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Complex and monosomal karyotype are distinct cytogenetic entities with an adverse prognostic impact in paediatric acute myeloid leukaemia. A NOPHO-DBH-AML study

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Summary

Data on occurrence, genetic characteristics and prognostic impact of complex and monosomal karyotype (CK/MK) in children with acute myeloid leukaemia (AML) are scarce. We studied CK and MK in a large unselected cohort of childhood AML patients diagnosed and treated according to Nordic Society for Paediatric Haematology and Oncology (NOPHO)-AML protocols 1993–2015. In total, 800 patients with *de novo* AML were included. CK was found in 122 (15%) and MK in 41 (5%) patients. CK and MK patients were young (median age 2·1 and 3·3 years, respectively) and frequently had FAB M7 morphology (24% and 22%, respectively). Refractory disease was more common in MK patients (15% vs. 4%) and stem cell transplantation in first complete remission was more frequent (32% vs. 19%) compared with non-CK/non-MK patients. CK showed no association with refractory disease but was an independent predictor of an inferior event-free survival (EFS; hazard ratio [HR] 1·43, $P = 0·03$) and overall survival (OS; HR 1·48, $P = 0·01$). MK was associated with a poor EFS (HR 1·57, $P = 0·03$) but did not show an inferior OS compared to non-MK patients (HR 1·14, $P = 0·62$). In a large paediatric cohort, we characterized AML with non-recurrent abnormal karyotype and unravelled the adverse impact of CK and MK on prognosis.

Keywords: Acute myeloid leukaemia, complex karyotype, monosomal karyotype, refractory disease, paediatrics.

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Approximately 80% of paediatric acute myeloid leukaemia (AML) patients harbour chromosomal abnormalities, and cytogenetics is considered one of the most important prognostic factors in this cohort (de Rooij *et al*, 2015). Although overall (OS) and event-free survival (EFS) rates have increased remarkably during the last 30 years and currently reach almost 70% for OS and 50% for EFS (Lie *et al*, 2005; Abrahamsson *et al*, 2007; Rubnitz *et al*, 2010; Creutzig *et al*, 2012), cure rates remain low in certain cytogenetic subgroups (Lie *et al*, 2005; Harrison *et al*, 2010; von Neuhoff *et al*, 2010; Creutzig *et al*, 2012, 2016). Relapse remains the most frequent cause of treatment failure and the main obstacle for further improvement of prognosis in paediatric AML (Sander *et al*, 2010; Karlsson *et al*, 2017).

A number of recurrent cytogenetic abnormalities are recognized in AML (Arber *et al*, 2016) and the prognostic impact of most of these balanced rearrangements have been established even though some of them are rare in children (Zwaan *et al*, 2015). The predictive influence of a broad spectrum of non-recurrent aberrations has not been resolved, and thus current algorithms of risk stratification may fail to identify high-risk disease entities among a large proportion of patients.

AML with numerous aberrations, designated as complex karyotype (CK), is associated with a poor prognosis in adults (Mrózek, 2008). CK is predominated by aberrations of chromosomal imbalances and is frequently detected in AML arising from myelodysplasia (Miesner *et al*, 2010) or secondary to antecedent cytotoxic therapy (Schoch *et al*, 2004; Kayser *et al*, 2011). Previous studies have used various definitions of CK and do not uniformly establish CK as an entity with an adverse outcome in children (Harrison *et al*, 2010; von Neuhoff *et al*, 2010). The prognostic contribution to outcome may vary between cytogenetic subgroups in patients with multiple non-recurrent aberrations and recently, the specific event of chromosome loss yielding a monosomal karyotype (MK) has been associated with an unfavourable outcome among adults (Breems *et al*, 2008; Medeiros *et al*, 2010; Perrot *et al*, 2011; Haferlach *et al*, 2012; Kayser *et al*, 2012; Voutiadou *et al*, 2013; Weinberg *et al*, 2014). MK is often associated with other unfavourable risk cytogenetics, such as inv(3), -5/del(5q), -7/del(7q), and rarely combined with *FLT3*-internal tandem duplication (ITD) or *NPM1* mutations (Kayser *et al*, 2012; Weinberg *et al*, 2014). The negative influence on prognosis has been identified in AML patients with MK independently of the co-occurrence of CK (Breems *et al*, 2008; Medeiros *et al*, 2010; Fang *et al*, 2011; Haferlach *et al*, 2012; Kayser *et al*, 2012; Weinberg *et al*, 2014) and may be less pronounced in younger patients (Breems *et al*, 2008; Kayser *et al*, 2012; Manola *et al*, 2013; Weinberg *et al*, 2014). A study of MK in children with AML by Manola *et al* (2013) indicated an adverse prognosis with OS of 52%, but included only 15 cases with MK. Another small study (Lee *et al*, 2016) found no dismal outcome among 10 children with MK and concomitant CK. The spectrum of genetic abnormalities in AML is highly age-dependent (Creutzig *et al*, 2016) and the adverse prognosis of CK and MK evident in adults may not mirror paediatric AML populations. To our knowledge, a recent study by Rasche *et al* (2017) is the only other study which has investigated CK and MK in a large cohort of paediatric AML (642 children with 22 cases of MK). They showed that MK was a strong and independent predictor of a dismal outcome. However, this study included both patients with core-binding factor (CBF) AML and normal karyotype in the comparison group, which may distort the interpretation of the influence of MK among children with AML.

