JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Complex, Not Monosomal, Karyotype Is the Cytogenetic Marker of Poorest Prognosis in Patients With Primary Myelodysplastic Syndrome

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Published online ahead of print at www.jco.org on January 14, 2013.

Written on behalf of the Spanish Group on Myelodysplastic Syndrome (GESMD).

Supported by unrestricted Grants No. FI 07/00107 (M.M.) and PI 11/02010 from Instituto de Salud Carlos III, El Fondo Europeo de Desarrollo Regional (FEDER), Ministerio de Sanidad y Consumo, Spain; Grants No. RD07/0020/2004, RD12/0036/0044, and COST BM0801 from Red Temática de Investigación Cooperativa en Cáncer, FEDER; and Grant No. PR(AG)28/2008 from a collaboration project between Institut de Recerca Vall d'Hebrón and Celgene.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/13/3199-1/$20.00

DOI: 10.1200/JCO.2012.41.6073

ABSTRACT

Purpose
Complex karyotype (CK) is the poorest risk factor in patients with myelodysplastic syndrome (MDS). It has recently been reported that monosomal karyotype (MK) worsens the prognosis of patients with CK.

Patients and Methods
We analyzed 1,054 adult patients with MDS with an abnormal karyotype from the Spanish Registry of MDS. The aim of the study was to describe the incidence, characteristics, and prognosis of MK; the main end points were overall survival (OS) and leukemia-free survival.

Results
MK was identified in 172 patients (16%), most of whom (88%) presented with CK. Variables significantly associated with OS were age (hazard ratio [HR], 1.90; $P < .001$), bone marrow (BM) blast percentage (HR, 1.05; $P < .001$), hemoglobin level (HR, 1.71; $P < .001$), platelet count (HR, 1.41; $P < .001$), karyotype complexity (CK [three abnormalities]: HR, 1.81; $P = .003$; very CK [> three abnormalities]: HR, 2; $P < .001$), and abnormalities of chromosome 5 and/or 7 (HR, 1.89; $P < .001$). Variables significantly related to the risk of transformation to acute myeloid leukemia (AML) were higher BM blast percentage (HR, 1.12; $P < .001$) and karyotype complexity (CK: HR, 2.53; $P = .002$; very CK: HR, 2.77; $P < .001$).

Conclusion
After accounting for karyotype complexity, MK was not associated with OS or evolution to AML. In conclusion, these results demonstrate that the prognostic value of MK in MDS is not independent and is mainly the result of its strong association with number of chromosomal abnormalities.

J Clin Oncol 31. © 2013 by American Society of Clinical Oncology

INTRODUCTION

Myelodysplastic syndrome (MDS) comprises a heterogeneous group of clonal hematologic disorders characterized by dysplasia in bone marrow (BM) and blood cells, presence of cytopenias, and variable risk of evolution to acute myeloid leukemia (AML).[1] The main prognostic factor for survival and risk of AML evolution is the presence of certain cytogenetic abnormalities (CAs), which are detected by conventional techniques in approximately 50% of patients,[2,3] most of them considered to be in the high-risk cytogenetic category of the International Prognostic Scoring System (IPSS).[4] Monosomal karyotype (MK) is defined as the presence of ≥ two autosomal monosomies or one monosomy with at least one additional structural abnormality and has been associated with worse prognosis in patients with AML,[5] MDS,[6,7] and primary myelofibrosis.[8]

In this retrospective study of the Spanish Group on MDS (GESMD), we analyzed the incidence, characteristics, outcome, and potential prognostic impact of MK in a large series of patients with MDS with at least one CA, focusing especially on assessing the ability of MK to independently predict outcome in patients with complex karyotype (CK).

PATIENTS AND METHODS

Patients

A total of 1,054 patients from the Spanish Registry of MDS, the common database of GESMD, were included in the study. Inclusion criteria were: diagnosis of de novo MDS according to WHO 2008 criteria and abnormal

Table 1. Univariate and Multivariate Analyses for OS

Variable	All Patients (N = 1,054)				Patients With CK (n = 203)				Patients With Two CAs (n = 124)			
	Univariate	Multivariate			Univariate	Multivariate			Univariate	Multivariate		
	P	HR	95% CI	*P*	*P*	HR	95% CI	*P*	*P*	HR	95% CI	*P*
Sex												
Male	.002*			NSS	NSS				NSS			
Age, years	< .001	1.92	1.51 to 2.42	< .001	< .001	1.80	1.21 to 2.7	.004	NSS			
> 60	< .001*				< .001*				NSS			
Bone marrow blasts†	< .001*	1.05	1.02 to 1.07	< .001	< .001*			NSS	.001			NSS
Peripheral blood blasts†	< .001*			NSS	NSS			NSS	.003			NSS
Hemoglobin level, g/L†	< .001				< .001	2.37	1.62 to 3.47	< .001	NSS			
< 100	< .001*	1.71	1.41 to 2.09	< .001	< .001*				NSS			
Platelet count, $\times 10^9$/L†	< .001	1.46	1.17 to 1.81	.001	< .001	1.68	1.19 to 2.39	.004	.001			NSS
< 100	< .001*				< .001*				.002*			
Neutrophil count, $\times 10^9$/L†	.012*			NSS	NSS		—	—	NSS			
WHO type‡	< .001*			NSS	< .001*	1.64	1.45 to 2.34	.007	.004*			NSS
IPSS risk group§	< .001*	1.48	1.10 to 1.99	.005	.096*			NSS	< .001*	2.53	1.45 to 4.42	< .001
Karyotype complexity	< .001*				—		—	—	—		—	—
Non-CK		1										
sCK		1.81	1.23 to 2.67	.003								
Very CK		2	1.51 to 2.64	< .001								
MK	< .001*			NSS	< .092*			NSS	.027*			NSS
No. of CAs†	< .001		—	—	< .001	1.64	1.18 to 2.28	.003	—		—	—
≥ 5 *v* < 5	< .001*				.006*							
Chromosome 5 and/or 7 alterations‖	< .001*	1.89	1.37 to 2.61	< .001	< .026*			NSS	NSS			

NOTE. For OS, the following variables were analyzed: age, sex, peripheral blood and bone marrow blast percentage (both as continuous variables), hemoglobin level, platelet count, neutrophil count, presence of MK, karyotype complexity (no complexity, sCK, and very CK), IPSS risk group, WHO morphologic subtype, and presence of chromosome 5 and/or 7 CAs. For patients with CK, number of CAs was also analyzed.

