (2)
CDA	9	2 (22%)	2	0	*KLF1* (1), *CDAN1* (1)
CSA	4	2 (50%)	2	0	*ALAS2* (1), *SLC25A38* (1)
CAMT	2	1 (50%)	0	1	*RUNX1* (1)
HLH	0	0	0	0	-
SCN	7	2 (29%)	2	0	*ELANE* (1), *HAX1* (1)
SDS	6	4 (67%)	4	0	*SBDS* (4)
Other neutropenia	3	0	0	0	-
Other thrombocytopenia	11	3 (27%)	1	2	*RUNX1* (2), *VWF* (1)
Other BMF	10	3 (30%)	0	3	*FANCG* (1), *RPL5* (1), *SBDS* (1)
Total cohort	121	53 (44%)	45	8	

BMF, bone marrow failure; CAMT, congenital amegaryocytic thrombocytopenia; CDA, congenital dyserythropoietic anemia; CHA, congenital hemolytic anemia; CSA, congenital sideroblastic anemia; DBA, Diamond-Blackfan anemia; DC, dyskeratosis congenita; FA, Fanconi anemia; HLH, hereditary hemophagocytic lymphohistiocytosis; SCN, severe congenital neutropenia; SDS, Shwachman-Diamond syndrome.

Table 2 Summary of genetic diagnoses by whole-exome sequencing

Categories of clinical diagnosis	Total cohort		Without previous genetic tests		With previous genetic tests		Clinical and genetic diagnoses		Identified gene mutations (*n*)
	N	Patients with genetic diagnosis	*n*	Patients with genetic diagnosis	*n*	Patients with genetic diagnosis	Matched	Unmatched	
FA	73	35 (48%)	16	10 (63%)	57	25 (44%)	35	0	*FANCG* (17), *FANCA* (14), *FANCB* (1), *FANCF* (1), *SLX4* (1), *BRCA2* (1)
DBA	61	11 (18%)	0	0	61	11 (18%)	11	0	*RPS26* (3), *RPS7* (2), *RPS19* (2), *RPL5* (2), *RPL35A* (1), *RPL11* (1)
HA	44	7 (16%)	44	7 (16%)	0	0	6	1	*SPTA1* (2), *SPTB* (2), *ANK1* (2), *CDAN1* (1)
DC	29	7 (24%)	2	1 (50%)	27	6 (22%)	7	0	*TERT* (3), *TINF2* (2), *DKC1* (2)
CDA	12	3 (25%)	0	0	12	3 (25%)	1	2	*CDAN1* (1), *SPTA1* (1), *ANK1* (1)
CSA	9	0	0	0	9	0	0	0	
CAMT	7	1 (14%)	0	0	7	1 (14%)	0	1	*TINF2* (1)
HLH	6	3 (50%)	0	0	6	3 (50%)	2	1	*UNC13D* (1), *XIAP* (1), *MVK* (1)
SCN	3	0	0	0	3	0	0	0	
SDS	0	0	0	0	0	0	0	0	-
Other BMF	6	1 (17%)	6	1 (17%)	0	0	1	0	*RUNX1* (1)
Total	250	68 (27%)	68	19 (28%)	182	49 (27%)	63	5	

BMF, bone marrow failure; CAMT, congenital amegaryocytic thrombocytopenia; CDA, congenital dyserythropoietic anemia; CSA, congenital sideroblastic anemia; DBA, Diamond-Blackfan anemia; DC, dyskeratosis congenita; FA, Fanconi anemia; HA, hemolytic anemia; HLH, hereditary hemophagocytic lymphohistiocytosis; SCN, severe congenital neutropenia; SDS, Shwachman-Diamond syndrome.

nondiagnostic in this study. For the specific pathogenicity of each variant, we used the Human Genome Mutation Database (http://www.hgmd.cf.ac.uk/, as of March 2014) and performed an extensive search of the literature in PubMed (http://www.ncbi.nlm.nih.gov/pubmed). The results of the literature search were discussed with physicians who were experts in that particular disease area and genetic diagnoses were made based on the mode of inheritance of each disease. Finally, causative variants were validated by Sanger sequencing.

Using targeted sequencing data, we performed copy-number analysis as described previously.[11] In brief, the coverage of each exon normalized by the mean coverage of the entire sample was compared with that of 12 unrelated reference samples. Exons exhibiting normalized coverage greater than 3 standard deviations from the coverage of reference samples were determined to be candidates for copy-number alterations. All candidate exons were visually inspected using the Integrative Genomics Viewer.