The aim of the present study was to elucidate the occurrence, genetic characteristics and prognostic impact of CK and MK at diagnosis in a large paediatric cohort of *de novo* AML.

Methods

Patients

The Nordic Society for Paediatric Haematology and Oncology (NOPHO) is a collaboration between the Nordic countries (Sweden, Denmark, Norway, Finland and Iceland). All children with AML in the Nordic countries are diagnosed, treated and subsequently followed in accordance with the same protocol. Hong Kong, Estonia and Latvia joined the

NOPHO-AML 2004 protocol. The Netherlands and Belgium joined a modified version of the 2004 protocol. All countries joined the NOPHO-DBH (Dutch-Belgian-Holland) AML 2012 protocol. Data on clinical presentation, genetics, morphology, treatment and outcome are registered in the NOPHO AML database.

This study included children up to 18 years of age diagnosed with *de novo* AML in the Nordic countries, Estonia, Latvia, Hong Kong, The Netherlands and Belgium and treated according to one of three consecutive protocols between 1993 and 2015 (NOPHO-AML 93, NOPHO-AML 2004, DB AML-01 and NOPHO-DBH AML 2012). Only patients with a complete G-band karyotype at diagnosis were included.

Patients with myeloid leukaemia of Down syndrome, acute promyelocytic leukaemia, juvenile myelomonocytic leukaemia, AML secondary to bone marrow failure syndromes or therapy-related AML were excluded.

Cytogenetics and classification

Classic metaphase karyotyping was performed according to standard protocols and registered in the NOPHO AML database. The karyotypes were described and reviewed according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013 (Shaffer *et al*, 2013). Results from interphase fluorescence *in situ* hybridization (FISH) and molecular diagnostic tests were also included. Chromosomal gains or structural aberrations detected in at least two metaphases and chromosomal losses detected in three metaphases were considered as clonal. Constitutional karyotypes were not considered as aberrant. Karyotypes have been centrally reviewed by the NOPHO cytogenetic working groups since 1995 for Sweden and since 2000 for the rest of the Nordic countries.

MK was defined as two or more distinct autosomal chromosome monosomies or one single autosomal monosomy in association with at least one structural chromosomal aberration and in the absence of CBF translocations (Breems *et al*, 2008; Kayser *et al*, 2012; Stölzel *et al*, 2016). In tri- and tetraploid karyotypes MK was defined after loss of two or more autosomal chromosomes or a single relative monosomy with at least one structural aberration in the absence of CBF translocations considered relative to the pure ploidy. Subclonality was not an exclusion criterion.

CK was defined as at least three unrelated cytogenetic aberrations in the absence of recurrent genetic abnormalities of AML, as defined by the WHO (Arber *et al*, 2016). Recurrent genetic abnormalities include t(8;21)(q22;q22), inv(16)(p13q22)/t(16;16)(p13;q22), t(9;11)(p21;q23), t(6;9)(p22;q34), inv(3)(q21q26)/t(3;3)(q21;q26) and t(1;22)(p13;q13). In tri- and tetraploid karyotypes the pure ploidy change was considered as a single abnormality. If there was an additional loss or gain of a chromosome it was considered as a single additional abnormality.

In addition, we defined a category of revised MK (MK-R), which included all MK with the exception of those with t(9;11), t(6;9), inv(3)/t(3;3) and t(1;22).

Statistics

To test the significance of differences between groups, the chi-square (χ^2) test was applied when appropriate. The Mann-Whitney U test was used for testing continuous variables. EFS and OS were estimated using the Kaplan-Meier method, and the log rank test was used for the comparison of survival distribution. Events were defined as induction failure, induction death, death in complete remission, refractory disease, relapse or secondary malignancy. OS was defined as time from diagnosis to death from any cause or to last follow-up. In calculations of cumulative incidence of relapse (CIR), death was considered a competing event and Gray's test was applied for comparison of cumulative incidence functions. In compliance with previous studies (Breems *et al*, 2008; Haferlach *et al*, 2012; Kayser *et al*, 2012), patients presenting either normal karyotype or CBF aberrations, including inv(16)/t(16;16) and t(8;21), were not included in the survival analysis, as the favourable prognostic influence of the latter independent from additional chromosomal abnormalities [e.g. sex chromosome loss (Klein *et al*, 2015) or del7q (Hasle, 2014)] will skew the differences in survival, and thus overestimate the

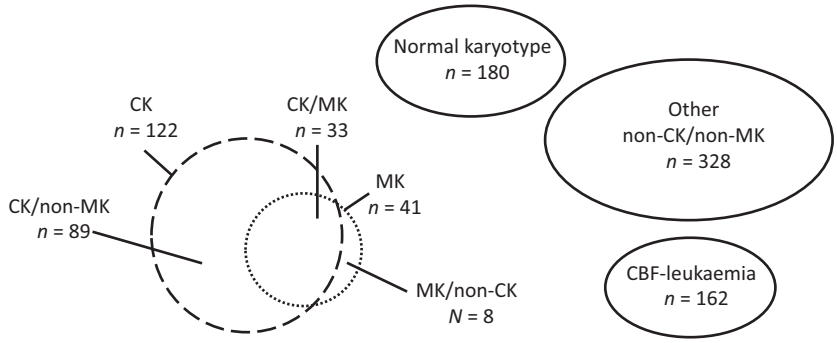

Fig 1. Distribution of the 800 patients according to karyotype classification. CBF, core binding factor; CK, complex karyotype; MK, monosomal karyotype.

