Complex karyotype newly defined: the strongest prognostic factor in advanced childhood myelodysplastic syndrome

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Running head: Complex karyotype in childhood MDS

Disclaimers: none
Abstract

To identify cytogenetic risk factors predicting outcome in children with advanced myelodysplastic syndrome (MDS), overall survival (OS) of 192 children prospectively enrolled in EWOG-MDS studies was evaluated with regard to karyotypic complexity. Structurally complex constitutes a new definition of complex karyotype characterized by ≥3 chromosomal aberrations including at least one structural aberration. Five-year OS in patients with ≥3 clonal aberrations which were not structurally complex did not differ from that observed in patients with normal karyotype. Cox regression analysis revealed the presence of a monosomal and structurally complex karyotype to be strongly associated with poor prognosis (Hazard Ratio, HR 4.6, P<0.01). Notably, a structurally complex karyotype without a monosomy was associated with a very short 2-year OS probability of only 14% (HR 14.5; P<0.01). The presence of a structurally complex karyotype was the strongest independent prognostic marker predicting poor outcome in children with advanced MDS.
**Introduction**

Karyotypic complexity has been reported to be associated with a poor prognosis in myeloid neoplasia \(^1\text{-}^3\). However, the definition of a complex karyotype remains a matter of debate. Most studies defined a complex karyotype as \(\geq 3\) independent abnormalities \(^2\text{-}^6\). In the MRC AML10 trial, \(\geq 5\) independent abnormalities were required \(^3\), since AML patients with \(\geq 5\) abnormalities had a significantly worse 5-year OS than those with 3 or 4 abnormalities \(^1\). Increased numbers of chromosomal abnormalities were also found to adversely influence median survival in adult MDS patients \(^7\). Breems et al. recently investigated the prognostic value of different cytogenetic components of a complex karyotype in adult AML and identified a monosomal karyotype, i.e. either one single autosomal monosomy in the presence of at least one structural aberration or at least 2 distinct autosomal monosomies, as a highly unfavorable risk category \(^8\). In order to better stratify children with advanced MDS, we evaluated the outcome in 192 children prospectively diagnosed and treated within the studies of the European Working Group of MDS in Childhood (EWOG-MDS) related to karyotypic complexity. This study is registered at clinicaltrials.gov as NCT00047268 and NCT00662090.

**Material and Methods**

All patients younger than 18 years with adequate cytogenetic studies and advanced MDS, i.e. RAEB or RAEB-T \(^9\text{-}^{10}\), enrolled in studies EWOG-MDS 98 (Clinical Trials.gov Identifier NCT00047268) or EWOG-MDS 2006 (NCT00662090) between July 1, 1998 and June 30, 2008 were included in this analysis. In both studies therapy recommendation consisted of upfront hematopoietic stem cell transplantation...
(HSCT). Institutional review board approval was obtained for both EWOG-studies from all participating institutions.

Cytogenetic analyses of bone marrow cells were performed according to standard procedures. Karyotypes were described according to ISCN 2009. At least 10 metaphases were analyzed (Tables S1-S3). Cytogenetic findings were centrally reviewed (G.G., H.B.B., D.B.). All cytogenetic studies were performed prior to administration of MDS-specific therapy. A structurally complex karyotype was defined as ≥3 chromosomal aberrations including at least one structural aberration (Table S4). Since we have never been able to detect differences in OS between patients with monosomy 7 as sole aberration, monosomy 7 and clonal evolution, or monosomy 7 and other aberrations (excluding those with structurally complex karyotypes, Table S5), we grouped all patients with monosomy 7. Thus, the monosomy 7 group included those with clonal evolution and additional aberrations.

The Kaplan-Meier method was used to estimate OS probabilities. The log-rank test was employed to compare survival between subgroups. For multivariate analysis, the Cox proportional hazard regression model was used. HSCT was included as a time-dependent covariate in the model. The different definitions of complex karyotype coded in a variable with k-categories were transformed into k-1 dummy variables and added to the model. All P values were two-sided and values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS for Windows 15.0.1.

