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ORIGINAL ARTICLE

Monosomal karyotype in MDS: explaining the poor prognosis?

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Monosomal karyotype (MK) is associated with an adverse prognosis in patients in acute myeloid leukemia (AML). This study analyzes the prognostic impact of MK in a cohort of primary, untreated patients with myelodysplastic syndromes (MDS). A total of 431 patients were extracted from an international database. To analyze whether MK is an independent prognostic marker in MDS, cytogenetic and clinical data were explored in uni- and multivariate models regarding overall survival (OS) as well as AML-free survival. In all, 204/431 (47.3%) patients with MK were identified. Regarding OS, MK was prognostically significant in patients with $\leqslant$4 abnormalities only. In highly complex karyotypes ($\geqslant$5 abnormalities), MK did not separate prognostic subgroups (median OS 4.9 months in MK+ vs 5.6 months in patients without MK, $P=0.832$). Based on the number of abnormalities, MK-positive karyotypes (MK+) split into different prognostic subgroups (MK+ and 2 abnormalities: OS 13.4 months, MK+ and 3 abnormalities: 8.0 months, MK+ and 4 abnormalities: 7.9 months and MK+ and $\geqslant$5 abnormalities: 4.9 months; $P<0.01$). In multivariate analyses, MK was not an independent prognostic factor. Our data support the hypothesis that a high number of complex abnormalities, associated with an instable clone, define the subgroup with the worst prognosis in MDS, independent of MK.

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INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal disorders of the hematopoietic stem cell, associated with peripheral cytopenias and the risk of transformation into acute myeloid leukemia (AML).[1,2] The commonly used prognostic classification systems[3-6] include the percentage of bone marrow blasts, the extent of peripheral cytopenias, the transfusion burden and the karyotype as major prognostic variables in MDS. However, the recently published revision of the International Prognostic Scoring System (IPSS-R)[4] revealed that the karyotype is the most influential prognostic parameter regarding overall survival (OS) as well as AML-free survival (AMLFS).

A complex karyotype (CK), defined as three or more abnormalities in one cell,[7] is clearly associated with an adverse prognosis.[3–5,8,9] However, little is known about prognostically relevant subgroups within this heterogeneous category. The revised IPSS (IPSS-R) separates two independent subsets of patients with complex abnormalities based on the number of abnormalities per clone. Those with exactly three abnormalities are classified as poor whereas patients with more than three abnormalities are assigned to the group with the worst prognosis.[4] Moreover, data from the German MDS study group demonstrated that the prognostically worse impact of complex abnormalities increases with the number of abnormalities per clone.[8]

In AML, an alternative approach was proposed by Breems *et al.*[10]: monosomal karyotype (MK), defined as at least two autosomal monosomies or one autosomal monosomy and one structural abnormality in one cell, was described as a better indicator for a worse prognosis than CK. Subsequently, associations of MK with treatment outcome,[11–18] occurrence of TP53-mutations,[19,20] specific copy number alterations[20] and multidrug resistance activity[13] were described. However, a recent publication by Haferlach *et al.*[21] revealed that the use of MK bears the risk of missing a significant subset of patients with an adverse prognosis in AML.

In MDS, MK was also described as an unfavorable risk factor.[17,22,23] In contrast, Itzykson *et al.*[24] found no significant differences between MK and non-MK in a group of patients with MDS, treated with 5-azacytidine. Recently, the Spanish Group of MDS[25] demonstrated that MK is not an independent risk factor for OS and the complexity of the karyotype is the most important factor predicting prognosis in this disease. Furthermore,

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investigations based on a patient cohort unbiased by therapy are not available as yet.

Hence, the main goal of this study was to analyze the relationship between the number and type of abnormalities, the occurrence of MK and their influence on OS and AMLFS in a cohort of primary, untreated MDS patients.

PATIENTS AND METHODS

Patient cohort

In total, 431 patients with $\geqslant 2$ abnormalities were extracted from an international MDS database and retrospectively analyzed. Owing to the fact that the definition of a MK needs at least two abnormalities, patients with single abnormalities were not considered for analysis. The database contains 2902 patients with primary, untreated MDS. Results from this database as well as details regarding the patient cohort were published elsewhere,[26] but did not focus on the question of MK. Inclusion criteria in the database were as follows: unambiguous morphologic diagnosis of MDS or oligoblastic AML following MDS (blast count $\leqslant 30\%$), age $\geqslant 16$ years, supportive care and International System for Human Cytogenetic Nomenclature formula available. The 431 patients with $\geqslant 2$ abnormalities were derived from the following databases: German-Austrian MDS study group ($n = 185$; 43%), International MDS Risk Analysis Workshop ($n = 121$; 28%), Spanish Hematological Cytogenetics working group ($n = 104$; 24%) and International Working Group on MDS Cytogenetics ($n = 21$; 5%). Further details regarding the study cohort are presented in Table 1. The study was conducted in accordance with the modified Declaration of Helsinki.