Table I. Baseline characteristics according to monosomal and complex karyotype.

	Non-CK/non-MK		CK/non-MK		MK	
	N	%	*N*	%	*N*	%
Patients, *n*	670		89		41	
Sex (male/female)	(365/305)	54/46	(43/46)	48/52	(16/25)	39/61
Median age (range), years	7·5 (0–18)		2·1 (0–17)*		3·3 (0–17)	
0–1 years	145	22	41*	46	14	34
2–9 years	262	39	34	38	13	32
10–18 years	263	39	14*	16	14	34
Median WBC count (range), $\times 10^9$/l	21 (1–567)		20 (1–687)		12 (1–193)	
CNS involvement (786 tested)	48	7	12	13	2	5
FAB classification						
M0	27	4	6	7	5*	12
M1	89	13	9	10	0*	0
M2	173	26	7*	8	2*	5
M4	142	21	8*	9	6	15
M5	141	21	23	26	12	29
M6	13	2	2	2	1	2
M7	27	4	21*	24	9*	22
Unclassified	21	3	5	6	3	7
Data missing	37	6	8	9	3	7
Cytogenetics						
Normal karyotype	180	27	0	0	0	0
t(8;21)	98	15	0	0	0	0
inv(16)	64	10	0	0	0	0
t(9;11)	77	11	0	0	2	5
Other *KMT2A* rearrangements	71	11	26*	29	11*	27
FLT3-ITD (505 tested)	64	10	0	0	1	2
NPM1 mutated (457 tested)	34	5	0	0	0	0
Stem cell transplantation						
SCT CR1	127	19	13	15	13*	32
SCT CR2	155	23	14	16	8	20
SCT CR1+ CR2	6	1	1	1	1	2
Protocol						
NOPHO-AML 1993	234	35	26	29	19	46
NOPHO-AML 2004	342	51	56	63	17	41
NOPHO-DBH AML 2012	94	14	7	8	5	12
Events						
No events	368	55	41	46	15	37
Induction failure, induction death or death in CR	42	6	7	8	2	5
Refractory disease	24	4	3	3	6*	15
Relapse	229	34	36	40	18	44
Secondary malignancy	7	1	2	2	0	0
Outcome % [SE]						
5-year EFS	50 [3]		46 [6]		34 [8]*	
5-year OS	69 [3]		59 [5]		64 [8]	

AML, acute myeloid leukaemia; CK, complex karyotype; CNS, central nervous system; CR, complete remission; DBH, Dutch-Belgian-Holland; EFS, event-free survival; FAB, French-American-British; *FLT3*, Fms-like tyrosine kinase 3; ITD, internal tandem duplication; MK, monosomal karyotype; NOPHO, Nordic Society for Paediatric Haematology and Oncology; *NPM1*, nucleophosmin 1; OS, overall survival; SCT, stem cell transplantation; SE, standard error; WBC, white blood cell.

*$P < 0{\cdot}05$ compared with the reference group (Non-CK/non-MK).

adverse influence of MK on prognosis.Refractory disease was defined as no remission (blasts ≥5%) after two induction courses. Relapse was defined as ≥5% blasts in blood or bone marrow blasts or development of extramedullary disease.

For multivariate analysis, the Cox proportional-hazard regression model was applied including the parameters of sex, age, white blood cell (WBC) count, MK and *KMT2A*-rearrangements other than t(9;11) as covariates.