Abbreviations: CA, cytogenetic abnormality; CK, complex karyotype; HR, hazard ratio; IPSS, International Prognostic Scoring System; MK, monosomal karyotype; OS, overall survival; NSS, not statistically significant.

*Included in multivariate analysis.

†Introduced as continuous variable in the analysis.

‡Refractory anemia with excess blasts *v* refractory anemia.

§Intermediate-2 and high risk *v* low and intermediate-1 risk.

‖Non-5/7 alterations *v* 5/7 alterations excluding isolated 5q deletion *v* isolated 5q deletion.

karyotype by conventional cytogenetic study. All patients were scrutinized and double-checked before inclusion to avoid duplication.

Cytogenetic Studies

Conventional G-banding cytogenetic studies were performed at diagnosis at the individual centers and described following the International System for Human Cytogenetic Nomenclature (2009).[9] Structural CAs or extrachromosomes were considered clonal when they were found in at least two metaphases. For monosomies, it was required the presence of the same loss in at least three complete metaphases.[9] Enumeration of the number of chromosome aberrations was performed according to the recommendations of Chun et al.[10] Cytogenetics G-banding data from GESMD were independently reviewed by five expert cytogeneticists (V.A., F.S., M.O., M.M., T.V.).

According to the criteria of Breems et al,[5] MK was defined as the presence of ≥ two autosomal monosomies or one monosomy with at least one additional structural CA. CK was defined following IPSS criteria as the presence of at least three CAs. Karyotype complexity was considered in accordance with Schanz et al,[11] including non-CK (< three CAs), sCK (to differentiate from CK used as classical definition in text; three CAs), and very CK (> three CAs). Sexual monosomies were not considered for MK because of their lack of impact on the evolution of the disease, as we have described previously[12] and confirmed again in our patient population (data not shown).

Statistical Analyses

The main end points of the study were overall survival (OS) and evolution to AML. OS was defined as time from diagnosis to death, censoring surviving patients at follow-up. Time to AML evolution was measured from diagnosis to development of AML, and patients free from AML were censored at last follow-up or death. Mean and median values as well as 95% CIs and ranges were calculated for each continuous variable. *t* and Pearson χ^2 tests were used to compare continuous and qualitative variables. Probability of death and evolution to AML were calculated using Kaplan-Meier curves and compared using log-rank tests.[13] Quantitative variables were tested first as continuous variables, and if they showed a statistically significant impact on outcome ($P < .05$), they were reanalyzed as categorical variables using a clinically meaningful cutoff (ie, age, 60 years; hemoglobin level, 100 g/L; and platelet count, 100×10^9/L) or the median value otherwise. Multivariate analyses were performed using the backward elimination Cox proportional hazards regression method,[14] including those variables with a *P* value < .1 in univariate testing, except for the presence of MK, which was always introduced into the multivariate analysis because it was considered the main investigation variable. Tests of significance were two sided, and a *P* value of ≤ .05 was considered statistically significant. All statistical analyses were performed with SPSS version 17.0 (SPSS, Chicago, IL). Variables analyzed are listed in Tables 1 and 2.

RESULTS

Patients

A total of 1,054 patients were included in the study. Median follow-up for survivors was 24 months (range, 0.3 to 210 months). Patient characteristics are summarized in Table 3. CK was identified in 203 patients (19.3%; CK patients), of whom 150 (73.9%) also fulfilled

Table 2. Univariate and Multivariate Analyses for Leukemia-Free Survival

Variable	All Patients				Patients With CK				Patients With Two CAs			
	Univariate	Multivariate			Univariate	Multivariate			Univariate	Multivariate		
	P	HR	95% CI	*P*	*P*	HR	95% CI	*P*	*P*	HR	95% CI	*P*
Sex												
Male	NSS				NSS				NSS			
Age, years	.039*			NSS	NSS				NSS			
> 60	NSS			—					NSS			
Bone marrow blasts†	< .001*	1.12	1.09 to 1.16	< .001	< .001*	1.09	1.045 to 1.14	< .001	< .001*	1.17	1.09 to 1.26	< .001
Peripheral blood blasts†	< .001*			NSS	.041*			NSS	NSS			
Hemoglobin level, g/L†	< .001			NSS	.026			NSS	.022			
< 100	.061*				.012*				NSS			
Platelet count, $\times 10^9$/L†	< .001			NSS	NSS			NSS	.004			
< 100	< .001*				.007*				.035*			
Neutrophil count, $\times 10^9$/L†	.002			NSS	NSS				.059			
WHO type‡	< .001*			NSS	.012*			NSS	.002			
IPSS risk group§	< .001*			NSS	.096*			NSS	< .001*			
Karyotype complexity	< .001*				—	—		—	—	—		—
Non-CK		1										
sCK		2.53	1.42 to 4.53	.002								
Very CK		2.77	1.77 to 4.35	< .001								
MK	< .001*			NSS	.65*			NSS	.20*			
No. of CAs†	< .001		—	—	.18							
≥ 5 *v* < 5	< .001*				.3							
Chromosome 5 and/or 7 alterations‖	< .001*	0.62	0.39 to 0.97	.038	.3				NSS			

NOTE. For OS, the following variables were analyzed: age, sex, peripheral blood and bone marrow blast percentage (both as continuous variables), hemoglobin level, platelet count, neutrophil count, presence of MK, karyotype complexity (no complexity, sCK, and very CK), IPSS risk group, WHO morphologic subtype, and presence of chromosome 5 and/or 7 CAs. For patients with CK, number of CAs was also analyzed.

Abbreviations: CA, cytogenetic abnormality; CK, complex karyotype; HR, hazard ratio; IPSS, International Prognostic Scoring System; MK, monosomal karyotype; OS, overall survival; NSS, not statistically significant.

*Included in multivariate analysis.

†Introduced as continuous variable in the analysis.

‡Refractory anemia with excess blasts *v* refractory anemia.

§Intermediate-2 and high risk *v* low and intermediate-1 risk.

‖Non-5/7 alterations *v* 5/7 alterations excluding isolated 5q deletion *v* isolated 5q deletion.

the criteria for MK (CK-MK patients). MK was observed in 172 patients (16.3%; MK patients), of whom 150 (87.2%) had CK. Both CK and MK patients had worse prognosis baseline characteristics compared with patients without these CAs (Table 3). After excluding those CK-MK patients, baseline characteristics of isolated CK (n = 53) and MK patients (n = 22) were similar, except for a higher number of monosomies (median, one; range, one to two *v* median, zero; range, zero to one; $P < .001$) and lower number of CAs (median, two; range, two to two *v* median, three; range, three to 10; $P < .001$) in patients with MK versus CK, respectively. For CK-MK patients (n = 150), these figures were two monosomies (range, one to eight) and six CAs (range, three to 18).

