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JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

New Comprehensive Cytogenetic Scoring System for Primary Myelodysplastic Syndromes (MDS) and Oligoblastic Acute Myeloid Leukemia After MDS Derived From an International Database Merge

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ABSTRACT

Purpose
The karyotype is a strong independent prognostic factor in myelodysplastic syndromes (MDS). Since the implementation of the International Prognostic Scoring System (IPSS) in 1997, knowledge concerning the prognostic impact of abnormalities has increased substantially. The present study proposes a new and comprehensive cytogenetic scoring system based on an international data collection of 2,902 patients.

Patients and Methods
Patients were included from the German-Austrian MDS Study Group (n = 1,193), the International MDS Risk Analysis Workshop (n = 816), the Spanish Hematological Cytogenetics Working Group (n = 849), and the International Working Group on MDS Cytogenetics (n = 44) databases. Patients with primary MDS and oligoblastic acute myeloid leukemia (AML) after MDS treated with supportive care only were evaluated for overall survival (OS) and AML evolution. Internal validation by bootstrap analysis and external validation in an independent patient cohort were performed to confirm the results.

Results
In total, 19 cytogenetic categories were defined, providing clear prognostic classification in 91% of all patients. The abnormalities were classified into five prognostic subgroups ($P < .001$): very good (median OS, 61 months; hazard ratio [HR], 0.5; n = 81); good (49 months; HR, 1.0 [reference category]; n = 1,809); intermediate (26 months; HR, 1.6; n = 529); poor (16 months; HR, 2.6; n = 148); and very poor (6 months; HR, 4.2; n = 187). The internal and external validations confirmed the results of the score.

Conclusion
In conclusion, these data should contribute to the ongoing efforts to update the IPSS by refining the cytogenetic risk categories.

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INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal hematopoietic stem-cell disorders[1,2] characterized by ineffective hematopoiesis and increased apoptosis,[3] resulting in peripheral cytopenias.[4] Acquired cytogenetic aberrations are well established as independent prognostic factors in MDS.[5-10] Initiated by the Bournemouth score in 1985,[11] several scoring systems for MDS were proposed[6,12,13] before culminating in the internationally accepted standard risk assessment system in MDS: the International Prognostic Scoring System (IPSS).[8] On the basis of the cytogenetic component of the IPSS, 86% of all cytogenetic findings can be explicitly classified according to their prognostic impact. The remaining 14% of patients show cytogenetic abnormalities with unknown prognostic significance. This finding underscores two major cytogenetic classification problems in MDS, namely, the profound heterogeneity of acquired cytogenetic aberrations in MDS, and the associated challenge of designing a comprehensive

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cytogenetic scoring system that predicts the prognostic impact of rare abnormalities. Furthermore, many abnormalities can occur as an isolated abnormality or in combination with one (ie, double abnormalities) or several other aberrations within a complex abnormal karyotype, defined as three or more abnormalities.[14] Within the past decade, analyses of large patient cohorts have led to an increasing knowledge of isolated abnormalities.[8-10] Nevertheless, few data exist about pairwise combinations of abnormalities or their prognostic relevance, resulting in prognostic uncertainty.[8,10]

Recent studies have provided evidence for the existence of prognostically different subgroups among patients with complex abnormal karyotypes.[9,10,15,16] Subsets of patients with various patterns of complex karyotypes, according to number of abnormalities, make it evident that prognosis in these patients deteriorates with increasing numbers of abnormalities, reflecting the clonal evolution and genetic instability of the clone.[10] The prognostic classification of rare abnormalities remains a challenge. The presence of rare cytogenetic abnormalities is not unusual in MDS and occurs most frequently in complex karyotypes.[17] In the absence of sufficient data, the IPSS combines rare abnormalities into the intermediate cytogenetic risk group. Hence, the objective of the present study was to develop a cytogenetic scoring system that incorporates the extensive variability of abnormalities in MDS, leading to an applicable tool to improve the prognostic impact of cytogenetic abnormalities in MDS and, possibly, providing a foundation for the upcoming IPSS revision.

PATIENTS AND METHODS

Patient Cohort

From four different databases, 2,902 patients were included. Databases used were those of the German-Austrian MDS Study Group (GA; n = 1,193; 41.1%),[10] International MDS Risk Analysis Workshop (IMRAW; n = 816; 28.1%),[8] Spanish Hematological Cytogenetics Working Group (n = 849; 29.3%),[9] and International Working Group on MDS Cytogenetics (n = 44; 1.5%).[17] GA patients were from four institutions in Germany (University of Düsseldorf, Düsseldorf; University of Göttingen, Göttingen; University of Freiburg, Freiburg; and Johannes-Hospital Duisburg, Duisburg) and four in Austria (University of Vienna; Hanusch Hospital, Vienna; Elisabethinen Hospital, Linz; Innsbruck Medical University, Innsbruck). The International Working Group on MDS Cytogenetics contributed patient cases involving rare abnormalities from the City of Hope Hospital (Duarte, CA), Tokyo Medical University (Tokyo, Japan), and Hospital del Mar Research Institute (Barcelona, Spain).[17] Only patients with primary MDS and oligoblastic AML after MDS meeting the following criteria were included: age 16 years or older; bone marrow blast count of 30% or less; and treated with supportive care, allowing for short (≤ 3 months) courses of oral chemotherapy or application of steroids, danazol, hematopooietic growth factors, or amifostine. Cytogenetic and clinical data for patients from IMRAW, GA, and Spanish Hematological Cytogenetics Working Group were reported previously.[8-10] To validate the score, an independent patient cohort including 1,632 patients with primary untreated MDS from the MD Anderson Cancer Center (MDA; Houston, TX) was analyzed.

The study was conducted in accordance with the modified Declaration of Helsinki. Additional data concerning the test and validation cohorts are listed in Table 1.

Bone Marrow Morphology and Cytopenia Classification

Bone marrow morphology studies were performed at the individual centers. Patients from the GA database were reviewed centrally (C.A., U.G.). Classification of MDS was performed according to French-American-British[18] and/or WHO classifications.[19] AML evolution was determined using the French-American-British definition (> 30% marrow blasts). Peripheral blood cytopenias were scored according to the IPSS.[8]

Cytogenetic Analysis

Cytogenetic analyses were performed at the time of first diagnosis, as described elsewhere.[1] Abnormalities diagnosed by fluorescent in situ hybridization only were not included. The median number of metaphases analyzed was 20 (range, 2 to 194). Karyotypes were documented according to the International System for Human Cytogenetic Nomenclature (ISCN).[14] All ISCN formulas were reviewed centrally (J.S., C.S., D.H.). The IPSS karyotype scores (ie, number of clonal abnormalities) were generated according to international guidelines.[20]

Composition of Cytogenetic Subgroups

Isolated anomalies occurring in at least five patients were judged as distinct subgroups of patients. Double abnormalities (ie, two distinct clonal MDS-related acquired karyotypic anomalies found within one cell) were classified into three subgroups: del(5q) with one additional clonal aberration, −7/del(7q) with one additional clonal aberration, and any other combination of two abnormalities. Complex karyotypes were subdivided into those with three unrelated karyotypic abnormalities and those with more than three karyotypic abnormalities. Independent clones, defined as two or more unrelated subclones in parallel, were calculated as a distinct group when none of the clones comprised complex abnormalities; otherwise, they were considered complex.

Statistical Analysis

Univariate analysis of overall survival (OS) and risk of AML transformation were calculated using the Kaplan-Meier method.[21] Differences were tested with the log-rank test.[22] Multivariate analysis was performed using a Cox proportional hazards regression model,[23] with OS or time to AML transformation as the end point. In multivariate analysis, age, sex, percentage of bone marrow blasts, peripheral cytopenia, cytogenetic pattern, site, and year of diagnosis (Appendix Fig A1, online only) were applied as covariables. Univariate and multivariate analyses were performed exclusively for groups with a minimum of five patients. Because of the high variability of time-to-event analyses in very rare abnormalities (n < 10), these were not considered as distinct abnormalities within the scoring system and were merged into one group labeled "any other single."

Group differences concerning numerical variables were tested for significance using the Kruskal-Wallis test for analysis of variance by rank and, in the case of ordered groups, using the significance test for Kendall's tau. Two-sided *P* values less than .05 were considered significant. In line with the essentially exploratory nature of the study, no adjustment for multiple testing was applied. The predictive power of the prognostic models was assessed using the generalization of the Dxy coefficient for censored data.[24]

Because the estimated risk attributable to a cytogenetic category is influenced by confounding characteristics like age, sex, and clinical features, several multivariable models including different combinations of possible confounders were calculated for survival and time to AML transformation. The resulting coefficients were combined in a weighted mean for each cytogenetic category, using number of events as weights.

On the basis of their mean coefficients, the cytogenetic categories could be divided into five separable risk groups, and the limits for the risk groups were equally spaced. Consequently, the five risk categories were considered as a numerical scale (1 to 5). A bootstrap analysis based on 2,000 repetitions was applied to validate the score.[25]

The external validation based on the MDA data was executed as a numeric scale by re-estimation of multivariate Cox models and comparison of the estimates and CIs. All statistical analyses were performed using the open-source software R version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria).

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Table 1. Patient Demographics and Clinical Characteristics

Characteristic	Database Test Set										Validation Set	
	Total (test set)		GA		GCECGH		IMRAW		IWCG		MDA	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total patients	2,902		1,193	41.1	849	29.3	816	28.1	44	1.5	1,632	
Sex												
Male	1,695	58.4	685	57.4	475	55.9	504	61.8	31	70.5	1,096	67.2
Female	1,207	41.6	508	42.6	374	44.1	312	38.2	13	29.5	536	32.8
Age, years												
< 60	686	23.9	298	25.3	169	20.2	205	25.1	14	31.8	554	34.0
≥ 60	2,188	76.1	879	74.4	668	79.8	611	74.9	30	68.2	1,078	66.0
Median	70		69		71.0		*		69.0		66.0	
Range	16-96		16-96		16-95		*		22-86		16-94	
Classification†												
FAB												
RA	650	22.4	184	15.4	169	19.9	294	36.0	3	6.8	0	0.0
RARS	296	10.2	80	6.7	90	10.6	126	15.4	0	0.0	0	0.0
RAEB	333	11.5	124	10.4	1	0.1	208	25.5	0	0.0	0	0.0
RAEB-T	138	4.8	77	6.5	0	0.0	61	7.5	0	0.0	0	0.0
CMML	314	10.8	189	15.8	0	0.0	125	15.3	0	0.0	0	0.0
WHO												
RA	89	3.0	48	4.0	19	2.2	*		22	50.0	314	19.2
RARS	62	2.1	29	2.4	32	3.8			1	2.3	112	6.9
RCMD	193	6.7	151	12.7	37	4.4			5	11.4	109	6.7
RCMD-RS	93	3.2	68	5.7	25	2.9			0	0.0	32	2.0
RAEB-1	158	5.4	61	5.1	89	10.5			8	18.2	293	18.0
RAEB-2	223	7.7	77	6.5	144	17.0			2	4.5	465	28.5
5q− syndrome	95	3.3	68	5.7	27	3.2			0	0.0	6	0.4
CMML-1	88	3.0	8	0.7	80	9.4			0	0.0	92	5.6
CMML-2	68	2.3	3	0.3	65	7.7			0	0.0	32	2.0
AML	79	2.7	15	1.3	64	7.5			0	0.0	153	9.4
Unclassified	10	0.3	0	0.0	7	0.8			3	6.8	12	0.7
No WHO or FAB classification	13	0.4	11	0.9	0	0.0	2	0.2	0	0.0	12	0.7
Bone marrow blasts, %												
< 5	1,599	57.3	634	56.4	483	56.9	482	59.1	*		699	42.8
5-10	577	20.7	224	19.9	170	20.0	183	22.5			439	26.9
11-20	429	15.4	171	15.3	143	16.8	114	14.0			370	22.7
21-30	184	6.6	95	8.4	53	6.2	36	4.4			124	7.6
Cytopenias												
Hb, g/dL												
Median	9.6		9.6		9.5		9.7		*		9.7	
Range	0.9-18.9		2.5-16.7		1.3-18.9		0.9-16.6				3.2-27.7	
ANC, ×10^3/μL												
Median	2.1		2.0		2.2		2.0				1.5	
Range	0-85.0		0.1-46.8		0-85.0		0-46.8				0-45.5	
PLT, ×10^3/μL												
Median	124		105		124		132				73	
Range	0-1371		3-1,371		4-1,240		0-999				1-1,200	
Observation time, months												
Median	50.0		55.9		37.5		67.2		40.9		53.2	
Range	0.1-326		0.1-326		0.1-289		0.1-206		0.2-262		0.1-258	
IPSS												
Low risk	574	29.5	77	20.8	224	29.1	273	34.0	*		301	18.4
Intermediate 1	763	39.2	141	38.0	295	38.3	327	40.7			606	37.1
Intermediate 2	377	19.4	90	20.0	154	20.0	133	16.6			470	28.8
High risk	230	11.8	63	12.6	97	12.6	70	8.7			255	15.6