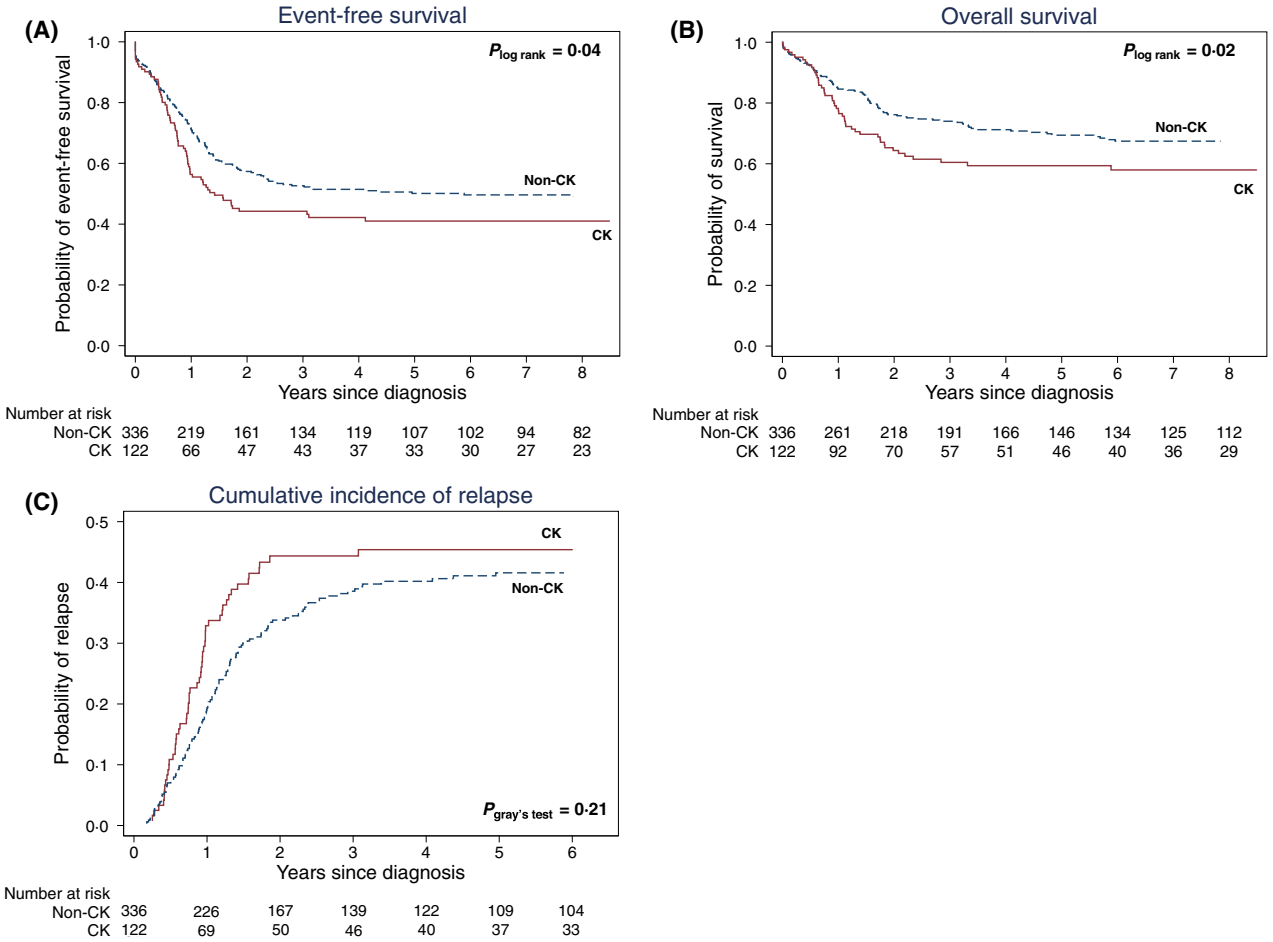

Fig 2. (A) Probability of event-free survival for patients with CK and non-CK. (B) Probability of overall survival for patients with CK and non-CK. (C) Cumulative incidence of relapse for patients with CK and non-CK. CK, complex karyotype; non-CK, non-complex karyotype. [Colour figure can be viewed at wileyonlinelibrary.com]

P-values were two-sided, and values less than 0·05 were considered statistically significant. Stata/IC Version 13 (StataCorp, College Station, TX, USA) was used to perform the statistical analysis.

Results

Clinical characteristics

In total, 853 paediatric AML patients met our inclusion criteria, 53 (6%) of which were excluded owing to missing or uninformative cytogenetic data. More karyotypes were considered non-informative than previously reported (Sandahl *et al*, 2014) due to higher standards for accuracy.

Figure 1 shows the distribution of patients according to karyotype and indicates that MK and CK overlap extensively. Of the 800 eligible patients, 41 patients (5%) had MK and 122 (15%) patients had a karyotype defined as complex. Eight patients (1%) had solely MK (MK/non-CK) and 89 (11%) patients showed CK in the absence of MK (CK/non-MK). The majority, of patients with MK (n = 33, 80%) also had complex karyotype (CK/MK). In total, 670 (84%) showed neither MK nor CK (non-CK/non-MK). Clinical characteristics for patients with MK, CK/non-MK and non-CK/non-MK are presented in Table I. Patients with CBF AML and normal karyotype were included in Table I, but excluded from the survival analysis.

Cytogenetic abnormalities were detected in 620 patients (78%). The most common recurrent aberrations involved 11q23 (n = 187, 23% of the total cohort) followed by t(8;21) (n = 98, 12%). *KMT2A* rearrangements other than t(9;11) were frequently part of CK (29% vs. 11%, $P < 0·001$) or part of MK (27% vs. 11%, $P = 0·002$).

FLT3-ITD and *NPM1* mutations were uncommon among both MK and CK. Only one case (2%) with *FLT3*-ITD was found in the MK group vs. 64 (10%) in the non-CK/non-MK group.

Patients with CK/non-MK were significantly younger than patients without either CK or MK (median age 2·1 vs. 7·5, $P < 0·001$). In particular the CK/non-MK group included more young children age 0–1 year in compared with non-CK/non-MK (46% vs. 22%, $P < 0·001$) and less patients aged

10–18 years (15% vs. 39%, $P < 0{\cdot}001$). Age distribution among patients with MK did not differ significantly either from CK/non-MK patients (median age 3·3 vs. 2·1) or non-CK/non-MK patients (median age 3·3 vs. 7·5).

No significant difference was found in sex distribution between the groups. However, the MK group had a female preponderance and the non-CK/non-MK group a male preponderance (male/female ratio: 0·63 vs. 1·2, $P = 0{\cdot}054$).

Among both CK/non-MK and MK patients, the French-American-British (FAB) type M7 dominated compared with non-CK/non-MK patients, constituting 24% and 22% vs. 4%, $P < 0{\cdot}001$, while FAB M2 was underrepresented with 8% among CK/non-MK and 5% among MK compared with 26% among non-CK/non-MK ($P < 0{\cdot}001$ and $P = 0{\cdot}003$, respectively). Additionally, more FAB M0 patients were seen among MK patients than among the non-CK/non-MK patients (12% vs. 4%, $P = 0{\cdot}014$) and no MK patients showed M1 morphology (0% vs. 13%, $P = 0{\cdot}013$). FAB M4 was underrepresented in the CK/non-MK group compared with the non-CK/non-MK group (9% vs. 21%, $P = 0{\cdot}007$).