Involved Chromosomes and Number of CAs

All chromosomes (except chromosome 1) were involved in at least one monosomy. A total of 227 patients (21.54%) had at least one monosomy, of whom 135 (59.47%) had only one monosomy, and 80 (60.10%) of these met the criteria for MK. Details regarding CAs are provided in Appendix Tables A1 to A3 and Appendix Figures A1 and A2 (online only).

The number of CAs was important in patient outcome; the higher the number of CAs, the lower the OS (HR, 1.22; 95% CI, 1.19 to 1.25; $P < .001$), and the higher the risk of AML evolution (HR, 1.20; 95% CI, 1.16 to 1.26; $P < .001$; Appendix Fig A2, online only). Median OS for non-CK, sCK, and very CK patients was 47.7 (95% CI, 41.70 to 53.67), 10.23 (95% CI, 6.53 to 13.95), and 7.56 months (95% CI, 6.16 to 8.97), respectively ($P < .001$; Fig 1A).

The importance of the number of CAs persisted in MK patients. Thus, the higher the number of CAs, the lower the OS (HR, 1.074; 95% CI, 1.026 to 1.125; $P < .001$). Median OS for non-CK, sCK, and very CK patients was 20.57 (95% CI, 12.24 to 28.91), 9.38 (95% CI, 7.12 to 11.63), and 7.62 months (95% CI, 6.99 to 9.39), respectively ($P = .02$; Fig 1B).

Abnormalities of chromosome 7 were observed in 165 patients (77 CK patients and 88 non-CK patients); this abnormality was associated with shorter OS in CK patients (chromosome 7 CA: median OS, 7.6; 95% CI, 5.7 to 9.5 *v* non–chromosome 7 CA: median OS, 8.8 months; 95% CI, 7.3 to 10.3; $P = .006$) as well as in non-CK patients (median OS: 19.6 months; 95% CI, 15.6 to 23.7 *v* 53.3 months; 95% CI, 49 to 59.7; $P < .001$). Presence of chromosome 5 abnormalities (excluding isolated 5q deletion) was observed in 169 patients (136 CK and 33 non-CK patients). Again, this abnormality was also associated with shorter OS in both CK patients (chromosome 5 CA: median OS, 7.9 months; 95% CI, 6.9 to 8.8 *v* non-chromosome 5 CA: median OS, 12.3 months; 95% CI, 6.8 to 17.8; $P = .013$) and non-CK patients (median OS, 19 months; 95% CI, 10.7 to 27.2 *v* 40.2 months; 95% CI, 33.9 to 46.4; $P = .023$).

Table 3. Patient Demographic and Clinical Characteristics

Characteristic	All Patients No.	%	CK Patients No.	%	MK Patients No.	%
Total patients	1,054		203	19.3	172	16.3
Sex						
Female	483	45.8	91	44.8	78	45.3
Male	571	54.2	112	55.2	94	54.7
Age, years						
Median	71.62		70		70.35	
Range	16.1-96.3		23-96.3		23-96.3	
> 60	814	77.2	157	77.3	133	77.3
Type of MDS				*		*
Refractory anemia	609	57.8	71	35	60	34.9
Refractory anemia with excess blasts	445	42.2	132	65	112	65.1
BM blasts percentage						
Median	4		7*		7*	
Range	0-19		0-19*		0-19*	
Hemoglobin level, g/L						
Median	95		88*		89*	
Range	22-166		45-152*		45-152*	
< 100	613	58.2	139*	68.5*†	124*	72.1*
Platelet count, $\times 10^9$/L						
Median	149		74.5*		75*	
Range	1-1,498		3-602*		3-586*	
< 100	353	33.5	126*	62.1*	104*	60.5*
ANC, $\times 10^9$/L						
Median	1.7		1.2*		1.2*	
Range	0-19.4		0-11.8*		0-15*	
MK	172	16.3	150*	73.9*	172*	100*
CK	203	19.3	203	100	150	87.2
No. of monosomies						
Median	0		1*		2*	
Range	0-8		0-8*		10-8*	
0	827	78.5	52	25.6	0	0
1	135	12.8	60	29.6	80	46.5
2	44	4.2	43	21.2	44	25.6
3	22	2.1	22	10.8	22	12.8
≥ 4	26	2.5	26	12.9	26	15.1
Monosomies of chromosomes 5 and 7						
7	62	5.9	14	6.9	25	14.5
7 plus other monosomies	23	2.2	22	10.8	23	13.4
5	13	1.2	5	2.4	9	5.2
5 plus other monosomies	12	1.2	12	5.9	12	7
5 and 7	14	1.2	14	6.9	14	8.1
Other monosomies	103	9.8	84	41.4	89	51.7
No monosomies	827	78.5	52	25.6	0	0
No. of CAs, median (range)						
Median	1		5*		5*	
Range	0-18		3-18*		2-18*	
1	727	69	0	0	0	0
2	124	11.8	0	0	22	12.8
3	56	5.3	56	27.6	27	15.7
4	36	3.4	36	17.7	23	13.4
5	26	2.5	26	12.8	23	13.4
≥ 6	85	8.1	85	41.9	77	44.7
Abnormalities of chromosomes 5 and 7	534	50.7	160	78.8	142	82.6
7 CA	106	10	24	11.8	29	16.9
5 CA	369	35.5	85	41.9	64	37.2
5 and 7 CAs	59	5.6	51	25.1	49	28.4
Other chromosomes involved	520	49.3	43	21.2	30	17.4
IPSS risk				*		*
Low	193	18.3	0	0	0	0
Intermediate-1	414	39.3	25	12.3	23	13.4
Intermediate-2	264	25	107	52.7	86	50
High	112	10.6	60	29.6	53	30
NA	71	6.7	11	5.4	10	5.8

(continued on following page)

Abbreviations: ANC, absolute neutrophil count; BM, bone marrow; CA, cytogenetic abnormality; CK, complex karyotype; IPSS, International Prognosis Scoring System; MDS, myelodysplastic syndrome; MK, monosomal karyotype; NA, not available.

*Statistically ($P < .001$) different compared with entire cohort of patients.

†$P = .004$.