RESULTS

Genetic diagnosis by targeted sequencing

Capture-based targeted sequencing covered 99.4% of the target region in 184 genes with more than 20 independent reads. With this coverage, our in-house pipeline detected 227 (201–267) coding variants per patient, of which common SNPs with >1% minor allele frequency accounted for 97% of all detected variants. In total, we identified 69 variants that were considered to be in category 1 (i.e., previously reported alleles) or category 2 (i.e., previously unknown but highly probable variants within known causative genes for each disease subtype). In addition, we were able to identify pathognomonic copy-number aberrations in 11 patients (*FANCA* ($n = 5$; UPN-1028, -1082, -1084, -1372, and -1373), *RPS17* ($n = 3$; UPN-1174, -1186, and -1304), *RUNX1* ($n = 1$, UPN-1222), *SBDS* ($n = 1$, UPN-1212), and *SPTB* ($n = 1$, UPN-1350) (**Figure 1**).

We calculated genetic diagnostic rates by our targeted sequencing pipeline based on the estimated mode of inheritance. For instance, we genetically diagnosed patients harboring homozygous or compound heterozygous mutations of known causative genes for autosomal recessive diseases such as FA. Patients with a clinical diagnosis of FA (15/22, 68%) and SDS (4/6, 67%) achieved relatively high genetic diagnostic rates. The genetic diagnosis was achieved in approximately half of the patients with DBA (12/26, 46%) and DC (5/13, 38%). Genetic diagnostic rates of other categories were as follows: SCN (2/7, 29%), other anemia (9/21, 43%), other neutropenia (0/3, 0%), other thrombocytopenia (4/13, 33%), and other BMF (3/10,