The distribution of FAB subtypes in the CK/non-MK group compared with the MK group was quite similar except for M1, which was only observed with CK/non-MK (10% vs. 0%, $P = 0{\cdot}035$).

MK was associated with refractory disease compared with non-CK/non-MK patients (15% vs. 4%, $P = 0{\cdot}001$) and CK/non-MK patients (15% vs. 3%, $P = 0{\cdot}019$). For all groups, relapse was the most frequent event (Table I).

MK patients more often received stem cell transplantation (SCT) in first complete remission (CR1) compared with other patients. MK vs. non-CK/non-MK (32% vs. 19%, $P = 0{\cdot}046$); MK vs CK/non-MK (32% vs. 15%, $P = 0{\cdot}024$) (Table I). For detailed information regarding karyotypes see Tables SI, SII and SIII.

Survival analysis

Median time of follow-up for patients alive was 7 years (range: 0–22).

The 5-year EFS was significantly inferior in CK (41%; 95% confidence interval [CI] 32–50%) compared with non-CK patients (50%; CI 44–56%, $P = 0{\cdot}04$). Patients with CK showed a lower 5-year OS compared with non-CK (59%; CI 50–68% vs. 69%; CI 64–74%, $P = 0{\cdot}02$, Fig 2B). The CIR at 5 years was 45%; CI 36–55% for patients with CK and 41%; CI 36–47% in non-CK patients, $P = 0{\cdot}21$. Among CK patients with five or more aberrations ($n = 56$) the 5-year OS was higher compared with CK patients with three or four aberrations ($n = 66$) (65%; CI 50–76% vs. 55%; CI 42–66%, $P = 0{\cdot}047$). No difference in 5-year EFS was observed between the groups (≥5 aberrations: 42%; CI 29–55% vs. 3–4 aberrations: 40%; CI 28–52%, $P = 0{\cdot}60$) (data not shown).

Table II. Results of Cox regression analysis according to (A) complex karyotype and (B) monosomal karyotype.

A	CK	Non-CK
EFS		
5-year EFS (95% CI)	41% (32–50%)	50% (44–56%)
Crude HR (95% CI)	1·34 (1·0–1·78)*	1
Adjusted HR (95% CI)†	1·43 (1·0–1·97)*	1
OS		
5-year OS (95% CI)	59% (50–68%)	69% (64–74%)
Crude HR (95% CI)	1·48 (1·1–2·1)*	1
Adjusted HR (95% CI)†	1·71 (1·2–2·5)*	1
Relapse risk		
5-year cumulative incidence (95% CI)	45% (36–55%)	41% (36–47%)
Crude HR (95% CI)	1·27 (0·92–1·75)	1
Adjusted HR (95% CI)†	1·41 (0·98–2·01)	1

B	MK	Non-MK
EFS		
5-year EFS (95% CI)	34% (20–49%)	49% (44–54%)
Crude HR (95% CI)	1·59 (1·06–2·40)*	1
Adjusted HR (95% CI)‡	1·57 (1·04–2·37)*	1
OS		
5-year OS (95% CI)	64% (47–77%)	67% (62–71%)
Crude HR (95% CI)	1·18 (0·70–1·99)	1
Adjusted HR (95% CI)‡	1·14 (0·68–1·93)	1
Relapse risk		
5-year cumulative incidence (95% CI)	46% (30–62%)	42% (37–47%)
Crude HR (95% CI)	1·22 (0·75–1·98)	1
Adjusted HR (95% CI)‡	1·16 (0·71–1·90)	1

95% CI, 95% confidence interval; CK, complex karyotype; EFS, event-free survival; HR, Hazard ratio; MK, monosomal karyotype; OS, overall survival.

*$P < 0{\cdot}05$.

†Adjusted for sex, age and *KMT2A* rearrangements other than t(9;11), white blood cell count and MK.

‡Adjusted for sex, age and *KMT2A* rearrangements other than t(9;11) and white blood cell count.

In a multivariate analysis with sex, age, WBC, MK and *KMT2A* rearrangements other than t(9;11) included as covariates, CK was a predictor of inferior EFS (HR 1·43; CI 1·0–1·97; $P = 0{\cdot}03$) and OS (HR 1·71; CI 1·2–2·5, $P = 0{\cdot}01$). The adjusted HR in risk of relapse was 1·41 (CI 0·98–2·01; $P = 0{\cdot}07$). Estimates of crude and adjusted HR are presented in Table IIA.

Outcome in the three groups (non-CK/non-MK; CK/non-MK; MK) was dichotomously compared and EFS and OS are presented in Fig 3. Due to the small number ($n = 8$), survival in patients with MK/non-CK was not evaluated separately. Four out of 8 patients experienced an event (2 with resistant disease, 2 with relapse) and six patients were alive at the end of follow-up.

Patients with CK/non-MK had a significantly lower 5-year OS compared with non-CK/non-MK (58%; CI 47–68% vs.