Impact of Chromosomal Aberrations on OS

At last follow-up, 471 patients were alive for a median OS of 35.8 months (95% CI, 30.7 to 40.8). Median OS for MK patients versus non-MK patients was 8.1 months (95% CI, 7.1 to 9.1) versus 47.9

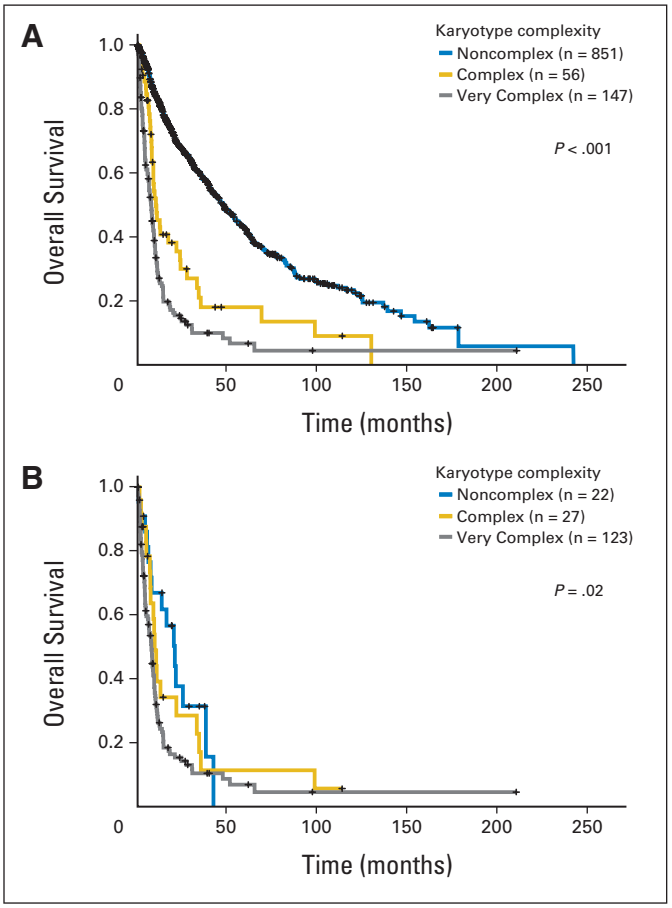

Fig 1. Overall survival according to karyotype complexity in (A) the whole population and (B) patients with monosomal karyotype.

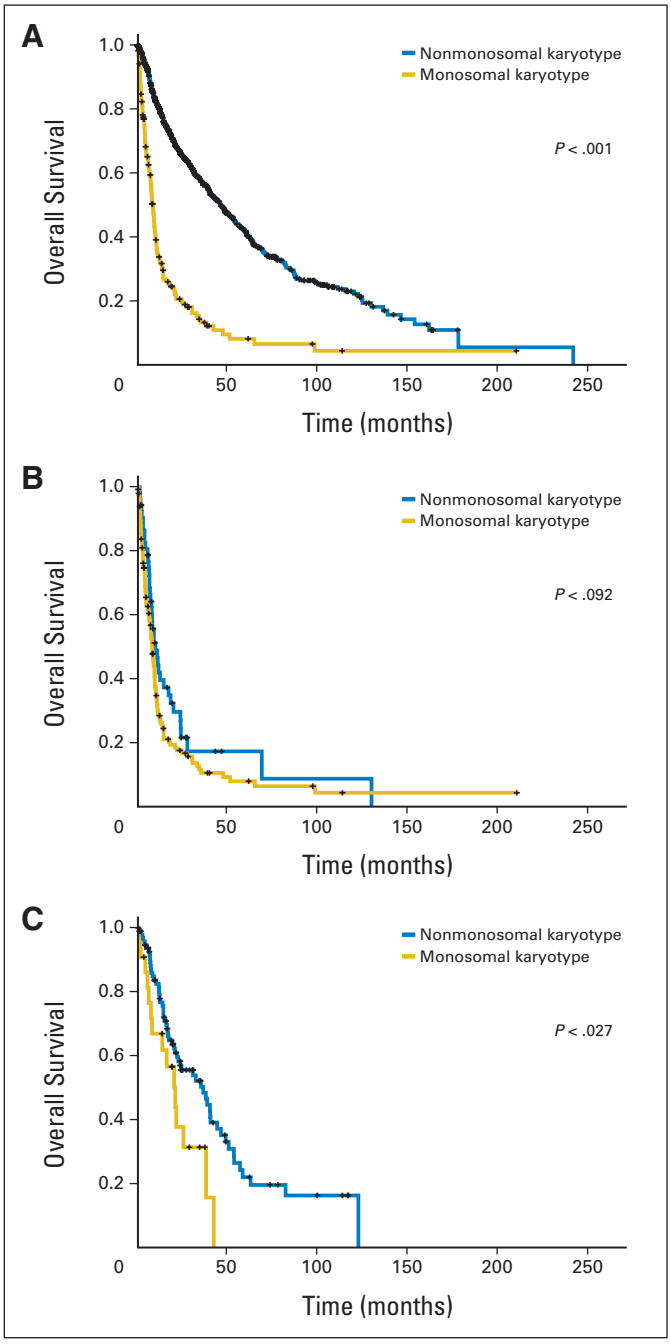

Fig 2. Overall survival for (A) monosomal (MK) and nonmonosomal karyotypes (non-MK), (B) MK and non-MK in complex karyotype patients, and (C) MK and non-MK in patients with two cytogenetic abnormalities.

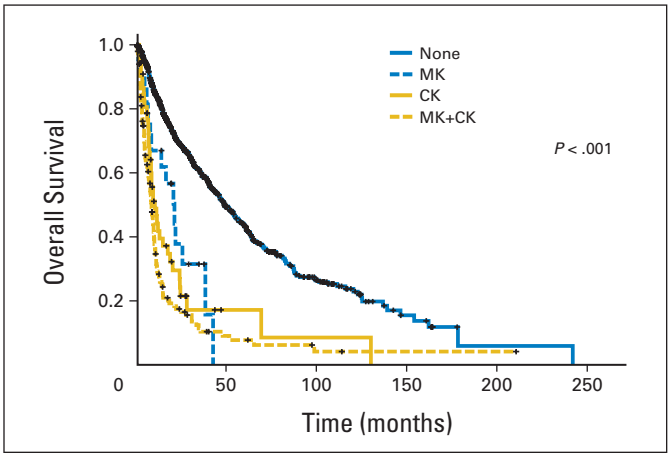

Fig 3. Overall survival according to presence of monosomal karyotype (MK), complex karyotype (CK), both, and none.