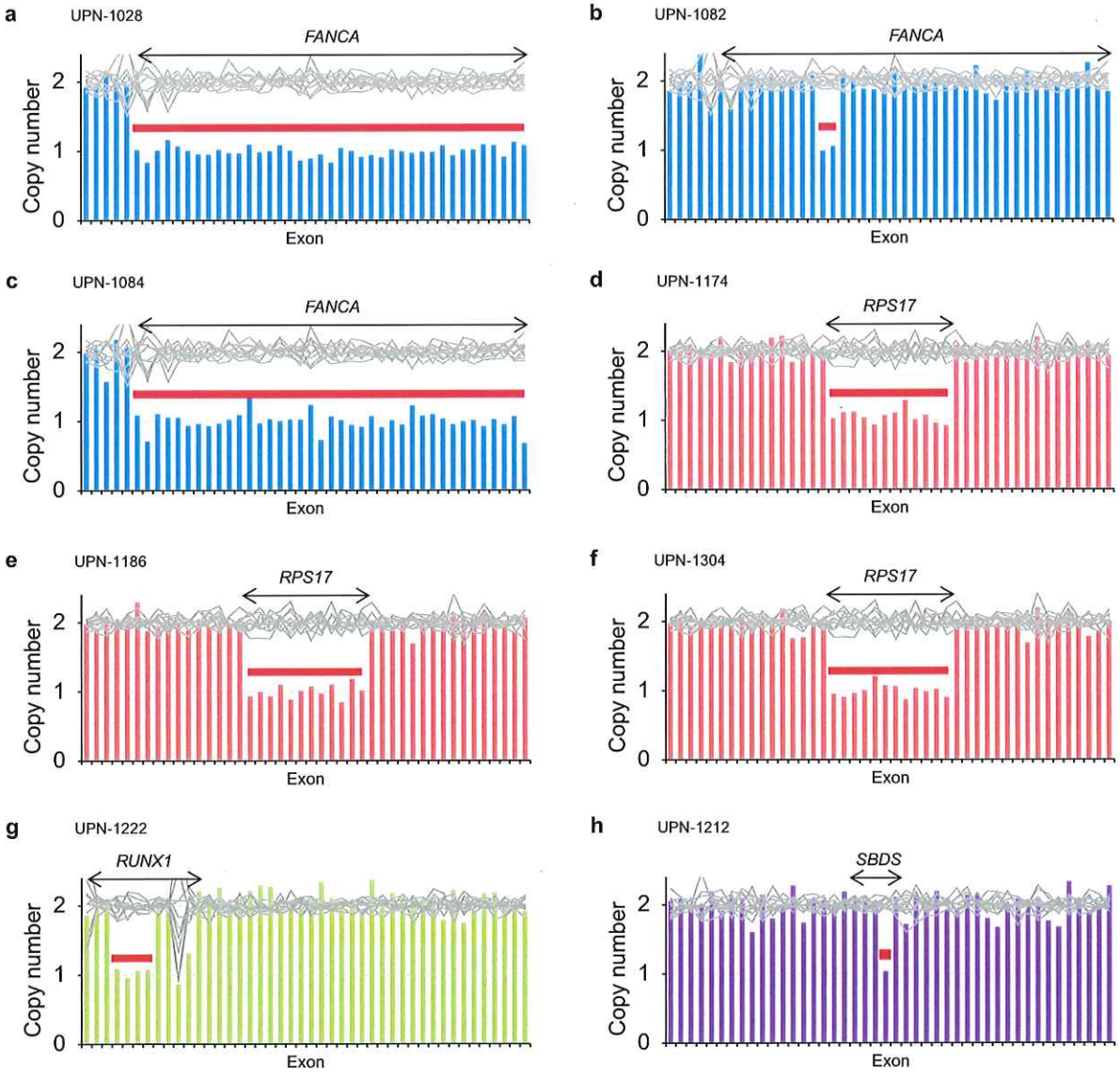

Figure 1 Copy-number analysis. The estimated copy number of each exon was based on the number of reads obtained from the targeted sequence. Analysis revealed pathogenic heterozygous gene deletion in eight patients. Each bar represents an exon and the y-axis represents the estimated copy numbers. Gray lines indicate estimated copy numbers obtained from 12 unrelated samples. Red bars indicate estimated deleted regions.

30%). In total, our targeted sequencing pipeline genetically diagnosed 53 of 121 patients (44%) (**Table 1**, **Supplementary Table S1** online). No patient had two or more genetic diagnoses.

Genetic diagnosis by whole-exome sequencing

WES covered more than 80% (56%–91% with SureSelect 50M) to 90% (79–98% with SureSelect V4 or V5) of the coding region with more than 20 independent reads (**Supplementary Figure S2** online). At this coverage level, our in-house pipeline detected 19,574 (13,811–21,945) coding variants per patient. Common SNPs with >1% minor allele frequency accounted for 97% of all detected variants.

We identified 64 category 1 variants (previously reported alleles) and 23 category 2 variants (previously unknown but highly probable variants within known causative genes for each disease subtype) (**Supplementary Table S3** online). These 87 variants established genetic diagnoses for 68 (27%) patients (**Table 2**). Diagnostic efficacy was comparable between patients with or without prior genetic testing (26 and 27%, respectively). No patient received more than one genetic diagnosis.

The highest diagnostic efficacy was achieved in patients with FA; in these patients, one or more highly putative causative variants were detected by WES in 35 of 73 patients (48%), followed by DBA (11/61, 18%), HA (7/44, 16%), DC (7/29, 24%), CDA (3/12, 25%), and HLH (3/6, 50%). In contrast, candidate variants were detected or otherwise not identified in only a small fraction of patients with CSA (0/9), CAMT (1/7), and SCN (0/3).

Discordance between clinical diagnosis and genetic variants by targeted sequencing and whole-exome sequencing

In the majority of cases (target sequencing: 45/53, 85%; WES: 63/68, 93%), the detected variants were concordant with, and therefore supported, the clinical diagnoses. However, for the remaining 13 cases (8 patients by target sequencing and 5 patients by WES), the clinical diagnoses were incompatible with the detected variants (**Table 3**, **Supplementary Data** online).

UPN-1350, one of the discordant patients clinically diagnosed with DBA, harbored a known pathognomonic *SPTB* gene deletion and was genetically diagnosed as hereditary spherocytosis. Two patients with thrombocytopenia (congenital amegakaryocytic thrombocytopenia (UPN-1222) and idiopathic thrombocytopenic purpura (ITP); UPN-1277) were reallocated as familial platelet disorder with propensity to myeloid malignancy (FPD/AML) with *RUNX1* gene alterations[12] (microdeletion (UPN-1222, **Figure 1**) and p.P330fs (UPN-1277)). Three patients had IBMFS but not otherwise specified obtained genetic diagnoses as a result of our analysis (UPN-1324, DBA; UPN-1348, SDS; and UPN-1355, FA). Similarly, genetic sequencing analysis corrected the diagnoses of two other patients (UPN-1289 (from DC to SDS) and UPN-1098 (from chronic ITP to von Willebrand disease (VWD) 2B)). In UPN-355, the initial clinical diagnosis was HA, but the detected variants (*CDAN1* p.N599S and p.P146fs) supported the diagnosis of CDA. Conversely, in UPN-216 and UPN-485, the suspected clinical diagnosis of CDA was revised to