Bone marrow morphology and peripheral blood count

Bone marrow morphology and peripheral blood count examinations were performed locally at the participating centers and reviewed as described elsewhere.[26] The classification of MDS was done according to the French–American–British (FAB) classification[27] and, if available, the World Health Organization (WHO) classification.[28]

Cytogenetic examinations

Cytogenetic analyses were performed, centrally reviewed and documented as described elsewhere.[26] Patients with missing, incomplete or invalid International System for Human Cytogenetic Nomenclature formula were excluded from the analysis. Results from fluorescent *in situ* hybridization were not included. The mean number of metaphase cells analyzed was 20 (range 2–194). The number of abnormalities per clone was calculated according to international guidelines.[29] MK was classified as defined by Breems *et al.*[10]: either two autosomal monosomies or one autosomal monosomy plus one structural abnormality in one clone. To analyze the impact of monosomies, trisomies and distinct structural abnormalities, a missing chromosome was classified as monosomy, an additional chromosome as trisomy, deletions as structural losses, additions, insertions and duplications as chromosomal gains, and balanced translocations, inversions or derivations as structural neutral. In unbalanced translocations or isochromosomes, the abnormalities were classified according to the resulting abnormalities: in der(1;7)(q10;p10), for example, the abnormality was calculated as a structural loss on chromosome 7 (deletion 7q) and a structural gain on chromosome 1 (trisomy 1q).

Statistical analyses

Statistical analyses were performed using the software SPSS 20.0 (IBM Corporation, Armonk, NY, USA) and Graph Pad Prism 4 (Graph Pad Software Inc., La Jolla, CA, USA). Univariate time-to-event analyses were calculated using the method of Kaplan–Meier.[30] OS was calculated from the time of first diagnosis to death or last contact, AMLFS from the time of diagnosis to AML transformation (as defined by the FAB classification) or last contact without transformation into AML. *P*-values for differences in time-to-event analyses were calculated by the log-rank test.[31] The multivariate analysis was done using a Cox proportional hazard model. In this model, origin of database, gender, age, date of diagnosis, hemoglobin, absolute neutrophil count, platelet count, bone marrow blast count, MK, the number of monosomies, trisomies, structural gains, structural losses, structural neutral abnormalities, the presence of markers and ring chromosomes were included. Differences in categorical variables were calculated using a χ^2 test and differences in continuous variables by means of the analysis of variance test. Two-sided *P*-values <0.05 were considered as significant. In view of the explanatory nature of the study, no adjustment for multiple testing was applied.

Table 1. Patient cohort

Number of patients	*Total* n *(%)* 431 (100.0)	*No MK* n *(%)* 227 (57.7)	*MK* n *(%)* 204 (47.3)	P-*value*
Database				
German-Austrian	185 (42.9)	99 (43.6)	86 (42.2)	0.440
IMRAW	121 (28.1)	57 (25.1)	64 (31.4)	
Spanish	104 (24.1)	57 (25.1)	47 (23.0)	
IWCG	21 (4.9)	14 (6.2)	7 (4.3)	
Gender				
Male	231 (53.6)	115 (50.7)	116 (56.9)	0.117
Female	200 (46.4)	112 (49.3)	88 (43.1)	
Age (years)				
Median	69	69	70	0.264
Range	21–90	21–90	24–89	
WHO classification[a]				
RA/RARS	18 (4.2)	16 (7.0)	2 (1.0)	0.025
RCMD/-RS	50 (11.6)	33 (14.5)	17 (8.3)	
RAEB-1	34 (7.9)	19 (8.4)	15 (7.4)	
RAEB-2	48 (11.1)	21 (9.3)	27 (13.2)	
CMML 1/2	8 (1.9)	6 (2.7)	2 (1.0)	
5q- Syndrome	3 (0.7)	3 (1.3)	0 (0.0)	
AML	20 (4.6)	10 (4.4)	10 (4.9)	
Unclassified	2 (0.5)	1 (0.4)	1 (0.5)	
No WHO classification	248 (57.5)	118 (52.0)	130 (57.5)	
FAB classification				
RA/RARS	158 (36.7)	110 (48.5)	48 (23.5)	<0.0001
RAEB	153 (35.5)	69 (30.4)	84 (41.2)	
RAEB-T	68 (15.8)	15 (6.6)	53 (26.0)	
CMML	33 (7.7)	21 (9.3)	12 (5.9)	
AML	1 (0.2)	1 (0.4)	0 (0.0)	
Unclassified	1 (0.2)	1 (0.4)	0 (0.0)	
No FAB classification	17 (3.9)	10 (4.4)	7 (3.4)	
Bone marrow blasts (%)				
Median	7	4	11	<0.0001
Range	0–30	0–30	0–30	
Cytopenias				
Hb (g/dl) median	9.0	9.5	8.5	0.007
Hb range	1.0–16.0	2.0–16.0	1.0–14.0	
ANC ($10^3/\mu l$) median	2.0	2.0	2.0	0.097
ANC range	0.0–35.0	0.0–35.0	0.0–27.0	
PLT ($10^3/\mu l$) median	93	109	75	0.001
PLT range	4–999	4–978	10–999	
IPSS				
Low	11 (4.0)	11 (8.0)	0 (0.0)	<0.0001
Intermediate-1	71 (25.9)	57 (41.6)	14 (10.2)	
Intermediate-2	89 (32.5)	43 (31.4)	46 (33.6)	
High	103 (37.6)	26 (19.0)	77 (56.2)	
IPSS-R				
Very good	1 (0.4)	1 (0.7)	0 (0.0)	<0.0001
Good	30 (11.1)	29 (21.3)	1 (0.7)	
Intermediate	39 (14.4)	34 (25.0)	5 (3.7)	
Poor	53 (19.6)	33 (24.3)	20 (14.8)	
Very poor	148 (54.6)	39 (28.7)	109 (80.7)	