Abbreviations: AML, acute myeloid leukemia; ANC, absolute neutrophil count; CMML, chronic myelomonocytic leukemia; CMML-1, chronic myelomonocytic leukemia with less than 10% bone marrow blasts; CMML-2, chronic myelomonocytic leukemia with 10% to 19% bone marrow blasts; FAB, French-American-British; GA, German-Austrian MDS Study Group; GCECGH, Spanish Hematological Cytogenetics Working Group; Hb, hemoglobin; IMRAW, International MDS Risk Analysis Workshop; IPSS, International Prognostic Scoring System; IWGC, International Working Group on MDS Cytogenetics; MDA, MD Anderson Cancer Center; MDS, myelodysplastic syndromes; PLT, platelet count; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RAEB-1, RAEB with blast level < 10%; RAEB-2, RAEB with blast level 10% to 20%; RAEB-T, RAEB in transformation; RARS, refractory anemia with ring sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ring sideroblasts.

*Not available in database.

†Specified as either WHO or FAB to avoid double classification.

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Table 2. Clinical Characteristics and Survival in Cytogenetic Subgroups

	Patients		Median						
Characteristic	No.	%	Age (years)	Hb (g/dL)	PLT ($\times 10^3/\mu L$)	ANC ($\times 10^3/\mu L$)	BLC	OS (months)	AML-Free Survival (months)
Karyotype									
Normal	1,543	55.1	70.0	9.9	130	2.2	3.0	47.4	NR
Abnormal	1,258	44.9	69.0	9.3	114	2.0	4.0	24.4	91.0
P			NS	< .01	< .01	< .01	< .01	< .01	< .01
Distribution of metaphases									
NA karyotype	500	18.3	70.4	9.5	117	2.0	3.0	29.7	NR
AA karyotype	693	25.3	67.5	9.1	118	2.1	4.0	27.0	84.3
P			< .01	< .05	NS	NS	< .01	< .05	< .05
Abnormalities per patient									
1	827	29.5	69.0	9.5	124	2.2	3.0	37.4	203.2
2	174	6.2	68.0	9.5	124	2.1	4.0	27.9	91.0
3	60	2.1	70.0	9.3	107	1.9	5.5	15.6	21.0
> 3	196	7.0	71.0	8.5	67	1.3	11.0	5.7	8.2
P			NS	< .01	< .01	< .01	< .01	< .01	< .01
Clonal evolution									
No (only abnormal patient cases)	780	86.6	69.2	9.4	111	2.0	4.0	28.9	121.2
Yes	121	13.4	69.7	9.4	85	1.8	5.0	11.4	33.0
P			NS	NS	NS	NS	NS	< .01	< .01
Abnormalities									
Single (n ≥ 10)									
inv(3)/t(3q)/del(3q)	12	0.4	67.6	8.2	130	2.1	4.0	23.8	7.9
del(5q)	180	6.4	66.8	8.7	251	2.1	3.0	57.8	203.2
−7	46	1.6	67.0	9.1	59	1.7	8.0	15.8	33.5
del(7q)	13	0.5	63.9	9.5	120	1.7	10.0	20.0	NR
+8	133	4.7	70.2	9.3	120	2.1	4.0	23.0	38.6
del(11q)	20	0.7	68.0	11.2	123	2.0	2.0	141.2	121.2
del(12p)	18	0.6	70.0	8.8	86	1.6	9.0	76.0	NR
i(17q)	11	0.4	74.5	8.4	89	2.7	10.0	18.0	16.8
+19	10	0.4	66.5	8.0	116	3.9	2.0	56.4	64.0
del(20q)	48	1.7	71.0	11.0	106	1.5	3.0	62.0	NR
−Y	63	2.2	73.0	11.2	150	2.7	3.0	60.8	NR
Rare single (n < 10)									
der (1;7)	9	0.3	59.5	7.8	75	0.9	3.0	NR	NR
+1q	5	0.2	53.5	11.2	32	2.2	8.0	21.3	19.2
−1/1p−	6	0.2	65.7	10.2	293	3.6	7.0	47.7	NR
t(5q)	6	0.2	62.1	13.3	59	4.4	3.0	NR	NR
+11	5	0.2	64.6	10.3	76	7.3	16.0	11.0	11.0
t(11q23)	7	0.2	65.4	7.9	140	6.3	4.0	26.7	78.0
−13/13q−	8	0.3	56.0	10.1	150	3.1	2.0	NR	NR
del(16q)	7	0.2	71.0	12.4	121	3.5	2.0	NR	NR
del(17p)	6	0.2	63.0	10.6	165	3.3	2.5	NR	NR
+21	9	0.3	67.0	9.1	105	1.9	6.0	21.5	100.7
−21	8	0.3	78.0	7.2	35	2.2	13.0	32.0	31.3
−X	9	0.3	66.0	9.4	108	2.3	5.0	16.0	14.0
+Mar	5	0.2	76.1	9.1	72	0.5	7.0	20.7	NR
Other	162	5.8	69.9	9.6	90	2.1	3.5	23.3	NR
P			< .05	< .01	< .01	NS	< .01	< .01	< .01
All rare abnormalities (n < 10) combined	252	9.0	67.2	9.6	105	2.4	4.0	30.5	100.7
Double abnormalities									
Including del(5q)	46	1.6	68.4	8.2	243	2.1	3.0	44.4	91.2
Including any other	94	3.4	69.0	9.8	117	2.0	3.0	28.0	NR
Including −7/del(7q)	33	1.2	66.4	9.4	124	2.2	8.0	13.4	19.3
P			NS	< .01	< .05	NS	NS	< .01	NS
No. of complex abnormalities									
3	60	2.1	70.0	9.3	107	1.9	5.5	15.6	21.0
> 3	196	7.0	71.0	8.5	67	1.3	11.0	5.7	8.2
P			NS	< .01	< .05	NS	< .01	< .01	< .01
Independent clones	25	0.9	71.6	7.9	160	3.1	6.0	18.5	84.3

Abbreviations: AA, only abnormal metaphases; AML, acute myeloid leukemia; ANC, absolute neutrophil count; BLC, bone marrow blast count; Hb, hemoglobin; NA, mosaic of normal and abnormal metaphases; NR, not reached; NS, not significant; OS, overall survival; PLT, platelet count.

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RESULTS

Patient Cohort and Clinical Findings

In total, 2,902 patients were analyzed retrospectively. The female to male ratio was 1:1.4. The median age was 70 years. Median hemoglobin was 9.6 g/dL; median absolute neutrophil count, 2.1×10^9/L; and median platelet count, 124×10^9/L. The median observation time was 50.0 months (range, 0.1 to 326 months; Table 1).

Cytogenetic Abnormalities

The ISCN karyotype description was evaluable and clearly interpretable in 2,801 (97%) of 2,902 patients. Clonal abnormalities were observed in 45% of patients (n = 1,258). Table 2 summarizes cytogenetic subgroups and related clinical features.

Distinct Abnormalities

Isolated abnormalities. Isolated abnormalities occurring in at least five patients included: del(5q) [n = 180], +8 [n = 133], −Y [n = 60], del(20q) [n = 48], −7 [n = 46], del(11q) [n = 20], del(12p) [n = 18], del(7q) [n = 13], i(17)(q10) [n = 11], inv(3)/t(3q)/del(3q) [n = 10], +19 [n = 10], +21 [n = 9], der(1, 7) [n = 9], −X [n = 9], −21 [n = 8], −13/del(13q) [n = 8], del(16q) [n = 7], t(11q23) [n = 7], −1/del(1p) [n = 6], t(5q) [n = 6], del(17p) [n = 6], +mar [n = 5], +1q [n = 5], and +11 [n = 5; Table 2].

Double abnormalities. Double abnormalities are characterized by profound variability. The only combination occurring in more than five patients was the combination of del(5q) and trisomy 8 (n = 6). Other combinations were found less than five times.

Complex abnormalities. Complex abnormalities were observed in 9% (n = 254) of all patients. The median number of abnormalities per patient with complex abnormalities was five (range, three to 20).

Analysis of Prognostic Factors

As possible confounders in the estimation of cytogenetic risk categories, host-related and clinical features were investigated. Female patients showed a lower OS risk (HR, 0.7; $P < .01$) but not a lower risk of AML transformation (HR, 0.9; P not significant). Likewise, age 60 years or older was found to be a risk factor for OS (HR, 1.6; $P < .01$) but not for transformation to AML (HR, 1.1; P not significant). The occurrence of anemia (hemoglobin < 10 g/dL) or thrombocytopenia (platelets < 100/μL) was significantly associated with a higher risk for both OS (anemia: HR, 1.6; $P < .01$; thrombocytopenia: HR, 1.3; $P < .01$) and AML evolution (anemia: HR, 1.7; $P < .01$; thrombocytopenia: HR, 1.3; $P < .05$). A lower absolute neutrophil count (< 1.8×10^9/L) did not significantly affect OS (HR, 0.9; P not significant) or risk of AML transformation (HR, 1.2; P not significant). As expected, the risks for OS and especially AML rise with increasing bone marrow blast counts. The HRs for OS (reference < 5%) were 1.9 for 5% to 10%, 2.8 for 11% to 20%, and 4.2 for 21% to 30% ($P < .01$