months (95% CI, 41.9 to 53.8), respectively ($P < .001$; Fig 2A). Table 1 lists the main variables associated with lower OS. In multivariate analysis, variables that retained a statistical significance were: age > 60 years (hazard ratio [HR], 1.92; 95% CI, 1.51 to 2.42; $P < .001$), BM blast percentage (HR, 1.05; 95% CI, 1.02 to 1.07; $P < .001$), hemoglobin < 100 g/L (HR, 1.71; 95% CI, 1.41 to 2.09; $P < .001$), platelet count < 100×10^9/L (HR, 1.46; 95% CI, 1.17 to 1.81; $P < .001$), higher IPSS risk group (HR, 1.48; 95% CI, 1.10 to 1.99; $P = .005$), karyotype complexity (sCK: HR, 1.81; 95% CI, 1.23 to 2.67; $P = .003$; very CK: HR, 2; 95% CI, 1.51 to 2.64; $P < .001$), and abnormalities of chromosome 5 and/or 7 (HR, 1.89; 95% CI, 1.37 to 2.61; $P < .001$; Table 1). MK was not independently associated with different OS in multivariate analysis. Figures 2 and 3 show OS curves for patients with and without MK.

Median OS was 8.1 months (95% CI, 7.1 to 9.14) for CK patients and 47.7 months (95% CI, 41.7 to 53.67) for non-CK patients ($P < .001$). In CK patients, presence of MK showed only a trend toward lower OS ($P = .092$; Fig 2B). Other variables associated with lower OS in the univariate analysis are listed in Table 1. In the multivariate analysis, in CK patients, variables associated with shorter OS were age > 60 years (HR, 1.8; 95% CI, 1.21 to 2.7; $P = .004$), WHO subtype (refractory anemia with excess blasts *v* refractory anemia; HR, 1.64; 95% CI, 1.45 to 2.34 $P = .007$), hemoglobin < 100 g/L (HR, 2.37; 95% CI, 1.62 to 3.47; $P < .001$), platelets < 100×10^9/L (HR, 1.68; 95% CI, 1.19 to 2.39; $P = .004$), and ≥ five CAs (HR, 1.64; 95% CI, 1.18 to 2.28; $P = .003$; Table 1). MK was not independently associated with different OS.

The total number of patients with two CAs was 124 patients, and their median OS was 30.5 months (95% CI, 17.4 to 43.7). In a univariate analysis restricted to patients with two CAs, presence of MK (n = 22) was associated with lower OS (median OS, 20.8 *v* 36.2 months for patients without MK [n = 100]; $P = .027$; Fig 2C). However, in a multivariate analysis, the only variable that retained statistical significance was higher IPSS risk group (HR, 2.53; 95% CI, 1.45 to 4.42; $P < .001$; Table 1).

Evolution to AML

At last follow-up, 221 patients (21%) had developed AML at a median time of 9 months (range, 1 to 125 months), and the 1- and 4-year probabilities of AML evolution were 14.2% (95% CI, 11.8 to 16.6%) and 28.6% (95 CI, 24.8% to 32.4%), respectively. Table 2 lists the variables showing a significantly higher risk of AML evolution. In multivariate analysis, the variables associated with higher risk of AML evolution were higher BM blasts (HR, 1.12; 95% CI, 1.09 to 1.16; $P < .001$) and karyotype complexity (sCK: HR, 2.54; 95% CI, 1.42 to 4.53; $P = .002$; very CK: HR, 2.77; 95% CI, 1.77 to 4.35; $P < .001$), whereas presence of isolated 5q deletion was a protective factor (HR, 0.62; 95% CI, 0.40 to 0.98; $P = .038$). MK was not independently

associated with higher risk of evolution to AML. Appendix Figure A3 (online only) plots the actuarial curves for AML evolution.

During the follow-up, 72 (35.5%) of 203 patients with CK developed AML, and the probabilities of 1- and 4-year AML evolution were 41.5% (95% CI, 32.9% to 50.1%) and 57.5% (95% CI, 25.5% to 60.5%), respectively. Variables associated with higher risk of AML evolution in patients with CK are listed in Table 2. Presence of MK did not show a significantly different risk of AML evolution, and the only factor associated with higher risk of AML evolution in multivariate analysis in CK patients was higher percentage of BM blasts (HR, 1.09; 95% CI, 1.05 to 1.14; $P < .001$).

Twenty-five (20.16%) of 124 patients with two CAs developed AML, and the 1- and 4-year probabilities of AML evolution were 11.6% (95% CI, 5% to 18.2%) and 32% (95% CI, 19.4% to 44.6%) respectively. Again, presence of MK was not associated with a different risk of AML evolution; higher percentage of BM blasts (HR, 1.17; 95% CI, 1.09 to 1.26; $P < .001$) was the sole factor clearly associated with higher risk of AML evolution.

Impact of Monosomies of Chromosome 5 and/or 7 on OS

Eighty-three patients had MK involving monosomies of chromosome 5 and/or 7 and 89 involving other monosomies. The univariate analysis including the whole population showed better OS for patients without MK versus MK without monosomy of chromosome 5 and/or 7 versus MK with monosomy of chromosome 5 and/or 7 (median OS, 46.17 months; 95% CI, 40.34 to 51.99 *v* 8.19 months; 95% CI, 6.36 to 10.02 *v* 8.12 months; 95% CI, 7.03 to 9.22, respectively; $P < .001$), and the impact persisted in a multivariate model (MK with monosomy 5 and/or 7: HR, 1.73; 95% CI, 1.14 to 2.64; $P < .01$ *v* MK without monosomy 5 and/or 7: HR, 1.05; 95% CI, 0.68 to 1.64; $P = .81$) compared with non-MK (HR, 1), whereas the other variables remain unchanged (Appendix Table A4; Appendix Fig A4, online only). The same analysis applied to leukemia-free survival did not show any impact of monosomies of chromosome 5 and/or 7 (data not shown).

Impact of Treatment

There were 431 patients who received at least one kind of therapy (AML-type chemotherapy, 103 patients; autologous stem-cell transplantation, eight patients; allogeneic stem-cell transplantation, 48 patients; lenalidomide, 141 patients; azacitidine, 108 patients; antithymocyte globulin ± cyclosporine A, six patients; and erythropoiesis-stimulating agents, 226 patients). Some of these patients received more than one treatment. Results of a multivariate analysis in untreated patients only were quite similar to results with the same variables entered into the different regression models (data not shown), strongly suggesting that those results were not influenced by treatment.