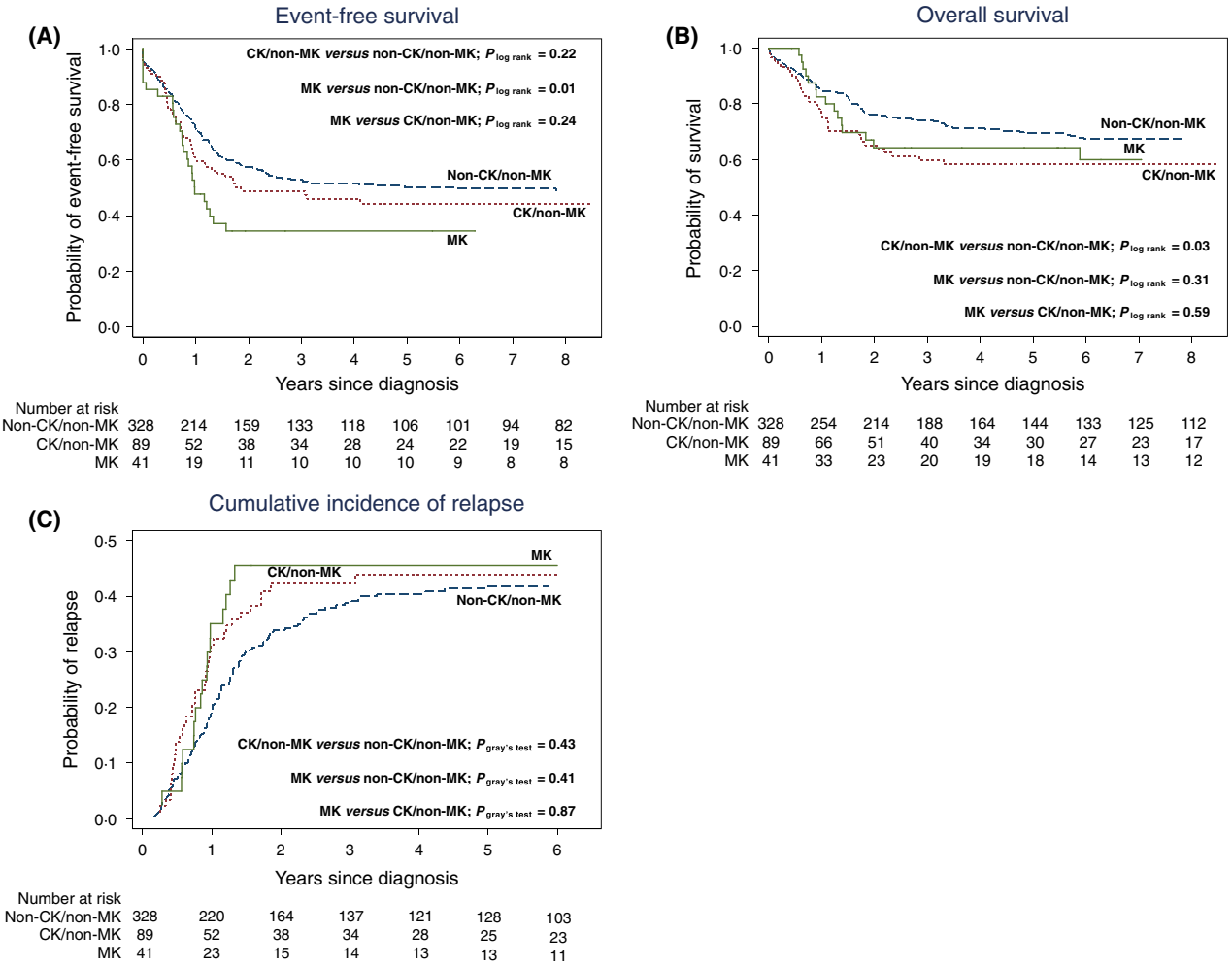

Fig 3. (A) Probability of event-free survival for patients with non-CK/non-MK, CK/non-MK and MK. (B) Probability of overall survival for patients with non-CK/non-MK, CK/non-MK and MK. (C) Cumulative incidence of relapse for patients with non-CK/non-MK, CK/non-MK and MK. CK, complex karyotype; MK, monosomal karyotype; non-CK, non-complex karyotype; non-MK, non-monosomal karyotype. [Colour figure can be viewed at wileyonlinelibrary.com]

69%; CI 64–74%, $P = 0{\cdot}03$), but no difference in OS was shown for MK compared with non-CK/non-MK (64%; CI 47–77% vs. 69%; CI 64–74%, $P = 0{\cdot}31$) or MK compared with CK/non-MK (64%; CI 47–77% vs. 58%; CI 47–68%, $P = 0{\cdot}59$). Patients with MK had a lower 5-year EFS compared with patients with non-CK/non-MK (34%; CI 20–49% vs. 50%; CI 44–56%, $P = 0{\cdot}01$) (Fig 3A). No significant difference was shown when CK/non-MK was compared to the non-CK/non-MK group (44%; CI 33–55% vs. 50%; CI 44–56%, $P = 0{\cdot}22$) or to the MK group (44%; CI 33–55% vs. 34%; CI 20–49%, $P = 0{\cdot}24$).

After exclusion of patients with CBF leukaemia and normal karyotype, refractory disease remained more frequent among patients with MK ($P = 0{\cdot}001$). Patients with MK had a lower 5-year EFS compared to patients without MK (34%; CI 20–49% vs. 49%; CI 44–54%, $P = 0{\cdot}03$). The 5-year OS among patients with MK was similar to patients without MK (64%; CI 47–77% vs. 67%; CI 62–71%, $P = 0{\cdot}52$). The CIR at 5 years was 46%; CI 30–62% for patients with MK and 42%; CI 37–47% in non-MK patients, $P = 0{\cdot}49$. The survival results are presented in Fig 4.