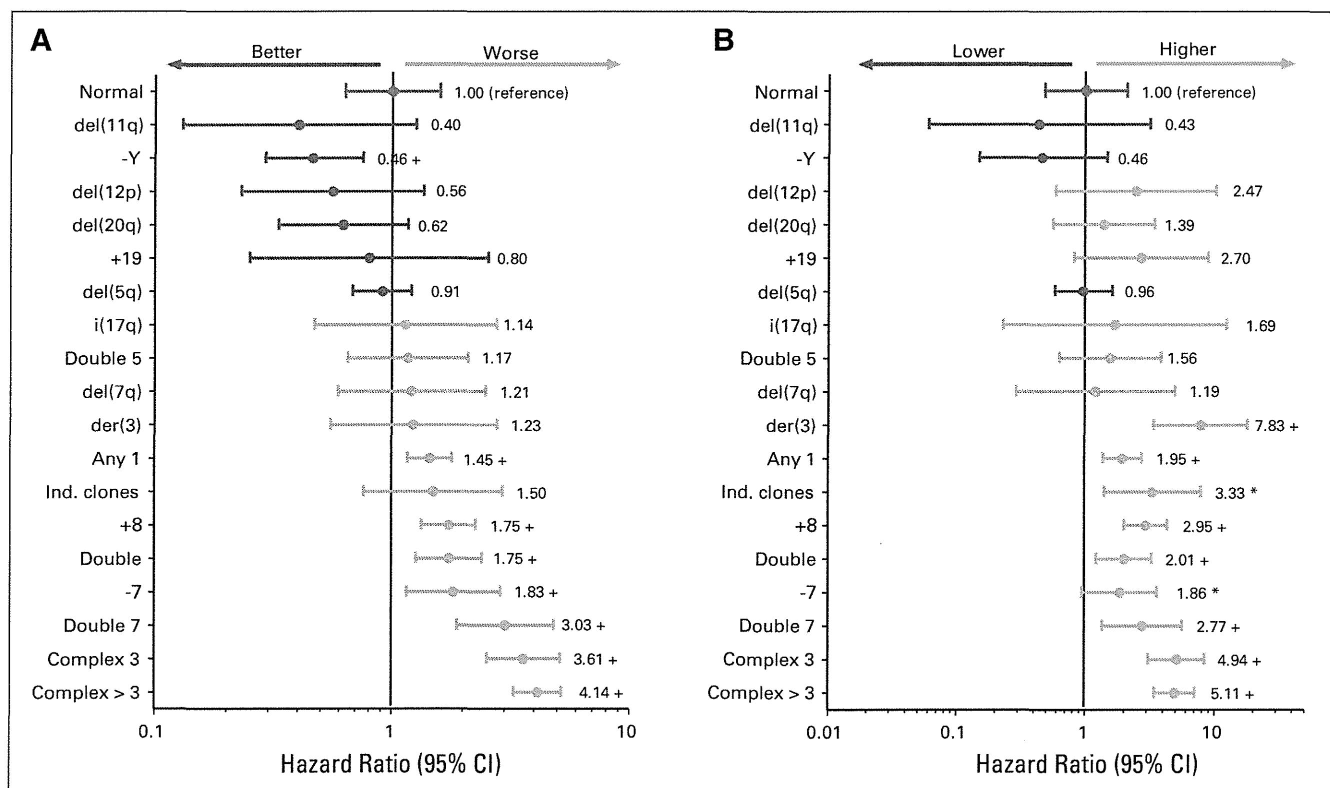

Fig 1. (A) Overall survival (n = 1,893) and (B) risk of acute myeloid leukemia transformation (n = 1,691) in distinct cytogenetic subgroups (abnormalities with n < 10 combined as Any 1). Any 1, any other single abnormality; double 5, double abnormalities including del(5q); double, any other combination of two abnormalities; double 7, double abnormalities including −7/7q−; complex 3, three abnormalities; complex > 3, four or more abnormalities; + indicates $P < .01$ (as compared with reference category); (*) indicates $P < .05$ (as compared with reference category). Ind., independent.

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for all groups). For AML transformation, HR increased with an increase in the percentage of bone marrow blasts: 3.0 for 5% to 10%, 5.5 for 11% to 20%, and 10.8 for 21% to 30% ($P < .01$ for all groups).

Karyotype Abnormalities

Univariate analysis was performed separately for each cytogenetic category. Table 2 and Figures 1A and 1B include additional information concerning OS and AML transformation in distinct single abnormalities. The presence of a second clonal aberration (ie, double abnormalities; Table 2; Appendix Figs A2A and A2B, online only) associated with del(5q) showed a median OS of 44.4 months and HR of 1.2 (AML transformation: median, 91.2 months; HR, 1.6). Gain of an additional clonal aberration with −7/del(7q) was associated with a significantly worse prognosis (OS: median, 13.4 months; HR, 3.0; AML: median, 19.3 months; HR, 2.8; $P < .01$). All other double abnormalities showed an intermediate prognostic impact (OS: median, 28.0 months; HR, 1.8; AML: median, not reached [NR]; HR, 2.0). Complex abnormalities (Table 2; Appendix Figs A2C and A2D, online only) showed a significant ($P < .01$) difference regarding prognosis between those with exactly three abnormalities (OS: median, 15.6 months; HR, 3.6; AML: median, 21.0; HR, 4.9) as compared with more than three abnormalities (OS: median, 5.7 months; HR, 4.1; AML: median, 8.2; HR, 5.1).

Proposal of a New Cytogenetic Prognostic Scoring System

All abnormalities were arranged according to OS and AML to classify their prognostic impact. The classification was based on the results of the multivariate model, considering OS and risk of AML transformation. The analyses suggested a five-armed model based on interpretability and predictive power (OS: Dxy, 0.48; AML: Dxy, 0.59). Using this model, the groups were formed as follows: very good (median OS, 60.8 months; HR, 0.5), del(11q) and −Y; good (reference category; median OS, 48.6 months; HR, 1.0), normal, del(5q), del(12p), and del(20q) [all as a single anomaly] and double abnormalities including del(5q); intermediate median OS, 26.0 months; HR,

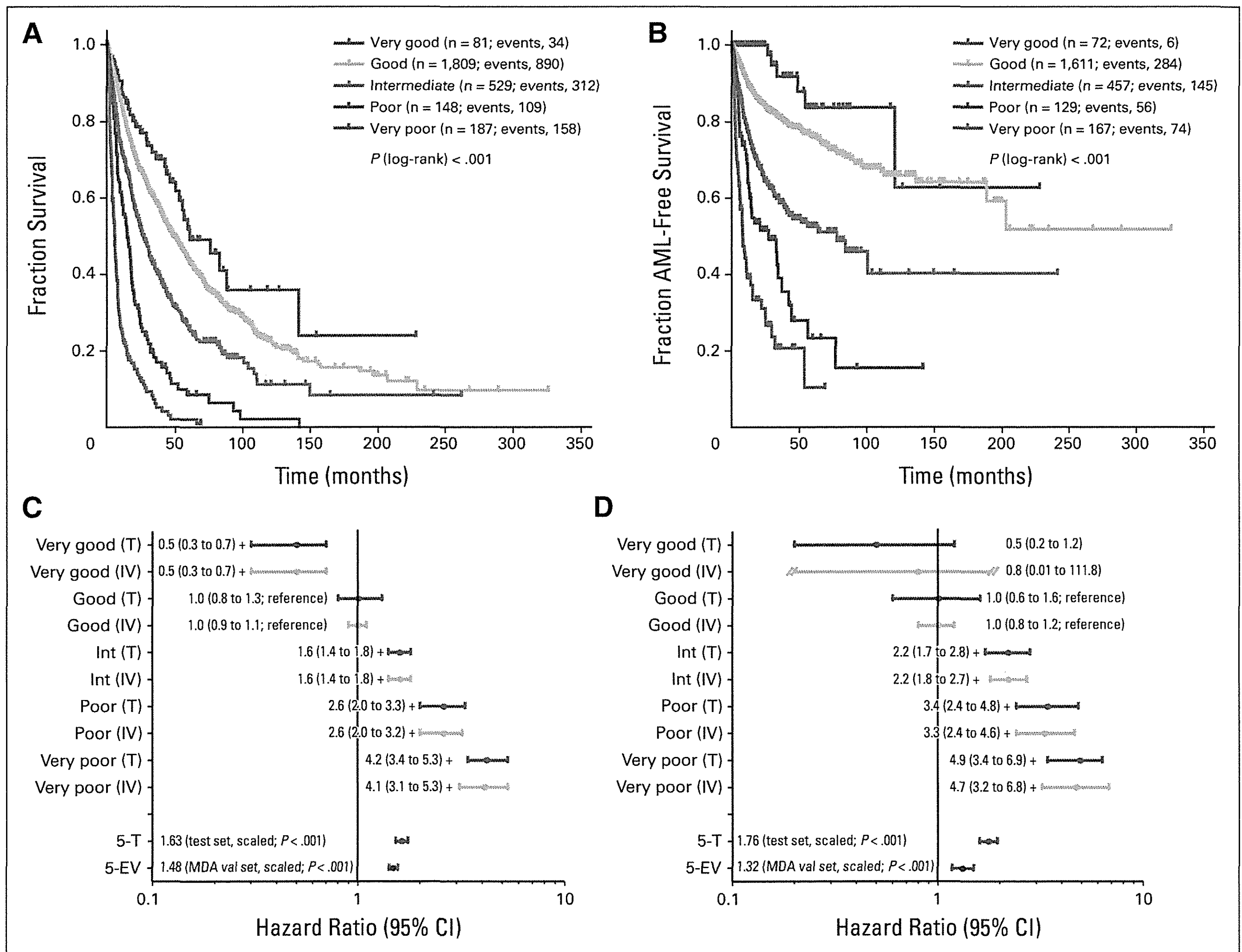

Fig 2. (A, B) Kaplan-Meier curves and (C, D) forest plots for (A, C) overall survival and (B, D) risk of acute myeloid leukemia (AML) transformation in new cytogenetic prognostic subgroups; + indicates $P < .01$ (as compared with reference category). 5-EV, external validation set (MD Anderson Cancer Center [MDA]), scaled, five groups; 5-T, test set, scaled, five groups; int, intermediate; IV, internal validation set; T, test set; val; validation.

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1.6), del(7q), +8, i(17)(q10), +19, +21, any other single abnormality, independent clones, double abnormalities not harboring del(5q) or −7/del(7q); poor (median OS, 15.8 months; HR, 2.6), inv(3)/t(3q)/del(3q), −7, double abnormalities including −7/del(7q), and complex (ie, three abnormalities); and very poor (median OS, 5.9 months; HR, 4.2), complex (ie, > three abnormalities). The HRs between groups differed markedly concerning OS as well as AML (Figs 2A to 2D; Table 3). Compared with the cytogenetic module of the IPSS, the risk group for 13% of all patients (n = 367) would change, 6% (n = 160) into a more favorable and 7% (n = 207) into a less favorable prognostic group (Fig 3).

To show the improvement of our score as compared with the original IPSS, we estimated models including the cytogenetic part of the IPSS and the five cytogenetic groups, together with all other possible confounders. In this model, the cytogenetic part of the IPSS did not reach significance ($P = .670$), whereas the five cytogenetic groups contributed significantly ($P < .001$).

Validation of the New Scoring System

Internal validation by bootstrap. To reassess our results, an internal validation was performed using bootstrap analysis. Here, the HRs regarding OS were 0.48 (95% CI, 0.31 to 0.74) for the vey good cytogenetic subgroup, 1.0 (reference; 95% CI, 0.92 to 1.1) for good, 1.6 (95% CI, 1.4 to 1.8) for intermediate, 2.6 (95% CI, 2.0 to 3.2) for poor, and 4.1 (95% CI, 3.1 to 5.3) for very poor (Dxy, 0.47). For AML, the HRs were 0.75 (95% CI, 0.01 to 111.8) for the vey good cytogenetic subgroup, 1.0 (reference; 95% CI, 0.84 to 1.2) for good, 2.2 (95% CI, 1.8 to 2.7) for intermediate, 3.3 (95% CI, 2.4 to 4.6) for poor, and 4.7 (95% CI, 3.2 to 6.8) for very poor (Dxy, 0.57).

External validation based on independent test data. In line with the intended use of the proposed cytogenetic categories as one component of a comprehensive prognostic scoring system, Cox proportional hazards models for survival and time to transformation were estimated based on the independently collected data from MDA. These models included the five cytogenetic categories as a numeric score and the same additional characteristics used in the multivariate models in development. In both models, for survival and time to transformation, respectively, the cytogenetic score showed strong and significant prognostic impact. The estimated mean HRs between each two adjacent categories were 1.48 (95% CI, 1.41 to 1.56) for survival (Dxy, 0.38) and 1.32 (95% CI, 1.17 to 1.49) for risk of AML transformation (Dxy, 0.30). The corresponding results for the development data were 1.63 (95% CI, 1.52 to 1.74) for survival (Dxy, 0.48) and 1.76 (95% CI, 1.59 to 1.94) for time to transformation (Dxy, 0.59).

