



Population-based age-specific incidences of cytogenetic subgroups of acute myeloid leukemia

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Background and Objectives. It is well known that the different cytogenetic subgroups of acute myeloid leukemia (AML) show different age-specific frequencies. For example, balanced translocations tend to be found in younger patients while complex aberrant karyotypes are usually found in elderly patients with AML. However, detailed data on the population-based age-dependent incidences of distinct cytogenetic subtypes as well as of molecular mutations are lacking.

Design and Methods. We evaluated the population-based age-specific incidences of different cytogenetic subgroups in 2555 patients with AML between 21 and 70 years of age. We also investigated the association of specific molecular markers (*FLT3-M*, *FLT3-TKD*, *MLL-PTD*, *NRAS*, *CEPBA*, *KITD816*).

Results. The incidence of balanced translocations was rather constant over lifetime. In contrast, the incidence of unbalanced aberrations and especially complex aberrant karyotypes increased sharply with age. There were also different age-specific incidences of some recurrent molecular mutations.

Interpretation and Conclusions. These results are suggestive of different mechanisms in the pathogenesis of AML.

Key words: acute myeloid leukemia (AML), age-specific incidences, cytogenetic subgroups, molecular markers

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Genetic abnormalities are the hallmark of cancer. Different mechanisms lead to different types of genetic alterations. These mechanisms might occur at different frequencies over lifetime.¹⁻⁶ To test this hypothesis we analyzed 2555 AML patients with respect to genetic abnormalities. AML is considered an interesting model disease as it is heterogeneous with respect to cytogenetics and molecular genetics. From the cytogenetic aspect AML can be divided into several major groups: (i) AML with a normal karyotype, which is observed in 40-45% of cases; (ii) AML with a primary balanced translocation, which occurs in 20-25% of cases; (iii) AML with an unbalanced karyotype without a primary (known) balanced abnormality characterized by gains or losses of rather large regions of the genome: this pattern is detected in 35-40% of all patients.^{7,8} Approximately 50% of patients in the last group have a complex aberrant karyotype, defined by ≥ 3 clonal abnormalities (excluding those with recurrent bal-

anced rearrangements).⁷⁻¹⁰

From a clinical point of view AML can be subdivided into three major categories according to cytogenetics; one group associated with a favorable prognosis, one with an intermediate prognosis, and one with an unfavorable prognosis.^{7,8,11,12} A normal karyotype is associated with an intermediate prognosis.^{7,13} Some balanced translocations (*PML-RARA*, *AML1-ETO*, and *CBFB-MYH11*) predict a favorable outcome.^{7,13} 11q23 rearrangements are also balanced translocations, but are associated with an unfavorable prognosis.¹⁴ Of all AML subtypes, complex aberrant karyotypes are associated with the most unfavorable prognosis.¹⁵

In addition, a variety of recurrent molecular markers have been described in AML. Mutations of the *FLT3* receptor tyrosine kinase can occur in the juxtamembrane domain (*FLT3*-length mutations; *FLT3-LM*) or in the protein tyrosine kinase domain (*FLT3-TKD*)¹⁶⁻²⁰ and represent the most frequent genetic alteration in AML.

FLT3 length mutations occur in 20-25% of all cases in adult AML and in 40% of cases with normal karyotype.¹⁶⁻²² In contrast, the incidence in childhood AML is only 11%.²³ *FLT3*-TKD mutations are detected in 6-8%.^{18,19} *MLL*-PTD are observed in 6.5% of AML patients.²⁴ Further mutations as *KITD816*, a mutation in the KIT receptor tyrosine kinase, have been described in AML²⁵⁻²⁷ and the mutations of the transcription factor *CEPBA* have been detected in 11-15% of all AML cases with a normal karyotype.²⁸⁻³⁰ Mutations of the *NRAS* proto-oncogene occur in 10% and are most frequently found in codons 12, 13 and 61.^{31,32} Mutations of nucleophosmin (NPM), a nucleocytoplasmic shuttling protein localized in the nucleolus, which regulates the ARF-p53-tumor suppressor pathway, are found in 35% of all patients with *de novo* AML.³³

While *FLT3* length mutations^{16,18,20,34} and *MLL*-PTD^{24,35} are associated with an unfavorable prognosis, *CEPBA* mutations represent a favorable molecular prognostic factor.^{28,36} The prognostic implications of *RAS* mutations are still under discussion.^{32,34}

It is well known that patients with balanced translocations tend to be younger while complex aberrant karyotypes are usually found in elderly patients with AML. Most studies on this aspect have analyzed the relative frequency of cytogenetic subtypes in different age decades or the proportions of cytogenetic subgroups within age decades. Detailed data on population-based age-dependent incidences of distinct cytogenetic subtypes and of molecular mutations are lacking. The mechanisms leading to balanced aberrations and to complex aberrant karyotypes are probably different from each other and might depend on age-associated factors. Indeed, we found an association between different cytogenetic mechanisms and age at onset of AML.⁹ Based on those results, in the present study we further evaluated the association of different cytogenetic subgroups with age in 2555 AML patients. We also investigated the association of specific molecular markers (*FLT3*-LM, *FLT3*-TKD, *MLL*-PTD, *NRAS*, *CEPBA*, *KITD816*) with age. We evaluated the relative frequencies and the relative proportions of the cytogenetic subtypes and the absolute incidence of distinct karyotype abnormalities with respect to different cytogenetic mechanisms over adult lifetime.