Abbreviations: AML, acute myeloid leukemia; ANC, absolute neutrophil count; CMML, chronic myelomonocytic leukemia; Hb, hemoglobin; IMRAW, International MDS risk analysis workshop; IPSS, International Prognostic Scoring System; IPSS-R, Revised International Prognostic Scoring System; IWCG, International work group on MDS cytogenetics of the MDS Foundation; FAB, French–American–British; MK, monosomal karyotype; PLT, platelet count; RA, refractory anemia; RARS, refractory anemia with ring sideroblasts; RAEB, refractory anemia with excess of blasts; RAEB-T, refractory anemia with excess of blasts in transformation; WHO, World Health Organization. [a]Only indicated in patients with missing FAB classification.

RESULTS

Patients

In total, 431 patients with at least two clonal abnormalities were analyzed; 231 (54%) were men and 200 (46%) were women.

The median age was 69 years (range 21–90 years). The median year of MDS diagnosis was 1992. Thus, most patients ($n=414$; 96%) were classified according to the FAB classification. Of these, 168 (39%) were additionally classified defined by the WHO system. In 15 (4%) patients, the classification was done by the WHO classification exclusively. According to the IPSS-risk group, 11 (4%) patients were classified as low risk, 71 (26%) as intermediate-1 risk, 89 (33%) as intermediate-2 risk and 103 (38%) as high-risk MDS. In 157 patients (36%), the IPSS classification was not available. In addition, the risk classification according to IPSS-R was calculated. Further details regarding the patient cohort are shown in Table 1.

Incidence of MK and correlation with clinical data

An MK-positive karyotype (MK+) was detected in nearly half of patients ($n=204$; 47%). Patients with MK showed a significantly higher percentage of bone marrow blasts (median 11%) as compared with those without MK (MK–; 4%; $P<0.001$). Accordingly, the distribution of FAB subtypes and IPSS-risk groups was also significantly different (refractory anemia with excess of blast/refractory anemia with excess of blast in transformation 67% in MK+ vs 37% in MK–; $P<0.0001$; intermediate-2/high-risk MDS 90% in MK vs 50% in MK–; $P<0.001$). The hemoglobin level (8.5 g/dl in MK+ vs 9.5 in MK–; $P=0.007$) and platelet count (75×10^3/μl in MK+ vs 109×10^3/μl in MK–; $P=0.001$) were also affected by the presence of MK, while no significant differences were observed in the absolute neutrophil count. Regarding age, gender or origin of database, significant differences were not detected.

Cytogenetic findings

The number of abnormalities per clone (A/C) was calculated in each patient. In total, 175 (41%) patients showed a non-CK with two abnormalities, 60 (14%) a complex abnormal karyotype with three abnormalities, 44 (10%) a complex abnormal karyotype with four abnormalities and 152 (35%) a highly CK with five or more abnormalities (Table 2). The incidence of MK increased with the number of abnormalities. In patients with two abnormalities, 13% were MK+, in complex abnormal patients with 3 abnormalities, 37% were MK+, in patients with 4 abnormalities, 73% were MK+ and in highly complex abnormal karyotypes with ⩾5 abnormalities, 84% showed MK ($P<0.01$). The median number of abnormalities was significantly higher in MK+ patients as compared with those with an MK– karyotype (5 vs 2 abnormalities; $P<0.01$).

MK, defined as one monosomy plus one structural abnormality, was found in only 20 patients (5%) and a combination of two monosomies with no additional abnormalities in 2 patients (0.5%). Furthermore, monosomy 5 and/or monosomy 7, both well-known poor prognostic markers, were observed in 58% of patients with MK and only 5% of patients without MK ($P<0.01$; Table 2). Marker chromosomes or double minutes, both indicative for an unstable clone and also associated with a high number of abnormalities and a poor prognosis, occurred in 44% of MK+ and 13% of MK– patients ($P<0.01$). Monosomy 7 was the most observed monosomy in MK ($n=87$; 43%), followed by -5 (54; 27%), -18 (42; 21%), -17 (33; 16%), -21 (32; 16%), -20 (29; 14%) and -13 (27; 13%). The mean number of abnormalities per clone is >5 in most of these monosomies (Supplementary Figure 5a), reflecting the fact that the majority of monosomies occur in highly complex abnormal karyotypes. An exception was seen in -7, which is also observed in patients with non-CK. This is also the fact in -X and, especially, -Y, but these abnormalities are excluded in the definition of MK. Interestingly, trisomies are more often associated with higher number of abnormalities per clone as compared with monosomies (Supplementary Figure 5b). This highlights the fact that highly complex abnormal karyotypes, which are MK+ in the majority of cases, also include a high number of distinct trisomies. In structural abnormalities, the mean number of abnormalities per clone is lower as compared with monosomies or trisomies (Supplementary Figure 5c). Furthermore, the number of monosomies increases over-proportional with the number of abnormalities per case (Supplementary Figure 5d). In patients with highly complex abnormal karyotypes (⩾5 abnormalities), the mean number is 2.9 for monosomies, 1.1 for trisomies, 0.8 for structural gains, 1.3 for structural losses and 1.3 for structural neutral abnormalities, respectively. This result indicates 2 or more