Finally, an external Italian working group further validated the accuracy of the score completely independently from us. The results confirmed that the score efficiently predicts outcome in patients with MDS.[26]

DISCUSSION

The present study was undertaken to improve cytogenetic classification in MDS and propose a more comprehensive cytogenetic scoring system for patients with primary untreated MDS by assembling cytogenetic and clinical data into a large multicenter project. We sought to create a system that clearly separates single and double abnormalities, defines a procedure to classify double abnormalities, and emphasizes prognostic subgroups within the heterogeneous category of patients showing complex abnormalities. The present study is based on, to our knowledge, the largest data set collected to date by focusing on these questions.

Regarding single abnormalities, inv(3)/t(3q)/del(3q), +8, del(11q), del(12p), i(17)(q10), +19, and +21 were newly integrated into the scoring system. Substantial differences were not seen in the

Table 3. Design of Cytogenetic Scoring System (n = 2,754)*

Prognostic Subgroup	Abnormality: No. of Patients	%	Single	Double	Complex	Overall Survival: Median (months)†	95% CI	HR	95% CI	AML Transformation: Median (months)†	95% CI	HR	95% CI
Very good	81	2.9	del(11q) −Y	—	—	60.8	50.3 to NR	0.5†	0.3 to 0.7	NR	121.2 to NR	0.5	0.2 to 1.2
Good (reference)	1,809	65.7	Normal del(5q) del(12p) del(20q)	Including del(5q)	—	48.6	44.6 to 54.3	1.0	0.9 to 1.1	NR	189.0 to NR	1.0	0.9 to 1.2
Intermediate	529	19.2	del(7q) +8 i(17q) +19 Any other Independent clones	Any other	—	26.0	22.1 to 31.0	1.6†	1.4 to 1.8	78.0	42.6 to NR	2.2†	1.8 to 2.7
Poor	148	5.4	inv(3)/t(3q)/del(3q) −7	Including −7/del(7q)	3	15.8	12.0 to 18.0	2.6†	2.1 to 3.2	21.0	13.4 to 42.2	3.4†	2.5 to 4.6
Very poor	187	6.8	—	—	> 3	5.9	4.9 to 6.9	4.2†	3.4 to 5.2	8.2	6.4 to 15.4	4.9†	3.6 to 6.7

Abbreviations: AML, acute myeloid leukemia; HR, hazard ratio; NR, not reached.
*Patients with complete data.
†$P < .01$.

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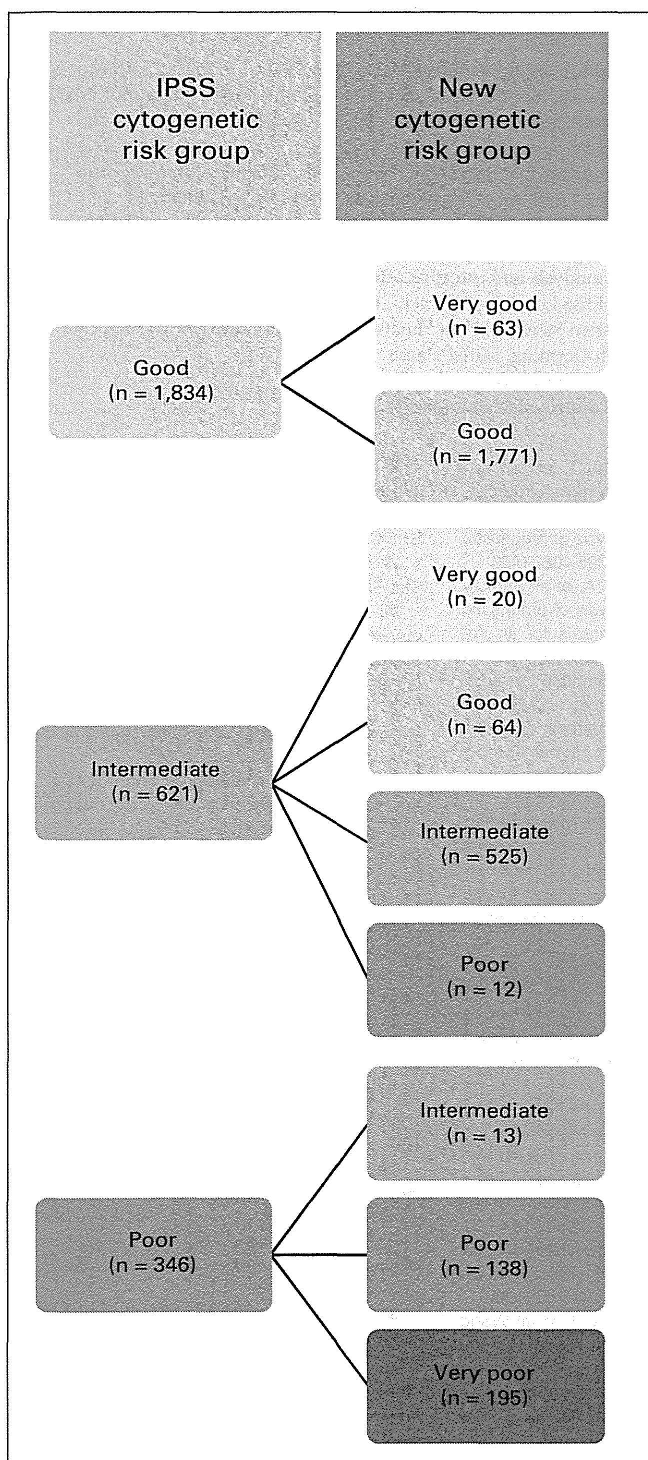

Fig 3. Change of cytogenetic prognostic subgroups in the new system as compared with the cytogenetic module of the International Prognostic Scoring System (IPSS).

prognostic impact of categories known from the IPSS [del(5q), del(20q), −Y] compared with the data based on large patient cohorts (n > 500) published to date.[6-10,27,28] An exception was seen in patients showing partial or total monosomy of chromosome 7 as a single abnormality, which was associated with poor prognosis in studies by IMRAW,[8] the Spanish MDS Cytogenetic Working Group,[9] and Podzdnyakova et al,[28] whereas in a previous analysis of our group based on the GA data set[10] as well as the findings of Bernasconi et al,[29] it was described as intermediate. The results are difficult to compare because of the fact that some studies also included patients treated with disease-altering therapy,[9,27] whereas others[8,10,29] included patients treated with supportive care exclusively. Our data revealed that a deletion of 7q was more favorable as compared with the loss of the whole chromosome 7 with regard to OS (HR: del (7q), 1.2; −7, 1.8; Appendix Fig A3A, online only) as well as risk of AML transformation (HR: del(7q), 1.2; −7, 1.9; Appendix Fig A3B), a finding described previously.[9,30] Consequently, we classified the deletion of 7q as intermediate, whereas the loss of the whole chromosome 7 was associated with poorer prognosis.

Double abnormalities were separated into three distinct subgroups clearly distinguishing risk with regard to OS as well as risk of AML transformation, which has not been described before. Recent publications assigned double abnormalities into one group[7-9,27] or merely separated noncomplex (one to two abnormalities) from complex (≥ three abnormalities).[6,10,31]

The subclassification of complex abnormalities in our study also defined new cytogenetic risk categories. We are aware that additional analyses are necessary to characterize distinct subgroups within the heterogeneous cluster of complex abnormalities; however, this proposal constitutes a classification that remains clinically applicable.

We included patients with refractory anemia with excess blasts in transformation (RAEB-T; ie, oligoblastic AML), although we are aware that this disease is classified as AML but not MDS in WHO classification. Statistically, the exclusion of patients with RAEB-T (8%) leads to a lower risk in the entire sample, whereas the relative position of the five prognostic subgroups remains unchanged. Additionally, the new score was designed to form the cytogenetic module of the upcoming IPSS revision, which is expected to include those with RAEB-T as well.

The results in the independent validation cohort were, as expected, somewhat less pronounced than those in the development data (Appendix Fig A4, online only). Still, the results for survival were within the CI of the original estimate. The risk estimation for time to transformation in the MDA data was weaker than that in the development data. This may partly stem from the optimism inherent in explorative model building and partly from differences in patient populations.

In summary, we propose a new and comprehensive cytogenetic scoring system. Ninety-one percent of all patients can be explicitly classified according to an estimated prognostic impact of the abnormality observed, which allows for a refined cytogenetic risk prediction. The present study is part of a multistep process to compose the cytogenetic module of the upcoming revision of the IPSS. Previous work of our group[10,32] focused on the prognostic impact of clonal abnormalities and their underrepresentation within the IPSS but did not include design and validation of a new scoring system. However, the system presented here should be viewed as a dynamic model, open to further refinement as our knowledge about karyotypic abnormalities in MDS evolves.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Manuscript writing: All authors
Final approval of manuscript: All authors

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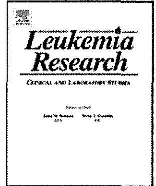

Will a peripheral blood (PB) sample yield the same diagnostic and prognostic cytogenetic data as the concomitant bone marrow (BM) in myelodysplasia?

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ABSTRACT

In patients with myelodysplastic syndromes (MDS), chromosome anomalies are detected by conventional cytogenetic studies (CCS) and/or interphase fluorescence in situ hybridization (FISH) of bone marrow (BM) samples and provide prognostic and diagnostic information, which can direct therapy. Whether peripheral blood (PB) can be substituted for bone marrow in these cases and can provide the same information remains unknown. Concurrent BM and PB specimens collected from 100 patients with recently diagnosed MDS were studied using both CCS and FISH. While 68% of BM samples showed an abnormal karyotype by CCS, only 31% of PB samples were abnormal by CCS. In 12% of patients, FISH and CCS were discordant due to the inability of the FISH panel to detect all possible abnormalities. However, only one case (1%) had a cryptic abnormality detected by FISH. BM and PB FISH were discordant in 3% of cases, most likely due to the smaller clone size in PB vs. BM. While PB should not be substituted for BM at diagnosis, it is a viable alternative for monitoring patients using the appropriate FISH probe(s).
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1. Introduction

The myelodysplastic syndromes (MDS) are a heterogeneous group of hematological malignancies characterized by ineffective hematopoiesis and a highly variable clinical course [1,2], for which novel treatments are beginning to emerge [3]. Conventional cytogenetic studies (CCS) of bone marrow (BM) are routinely used in clinical practice to detect abnormal clones in proliferating (metaphase) cells, identifying clonal aberrations in approximately 50% of de novo MDS cases [4]. Cytogenetics is also one of the key International Prognostic Scoring System (IPSS) components used to estimate overall survival and leukemia-free survival in MDS [5,6].

Chromosome abnormalities may be quantified at presentation and during treatment by the use of fluorescence in situ hybridization (FISH) with DNA probes for specific chromosome loci (e.g. chromosomes 5, 7, 8 and 20) in non-proliferating (interphase) nuclei [7,8]. However, it is not clear whether or not peripheral blood

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CCS and/or FISH will yield the same diagnostic and prognostic data as bone marrow CCS and/or FISH at disease presentation or for disease monitoring. It is also not clear whether patients without apparent abnormalities by CCS have occult abnormalities that can be identified by interphase FISH [9].

Conventional chromosome studies are usually performed on BM because PB studies of patients with MDS often lack mitotic cells. The utility of interphase FISH studies on PB from patients with MDS has not yet been established. However, one study involving 22 MDS patients suggested that interphase FISH of PB may be useful [10]. As well, Braulke et al. [11], performed FISH analysis on CD34+ enriched PB from 27 MDS patients in comparison with unenriched PB [11]. They showed that enrichment increased the percentage of FISH positive cells. Recently, Coleman et al. [12] compared the FISH results of PB and BM on the 48 patients and found comparable results, when the clone size was adequate.