Table 3 Genetic diagnoses unmatched with clinical diagnoses

UPN	Sequencing	Clinical Dx	Genetic Dx	Gene mutation (1)		Zygosity	Gene mutation (2)		Zygosity
UPN-1350	Target	DBA	Hereditary spherocytosis	*SPTB*	Deletion	Hetero			
UPN-1289	Target	DC	SDS	*SBDS*	p.K33E	Hetero	*SBDS*	p.244_245del	Hetero
UPN-1098	Target	Chronic ITP	VWD 2B	*VWF*	p.A1461D	Hetero			
UPN-1222	Target	CAMT	FPD/AML	*RUNX1*	Deletion	Hetero			
UPN-1277	Target	ITP	FPD/AML	*RUNX1*	p.P330fs	Hetero			
UPN-1324	Target	IBMFS, NOS	DBA	*RPL5*	p.Y219X	Hetero			
UPN-1348	Target	IBMFS, NOS	SDS	*SBDS*	p.K62X	Hetero	*SBDS*	Splice site (c.258+2T>C)	Hetero
UPN-1355	Target	IBMFS, NOS	FA	*FANCG*	p.Q356X	Hetero	*FANCG*	Splice site (c.307+1G>C)	Hetero
UPN-355	WES	HA	CDA	*CDAN1*	p.N599S	Hetero	*CDAN1*	p.P146RfsX9	Hetero
UPN-216	WES	CDA	HA	*SPTA1*	p.R28H	Hetero			
UPN-485	WES	CDA	HA	*ANK1*	p.R935X	Hetero			
UPN-83	WES	CAMT	DC	*TINF2*	p.R276X	Hetero			
UPN-312	WES	HLH	Hyper IgD syndrome	*MVK*	Splice site (c.227-1G>A)	Hetero			

CAMT, congenital amegakaryocytic thrombocytopenia; DBA, Diamond-Blackfan anemia; DC, dyskeratosis congenita; Dx, diagnosis; FA, Fanconi anemia; FPD/AML, familial platelet disorder with propensity to myeloid malignancy; Hetero, heterozygous; ITP, idiopathic thrombocytopenic purpura; IBMFS, inherited bone marrow failure syndrome; NOS, not otherwise specified; SDS, Shwachman-Diamond syndrome; UPN, unique patient number; VWD, von Willebrand disease; WES, whole-exome sequencing.

HA as a result of identification of mutations (*SPTA1* p.R28H in UPN-216 and *ANK1* p.R935X in UPN-485) that are typically associated with HA. In these cases, the inconsistency was most likely due to an overlap of clinical phenotypes between HA and CDA; both of which are characterized by hemolysis. In UPN-83, a patient clinically diagnosed with possible CAMT, the genetic diagnosis of DC was reached with causative variants in *TINF2* (p.R276X). In UPN-312, the clinical diagnosis of HLH was revised to hyperimmunoglobulin D syndrome, a type of hyperinflammatory syndrome, based on the causative variants in *MVK* (p.A147T and c.227-1G>A).

DISCUSSION

Recent advances in genetic research identified a large number of causative genes of IBMFS and reinforced the need for a comprehensive genetic diagnostic system in both clinical practice and research.[6] Here, we developed a molecular diagnostic system using WES and a targeted sequencing pipeline covering 184 associated genes for IBMFS. We were successful in providing genetic diagnoses for 53 of 121 patients (44%) by targeted sequencing (**Table 1**) and for 68 of 250 patients (27%) by WES (**Table 2**). Although the possibility of concomitant diagnoses remains, 13 patients with discordant clinical and genetic diagnoses clearly demonstrated the clinical value of next-generation sequencing (**Table 3**).