Table 2. Cytogenetic abnormalities

	Total (n = *431*)	*MK,* n *(%)*		P-*value*
	n *(%)*	*MK–* (n = *227*)	*MK+* (n = *204*)	
Number of abn.				
2 Abn.	175 (40.6)	153 (67.4)	22 (10.8)	<0.01
3 Abn.	60 (13.9)	38 (16.7)	22 (10.8)	
4 Abn.	44 (10.2)	12 (5.3)	32 (15.7)	
⩾5 Abn.	152 (35.3)	24 (10.6)	128 (62.7)	
Involvement of -5/-7	130 (30.2)	12 (5.3)	118 (57.8)	<0.01
Median number of abn./clone (range)	3.0 (2–20)	2.0 (2–20)	5.0 (2–18)	<0.01
Marker/dminutes present	119 (27.6)	30 (13.2)	89 (43.6)	<0.01
Ring chromosomes	10 (2.3)	4 (1.8)	6 (2.9)	NS
IPSS cytogenetic subgr.				
Good	0 (0.0)	0 (0.0)	0 (0.0)	<0.01
Intermediate	144 (33.4)	136 (59.9)	8 (3.9)	
Poor	287 (66.6)	91 (40.1)	196 (96.1)	
IPSS-R cytogenetic subgr.				
Very good	0 (0.0)	0 (0.0)	0 (0.0)	<0.01
Good	45 (10.4)	43 (18.9)	2 (1.0)	
Intermediate	99 (23.0)	93 (41.0)	6 (2.9)	
Poor	91 (21.1)	55 (24.2)	36 (17.6)	
Very poor	196 (45.5)	36 (15.9)	160 (78.4)	
Mean number and range				
Monosomies	1.4 (0–10)	0.2 (0–2)	2.7 (1–10)	<0.01
Trisomies	0.7 (0–14)	0.9 (0–14)	0.4 (0–8)	<0.01
Structural gains	0.5 (0–5)	0.3 (0–5)	0.6 (0–5)	<0.01
Structural losses	1.0 (0–6)	0.9 (0–4)	1.1 (0–6)	<0.05
Structural neutral abn.	0.8 (0–6)	0.7 (0–4)	1.0 (0–6)	<0.01
Monosomy				
-1	7 (1.6)	0 (0.0)	7 (3.4)	<0.01
-2	5 (1.2)	1 (0.4)	4 (2.0)	<0.01
-3	20 (4.6)	0 (0.0)	20 (9.8)	<0.01
-4	18 (4.2)	0 (0.0)	18 (8.8)	<0.01
-5	60 (13.9)	6 (2.6)	54 (26.5)	<0.01
-6	17 (3.9)	1 (0.4)	16 (7.8)	<0.01
-7	93 (21.6)	6 (2.6)	87 (42.6)	<0.01
-8	18 (4.2)	0 (0.0)	18 (8.8)	<0.01
-9	17 (3.9)	0 (0.0)	17 (8.3)	<0.01
-10	10 (2.3)	0 (0.0)	10 (4.9)	<0.01
-11	13 (3.0)	0 (0.0)	13 (6.4)	<0.01
-12	17 (3.9)	0 (0.0)	17 (8.3)	<0.01
-13	29 (6.7)	2 (0.9)	27 (13.2)	<0.01
-14	15 (3.5)	0 (0.0)	15 (7.4)	<0.01
-15	20 (4.6)	0 (0.0)	20 (9.8)	<0.01
-16	16 (3.7)	0 (0.0)	16 (7.8)	<0.01
-17	33 (7.7)	0 (0.0)	33 (16.2)	<0.01
-18	43 (10.0)	1 (0.4)	42 (20.6)	<0.01
-19	14 (3.2)	0 (0.0)	14 (6.9)	<0.01
-20	29 (6.7)	0 (0.0)	29 (14.2)	<0.01
-21	33 (7.7)	1 (0.4)	32 (15.7)	<0.01
-22	14 (3.2)	1 (0.4)	13 (6.4)	<0.01
-X	14 (3.2)	7 (3.1)	7 (3.4)	NS
-Y	35 (8.1)	22 (9.7)	13 (6.4)	NS

Abbreviations: Abn., abnormalities; IPSS, International Prognostic Scoring System; IPSS-R, Revised International Prognostic Scoring System; MK–, monosomal karyotype absent; MK+, monosomal karyotype present; Marker/dminutes, marker chromosomes or double minutes; NS, not significant; subgr., subgroup; NS (>0.05).

monosomies are usually associated with a number of karyotypes with ⩾5 abnormalities (Supplementary Figure 5d).