If FISH could be used to examine PB specimens in lieu of BM, it would reduce the frequent need for BM examinations in clinical practice. As well, any occult anomalies might also be identified by FISH. To address the question of whether or not cytogenetic studies on PB will yield the same diagnostic and prognostic data as the BM in a multi-institutional, international setting, 15 members of the MDS Foundation's International Working Group on Cytogenetics in MDS agreed to perform CCS and FISH in parallel on both PB and BM samples collected from consecutive MDS patients during a two year time period.

2. Materials and methods

This study was performed in two phases. In phase I or the familiarization phase of this study, the 15 participating laboratories independently studied the bone marrow cells from the same two patient-derived samples using the same set of FISH probes. The familiarization specimens were derived from patients with known chromosomal abnormalities who had undergone apheresis. These samples were provided by Mayo Clinic (Rochester, MN). All sites received identical packets containing a set of specific instructions for FISH testing and scoring, five microscope slides labeled "MDS Pt #1", five microscope slides labeled "MDS Pt #2", eight labeled probe sets to study the test patients and database entry instructions. While the laboratories were blinded to the karyotypes during this phase of the study, patient #1 was known to have a long arm deletion of chromosome #5 (deletion 5q), monosomy 7 and deletion of the short arm of chromosome #17 (deletion 17p), while patient #2 was known to have trisomy 8, deletion of the long arm of chromosome #20 (deletion 20q) and monosomy 21. Each participant was asked to score 200 consecutive qualifying nuclei for each probe set. A FISH panel consisting of eight Vysis probe sets [−5/5q−, −7/7q−/der(1;7), +8/8q−, −11/+11/11q−/add(11q), 12p−/+21/*t*(12;21), −13/13q−, 17p− and 20q−/i(20q)/i(20p)], supplied by Abbott Molecular, Inc. (Des Plaines, IL, USA) was used (Table 1). Each specimen was scored independently by two individuals (100 cells each) and their results were combined. Possible interpretations of signal patterns with these probes were provided to aid participants in their work (Fig. 1). Data were also collected regarding each participating laboratory's established normal cut-off values and their method of determining these figures. This phase of the study helped the participants gain experience with the FISH probe panel including processing, scoring and interpreting FISH data, as well as to determine the appropriate cut-off values to be used in phase II of this study (Supplemental Data, Appendix).

In the second phase of the study, each participating laboratory performed prospective CCS and FISH analyzes in recently diagnosed MDS patients whose PB and BM specimens were drawn within a maximum of 3 days of each other. Both CCS and FISH were performed using standard protocols. The WHO classification (2008), as well as prior treatment, percent blasts, patient age and gender were recorded (Table 2). Each pair of BM and PB specimens had CCS performed with analysis of 20 metaphase cells where possible. As well, interphase FISH was performed on these parallel samples where material permitted, using the FISH panel as described in phase I of the study (Table 1). Two hundred interphase nuclei were scored for each probe set. As in phase I, participating laboratories were asked to use their own laboratory cut-off values in determining which probe sets were positive or negative. The initial intent was for each of the 15 participating laboratories to recruit 10 consecutive patients, but not all reached this goal. A total of 100 MDS patients were accrued to the study. A FISH panel consisting of eight probe sets, as described in phase I of this study, was performed on both specimen types. Each participating laboratory had approval of their individual Institutional Review Board (IRB) and informed consent of patients in phase II was obtained in accordance with the declaration of Helsinki.

Examples of 2-color ND-FISH Scoring Criteria for Chromosome 8 Abnormalities[a]

	8q24 Green (G) 8cen Orange (R)
Normal 2R2G	
Normal 2R2G Normal Overlap	Abnormal 2RG3 Gain = unbal t(8;?) or i(8q)
Abnormal 1R1G Loss = -8	Abnormal 2R≥6G Gain = amp 8q24
Abnormal 3R3G Gain = +8	Do not score overlapping cells
Abnormal 2R1G Loss 8q24 = del(8q)	Score cells with ≥1 signals
Abnormal 3R2G Gain 8cen & loss 8q24 = +del(8q)	

[a]ND-FISH detects numeric and structural abnormalities

Examples of BAP-FISH Scoring Criteria for Chromosome 11 Abnormalities[a]

	MLL 5' Green (G) MLL 3' Red (R) Touching R & G (F)
Normal 2F	
Abnormal 1F Loss = -11 or del(11q)	
Abnormal 3F Gain = +11	Abnormal 1R1F MLL 5' Loss = der t(11;?)
Abnormal 1R1G1F Bal = t(11;?)	Abnormal ≥6F Gain = 11q23 amp
Abnormal 1R2G1F Gain = t(11;?),+der t(11)	Do not score overlapping cells
Abnormal 1G1F MLL 3' Loss = der t(11;?)	Score cells with ≥1 signals

[a]BAP-FISH indicates break-apart of a set of R & G probes (the fused R and G signal often appears yellow)

Fig. 1. FISH scoring criteria and examples of possible patterns and their interpretations for ND (numeric deletion), BAP (break apart) and ES (extra signal) probe sets.

3. Results

3.1. Phase I

To determine to what extent the methods, nomenclature, interpretation and data entry aspects may vary among the laboratories,

Table 1
Probes in the MDS FISH panel test.

Common chromosome abnormalities detected	FISH strategy[a]	Cells	FISH probe and color[b]	Hybridization loci[b]
5q−, −5	2 color ND-	200	EGR1orange/D5S721;D5S23 green	5q31/5p15.2
7q−, −7, der(1;7)	3 color ND-	200	D7S486 orange/D7Z1 green/1q25 aqua	7q31/7 centromere/1q25
+8, 8q−	2 color ND-	200	D8Z2 orange/c-MYC green	8 centromere/8q24
11q−, +11, add(11), *t*(11q23)	2 color BAP-	200	MLL 5′ green/3′ orange	11q23
12p−, +21, *t*(12;21), −21	2 color ES-	200	ETV6(TEL) green/RUNX1(AML1) orange	12p13/21q22
13q−, −13	2 color ND-	200	D13S319 orange/13q34 green	13q14.3/13q34
17p−, −17	2 color ND-	200	TP53(P53) orange/D17Z1 green	17p13.1/17 centromere
20q−, i(20q), i(20p)	2 color ND-	200	D20S108 orange/20qter green	20q12.1/20q subtel

[a] ND-FISH; target and control locus are linked together on the same chromosome. ND = numeric and deletion; BAP = break apart; ES = extra signal.
[b] FISH probe nomenclature and location per Abbott Molecular Diagnostics.

Table 2
Patient summary.

	Number of patients
Gender	64 male, 36 female
Age	
7 years old	1
17–49	8
50–59	16
60–69	26
70–79	31
≥80	18
Prior treatment	43
Percent blasts	
0–4	27
5–20	70
>20	3
WHO classification	
5q−	2
RA/RARS	4
RAEB-1	24
RAEB-2	23
RAEB	4
RCMD	26
Unknown/other	17

all participants were asked to evaluate two identical MDS patient samples using the same FISH probes to process, score and interpret interphase cells. The participants were also asked to enter their data into a newly constructed MDS Foundation web-based data collection site. Results for patient #1 and patient #2 are shown in Supplemental Data, Tables 1 and 2. For patient #1, each participant correctly identified a deletion 5q, −7 and deletion 17p. There was good agreement among participants in this part of the study, with the mean percentages of cells with del(5q), −7 and del(17p) at 44 ± 7.3%, 47 ± 7.1% and 41 ± 5.1%, respectively.

For patient #2, most participants correctly identified a trisomy 8, deletion 20q and −21. False-negative FISH abnormalities for patient #2 were received from four participating laboratories. False-positive FISH abnormalities for patient #2 were received from three participating laboratories. Excluding results for the four participants who made data entry or reporting errors, the mean percentage of cells with +8, −21 and del(20q) were 13 ± 6.3%, 79 ± 15.1% and 82 ± 7.5%. These data indicate a close agreement among participants for percentage of abnormal nuclei.

A good deal of variation was reported by participants for normal cut-off values (Supplemental Data, Appendix I). For example, the normal cut-off values to detect deletion 5q, trisomy 8 and deletion 20q ranged from 0.6 to 6.0%, 0.6 to 3.0% and 1.2 to 6.0%, respectively. A variety of different statistical methods were used by participants to calculate their normal cut-offs, including mean +/−2 or 3 standard deviations, Student's *t* test, beta inverse function and binomial distribution (data not shown). Despite the variability, it was felt it was beyond the scope of this study to standardize normal cut-off values. Each participant was instructed to use their own established normal cut-offs wherever possible. These variations are no doubt related to a variety of issues ranging from how technologists interpret overlapping signals, number of cells analyzed, number of normal individuals studied, statistical methods used to establish the cut-off and so on. For the new 3-color ND-FISH (N = numeric; D = deletion) method to detect abnormalities of chromosomes 1 and 7, the participants were instructed to use the same cut-off rate that they have established for the −7/7q− probe set and for the new chromosome 8 and 20 ND-FISH probes, the participants were to use the same normal cut-offs that they use for the −5/5q− probe set, as they are similar techniques.

3.2. Phase II

As in Phase I of this study, participating laboratories were instructed to use their own established cut-off values for FISH, and to report results accordingly. For the purposes of this study, a case was called discordant if the data, either CCS and/or FISH, did not match (e.g. abnormal CCS results and normal FISH results). When comparing FISH results between BM and PB, cases were concordant if at least one abnormality was recognized in both (e.g. 5q− and 20q− FISH positive on BM, but only 5q− FISH positive on PB).

A total of 100 MDS patients were accrued to the study with 68% having an abnormal BM karyotype by CCS. While CCS was frequently unsuccessful (54%) in the PB specimens, 31% of PB samples had an abnormal karyotype by CCS. When CCS was successful (46 cases), BM and PB CCS were discordant in 6.5% (or 3 of 46 cases). As well, FISH was discordant in 3% of BM and PB samples, while CCS and FISH on BM were discordant in 12% of samples (mostly due to non-coverage in the targeted FISH panel). When PB CCS was successful, CCS and FISH on PB were discordant in 17.3% (or 8 of 46 cases) of samples (Tables 3 and 4). Interestingly, in cases where PB CCS was unsuccessful or discordant with BM CCS results, BM FISH and PB FISH were often concordant, with the exception of three cases (Tables 3 and 4).

3.3. CCS: BM vs. PB

PB CCS was frequently unsuccessful (54%), however, when PB CCS was successful, three cases (Cases 26, 29 and 83) were discordant with BM CCS. All three cases had normal metaphases by PB CCS (in 20, 4 and 4 metaphase cells, respectively), however BM CCS was abnormal. Case 26 had two of 20 metaphases on BM CCS which had trisomy 8, while case 29 had three of 19 metaphases on BM CCS which had +1, der(1;7). Case 83 had a complex karyotype (Table 3) in 11 of 21 metaphases examined on BM CCS, but normal PB CCS in the four metaphases available for analysis.

3.4. FISH: BM vs. PB

There were three cases with discordant BM and PB FISH (Cases 12, 25 and 32). Case 12 showed trisomy 8 by CCS on both PB (1 metaphase cell analyzed) and BM (9 metaphase cells analyzed) and confirmed positive by BM FISH in 17.5% of the interphase cells scored; however the PB FISH result was negative for trisomy 8. It should be noted that there was only one metaphase cell on the PB CCS and it was abnormal (trisomy 8). Case 25, which had a der(1;7) by BM CCS and confirmed by BM FISH in 21.5% of the interphase cells scored, had no analyzable metaphases (NAM) by PB CCS and was negative by PB FISH. Case 32 also had a der(1;7) by CCS on BM (14% positive by BM FISH), but NAM by PB CCS and negative by PB FISH (Table 3).