The adjusted HR in EFS for MK compared with non-MK patients was 1·57 (CI 1·04–1·37, $P = 0{\cdot}03$) and in OS 1·14 (CI 0·68–1·93, $P = 0{\cdot}62$). The adjusted HR in risk of relapse was 1·16 (CI 0·71–1·90, $P = 0{\cdot}56$). Estimates of crude and adjusted HR are presented in Table IIB.

Monosomy 7 was present in 11 patients, loss of chromosome 5, 10, 11 and 13 in 6 patients each, loss of chromosome 12 in 5 patients, other chromosomes were each lost in less than 5 patients. Survival for patients with loss of chromosome 7 in MK did not differ from other patients with MK.

No significant differences in either EFS or OS between MK-R ($n = 23$) *versus* MK other ($n = 18$) were observed (data not shown).

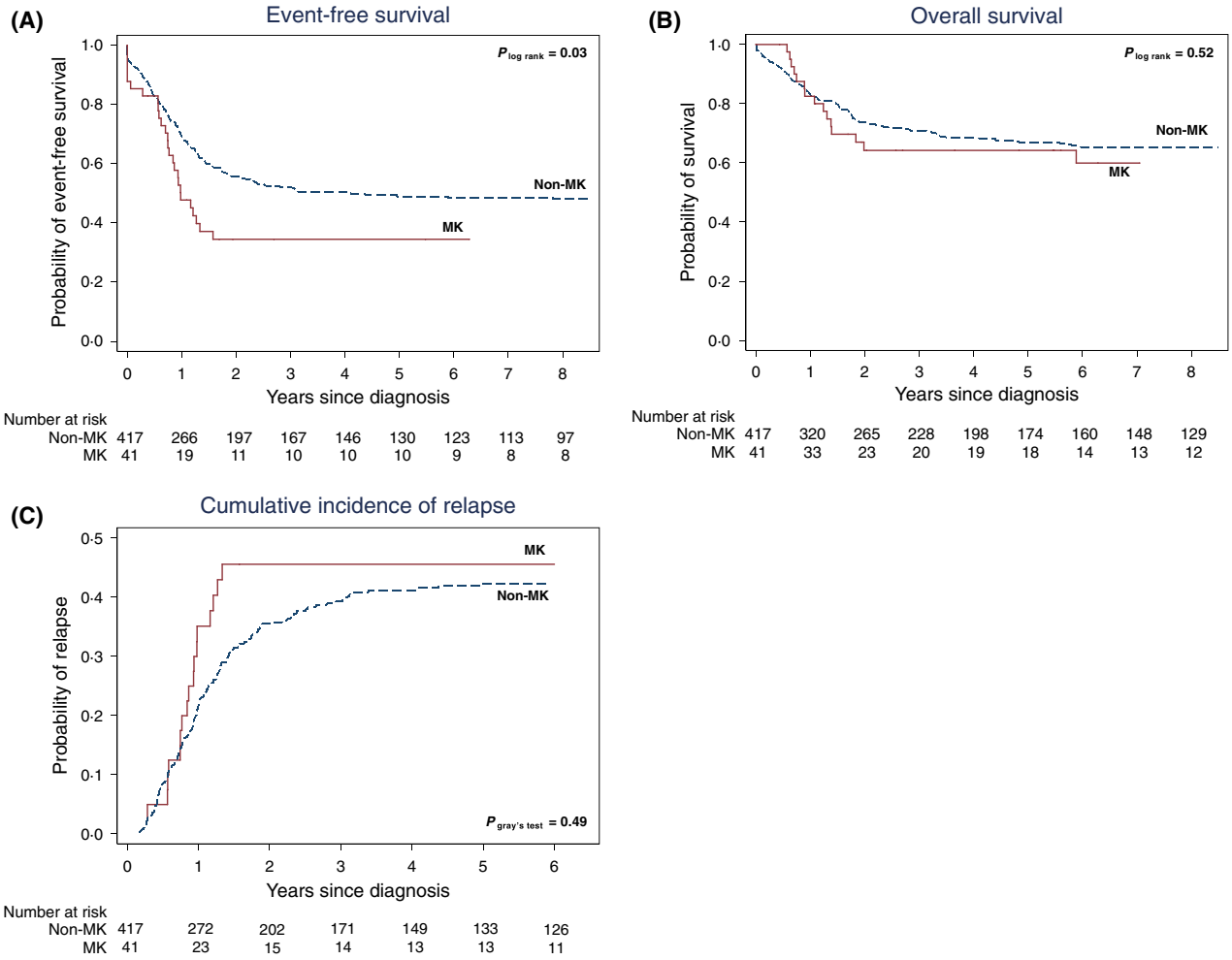

Fig 4. (A) Probability of event-free survival for patients with MK and non-MK. (B) Probability of overall survival for patients with MK and non-MK. (C) Cumulative incidence of relapse for patients with MK and non-MK. MK, monosomal karyotype; non-MK, non-monosomal karyotype. [Colour figure can be viewed at wileyonlinelibrary.com]

Discussion

Our study was initiated to investigate the occurrence, clinical characteristics and prognostic impact of CK and MK among children diagnosed with *de novo* AML. This was undertaken on a large cohort of 800 patients with eligible cytogenetic information. The size of this study exceeds other large studies on cytogenetics in children with AML, e.g. Harrison *et al* (2010) and von Neuhoff *et al* (2010), which included 729 and 454 children, respectively.