DISCUSSION

Presence of MK has recently been associated with worse prognosis in patients with MDS,[6,7] but our study shows that this is because MK is closely associated with a more complex karyotype, suggesting that it is greater complexity that explains the poor prognosis of these patients.

Incidence of MK in our series was 16.3%, and its presence was strongly related to CK; 87.2% of MK patients also met criteria for CK, and 73.9% of CK patients also fulfilled criteria for MK, which is in agreement with previous data from Patnaik et al.[6] Both MK and CK patients had worse prognosis baseline characteristics compared with the global population, as summarized in Table 3.

In a recent study including only CK patients, Patnaik et al[6] suggest that MK is associated with lower OS. Our results differ from those of Patnaik et al; in our study, MK only showed an association with lower OS in univariate analysis, but it no longer retained its significance in multivariate analysis, suggesting that its importance is related to some other variable, which seems to be CK. In fact, when patients were stratified into four groups (Fig 3), isolated MK patients showed higher OS compared with isolated CK patients, although the difference was not statistically significant ($P = .3$; data not shown), and the worse prognosis was for MK-CK patients. Trying to clarify the relevance of the presence of MK, we analyzed its impact on both CK patients and patients with only two CAs, which is the minimum necessary to fulfill the criteria for MK. In CK patients, MK was not statistically associated with lower OS in univariate or multivariate analysis, but the risk factors associated with lower OS in patients with CK were the classic variables (refractory anemia with excess blasts *v* refractory anemia, high IPSS, low hemoglobin level, and low platelet count) and higher number of CAs. In patients with only two CAs, although in univariate analysis patients with MK showed lower OS, this effect did not persist in multivariate analysis, in which the only variable associated with lower OS was higher IPSS risk group. In contrast, the karyotype complexity adverse prognostic value was retained in MK patients. As illustrated in Figure 1 and Appendix Figure A2 (online only), increase of karyotype complexity led to worse prognosis. This idea supports the hypothesis regarding the predominant role of karyotype complexity to determine prognosis in patients with MDS.

Belli et al[7] suggest that MK is associated with poor prognosis, and although they did not specifically analyze the impact of MK in CK patients in a multivariate model, their survival curves suggest that MK did not further adversely affect prognosis. Finally, presence of MK was not associated with a different outcome in a recent study by Itzykson et al[15] in patients with MDS who received azacitidine; the same group in a previous report[16] identified presence of unfavorable cytogenetics (including CK and abnormalities of chromosome 7) as a poor prognosis factor for OS.

Although in our study the classic definition of CK was able to differentiate a worse prognostic group, we also included the new definition of very CK (≥ four CAs), as described by Schanz et al.[12] We confirmed its applicability; it was associated with worse OS and higher AML evolution compared with non-CK or sCK patients (three CAs) in multivariate analyses. This is especially of interest because this score was used in the development of the new revised version of the IPSS and has not been previously validated in a large independent cohort.

From a physiopathologic point of view, presence of multiple CAs could be related to multiple genes alterations and thus worse prognosis. Conventional G-banding cytogenetic studies have some limitations in detecting CAs. Therefore, the introduction of newer and more sensitive techniques could be of great use in identifying CAs, leading to the identification of complex (higher-risk) karyotypes, as suggested by Tiu et al.[17] In their study, the single-nucleotide polymorphism array application combined with conventional karyotyping found CAs in

74% of patients versus 44% using only conventional techniques ($P = .001$). Moreover, they could identify new alterations associated with lower OS in low-risk (IPSS) patients.

In addition to the number, the location of CAs seems to be of prognostic importance. The most frequent MK (ie, involving chromosomes 7 and/or 5) was exceedingly high, suggesting that such abnormalities could play a key role in the pathogenesis of MDS in that subset of patients. Moreover, MK involving these chromosomes was associated with worse prognosis (compared with non-MK or MK involving other chromosomes; Appendix Table A4, online only), and presence of CAs in these chromosomes (monosomies or not) was independently associated with lower OS and higher risk of AML evolution.

In conclusion, our study shows that although MK is closely associated with CK, it is the complexity of the karyotype (ie, number of chromosomal abnormalities) that is associated with lower OS and higher AML evolution. Taking into account the number of CAs, MK is not independently associated with poorer prognosis in patients with MDS. Finally, our findings support the inclusion of very CK but not MK as the poorest risk category in the ongoing effort to update the IPSS.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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Acknowledgment

We thank all the participating investigators of the Spanish Registry of Myelodysplastic Syndrome and its central data manager, Lluis Benlloch, for collecting patient data. We thank Dr Francesc Bosch for his critical review of the manuscript.

Appendix

Table A1. Description of CAs in the Whole Population and in Patients With MK

CA	All Patients	MK Patients
CK	203	150
Including −7	85	58
Including −5	25	21
Including −5 and −7	14	14
Inversion 3q	5	2
Monosomy 7 (noncomplex)	49	10
Deletion 7q	22	16
Monosomy 5 (noncomplex)	8	4
Isolated 5q−	258	0
5q− and other CA	15	3*
Isolated trisomy 8	104	0
Trisomy 8 and other CA	21	
Deletion 11q	27	6
Deletion 12p	19	5
Deletion 17p	3	0
Isochromosome 17q	23	8
Deletion 20q	55	2
Trisomy 21	9	0
Monosomy 21	6	2
−Y	83	3
Other trisomies	34	7
Other monosomies	47	33
Translocations	80	37
Other	93	1

Abbreviations: CA, cytogenetic abnormality; CK, complex karyotype; MK, monosomal karyotype.
*All with associated monosomy 7 (not included in monosomy 7 noncomplex category).

Table A2. Detailed Information of Chromosomes Involved in Monosomies

Chromosome in Monosomy	Frequency	Isolated	Combined
1	0	0	0
2	7	2	5
3	15	3	12
4	8	1	7
5	36	10	26
6	15	1	14
7	62	27	35
8	7	1	6
9	8	1	7
10	4	1	3
11	12	3	9
12	14	1	13
13	17	4	13
14	15	2	13
15	17	1	16
16	15	4	11
17	22	5	17
18	26	4	22
19	9	1	8
20	18	4	14
21	25	1	24
22	9	3	6

Table A3. Detailed Information of CAs Found in Patients With MK

Alteration	Frequency
−2	7
−3	15
inv(3)(q21q26)	2
−4	8
−5	36
5q−	79
−6	15
−7	62
7q−	15
+8	23
−8	7
−9	8
−10	4
+11	4
−11	12
11q−	6
−12	14
12p−	5
+13	1
−13	17
13q−	5
−14	15
−15	17
−16	15
i(17)(q10)	7
−17	22
−18	26
−19	9
−20	18
del(20)(q11q13)	7
+21	3
−21	25
−22	9
−X	4
−Y	6

Abbreviations: CA, cytogenetic abnormality; MK, monosomal karyotype.