Results from univariate survival analyses

In total, we observed a clear difference in OS as well as AMLFS in MK+ as compared with MK– (Table 3). The median OS was 24.0 months in MK– vs 6.7 months in MK+ ($P<0.01$), the median time to AML transformation was 22.5 months in MK– and 5.0 months in MK+ ($P<0.01$). However, classifying the patients according to the number of abnormalities per case revealed that the poor impact of MK+ regarding prognosis is only observable in patients with <5 abnormalities (Figure 1a). In highly complex abnormal karyotype with 5 or more abnormalities, the presence of MK was not associated with a poorer prognosis as compared with the absence of MK (Figure 1b). Similar results were observed regarding AMLFS (Figures 1 c and d). Furthermore; the prognosis of MK+ patients is clearly influenced by the number of abnormalities. MK+ patients with 2 abnormalities show a better prognosis as those with 3, 4 or ⩾5 abnormalities. The median OS was 13.4, 8.0, 7.9 and 4.9 months, respectively ($P<0.001$). Finally, MK– patients with ⩾5 abnormalities show a worse prognosis (OS 5.6 months) as MK+ with two abnormalities (13.4 months) or three or four abnormalities (8.0 and 7.9 months). This highlights the fact that MK+ is not necessarily associated with the worst prognosis in MDS patients (Table 3, Figures 2a and b).

Impact of -5/-7 on survival in patients with MK

Monosomy 5 and/or monosomy 7 are the most frequent monosomies in patients with MK. In total, 69% of patients showed

Table 3. Survival in cytogenetic subgroups

	Univariate analysis							
	OS (months)				*AMLFS (months)*			
	n	*Median*	*95% CI*	P-*value*	n	*Median*	*95% CI*	P-*value*
Total								
MK–	225	24.4	19.0–29.8	<0.001	198	91.0	26.0–156.0	<0.001
MK+	197	6.7	5.8–7.6		178	9.0	6.2–11.8	
MK– karyotypes								
MK– and 2 abn.	153	31.3	24.7–37.9	<0.001	133	91.0	–	0.010
MK– and 3 abn.	38	17.4	13.2–21.6		34	33.0	8.8–57.2	
MK– and 4 abn.	12	22.8	7.0–38.6		12	22.0	3.3–40.7	
MK– and ⩾5 abn.	22	5.6	0.8–10.4		19	8.3	4.2–12.4	
MK– without -5/-7	213	24.4	19.7–29.1	NS	187	91.0	13.0–169.0	NS
MK– with -5/-7	12	17.0	12.6–21.4		11	17.2	8.3–26.1	
MK+ karyotypes								
MK+ and 2 abn.	22	13.4	10.6–16.2	<0.001	20	12.0	8.0–16.0	NS
MK+ and 3 abn.	21	8.0	5.2–10.8		21	14.7	0.0–44.0	
MK+ and 4 abn.	31	7.9	4.6–11.2		27	8.2	2.2–14.2	
MK+ and ⩾5 abn.	123	4.9	3.9–5.9		110	7.7	5.7–9.7	
MK+ without -5/-7	85	6.6	4.6–8.6	NS	74	7.7	5.3–10.1	NS
MK+ with -5/-7	112	6.8	5.7–7.9		104	9.7	6.5–12.9	
Category	*OS*				*AMLFS*			
	n = *269 pts. with complete data*				n = *254 pts. with complete data*			
	n	*HR*	*95% CI*	P-*value*	n	*HR*	*95% CI*	P-*value*
Monosomal karyotype	134	1.1	0.6-1.9	NS	129	1.1	0.5-2.8	NS
-5 and/or -7 present	88	1.6	1.1–2.5	0.023	86	1.9	1.0–3.5	NS
No monosomy (Ref.)	108	1.0			102	1.0		
1 Monosomy	78	1.0	0.6–1.7	NS	76	1.0	0.4–2.2	NS
2 Monosomies	26	1.7	0.7–3.8	NS	25	2.3	0.7–7.9	NS
⩾3 monosomies	57	1.4	0.7–3.1	NS	54	1.3	0.4–4.3	NS
No trisomy (Ref.)	164	1.0			155	1.0		
1 Trisomy	73	1.2	0.9–1.7	NS	70	1.4	0.8–2.3	NS
2 Trisomies	16	1.4	0.8–2.6	NS	16	0.8	0.3–2.5	NS
⩾3 Trisomies	16	2.9	1.5–5.5	0.001	16	5.0	2.0–12.2	0.000
No structural gain (Ref.)	186	1.0			176	1.0		
1 Structural gain	65	1.2	0.8–1.7	NS	63	1.5	0.9–2.5	NS
2 Structural gains	12	1.5	0.7–3.3	NS	11	4.0	1.5–10.8	0.006
⩾3 Structural gains	6	0.8	0.3–2.0	NS	7	2.0	0.6–6.7	NS
No structural loss (Ref.)	98	1.0			95	1.0		
1 Structural loss	114	1.0	0.7–1.4	NS	105	1.1	0.6–2.0	NS
2 Structural losses	44	1.0	0.6–1.7	NS	44	1.1	0.5–2.3	NS
⩾3 Structural losses	13	1.1	0.5–2.4	NS	13	1.4	0.4–4.8	NS
No structural neutral abn. (Ref.)	169	1.0			158	1.0		
1 Structural neutral abn.	37	1.4	0.9–2.2	NS	36	1.0	0.5–2.0	NS
2 Structural neutral abn.	46	1.4	0.9–2.1	NS	45	1.4	0.8–2.7	NS
⩾3 Structural neutral abn.	17	1.7	0.9–3.1	NS	18	2.2	1.0–4.9	NS
Marker chromosomes/dmin	79	1.8	1.3–2.5	0.001	77	1.5	0.9–2.5	NS
Ring chromosomes	7	2.6	1.0–6.6	0.042	7	4.7	1.5–15.1	0.009