3.5. BM: CCS vs. FISH

Twelve cases showed discordant BM CCS and FISH results. In these 12 cases (Cases 1, 8, 9, 11, 14, 27, 31, 43, 52, 54, 58 and 91), abnormal CCS findings with normal or discordant FISH findings can be explained by the inability of the targeted FISH panel to pick up the described clonal chromosomal abnormalities not represented by FISH probes, for example *t*(8;10), trisomy 19, -Y. There was one case where CCS and FISH differed. Case 91 showed a trisomy 19 (not detectable by the FISH panel) clone by both BM and PB CCS, but was positive for a 12p/ETV6 deletion (TEL gene) by BM and PB FISH (Tables 3 and 4).

Table 3
CCS and FISH results on 100 BM and PB samples from patients with MDS.

Case#	Karyotype	5/5q	1/7/7q	8	11q	12;21	13q	17p	20q
1 bm	46,XY,t(8;10)(q24;q24)[20]	0	0	0	0	0	0	0	0
1 pb	46,XY,t(8;10)(q24;q24)[20]	0	0	0	0	0	0	0	0
2 bm	46,X,−Y,+8[12]/46,idem,del(1)(p36.1p36.3)[5]/ 46,idem,del(1)(p36.1p36.3),del(5)(q31)[3]	4	0	89.5	0	0	0	0	0
2 pb	46,X,−Y,+8[10]/46,idem,del(1)(p36.1p36.3)[7]/ 46,idem,del(1)(p36.1p36.3),del(5)(q31)[3]	2.5	0	84.5	0	0	0	0	0
3 bm	46,XY[20]	0	0	0	0	0	0	0	0
3 pb	46,XY[5]	0	0	0	0	0	0	0	0
4 bm	46,XY[9]	0	0	0	0	0	0	0	0
4 pb	NAM	0	0	0	0	0	0	0	0
5 bm	47,XY,+19[4]/48,idem,+8[16]	0	0	45.5	0	0	0	0	0
5 pb	47,XY,+19[6]/48,idem,+8[2]	0	0	23	0	0	0	0	0
6 bm	46,XY[20]	0	0	0	0	0	0	0	0
6 pb	46,XY[3]	0	0	0	0	0	0	0	0
7 bm	46,XY,del(20)(q11.2)[18]/47,idem,+8[2]	0	0	6.5	0	0	0	0	98.5
7 pb	46,XY,del(20)(q11.2)[22]/47,idem,+8[1]	0	0	7.5	0	0	0	0	51
8 bm	46,XX,t(14;18)(q32;q21.1)[20]	0	0	0	0	0	0	0	0
8 pb	46,XX,t(14;18)(q32;q21.1)[13]	0	0	0	0	0	0	0	0
9 bm	47,XX,+15[3]/46,XX[21]	0	0	0	0	0	0	0	0
9 pb	NAM	0	0	0	0	0	0	0	0
10 bm	46,XY[9]	0	0	0	0	0	0	0	0
10 pb	46,XY[1]	0	0	0	0	0	0	0	0
11 bm	46,X,idic(X)(q13)[9]/46,XX[10]	0	0	0	0	0	0	0	0
11 pb	NAM	0	0	0	0	0	0	0	0
12 bm	46,XY,+8,der(13;14)(q10;q10)?c[9]/ 45,XY,der(13;14)(q10;q10)?c[11]	0	0	17.5	0	0	0	0	0
12 pb	46,XY,+8,der(13;14)(q10;q10)?c[1]	0	0	0	0	0	0	0	0
13 bm	45,XY,−7[17]/45,idem,del(12)(p11.2p13)[3]	0	79			20	0	0	0
13 pb	NAM	ND	ND	ND	ND	ND	ND	ND	ND
14 bm	45,X,−Y[17]/46,XY[18]	0	0	0	0	0	0	0	0
14 pb	NAM	0	0	0	0	0	0	0	0
15 bm	46,XX[20]	0	0	0	0	0	0	0	0
15 pb	46,XX[20]	0	0	0	0	0	0	0	0
16 bm	46,XX[20]	0	0	0	0	0	0	0	0
16 pb	NAM	0	0	0	0	0	0	0	0
17 bm	46,XY[14]	0	0	0	0	0	0	0	0
17 pb	NAM	0	0	0	0	0	0	0	0
18 bm	46,XY[20]	0	0	0	0	0	0	0	0
18 pb	46,XY[5]	0	0	0	0	0	0	0	0
19 bm	46,XY[20]	0	0	0	0	0	0	0	0
19 pb	NAM	0	0	0	0	0	0	0	0
20 bm	46,XX,der(11)add(11)(p11.2)add(11)(q13)[18]/46,XX[2]	0	0	0	74	0	0	0	0
20 pb	46,XX,der(11)add(11)(p11.2)add(11)(q13)[1]	0	0	0	56	0	0	0	0
21 bm	46,XX,del(11)(q13q23)[17]/46,XX[3]	0	0	0	70	0	0	0	0
21 pb	NAM	0	0	0	36.5	0	0	0	0
22 bm	47,XX,+21[17]/46,XX[3]	0	0	0	0	40	0	0	0
22 pb	47,XX,+21[8]	0	0	0	0	23.5	0	0	0
23 bm	46,XY,del(20)(q11.2)[17]/46,XY[3]	0	0	0	0	0	0	0	92.5
23 pb	NAM	0	0	0	0	0	0	0	72.5
24 bm	46,XY[20]	0	0	0	0	0	0	0	0
24 pb	NAM	0	0	0	0	0	0	0	0
25 bm	47,XY,+1,der(1;7)(q10;p10)[9]/46,XY[9]	0	21.5	0	0	0	0	0	0
25 pb	NAM	0	0	0	0	0	0	0	0
26 bm	47,XX,+8[2]/46,XX[18]	0	0	10	0	0	0	0	0
26 pb	46,XX[20]	0	0	7.5	0	0	0	0	0
27 bm	46,XY,t(1;20)(q25;q11.2)[17]/46,XY[3]	0	0	0	0	0	0	0	0
27 pb	NAM	0	0	0	0	0	0	0	0
28 bm	48,XY,+8,+21[20]	0	0	100	0	100	0	0	0
28 pb	48,XY,+8,+21[9]/46,XY[6]	0	0	54.5	0	53	0	0	0
29 bm	46,XY,+1,der(1;7)(q10;q10)[cp3]/46,XY[16]	0	6	0	0	0	0	0	4
29 pb	46,XY[4]	0	1.5	0	0	0	0	0	17
30 bm	46,XX,der(15)t(11;15)(q13;p11)[12]/46,XX[7]	0	0	0	94	0	0	0	0
30 pb	46,XX,der(15)t(11;15)(q13;p11)[4]/46,XX[16]	0	0	0	65	0	0	0	0
31 bm	46,XY,del(11)(q21q23)[5]/46,XY[17]	0	0	0	0	0	0	0	0
31 pb	NAM	0	0	0	0	0	0	0	0
32 bm	46,XY,+1,der(1;7)(q10;p10)[14]/46,XY[3]	0	14	0	0	0	0	0	0
32 pb	NAM	0	0	0	0	0	0	0	0
33 bm	46,XX[2]	0	0	0	0	0	0	0	0
33 pb	NAM	0	0	0	0	0	0	0	0
34 bm	46,XX,del(5)(q15q31)[4]/45~47,idem,add(6)(q27),−15,−18, hsr(19)(q13),add(21)(p13),+1~3mar[cp16]	86	0	0	8	0	0	0	0
34 pb	46,XX,del(5)(q15q31)[3]/45~47,idem,add(6)(q27),−15,−18, hsr(19)(q13),add(21)(p13),+1~3mar[cp5]/46,XX[12]	19.5	0	0	0	0	0	0	0
35 bm	45,XY,del(5)(q15q31),add(6)(p24),−20[cp19]/46,XY[1]	62.5	0	0	0	0	0	0	0
35 pb	NAM	70	0	0	0	0	0	0	0

Table 3 (*Continued*)

Case#	Karyotype	5/5q	1/7/7q	8	11q	12;21	13q	17p	20q
36 bm	48,XY,−5,+6,+add(8)(p15),+9,del(16)(q12q22),add(17)(p13)[16]/46,XY[4]	41	0	0	0	0	0	46	0
36 pb	NAM	29.5	0	0	0	0	0	29.5	0
37 bm	46,XX,−7,+mar[cp13]/46,XX[2]	0	52.5	0	0	0	0	0	0
37 pb	46,XX,−7,+mar[4]/46,XX[16]	0	22	0	0	0	0	0	0
38 bm	45,XY,−7[19]/46,XY[1]	0	97	0	0	0	0	0	0
38 pb	NAM	0	62	0	0	0	0	0	0
39 bm	46,XX[20]	0	0	0	0	0	0	0	0
39 pb	46,XX[5]	0	0	0	0	0	0	0	0
40 bm	46,XX,del(5)(q14q34)[20]	60.5	0	0	0	0	0	0	0
40 pb	46,XX,del(5)(q14q34)[10]	14.5	0	0	0	0	0	0	0
41 bm	46,XX,del(20)(q11)[18]/46,XX[3]	0	0	0	0	0	0	0	78
41 pb	NAM	0	0	0	0	0	0	0	71.5
42 bm	46,XY[20]	0	0	0	0	0	0	0	0
42 pb	46,XY[5]	0	0	0	0	0	0	0	0
43 bm	46,XY,t(1;14)(p35;q32)[20]	0	0	0	0	0	0	0	0
43 pb	46,XY,t(1;14)(p35;q32)[2]	0	0	0	0	0	0	0	0
44 bm	46,XY[20]	0	0	0	0	0	0	0	0
44 pb	46,XY[20]	0	0	0	0	0	0	0	0
45 bm	47,XX,+8[16]/46,XX[4]	0	0	86.5	0	0	0	0	0
45 pb	47,XX,+8[20]	0	0	90	0	0	0	0	0
46 bm	46,XX,del(5)(q14q34),del(11)(q21q24)[7]/46,XX[18]	6	0	0	5	0	0	0	0
46 pb	46,XX,del(5)(q14q34),del(11)(q21q24)[4]/46,XX[11]	0.5	0	0	0	0	0	0	0
47 bm	46,XY[20]	0	0	0	0	0	0	0	0
47 pb	46,XY[15]	0	0	0	0	0	0	0	0
48 bm	46,XY[20]	0	0	0	0	0	0	0	0
48 pb	46,XY[1]	0	0	0	0	0	0	0	0
49 bm	45,XY,der(2)t(2;3)(p13;p13)ins(2;3)(q33;p?p?),dic(3;20)(p11;q12),del(11)(q14q24)[19]/46,XY[1]	0	0	0	73	0	0	0	79
49 pb	NAM	0	0	0	33	0	0	0	29
50 bm	46,XY[20]	0	0	0	0	0	0	0	0
50 pb	NAM	0	0	0	0	0	0	0	0
51 bm	46,XY[20]	0	0	0	0	0	0	0	0
51 pb	NAM	0	0	0	0	0	0	0	0
52 bm	45,X,−Y[14]/46,XY[6]	0	0	0	0	0	0	0	0
52 pb	NAM	0	0	0	0	0	0	0	0
53 bm	45,XY,−7[20]	0	61	0	0	0	0	0	0
53 pb	NAM	0	34	0	0	0	0	0	0
54 bm	45,X,−Y[3]/46,XY[17]	0	0	0	0	0	0	0	0
54 pb	NAM	0	0	0	0	0	0	0	0
55 bm	47,XX,+20,del(20)(q11.2q13.3)x2[20]	0	0	0	0	0	0	0	55
55 pb	NAM	0	0	0	0	0	0	0	47
56 bm	46,XX[6]	0	0	0	0	0	0	0	0
56 pb	46,XX[3]	0	0	0	0	0	0	0	0
57 bm	48~59,XX,+X,+1,+2,del(5)(q13q33),+9,+10,+11,−13,+15,+16,+19,+21,+22,+22,+2mar[cp20]	76	57.5	0	48.5	38	0	0	0
57 pb	NAM	10	6	0	3	7.5	0	0	0
58 bm	45,X,−Y[20]	0	0	0	0	0	0	0	0
58 pb	NAM	0	0	0	0	0	0	0	0
59 bm	46,XX[20]	0	0	0	0	0	0	0	0
59 pb	NAM	0	0	0	0	0	0	0	0
60 bm	46,XY[20]	0	0	0	0	0	0	0	0
60 pb	46,XY[10]	0	0	0	0	0	0	0	0
61 bm	46,XX[25]	0	0	0	0	0	0	0	0
61 pb	NAM	0	0	0	0	0	0	0	0
62 bm	46,XX[27]	0	0	0	0	0	0	0	0
62 pb	NAM	0	0	0	0	0	0	0	0
63 bm	46,XY,del(5)(q14q33)[2]/46,XY[10]	11	0	0	0	0	0	0	0
63 pb	NAM	11	0	0	0	0	0	0	0
64 bm	45,XY,−7[6]/46,XY[3]	0	12.5	0	0	0	0	0	0
64 pb	NAM	0	11	0	0	0	0	0	0
65 bm	46,XY,+1,der(1;7)(p12;q10)[23]/46,XY[2]	0	19	0	0	0	0	0	0
65 pb	NAM	0	4.5	0	0	0	0	0	0
66 bm	46,XX,del(5)(q13q33)[19]/46,XX[6]	44	0	0	0	0	0	0	0
66 pb	NAM	22.2	0	0	0	0	0	0	0
67 bm	47,XY,+8[30]/46,XY[1]	0	0	77.5	0	0	0	0	0
67 pb	47,XY,+8[8]/46,XY[2]	0	0	30.5	0	0	0	0	0
68 bm	46,XY,+1,der(1;7)(p10;q10)[2]/46,XY[20]	0	11	0	0	0	0	0	0
68 pb	NAM	0	1.5	0	0	0	0	0	0
69 bm	45,XX,del(5)(q13q31),−21[2]/46,XX[6]	43	0	0	0	9	0	0	0
69 pb	46,XX,del(5)(q13q31)[7]/45,idem,−21[2]/46,XX[7]	46	0	0	0	7	0	0	0
70 bm	46,XY,del(5)(q22q31)[2]/46,XY[25]	14	0	0	0	0	0	0	0
70 pb	NAM	0.5	0	0	0	0	0	0	0
71 bm	46,XX[30]	0	0	0	0	0	0	0	0
71 pb	NAM	0	0	0	0	0	0	0	0
72 bm	46,XX,der(5)t(5;17)(q11.2;q11.2),+8,−17[20]/46,XX[20]	60.5	0	52.5	0	0	0	55	0
72 pb	NAM	10	0	3.5	0	0	0	8	0