The diagnostic rate of our targeted sequencing platform was significantly higher than those demonstrated in a previous study by Zhang *et al.*[6] (53/121 (44%) vs. 17/85 (20%); $P < 0.001$), thus reflecting the difference between patient characteristics and genetic regions covered. Although we used a nonbiased approach analyzing 121 consecutive patients who were clinically suspected to have IBMFS without preceding genetic screening, Zhang *et al.* resequenced patients who remained unclassified after a conventional genetic workup using a sequencing platform with a smaller gene number (85 genes) and a lower coverage rate (>10× coverage in 98.2% of bases).[6] Nine patients in our study had diagnostic variants in four genes that were not included in the gene panel of Zhang *et al.* (*PIEZO1* ($n = 2$), *RPS17* ($n = 3$), *SPTB* ($n = 3$), and *VWF* ($n = 1$)). Ghemla *et al.* reported similar diagnostic rates (59/158, 37%) using a multiplex polymerase chain reaction (PCR) platform covering 72 genes[7]; however, the detection rate of copy-number variants was significantly lower than that of our study (2/158 (2%) vs. 11/121 (9%); $P = 0.003$), thus reflecting the superiority of the capture-based platform over multiplex PCR in copy-number analysis. In addition, 10 patients in our study had diagnostic variants in 7 genes that were not included in the gene panel utilized by Ghemla *et al.* (*ALAS2* ($n = 1$), *CDAN1* ($n = 1$), *KLF1* ($n = 1$), *PIEZO1* ($n = 2$), *SLC25A38* ($n = 1$), *SPTB* ($n = 3$), and *VWF* ($n = 1$)).

Unsuspected genetic diagnosis has a positive effect on the clinical management of patients, particularly for gene mutations conferring cancer predisposition. Patients with most categories of IBMFS, including DBA,[13] DC,[14] FA,[15] SDS,[16] and SCN,[17] have a significantly higher probability of hematological malignancies than the general population. In addition, we identified three *RUNX1* gene alterations (mutation/deletion) among four patients with thrombocytopenia in the targeted sequencing cohort who were diagnosed with FPD/AML. All three patients developed thrombocytopenia during infancy, suggesting that FPD/AML should be included in the differential diagnosis of infantile thrombocytopenia. In total, we genetically diagnosed 43 patients with cancer predisposition in the targeted sequencing cohort (DBA, $n = 12$; DC, $n = 4$; FA, $n = 16$; SDS, $n = 6$; SCN, $n = 2$; FPD/AML, $n = 3$) and 55 patients in the WES cohort (DBA, $n = 11$; DC, $n = 8$; FA, $n = 35$; FPD/AML, $n = 1$). These patients need to be continually and regularly evaluated by complete blood counts and physical examination to screen for the development of hematological malignancies. In addition, appropriate genetic counseling and familial genetic screening are mandatory.

The diagnostic rate of WES was inferior to that of target sequencing (53/121 (44%) vs. 68/250 (27%); $P = 0.002$), mainly due to the conventional genetic testing conducted before enrollment in the WES cohort. For example, the highest diagnostic rate was demonstrated for FA (15/22, 68%) in the target sequencing cohort, reflecting the ability of the chromosomal breakage test to achieve a precise clinical diagnosis. In contrast, the diagnostic efficacy of WES in patients with FA and prior genetic testing tended to be lower (43%, 25/57), whereas WES in patients with FA without prior genetic testing showed diagnostic rates (62%, 10/16) comparable to that of target sequencing. In addition, our target sequencing platform was able to identify copy-number variants in 11 of 121 patients (9%); however, we could not perform a reliable copy-number analysis in the WES cohort due to relatively low coverage.

Compared to WES, targeted sequencing can achieve similar results at a lower cost for sequencing and computing resources. Although we did not identify any incidental, medically actionable genetic discovery that was not associated with the targeted hematological disease categories in our WES cohort, targeted sequencing could decrease the risk of incidental genetic discovery, which may cause serious ethical problems in a clinical setting.[18]

Future accumulation of data regarding genotype–phenotype correlation and functional studies of variants with unknown significance will promote the accuracy of genetic testing for IBMFS. The diagnostic yield of our WES analysis (27%) suggested the insufficiency of current knowledge about missense mutations. A combination of WES and array comparative genomic hybridization, RNA sequencing, and capture sequencing of intron lesions may complement efforts to find small deletion and splicing defects caused by missense, synonymous, and deep-in-intron variants in known causative genes.[19] In addition, WES/WGS applications will certainly identify novel IBMFS causative genes, which should continue to increase the genetic diagnostic rate of next-generation sequencing.[20,21] In this context, periodic reanalysis of the results is desirable. Furthermore, a meticulous combination of clinical judgment and analysis with genetic information is required.

In summary, we analyzed 371 IBMFS patients with two next-generation sequencing platforms and successfully diagnosed 53 of 121 (44%) and 68 of 250 (27%) patients using target sequencing and WES, respectively. Our results demonstrate the efficacy of massive parallel sequencing as a diagnostic tool for IBMFS in clinical practice.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

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