Abbreviations: Abn., abnormalities; AMLFS, AML-free survival; 95% CI, 95% confidence interval; HR, hazard ratio; MDS, myelodysplastic syndrome; MK–, monosomal karyotype absent; MK+, monosomal karyotype present; NS, not significant; OS, overall survival; Pts., patients; struct., structural.

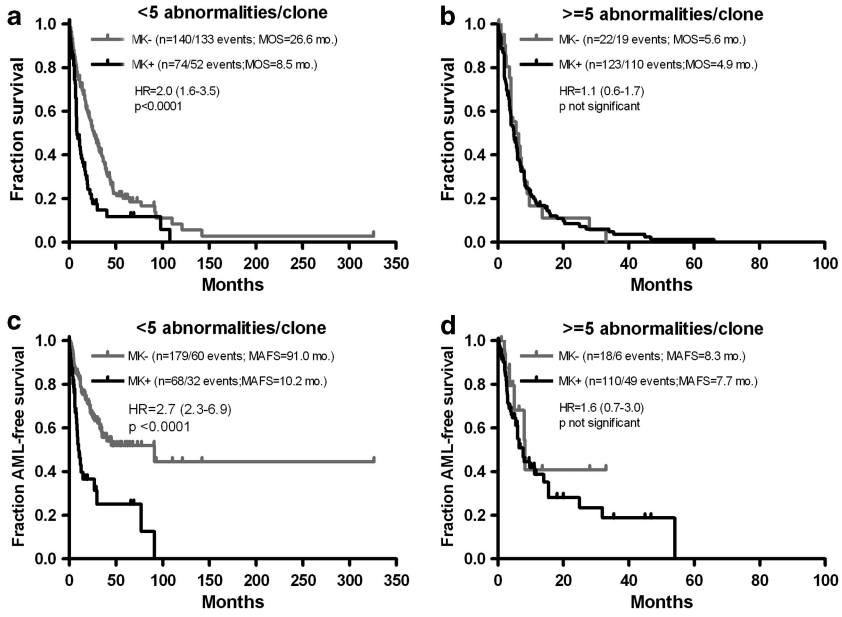

Figure 1. Impact of MK in patients with <5 or $\geqslant 5$ abnormalities per clone regarding OS (**a, b**) and AMLFS (**c, d**). HR, hazard ratio (and 95% confidence interval); MAFS, median AMLFS; mo., months; MOS, median OS.

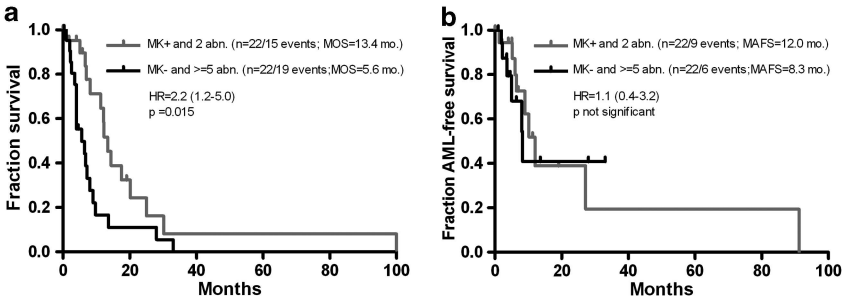

Figure 2. OS (**a**) and AMLFS (**b**) in MK+ patients with 2 abnormalities as compared with MK– patients with highly complex abnormal ($\geqslant 5$) abnormalities. Abn., abnormalities; HR, hazard ratio (and 95% confidence interval); MAFS, median AMLFS; mo., months; MOS, median OS.

one of these abnormalities. The median survival in patients with MK in total did not differ between patients with or without monosomy 5/7 (median OS 6.8 vs 6.6 months, *P*-value not significant; Table 3). However, by separating MK+ patients according to the number of abnormalities per case, it became obvious that the presence of -5/-7 shows a different prognostic value depending on the complexity of the clone. Owing to the fact that nearly all patients with double ($=2$) abnormalities and MK show -5 or -7 (17/22 patients; 77%) all patients with <5 abnormalities were coalesced into one group and compared with patients with $\geqslant 5$ abnormalities in the following analyses. In patients with <5 abnormalities, MK without the involvement of -5/-7 was associated with a better OS as compared with those with -5/-7 (12.2 vs 8.2 months, $P=0.053$), while the presence of -5/-7 became irrelevant in highly CKs with $\geqslant 5$ A/C (4.0 vs 5.0 months, $P=$ not significant; Figures 3a and b). Regarding AMLFS, no significant differences were found (Figures 3c and d).