Results and Discussion

Cytogenetic data from 192 patients with RAEB and RAEB-T were analyzed (Table S3). HSCT was performed in 143 patients (74%) resulting in a probability of OS at 5
years of 0.58 for all children with primary MDS and 0.47 for those with secondary MDS (P=0.09). Here we analyze the prognostic significance of a novel simple definition of a complex karyotype termed structurally complex, i.e. ≥ 3 chromosomal aberrations including at least one structural aberration, introduced for the following reasons: The most frequent chromosomal aberrations of highly complex clones are deletions, unbalanced translocations and dicentric chromosomes. Monosomies are also often noted in karyotypes with multiple aberrations. However, it may be difficult to be certain that a particular chromosome is lost. Using more advanced molecular cytogenetic techniques such as mFISH or array CGH, it often transpires that parts of “missing” chromosomes are involved in structural aberrations. In addition, structural aberrations such as deletion 5q, deletion 7q, deletion 17p or aberrations involving 3q, 11q and 12p are known to be associated with a poor prognosis in AML and MDS.1,16,17

The number of patients identified by the traditional definition of complex karyotype with ≥3 clonal aberrations, complex karyotype with ≥5 clonal aberrations, and structurally complex karyotype was 35 (18%), 19 (10%) and 28 (15%), respectively (Table S6). With the exception of one patient, all patients with ≥ 5 clonal aberrations were also recognized as structurally complex. Seven patients with ≥ 3 clonal aberrations did not fulfill the criteria of a structurally complex karyotype, due to the absence of structural aberrations by standard cytogenetics. Notably, 5 of these 7 patients are still alive at a median time of 6.9 years (0.2-8.7) after diagnosis (Figure 1A). Children with a structurally complex karyotype had a highly unfavorable prognosis with a 2-year OS probability of 0.14 [0.00-0.30] (Figure 1B, 1C). The group of patients with a structurally complex karyotype given HSCT had a significantly shorter event-free survival (P<0.01, Figure 1D) and OS (data not shown) than
children with other karyotypes due to a higher risk of relapse following HSCT (0.48 [0.27-0.82] versus 0.28 [0.20-0.38], \( P < 0.01 \)). Whether novel therapy approaches prior to HSCT can improve outcome following HSCT for children with structurally complex karyotype remains to be determined.

Recently, patients with a monosomal karyotype were shown to have a dismal prognosis\(^8\). Here, a monosomal karyotype with at least 2 autosomal monosomies was seen in 12 (6%) patients. Of these 12 patients, all but one were included in the group with structurally complex karyotype, while 17 of the 28 patients with a structurally complex karyotype did not have 2 autosomal monosomies. A monosomal karyotype with at least one autosomal monosomy and one or more structural aberrations was noted in 30 (16%) patients (Tables S7 and S8). In summary, of the 30 monosomal karyotypes with at least one autosomal monosomy and one or more structural aberrations, 19 fulfilled the definition of a structurally complex karyotype. The 5-year OS of these patients was significantly worse than that of the 11 monosomal patients not coded as structurally complex (\( P < 0.01 \), Figure 1B). Thus, a monosomal karyotype in childhood MDS identifies a group of patients with a heterogeneous prognosis. In contrast, using the new definition of a structurally complex karyotype, only children with a highly unfavorable prognosis were identified.

The Cox model included demographic data (gender, age) and all variables with a \( P \) value <0.1 in the univariate analysis. Age \( \geq 15 \) years was associated with an increased risk of patient death (HR 1.7; \( P = 0.05 \), Table 1). HSCT improved the outcome significantly (HR 0.4; \( P = 0.05 \)). Statistical analysis of the different definitions of complex karyotype had to take into account, that some patients fulfilled the criteria of more than one definition. The presence of a monosomal karyotype was an
independent adverse prognostic factor only when it also fulfilled the criteria of a structurally complex karyotype (HR 4.6; $P<0.01$, Table 1). In contrast, a monosomal karyotype that did not fulfill the criteria of a structurally complex karyotype (“monosomal karyotype only”, see Table S8) did not identify a group with inferior outcome. Notably, a structurally complex karyotype without a monosomy (“structurally complex only”) was associated with a poor probability of OS (HR 14.4; $P<0.01$). This held for both primary and secondary MDS (primary MDS n=9, secondary MDS n=19, Table S6). Thus, the presence of a structurally complex karyotype was a better predictor of a very unfavorable prognosis in children with MDS than the presence of $\geq 3$ clonal aberrations or a monosomal karyotype.

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**Authorship**

Gudrun Göhring, Brigitte Schlegelberger, Franco Locatelli, and Charlotte Niemeyer designed the study and wrote the manuscript.