Design and Methods

Patients

Within a 4-year period from January 1999 to January 2003 blood or bone marrow samples from 3734 consecutive patients with AML at diagnosis were analyzed by standard cytogenetics. All cases

had a diagnosis of AML proven cytomorphologically and/or immunophenotypically according to the French-American-British (FAB) classification.³⁷

Only cases with *de novo* or secondary AML after a myelodysplastic syndrome were included in this study; 231 cases with therapy-related AML were excluded. Our laboratory focuses mainly on adult patients. As cytogenetic analyses are not always requested for elderly patients, we excluded 948 patients <21 years or >70 years of age resulting in a cohort of 2555 cases for this study. The age distribution of this cohort corresponded with that of a standard cohort based on the comparison with the SEER project for patients with AML between 21 and 70 years of age (*SEER Cancer Statistics Review, 1975-2001*, <http://seer.cancer.gov/csr/1975-2001/>).

Cytogenetic analyses and polymerase chain reaction

Cytogenetic analyses were performed in all 2555 cases according to standard protocols.³⁸ The definition of a cytogenetic clone followed the International System for Human Cytogenetic Nomenclature (Mitelman, ISCN, Guidelines for Cancer Cytogenetics, Karger, Basel, 1995). Cases with ≥ 3 clonal aberrations without a known balanced chromosomal rearrangement were defined as complex aberrant karyotypes. The cases included in the evaluation were categorized into the following subgroups: normal karyotype, balanced translocations, unbalanced not complex aberrant karyotype, or complex aberrant karyotypes.

We further analyzed the mutations status of molecular markers: *FLT3*-LM (n=1799), *FLT3*-TKD (n=1565), *MLL*-PTD (n=1881), *NRAS* (n=2029), *KITD816* (n=1379), and *CEPBA* (n=295). Polymerase chain reactions were performed as described previously.^{17,24}

Statistics

Patients were divided into five age groups (age group 1: 21-30 years; age group 2: 31-40 years, age group 3: 41-50 years, age group 4: 51-60 years, age group 5: 61-70 years). The age-specific incidences were calculated on the basis of the distribution of the general population of Germany in 1998 (Statistisches Bundesamt, Fachserie 12 Reihe 4, Bonn, Germany, 1998). We then calculated the incidence of cytogenetic subtypes in the different age groups. Differences in incidences between age cohorts were analyzed by the χ^2 test.

Results

Cytogenetic results

Of the 2555 patients evaluated, 1220 (47.7%) had a

Table 2. Proportions of different age groups within the different cytogenetic subgroups.

Cytogenetic subgroups	n	Age group 1 (%)	Age group 2 (%)	Age group 3 (%)	Age group 4 (%)	Age group 5 (%)	All age groups (%)
t(8;21)	106	11.3	20.8	23.6	25.5	18.9	100.0
inv(16)	102	14.7	26.5	17.6	17.6	23.5	100.0
t(15;17)	136	15.4	20.6	19.9	20.6	23.5	100.0
11q23 rearrangements	181	12.3	27.2	28.4	17.3	14.8	100.0
Other balanced translocations	124	12.1	16.1	18.5	21.0	32.3	100.0
Balanced translocations	549	13.3	21.7	21.1	20.6	23.3	100.0
Normal karyotype	1220	5.2	11.8	17.2	25.1	40.7	100.0
Trisomies	188	4.8	4.8	8.5	29.3	52.7	100.0
-7/del(7q)	49	8.2	6.1	12.2	26.5	46.9	100.0
del(5q)	31	0.0	3.2	3.2	35.5	58.1	100.0
Other monosomies	8	12.5	12.5	12.5	25.0	37.5	100.0
Unbalanced aberrations	276	5.1	5.1	8.7	29.3	51.8	100.0
Complex aberrant	330	2.4	5.8	10.6	28.5	52.7	100.0
Other aberrations	180	0,0	6,1	18,9	22,2	51,1	100,0
Total	2555	6.3	12.0	16.4	24.8	40.5	100.0

(Age group 1: 21-30 years; 2: 31-40 years, 3: 41-50 years, 4: 51-60 years, 5: 61-70 years).