In the current cohort we found 41 (5%) patients with MK. All patients except 8 (1%) also had CK. Clinically, MK patients were younger (median age 3·3 years) and with a female preponderance. They were morphologically associated with M7 as well as the CK/non-MK patients and there were no cases of M1. Only one MK case had *FLT3*-ITD and no *NPM1* mutations were detected. MK patients more often received SCT in CR1 compared with other patients (P = 0·046), which confirmed the results reported by Lee *et al* (2016).

The CK-group constituted 122 (15%) patients of the total cohort, of which 89 (73%) did not have MK. CK was clinically associated with young age (median age 2·1 years). Furthermore, there was an association with FAB M7, which could be explained by the known correlation between M7 and young age (Webb *et al*, 2001; Manola *et al*, 2013). Consistent with previous findings in adult AML populations (Grimwade *et al*, 1998; Slovak *et al*, 2000; Byrd *et al*, 2002; Haferlach *et al*, 2012), CK predicted an adverse prognosis in the present study and showed inferior EFS and OS compared to non-CK patients. The poor EFS associated with CK was mitigated by the omission of patients with MK and corresponds to the observations in the recent report from the AML-Berlin-Frankfürt-Muenster (BFM) 2004 trial (Rasche *et al*, 2017). Taken together, our data suggest that, in patients with multiple non-recurrent chromosomal abnormalities, the event of chromosome loss in particular heralds an increased risk of early events, such as primary refractoriness to chemotherapy.

The 5-year OS was higher among patients with five or more aberrations in CK compared with patients with three or four aberrations (65%; CI 50–76% vs. 55%; CI 42–66%, $P = 0{\cdot}047$). This is in line with previous studies showing that ≥4 aberrations did not adversely affect outcome (Harrison *et al*, 2010). A possible explanation for this finding might be that high hyperdiploidy could have happened as one event in a single abnormal mitosis, which has been suggested as a mechanism in childhood ALL (Paulsson *et al*, 2005), and not as a series of cumulative events albeit still included in the group of 5 or more aberrations. Hyperdiploidy is not of prognostic significance in childhood AML (Sandahl *et al*, 2014).

Our study distinguishes MK as an independent predictor of inferior EFS but, in contrast to the findings by Rasche *et al* (2017), the high proportion of events did not translate into a poor OS. This discrepancy may be explained by the fact that the comparison group of the current study did not include patients with CBF abnormalities who, according to previous reports, fare exceedingly well after relapse (Kaspers *et al*, 2013; Karlsson *et al*, 2017).

It has been proposed that SCT increases OS among adults with MK (Fang *et al*, 2011) and this finding may also mirror the childhood setting. No patients received SCT based on adverse morphology alone (M7) or monosomy 7 or 5 in any of the NOPHO-AML protocols. The poor prognosis of refractory disease may be overcome by intensive timing of induction and early SCT (Wareham *et al*, 2013). These data do not allow any conclusion on whether SCT is of benefit in those patients with MK and a favourable response to induction.

Unlike previous studies (Breems *et al*, 2008; Rasche *et al*, 2017), we included marker and ring chromosomes as individual aberrations in both CK and MK, as it has been shown that extensive cytogenetic tests, such as spectral karyotyping, can identify marker chromosomes (Kerndrup & Kjeldsen, 2001). We only found marker chromosomes in 38 patients (5%), which indicates a high quality of the cytogenetic tests used.

The pathogenesis of MK is unknown. However, it is known that AML with MK shares some clinical characteristics with myelodysplastic syndrome (MDS), including low WBC count and poor response to induction chemotherapy, which suggests similar pathogenetic features with MDS rather than with AML without MK.

Our study was based on the NOPHO-AML registry, which facilitated the collection of a large group of unselected, uniformly treated patients compared to similar studies (Manola *et al*, 2013; Lee *et al*, 2016). However, only 41 patients with MK were found and, out of these, only 8 presented MK without CK. Due to the small number of patients it was not possible to identify if the poorer prognosis was associated with the loss of specific chromosomes.

In conclusion, this study characterizes in detail the biological and clinical features of CK and MK and reports the adverse prognostic impact of these cytogenetic entities among paediatric AML patients with non-CBF abnormal karyotype. CK and MK were associated with young age and FAB M7 morphology. MK patients frequently suffered from refractory disease but the poor prognostic impact on EFS seemed to be partly overcome by the use of SCT. Most current treatment protocols employ treatment response to guide the intensity of post-induction therapy and accurate risk-stratification of CK and MK patients with an inadequate treatment response may further improve the prognosis in these patients.

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Authorship contributions

N.B., H.H., K.L.J-D. and J.D.S. designed the study and wrote the manuscript. N.B., H.H., K.L.J-D., J.D.S. and E.K. analysed and interpreted data. J.A., B.B., E.S.J.M.de B., S-Y.H., K.J., Ó.G.J., G.L.K., Z.K., B.L., B.De M., U.N-N., J.P., K.S. and B.Z. contributed data. All authors critically reviewed the paper and approved the final version.

Conflict of interest

The authors have no competing interests.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Karyotypes of the 8 Cases with MK/non-CK.

Table SII. Karyotypes of the 33 Cases with CK/MK.

Table SIII. Karyotypes of the 89 Cases with CK/non-MK.

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