Gudrun Göhring, Brigitte Schlegelberger, Kyra Michalova, H. Berna Beverloo, David Betts, Jochen Harbott, Oskar A. Haas, Gitte Kerndrup, Laura Sainati, Elisabeth R. van Wering performed the Cytogenetic Analysis.

Eva Bergsträsser, Henrik Hasle, Jan Starý, Monika Trebo, Marry M. van den Heuvel-Eibrink, Marco Zecca are the Regional Coordinators of the EWOG MDS Study Group and performed the collection of the clinical data.
Peter Noellke, Alexandra Fischer, Gudrun Göhring, Brigitte Schlegelberger, Brigitte Strahm, Franco Locatelli, and Charlotte Niemeyer analyzed the data.

All authors critically reviewed the manuscript.

Disclaimer

We have no competing conflict of interest to declare.

Reference List


### Table 1. Multivariate analysis for probability of overall survival in 192 consecutive children with advanced MDS and different karyotypic complexities

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative Risk of Death</th>
<th>95% CI</th>
<th>p-value</th>
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<td><strong>Age at diagnosis</strong></td>
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</tr>
<tr>
<td>&gt;=15 years</td>
<td>1.7</td>
<td>1.0-2.9</td>
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<td><strong>Gender</strong></td>
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<td><strong>Primary/ secondary MDS</strong></td>
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<td></td>
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<tr>
<td>secondary</td>
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<td>0.6-1.8</td>
<td>n.s.</td>
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<td><strong>Different karyotypic complexities</strong></td>
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<td>Monosomal karyotype but not structurally complex (n=11)</td>
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<td>0.2-1.9</td>
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<td>0.2-1.0</td>
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Figure legends

Figure 1:

Panel A: Probability of 5-year overall survival for children with advanced primary or secondary MDS according to cytogenetic stratification.

Traditionally, a complex karyotype is defined by the presence of ≥ 3 or ≥ 5 aberrations. Structurally complex karyotype is defined by at least 3 chromosomal aberrations including at least one structural aberration, excluding those with clonal evolution of monosomy 7. Karyotypes were grouped in the following hierarchical order: ≥ 5 aberrations, 3-4 aberrations, structurally complex, monosomy 7 (-/+ other aberrations), normal karyotype, other karyotypes.

Panel B: Probability of 5-year overall survival for children with: advanced primary or secondary MDS with structurally complex karyotype; monosomal karyotype with at least one autosomal monosomy and one or more structural aberrations that did not fulfill the criteria of a structurally complex karoytpe (“monosomal karyotype only”); monosomy 7 with or without additional aberrations, including clonal evolution of monosomy 7; normal karyotype; other karyotypes.

Panel C: Probability of 5-year overall survival for patients according to cytogenetic subgroup. Patients classified as monosomal karyotype only in panel B are now included in the group of monosomy 7 (-/+ additional aberrations) or other karyotypes.

Panel D: Probability of 5-year event-free survival for patients who received allogeneic hematopoietic stem cell transplantation (N=143).
Figure 1

A

- 3-4 aberrations: 0.80 [0.44-1.50]
- -7 (+/− other aberrations): 0.57 [0.39-0.76]
- Other karyotypes: 0.57 [0.38-0.76]
- Normal karyotype: 0.51 [0.37-0.65]
- ≥5 aberrations: 0.20 [0.09-0.40]
- Structurally complex: 0.00

Log rank: P<0.01

B

- Monosomal karyotype only: 0.69 [0.39-0.99]
- Other karyotypes: 0.58 [0.39-0.77]
- -7 (+/− other aberrations): 0.58 [0.40-0.76]
- Normal karyotype: 0.51 [0.37-0.65]
- Structurally complex (2-year survival): 0.14 [0.00-0.30]

Log rank: P<0.01

C

- -7 (+/− other aberrations): 0.62 [0.44-0.76]
- Other karyotypes: 0.58 [0.39-0.77]
- Normal karyotype: 0.51 [0.37-0.65]
- Structurally complex (2-year survival): 0.14 [0.00-0.30]

Log rank: P<0.01

D

- -7 (+/− other aberrations): 0.54 [0.37-0.71]
- Other karyotypes: 0.53 [0.39-0.67]
- Normal karyotype: 0.48 [0.28-0.68]
- Structurally complex (2-year survival): 0.19 [0.00-0.41]

Log rank: P<0.01
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