Table 3 (*Continued*)

Case#	Karyotype	5/5q	1/7/7q	8	11q	12;21	13q	17p	20q
73 bm	46,XX,del(1)(p13p21),del(5)(q21q34)[6]/46,XX[14]	10.5	0	0	0	0	0	0	0
73 pb	NAM	3.5	0	0	0	0	0	0	0
74 bm	46,XY[20]	0	0	0	0	0	0	0	0
74 pb	NAM	0	0	0	0	0	0	0	0
75 bm	46,XX,idic(17)(p11.2)[2]/45,sl,−7[27]/90,sdl1x2[3]	0	87	0	0	0	0	90.5	0
75 pb	46,XX,idic(17)(p11.2)[1]/45,sl,−7[29]	0	93.5	0	0	0	0	85.5	0
76 bm	46,XX,del(5)(q13q33)[19]/46,XX[1]	46	0	0	0	0	0	0	0
76 pb	NAM	50.5	0	0	0	0	0	0	0
77 bm	42~43,X,−Y,−4,−5,−7,−8,−12,add(12)(q24),add(16)(q11.2),−17,−18, +r,+mar1,+mar2,+mar3,+mar4,+mar[cp15]/42~43,sl,−mar4, +mar5,+1~2mar[cp3]/46,XY[2]	54.5	0	32.5	0	64.5	0	62	0
77 pb	42~43,X,−Y,−4,−5,−7,−8,−12,add(12)(q24),add(16)(q11.2),−17,−18, +r,+mar1,+mar2,+mar3,+mar4,+mar[cp12]/42~43,sl,−mar4,+mar5,+1 ~2mar[cp5]/46,XY[3]	64.5	0	42.5	0	71	0	57	0
78 bm	NAM	0	0	0	0	0	0	0	0
78 pb	NAM	0	0	0	0	0	0	0	0
79 bm	46,XX[21]	0	0	0	0	0	0	0	0
79 pb	NAM	0	0	0	0	0	0	0	0
80 bm	44~45,XY,−4,del(5)(q23),−14,+1~2mar[10]/46,XY[11]	89	0	0	0	0	0	0	0
80 pb	44~45,XY,−4,del(5)(q23),−14,+1~2mar[12]	45	0	0	0	0	0	0	0
81 bm	46,XY[21]	0	0	0	0	0	0	0	0
81 pb	NAM	0	0	0	0	0	0	0	0
82 bm	46,XY,del(20)(q11)[10]/47,XY,+20,del(20)(q11)x2[7]/ 48,XY,+9,+20,del(20)(q11)x2[3]	0	0	0	0	0	0	0	79.5
82 pb	46,XY,del(20)(q11)[2]/47,XY,+20,del(20)(q11)x2[1]/ 49,XY,+9,+20,del(20)(q11)x2[5]/46,XY[2]	0	0	0	0	0	0	0	75
83 bm	43~44,X,add(Y)(q12),−5,add(6)(p22),−14,−15,add(17)(p10),−18, +1~2mar[11]/46,XY[10]	76.5	0	0	0	0	0	0	0
83 pb	46,XY[4]	9	0	0	0	0	0	0	0
84 bm	42~47,XY,del(1)(p13),−5,add(17)(p12),+1~5mar[11]/ 39~44,XY,del(1)(p13),−5,add(17)(p12),−18,−20,−21,+1~5mar[11]/ 46,XY[1]	68.5	0	0	0	0	0	0	0
84 pb	42~47,XY,del(1)(p13),−5,add(17)(p12),+1~5mar[12]	10.5	0	0	0	0	0	0	0
85 bm	46,XX[21]	0	0	0	0	0	0	0	0
85 pb	NAM	0	0	0	0	0	0	0	0
86 bm	46,XY[21]	0	0	0	0	0	0	0	0
86 pb	NAM	0	0	0	0	0	0	0	0
87 bm	46,XX,del(5)(q23),del(7)(q21)[21]	74	83	0	0	0	0	0	0
87 pb	46,XX,del(5)(q23),del(7)(q21)[3]	84	83	0	0	0	0	0	0
88 bm	44~45,XY,−3,add(5)(q33),del(5)(q22q33),del(7)(q13), der(7;12)(q10;q10),add(11)(p11),−12,−16,de1(21)(q22), +1~2mar[20]	84.5	86.5	0	29.5	79	0	0	0
88 pb	44~45,XY,−3,add(5)(q33),del(5)(q22q33),del(7)(q13), der(7;12)(q10;q10),add(11)(p11),−12,−16,de1(21)(q22), +1~2mar[20]	81	77	0	24.5	75.5	0	0	0
89 bm	47,XX,+8[23]	0	0	66	0	0	0	0	0
89 pb	47,XX,+8[12]/46,XX[8]	0	0	31	0	0	0	0	0
90 bm	46,XY,+1,der(1;7)(q10;p10)[6]/46,XY[16]	0	40.5	0	0	0	0	0	0
90 pb	46,XY,+1,der(1;7)(q10;p10)[3]/46,XY[3]	0	20.5	0	0	0	0	0	0
91 bm	47,XY,+19[25]	0	0	0	0	85	0	0	0
91 pb	47,XY,+19[18]/46,XY[5]	0	0	0	0	18	0	0	0
92 bm	46,XY,r(7)(p13q11.2)[18]/46,XY[2]	0	86	0	0	0	0	0	0
92 pb	46,XY,r(7)(p13q11.2)[20]	0	41.5	0	0	0	0	0	0
93 bm	45~48,XY,−3,del(5)(q14q33),del(7)(q22),add(7)(q32),+8, del(9)(p22),der(10)?t(3;10)(q12;q22),+11,−15,−18,del(20)(q11), −21,+2~4mar[cp20]/46,XY[1]	94	0	84.5	75	78.5	0	0	9
93 pb	45~48,XY,−3,del(5)(q14q33),del(7)(q22),add(7)(q32),+8, del(9)(p22),der(10)?t(3;10)(q12;q22),+11,−15,−18,del(20)(q11), −21,+2~4mar[cp22]/46,XY[1]	81	0	63	61	72.5	0	0	0
94 bm	45,XY,del(3)(p13),del(5)(q13q33),−7,del(12)(p13),der(12)t(7;12)(q11; p13),der(12)t(12;21)(p13;q22),−21,+mar[8]/44,sl,−Y[8]/43,sdl1, −der(12)[2]/46,XY[3]	62	0	0	0	40	0	0	0
94 pb	NAM	17	0	0	0	27	0	0	0
95 bm	46,XY[24]	0	0	0	0	0	0	0	0
95 pb	NAM	0	0	0	0	0	0	0	0
96 bm	46,XX,del(5)(q13q33)[4]/44,XX,der(5)t(1;5)(p31;q13), −7,dup(8)(q13q24),der(14;16)(q10;p1?)[10]/ 47~48,XX,der(5)t(1;5)(p31;q13),16,+1~2tas,+mar[cp6]/ 46,XX[2]	73.5	13	22	0	0	0	0	0
96 pb	NAM	48	11.5	4	0	0	0	0	0
97 bm	48,XY,+13,+14[2]/46,XY[18]	0	0	0	0	0	32	0	0
97 pb	NAM	0	0	0	0	0	24	0	0

Table 3 (*Continued*)

Case#	Karyotype	5/5q	1/7/7q	8	11q	12;21	13q	17p	20q
98 bm	45,XY,del(5)(q13q33),−17,del(20)(q12.1), der(20)t(17;20)(p11;q12)[3]/44,sl,−16[9]/44,sl,−18[4]/43,sl,−16, −18[3]/45,sl,der(4)t(4;17)(q35;p11)[2]/46,sl,+8[2]	72	0	11.5	0	0	0	16	53
98 pb	NAM	42	0	4	0	0	0	0	36
99 bm	48,XX,+8,+9[13]	0	0	86.5	0	0	0	0	0
99 pb	48,XX,+8,+9[7]	0	0	82	0	0	0	0	0
100 bm	46,XY[20]	0	0	0	0	0	0	0	0
100 pb	NAM	0	0	0	0	0	0	0	0

Table 4
Discordant Cases.

	Discordant cases	Comments
BM CCS vs. PB CCS	3/46 (or 6.5%)	54 PB CCS cases were NAM; 1 BM CCS was NAM
BM FISH vs. PB FISH	3/99 (or 3%)	Low level in BM FISH; 1 PB FISH not done
BM CCS vs. BM FISH	12/99 (or 12%)	Not detectable by FISH panel in 12 cases; 1 BM CCS was NAM
PB CCS vs. PB FISH	8/46 (or 17.3%)	54 PB CCS cases were NAM; 1 PB FISH not done

3.6. PB: CCS vs. FISH

Discordant results between PB CCS and FISH were more difficult to troubleshoot, as a large percentage (54%) of PB CCS was unsuccessful. Five cases showed an abnormal PB CCS result but were negative by PB FISH (Cases 1, 8, 12, 43 and 91). In four cases (Cases 1, 8, 43 and 91), abnormalities were not detectable by the FISH panel (*t*(8;10), *t*(14;18), *t*(1;14), +19). Case 12 showed only one trisomy 8 metaphase cell by PB CCS. While BM FISH was positive for trisomy 8 (17.5%), PB FISH was negative. Case 91 had trisomy 19 by BM and PB CCS, but was positive for a 12p/ETV6 deletion (TEL gene) by BM and PB FISH (85% and 18%, respectively).