Results from multivariate survival analyses

In order to analyze the impact of monosomies, trisomies or structural abnormalities on survival, a multivariate analysis was performed. In this analysis, database, age, cytopenias, blast count, the presence of -5 or -7, 3 or more trisomies, the presence of marker chromosomes, and ring chromosomes were associated with a higher risk regarding OS. Concerning AMLFS, database, bone marrow blast count, three or more trisomies, two structural gains and the presence of ring chromosomes was identified as unfavorable. MK was not identified as an independent risk factor for OS or AMLFS (Table 3, Figures 4a and b, Supplementary Table 4). In addition, three or more trisomies were associated with a higher risk (hazard ratio 2.9 for OS) as compared with three or more monosomies (1.4). As mentioned above, three or more trisomies but not monosomies were an independent risk factor for AML transformation (Table 3, Figures 4a and b).

DISCUSSION

The presence of a MK defines an adverse prognostic factor in AML and MDS.[10–18,22,23] However, recent publications addressed some reasonable doubts concerning the clinical value[21,24] and the independent prognostic impact[25] of this karyotype category. Furthermore, no data on primary, untreated MDS patients, determining the effect of MK uninfluenced by disease altering therapy or etiology, has been published to date.

Although MK was developed to identify prognostically adverse subgroups within complex abnormal karyotype abnormalities, its definition allows the diagnosis of MK also in patients with two

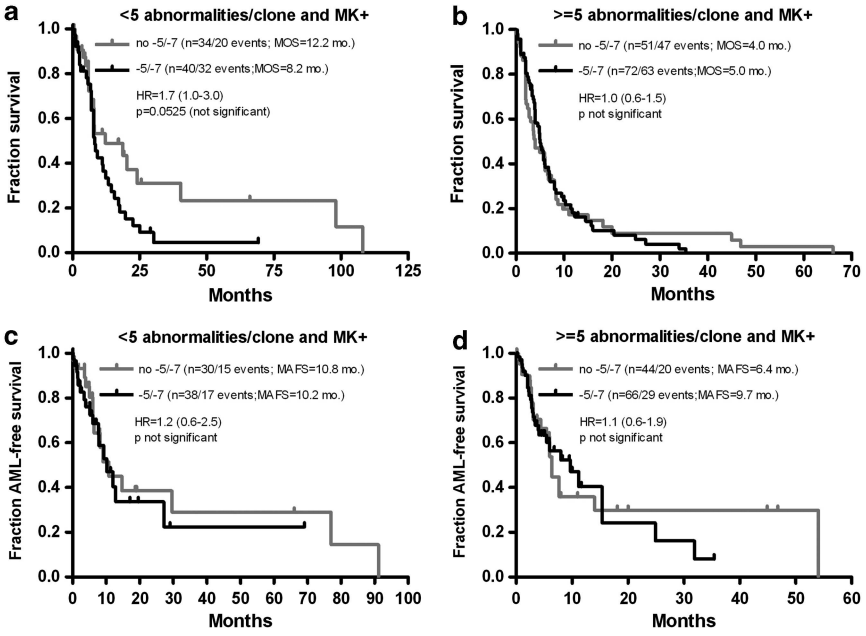

Figure 3. Impact of monosomy 5 and/or 7 in MK+ patients with <5 or ⩾5 abnormalities per clone regarding OS (**a**, **b**) and AMLFS (**c, d**). Abn., abnormalities; HR, hazard ratio (and 95% confidence interval); MAFS, median AMLFS; MOS, median overall survival; no -5/-7, no monosomy 5 and/or 7 present; -5/-7, monosomy 5 and/or 7 present.

abnormalities, which are, by definition, not assignable to complex chromosomal abnormalities. Hence, the prognostic impact of MK in this group was also investigated in the present study. In addition, in order to examine the effect of the number of abnormalities per clone, its interaction with MK was analyzed. The results clearly show that MK is associated with a high number of abnormalities, and the presence of distinct unfavorable cytogenetic abnormalities. Furthermore, the multivariate analyses revealed that MK is not independent from these parameters. Remarkably, the distinction between MK+ and MK– does not add any prognostic information in the group of patients with highly unstable clones. Remarkably, MK mainly occurs in this group: 63% of patients with MK have ⩾5 abnormalities while only 13% of patients with 2 abnormalities show MK. This finding is also underlined by the fact that the median number of abnormalities in MK+ patients is 5.0 as compared with 2.0 abnormalities in non-MK patients. Furthermore, the MK+ patient group is heterogonous and its prognostic impact is strongly influenced by the number of abnormalities. An MK with 2 abnormalities is associated with a significant better prognosis as compared with MK+ and ⩾5 abnormalities (median OS 13.4 vs 4.9 months; $P<0.01$) and associated with a better prognosis as compared with an MK– karyotype with ⩾5 abnormalities (13.4 vs 5.6 months, $P<0.01$; Figure 2). This finding demonstrates that MK is not necessarily the group with the worst prognosis, as found elsewere.[22] Our data support the hypothesis that a high number of complex abnormalities, associated with an unstable clone, define the subgroup with the worst prognosis in MDS, independent of MK. The results from the multivariate analysis underline this by showing that MK is not an independent prognostic factor in MDS. Interestingly, the results from the Cox regression revealed that two monosomies are not prognostically worse as compared with two trisomies or two structural abnormalities. Actually, trisomies, mostly accompanied by structural abnormalities, are often associated with a high number of abnormalities per clone (Supplementary Figure 5b), a shorter survival and a higher risk of AML transformation (Figures 4a and b). This is in accordance with the results from Solé *et al.*,[32] showing that a hyperdiploid karyotype is associated with a very poor prognosis and a 100% 5-year cumulative risk of transformation to AML.