Table A4. Multivariate Analysis Including MKs Involving Chromosome 5 and/or 7 Monosomy Instead of All MKs

Variable	OS				AML-Free Survival				
	Univariate	Multivariate			Univariate		Multivariate		
	P	HR	95% CI	*P*	HR	95% CI	HR	95% CI	*P*
Sex									
Male	.002*			NSS		NSS			NSS
Age, years	< .001	1.90	1.51 to 2.42	< .001		.039*			NSS
> 60	< .001*					NSS			
Bone marrow blasts†	< .001*	1.046	1.02 to 1.07	< .001		< .001*	1.12	1.09 to 1.15	< .001
Peripheral blood blasts†	< .001*			NSS		< .001*			NSS
Hemoglobin level, g/L†	< .001					< .001			NSS
< 100	< .001*	1.70	1.40 to 2.07	< .001		.061*			
Platelet count, $\times 10^9$/L†	< .001	1.48	1.19 to 1.83	.001		< .001			NSS
< 100	< .001*					< .001*			
Neutrophil count, $\times 10^9$/L†	.012*			NSS		.002			NSS
WHO type‡	< .001*			NSS		< .001*			NSS
IPSS risk group§	< .001*	1.52	1.12 to 2.07	.006		< .001*			NSS
Karyotype complexity	< .001*					< .001*			
Non-CK		1					1		
sCK		1.54	0.99 to 2.4	.052			2.53	1.42 to 5.53	.002
Very CK		1.76	1.15 to 2.69	< .001			2.77	1.77 to 4.35	< .001
MK	< .001*					< .001*			NSS
No		1							
Yes, involving chromosomes 5 and/or 7		1.73	1.14 to 2.64	.01					
Yes, not involving chromosomes 5 and/or 7		1.05	0.68 to 1.64	.81					
Chromosome 5 and/or 7 alterations‖	< .001*	1.74	1.25 to 2.42	< .001		< .001*	0.62	0.39 to 0.97	.03

Abbreviations: AML, acute myeloid leukemia; CA, cytogenetic abnormality; CK, complex karyotype; HR, hazard ratio; IPSS, International Prognostic Scoring System; MK, monosomal karyotype; OS, overall survival; NSS, not statistically significant.

*Included in multivariate analysis.

†Introduced as continuous variable in the analysis.

‡Refractory anemia with excess blasts *v* refractory anemia.

§Intermediate-2 and high risk *v* low and intermediate-1 risk.

‖Non-5/7 alterations *v* 5/7 alterations excluding isolated 5q deletion *v* isolated 5q deletion.

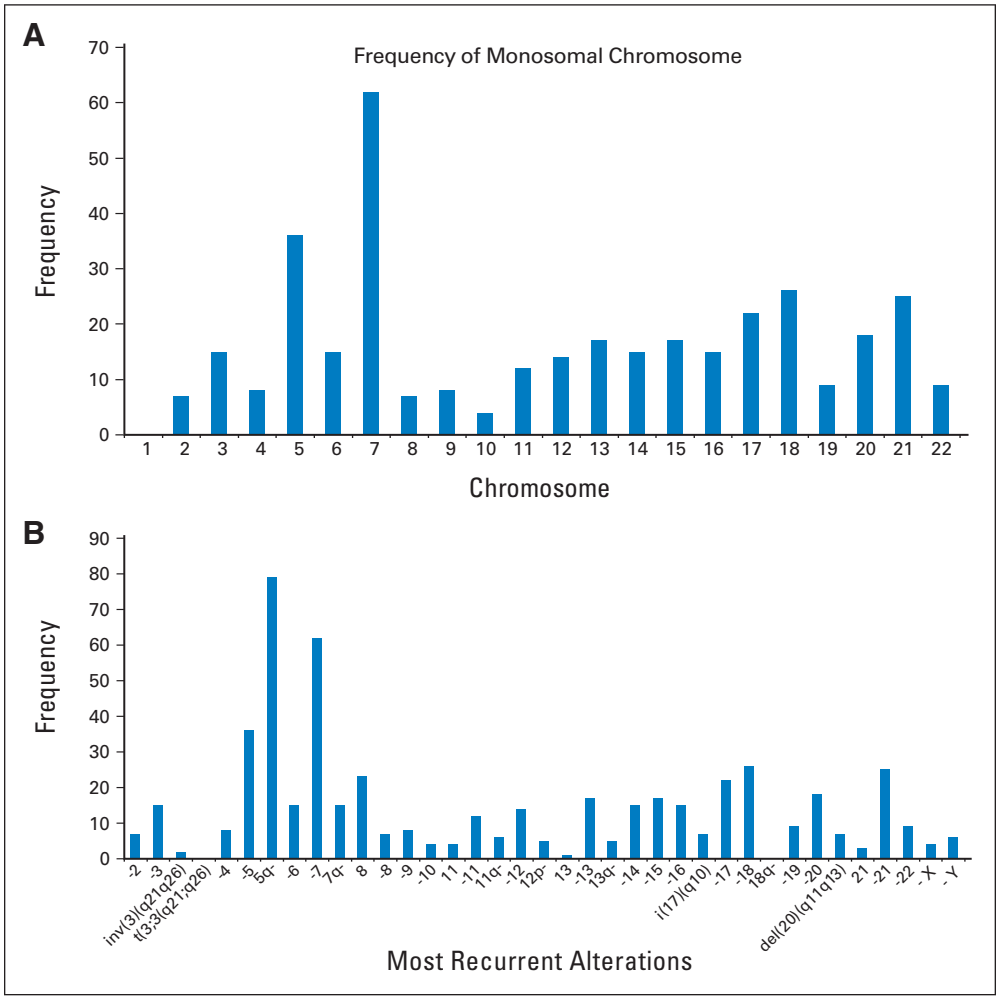

Fig A1. Involved chromosomes in (A) monosomies and (B) most frequent cytogenetic abnormalities.