Table 1. Cytogenetic results in 2555 patients with AML.

Cytogenetic subtype	n	% of all patients
Normal karyotype	1220	47.7
Balanced translocations	549	21.5
Complex aberrant karyotype	330	12.9
Unbalanced aberrations*	276	10.8
Other aberrations°	180	7.0
All patients	2555	100.0

*Trisomies, monosomy 7 and deletions of 7q, other monosomies, and deletions of 5q; °combination of different cytogenetic mechanisms or structural deletions.

normal karyotype (Table 1). Balanced translocations were observed in 549/2555 cases (21.5%). This cohort comprised 106 cases with t(8;21), 136 cases with t(15;17), 102 cases with inv(16), 81 cases with 11q23 rearrangements, and 124 cases with other rare balanced translocations, e.g. t(6;9)(p23;q34), t(3;3)(q21;q26), or inv(3)(q21q26) or other balanced rearrangements. The cohort with MLL rearrangements was heterogenous. The largest subgroups were t(9;11) with MLL-AF9 including 24 patients, t(10;11) with MLL-AF10 including 14 patients, and t(6;11) with ML-AF6 including 10 patients.

The cohort of 276 patients (10.8%) with unbalanced but non-complex aberrations comprised cases with single trisomies (n=188), single monosomies of chromosome 7 or deletions of 7q as the sole abnormality (n=49), deletions of 5q alone (n=31), and other single monosomies (n=8). Of the total 2555 cases 330 (12.9%) had a complex aberrant karyotype and 180 cases (7.6%) had single structural aberrations

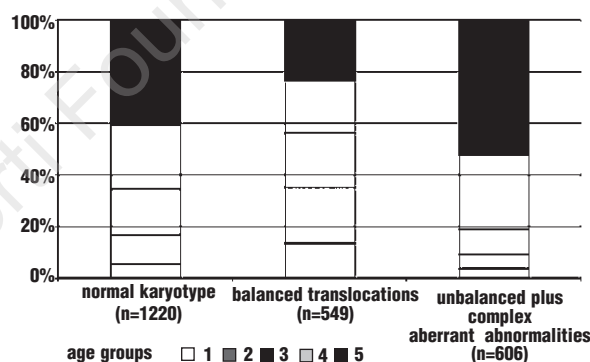


Figure 1. Proportions of age groups in different cytogenetic subtypes in AML. The distribution of different age groups in the cytogenetic subtypes with normal karyotype, balanced aberrations and unbalanced aberrations including complex aberrant karyotypes is illustrated. The y-axis shows the percentage of cases of the respective age groups found in the specific cytogenetic groups. The different cytogenetic groups are shown on the x-axis. (age group 1: 21-30 years; 2: 31-40 years, 3: 41-50 years, 4: 51-60 years, 5: 61-70 years).

or a combination of different cytogenetic abnormalities and were therefore excluded from further evaluation.

Proportions of the five age groups within the different cytogenetic subgroups

We compared the distribution of age of our patients with the SEER data (SEER Cancer Statistics Review, 1975-2001, <http://seer.cancer.gov/csr/1975-2001/>) and were able to demonstrate that the age distribution of our cohort was representative for a standard cohort.

We first analyzed the proportion of the five age

Table 3. Proportions of the different cytogenetic subtypes in each age group.

Cytogenetic subgroups	n	age group 1	age group 2	age group 3	age group 4	age group 5
t(8;21)	106	12 (7.5%)	22 (7.2%)	27 (6.0%)	27 (4.3%)	20 (1.9%)
inv(16)	102	15 (9.3%)	27 (8.8%)	18 (4.3%)	18 (2.8%)	24 (2.3%)
t(15;17)	136	21 (13.0)	28 (9.1%)	27 (6.4%)	28 (4.4%)	32 (3.1%)
11q23 rearrangements	181	10 (9.3%)	22 (6.5%)	23 (5.5%)	14 (4.1%)	12 (3.9%)
Other balanced translocations	124	15 (6.2%)	20 (7.2%)	23 (5.5%)	26 (2.2%)	40 (1.2%)
Balanced translocations	549	73 (45.3%)	119 (38.8%)	116 (27.7%)	113 (17.8%)	120 (12.4%)
Normal karyotype	1220	63 (39.1%)	144 (46.9%)	210 (50.1%)	306 (48.3%)	497 (48.1%)
Trisomies	188	9 (5.6%)	9 (2.9%)	16 (3.8%)	55 (68.7%)	99 (9.6%)
-7/del(7q)	49	4 (2.5%)	3 (1.0%)	6 (1.4%)	13 (2.1%)	23 (2.2%)
del(5q)	31	0 (0.0%)	1 (0.3%)	1 (0.2%)	11 (1.7%)	18 (1.7%)
Other monosomies	8	1 (0.6%)	1 (0.3%)	1 (0.2%)	2 (0.3%)	3 (0.3%)
Unbalanced aberrations	276	14 (8.7%)	14 (4.6%)	24 (5.7%)	81 (12.8%)	143 (13.8%)
Complex aberrant	330	8 (5.0%)	19 (6.2%)	35 (8.4%)	94 (14.8%)	174 (16.8%)
Other aberrations	180	3 (1.9%)	11 (3.6%)	34 (8.1%)	40 (6.3%)	92 (8.9%)
Total	2555	161 (100.0%)	307 (100.0%)	419 (100.0%)	634 (100.0%)	1034 (100.0%)