As mentioned above, Case 25 had a der(1;7) by BM CCS, confirmed by BM FISH in 21.5% of the interphase cells scored, however the PB CCS was NAM and negative by PB FISH. Case 32 also had a der(1;7) by CCS on BM (14% positive by BM FISH), but NAM by PB CCS and negative by PB FISH (Table 3).

Three cases showed abnormal FISH findings with normal PB CCS (Cases 26, 29 and 83). In all three cases, the abnormal FISH findings are concordant with their BM CCS and FISH. In total there are 23 cases with NAM by CCS on PB, but abnormal PB FISH. All of the cases with NAM by PB CCS but abnormal PB FISH had BM CCS and BM FISH abnormalities, which were concordant with these findings (Table 5).

3.7. Comparison of clone size by FISH

A side-by-side comparison was made of the percent positive cells detected by FISH in both the BM and the PB for each probe combination (Table 3; Supplemental Data, Table 3). In general, for each probe set used, the percentage of FISH positive cells was typically higher in the BM than it was in the PB. Coefficients of correlation were calculated for each probe set, with the exception of the 13q probe set, as only one case had abnormalities of chromosome #13 (trisomy 13). The data show that all probe sets have a strong association between the BM FISH results and the PB FISH results (all regressions are significant at the 0.05 level), with the exception of the 5/5q probe set (Table 6).

A Student's *t*-test was also performed on the differences between BM and PB FISH for the different probe sets. This revealed that for the 5/5q, 1/7/7q, +8 and MLL probe sets, there was a significant difference ($p < 0.01$), while the p values for the *t*12;21, 17p and 20q probe sets were not statistically significant (Table 7). There did not appear to be any correlation with MDS classification, percent blasts, age, gender or previous treatment. Other clinical data were not available.

There were 24 cases with −5/5q− positive FISH (Supplemental Data, Fig. 1a). Cases 46 and 70, both positive by BM FISH (6% and 14%), were also called positive on PB FISH by the participating laboratories, however they were scored at 0.5% (or 1/200 nuclei analyzed). The majority of participating laboratories would not have called these results positive. However, as stated earlier, laboratories were to make their own cut-off value calls.

There were 16 cases, which were FISH positive for the 1/7/7q probe set (Supplemental Data, Fig. 1b). Cases 25 and 32 both had positive BM FISH (21.5% and 14%, respectively), but were negative on PB FISH (Table 3).

Table 5
Cases with NAM PB CCS and concordant BM and PB FISH.

Case #	Probe set	% Abn. BM FISH	% Abn. PB FISH
21	MLL	70	36.5
23	20q	92.5	72.5
35	5/5q	62.5	70
36	5/5q	41	29.5
	17p	46	29.5
38	1/7/7q	97	62
41	20q	78	71.5
49	MLL	73	33
	20q	79	29
53	1/7/7q	61	34
55	20q	55	47
57	5/5q	76	10
	1/7/7q	57.5	6
	MLL	48.5	3
	12;21	38	7.5
63	5/5q	11	11
64	1/7/7q	12.5	11
65	1/7/7q	19	4.5
66	5/5q	44	22.2
68	1/7/7q	11	1.5
70	5/5q	14	0.5
72	5/5q	60.5	10
	+8	52.5	3.5
	17p	55	8
73	5/5q	10.5	3.5
76	5/5q	46	50.5
94	5/5q	62	17
	12;21	40	27
96	5/5q	73.5	48
	1/7/7q	13	11.5
	+8	22	4
97	13q	32	24
98	5/5q	72	42
	+8	11.5	4
	17p	16	0
	20q	53	36

Table 6
Coefficient of correlation for each probe set comparing BM and PB FISH.

Probe set	Number of cases	Coefficient of correlation
5/5q	24	0.553
1/7/7q	16	0.876*
+8	15	0.832*
MLL	9	0.878*
12;21	9	0.655*
13q	1	Not done
17p	5	0.882*
20q	9	0.804*

* Regression is significant at the 0.05 level Graphs are shown in Supplemental Data.

Table 7
BM vs. PB FISH, *t*-test for significant differences.

Probe set	*p* value	Mean BM FISH	Mean PB FISH
5/5q	<0.01*	54.7	32.2
1/7/7q	<0.01*	46.8	29.3
+8	<0.01*	52.6	35.2
MLL	<0.01*	53	31
t(12;21)	0.02	59.3	39.4
13q	Not done	Not done	Not done
17p	0.04	53.9	36
20q	0.02	60.9	44.3

* Significantly different.

Fifteen cases were FISH positive for the chromosome 8 probe set (Supplemental Data, Fig. 1c). As discussed above, 17.5% of the interphase BM cells in case 12 were positive for trisomy 8 whereas the PB FISH study was negative. Concurrent BM CCS was abnormal (nine of 20 metaphase cells), with only a single metaphase cell observed in the PB, which had trisomy 8.

In all, there were nine cases with FISH positive results for the 11q probe set (Supplemental Data, Fig.1d). Case 34 had a complex karyotype on BM and PB CCS (Table 3), but was FISH positive in 8% of the BM cells and FISH negative by PB FISH (this case was positive by FISH and CCS on both BM and PB for deletion 5q). Lastly, Case 46 was FISH positive in 5% of the BM cells scored, negative by PB FISH and had deletion 5q and 11q by BM and PB CCS.

Nine cases showed a positive FISH result using the 12;21 (TEL(ETV6)/AML1(RUNX1)) dual fusion probe set (Supplemental Data, Fig.1e). This probe set was used to detect monosomy 21/trisomy 21 or deletion 12p/ETV6 (TEL). It is interesting to note that Case 69 had deletion 5q and monosomy 21, both of which were observed on CCS on BM and PB. While the deletion 5q was detectable by FISH in both BM and PB (43% vs. 46%), the monosomy 21 was also detectable, but at a much lower level (9% vs. 7%), consistent with a secondary aberration (Table 3).

There was only one case with FISH positive results using the monosomy 13/deletion 13q probe set (data not shown). One case, Case 97, is positive for trisomy 13 by FISH in both BM and PB (32% vs. 24%).

There were five cases of FISH positive results using the −17/17p− probe set (Supplemental Data, Fig.1f). Case 98 had a complex karyotype on BM CCS. While other probes were informative by FISH on both BM and PB, 16% of cells were positive for loss of D17Z1 (with two copies of P53) by BM FISH, but negative on PB FISH (Table 3).

Finally, there were nine cases with FISH positive results using the deletion 20q probe set (Supplemental Data, Fig.1g). Case 93, also had a complex karyotype on both BM and PB by CCS and was concordant for FISH results using other probe sets (5/5q−, +8, 11q, *t*(12;21)), deletion 20q was observed in 9% of BM cells by FISH, but was negative by PB FISH (Table 3).

4. Discussion

Phase I of this study showed that there was very good agreement between the 15 participating laboratories when studying two samples with known clonal chromosomal abnormalities (Supplemental Data, Tables 1 and 2). Although all participating sites are designated MDS Centers of Excellence and have considerable FISH experience, the goal of the familiarization phase was to determine to what extent the methods, nomenclature, interpretation of the results and data entry aspects may vary among the laboratories. This phase proved the participants used similar procedures for processing and reporting FISH data and provided data entry experience into a secured database; however cut-off value variability and methods used to determine cut-off values resulted in a few minor discrepancies (Supplemental Data, Appendix I). A review of the data to clear up the data entry errors and standardization of the FISH scoring criteria was implemented by distributing FISH scoring diagrams.

Phase II of our study showed good concordance between BM and PB, both by CCS and FISH (Tables 3 and 4). Three discordant results were clear when comparing BM and PB FISH – Cases 12, 25 and 32. All of these discordant cases had a relatively low rate of abnormal cells (17.5%, 21.5% and 14%, respectively) (Table 3). This is in agreement with earlier studies [10–12], which showed that the sensitivity of PB FISH was largely determined by the size of the abnormal clone in the BM, with lower rates of PB FISH positive cells, even when the PB is enriched for CD34+ cells [11].

There have been numerous studies regarding the ability of MDS FISH panels to detect occult or cryptic anomalies in the bone marrow of MDS patients [7,8,13–16]. These studies described abnormalities in 3–18% of cases, however the clinical significance of these cryptic anomalies has never been established. More recently, the effectiveness and efficiency of MDS FISH panels has been questioned, since the vast majority of MDS FISH panel studies do not find occult anomalies when a full analysis of 20 metaphase cells is performed [17–19]. In the second phase of our study, we found one case with an apparently cryptic anomaly detected by our FISH panel – Case 91 (Table 3). Case 91 had trisomy 19 by both BM and PB CCS, but both BM and PB FISH were positive for deletion 12p13/ETV6 in 85% and 18% of cells, respectively. Therefore, our study's rate of cryptic anomalies detected by FISH is 1%.

In 54% of the cases, PB CCS was unsuccessful. This finding was not unexpected, as the tumor burden in MDS is expected to be relatively low in the periphery. In this study, lower grade MDS samples with single abnormalities of trisomy 8, del(20q) and der(1;7) in ≤20% bone marrow cells were not generally detectable in the circulation (Supplemental Data, Table 3). However, abnormalities were detected by PB FISH when the clone was large enough. As shown by Li et al. [10] when studying 60 cases of MDS, the percentage of clonal bone marrow cells and the percentage of blasts (or mean clonal/blast disparity) was very different (50.1% vs. 7.0%, respectively). This disparity was much lower when looking at AML patients, as well as advanced MDS patients. FISH was performed on 22 MDS patients in the Li et al. study. FISH showed that the percentage of clonal cells was not very different when comparing BM and PB on the same patient (49.8% vs. 39.1%, respectively).

When comparing successful PB CCS with negative PB FISH, four of the five discordant cases had abnormalities, which could not be detected by the FISH panel (Cases 1, 8, 43 and 91). The fifth case, Case 12, had only one trisomy 8 metaphase cell by PB CCS, while BM FISH confirmed trisomy 8 in 17.5% of the cells scored, PB FISH was negative. Clearly, low-level clonality in the PB is a major concern in MDS patients. In addition, Case 91 had trisomy 19 by both BM and PB CCS, but both BM and PB FISH showed deletion 12p. As stated earlier, the finding of a hidden or cryptic clone occurred in only one of our cases (or 1%).

As in the limited number of cases in the literature, it appears that the lack of detection of an abnormal clone by PB FISH is mostly correlated with lower grade MDS with single aberrations and the size of the abnormal clone in the BM detected by FISH [10–12]. In most cases, when there are approximately 20% FISH positive cells in the BM, it is possible to detect FISH positive cells in the PB, albeit at a typically reduced rate.

While array comparative genomic hybridization (aCGH) has been used in studying the BM of MDS patients [20–24] and has shown some utility in detecting otherwise cryptic abnormalities, there is no evidence to date that PB could be substituted for BM unless the tumor burden is high enough in the periphery. Low levels of mosaicism may be missed in the PB.

In summary, our study showed that there is very good correlation between BM and PB FISH (3% discordance). Overall, from our study, we conclude that while PB cannot be substituted for BM at diagnosis, it could be used when serial bone marrow samples are not possible to monitor the clinical course when a FISH detectable clone has been established. Following such a clone over time, as in Braulke et al. [11], might also help to determine when to treat and/or the effectiveness of treatment. As well, it would replace the need for serial bone marrows with the ease of a blood draw for clinical follow-up.

Conflict of interest statement

There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.leukres.2012.03.013.

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