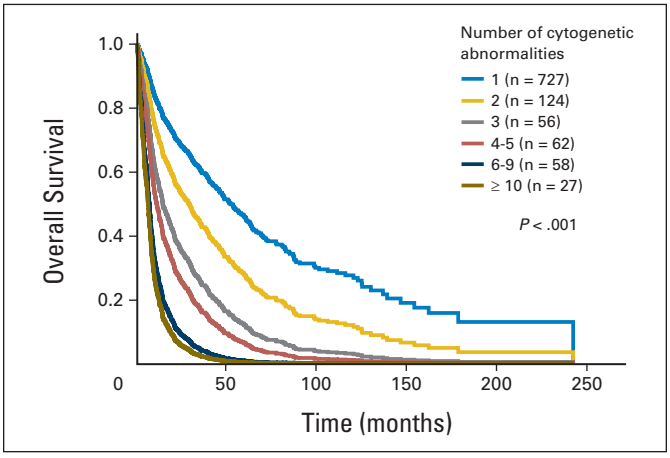

Fig A2. Impact of the number of cytogenetic abnormalities.

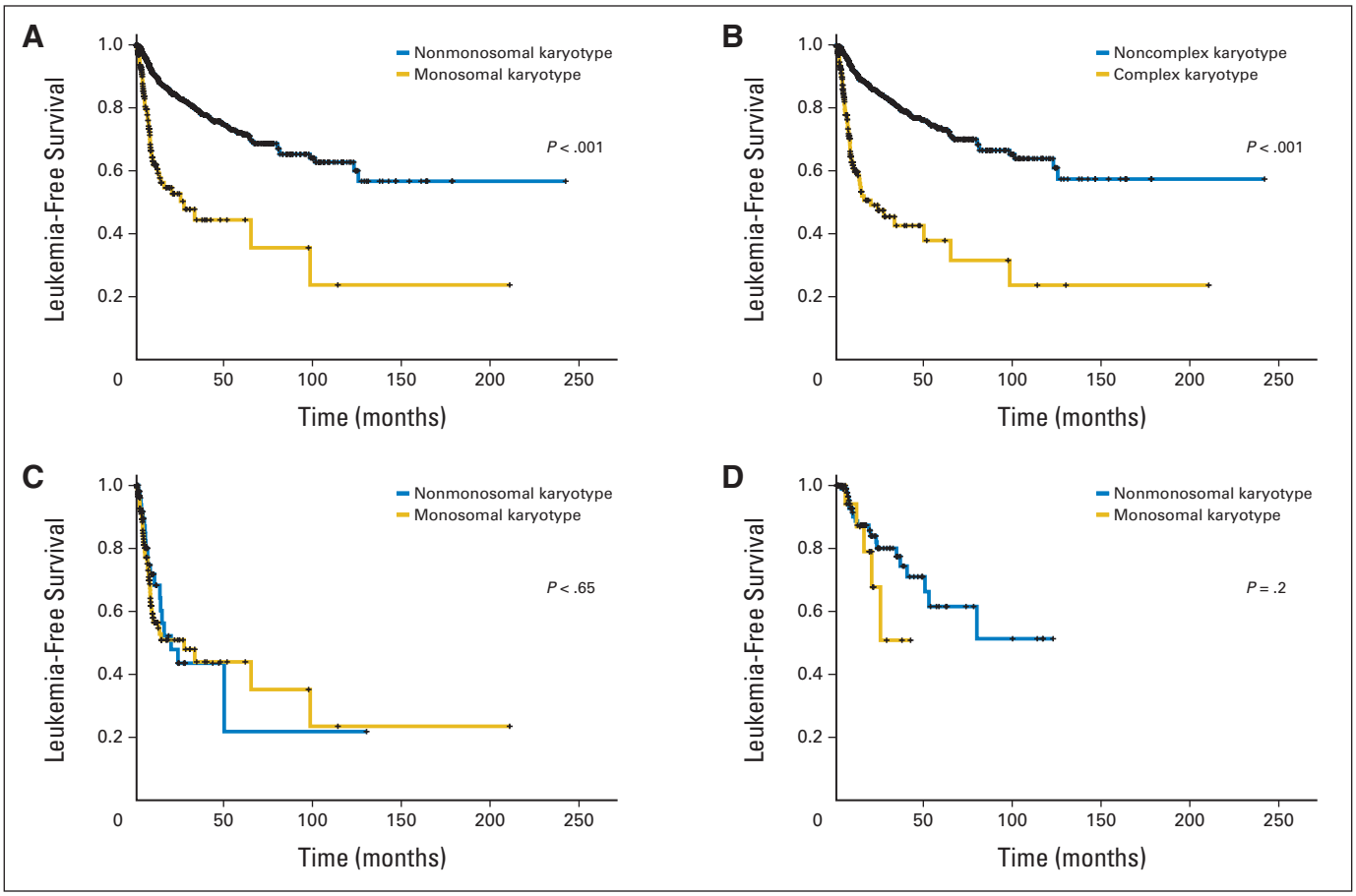

Fig A3. Leukemia-free survival for (A) patients with monosomal (MK) and nonmonosomal karyotypes (non-MK), (B) patients with complex (CK) and noncomplex karyotypes (non-CK), (C) MK and non-MK in CK patients, and (D) MK and non-MK in patients with two cytogenetic abnormalities.

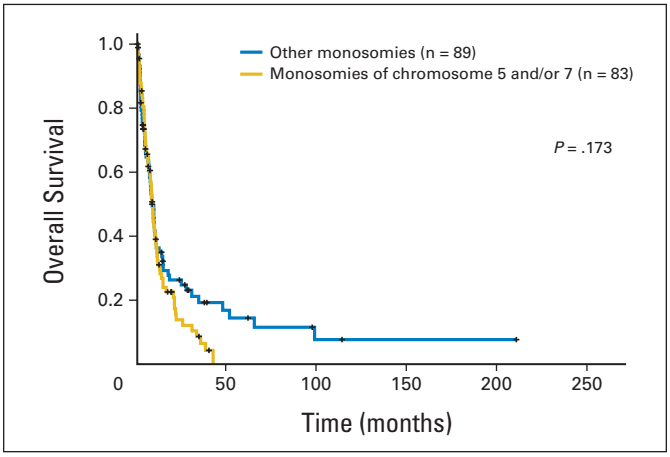

Fig A4. Overall survival in monosomal karyotype for patients with chromosome 5 and/or 7 monosomies versus other monosomies.