Taken these results together, we conclude that the adverse prognostic impact of MK in MDS is predominantly based on contingencies with other, biologically more conclusively interpretable factors. First, the presence of two monosomies is associated with a high number of abnormalities in the entire clone: patients with at least two monosomies show a mean number of >4 abnormalities in total (Supplementary Figure 5d). Thus, in the case of MK, there is a high probability that these monosomies are part of a highly complex abnormal karyotype. In these patients, the adverse prognosis is well known.[3–5,8] Second, MK detects mainly monosomies 5 and/or 7. This is of prognostic relevance in patients with a non-highly complex abnormal karyotype (Figure 3a). In this group of patients, the poor impact of MK is mainly based on these abnormalities. In the absence of −5/−7, MK is associated with a median OS of 12.2 months, which matches the results from the poor, but not very poor cytogenetic prognostic subgroup of the IPSS-R.[4,26] Owing to the over-representation of highly complex abnormal karyotypes in the MK group, this effect remains undetected if the number of abnormalities per clone is not considered in the analyses. Third, it is well known that monosomy 5, the second most frequent monosomy found in MK, is often not a real monosomy, but is a marker of pronounced clonal instability and is a masked del(5q), or another structurally rearranged chromosome.[33,34] The Supplementary Figure 6 shows an example: the chromosome banding analysis detects a MK including monosomy 5, with a total number of five abnormalities. However, the accompanying multicolor fluorescent *in situ* hybridization uncovered a highly unstable clone with several unbalanced structural rearrangements. Finally, in highly complex abnormal karyotypes abnormalities, the presence or absence of MK is irrelevant. In these patients, clonal instability predicts the prognosis.

We conclude that the number of abnormalities, rather than MK, describes the biological background more precisely. However, the prognostic heterogeneity of complex abnormalities remains a challenge that needs further investigations in subsequent

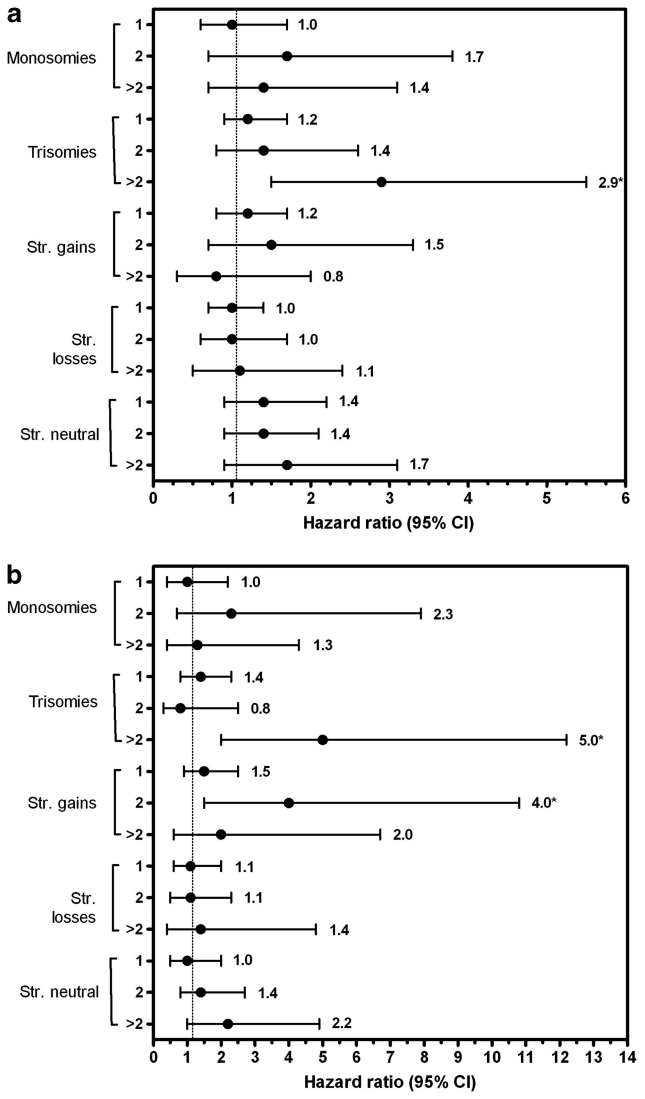

Figure 4. Prognostic impact on OS (**a**) and AML transformation (**b**) according to the number of monosomies, trisomies and distinct structural abnormalities (multivariate analysis). CI, confidence interval; Str., structural; $*P<0.001$ as compared with the reference category (reference was defined as the absence of a monosomy, trisomy or structural abnormality in the respective group).

studies. It is likely that more sophisticated analytical methods will lead to more precise genomic profiling, which has the potential to provide a more refined classification system.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)