(Age group 1: 21-30 years; 2: 31-40 years, 3: 41-50 years, 4: 51-60 years, 5: 61-70 years).

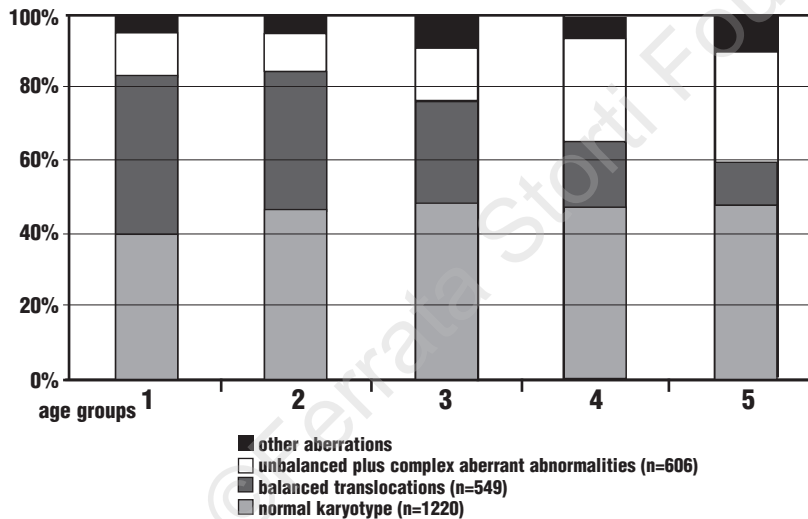


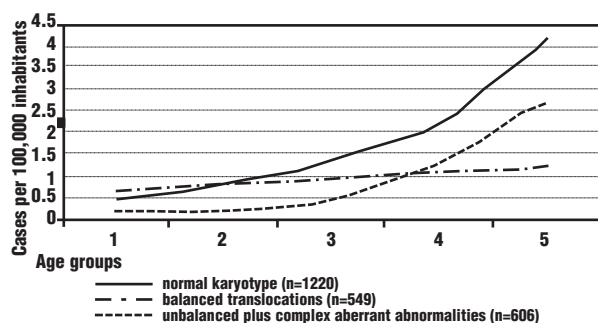
Figure 2. Proportions of cytogenetic subtypes in the different AML age groups. The percentages of the different cytogenetic subgroups within a certain age group are shown on the y-axis. The different age groups are shown on the x-axis, (age group 1: 21-30 years; 2: 31-40 years, 3: 41-50 years, 4: 51-60 years, 5: 61-70 years).

groups within the different cytogenetic subgroups (Table 2, Figure 1). Within the cohort of AML with balanced translocations the proportions of the different age groups were rather constant.

AML patients with a normal karyotype were more frequently older. While only 5.2% of all cases with a normal karyotype were aged 21-30 years, 40.7% were aged 61-70 years. However, the most dramatic changes with age were detected in AML with unbalanced aberrations (5.1% to 51.8%), and in AML with complex aberrant karyotype in particular. In the latter cohort only 2.4% were aged 21-30 years but 52.7% were 60 years and older ($p < 0.0001$).

Relative proportions of the different cytogenetic subtypes within the five age groups

The relative proportions of cytogenetic subgroups within the different age decades were also analyzed (Table 3, Figure 2). The proportion of cases with balanced translocations decreased from 45.3% to 12.4% from age group 1 to age group 5. Decreasing proportions with higher age were observed in all cytogenetic subgroups: t(8;21): from 7.5% to 1.9%; inv(16): from 9.3% to 2.3%; t(15;17): from 13.0% to 3.1%; 11q23 rearrangements: from 9.3% to 3.9%; other balanced translocations: from 6.2% to 1.2% ($p < 0.0001$ for all).



The proportion of patients with a normal karyo-

Figure 3. Population-based incidences of the cytogetic subtypes in AML. The absolute incidence of the different cytogetic subgroups in AML on the basis of German population data is shown. The y-axis shows the absolute incidence of the respective cytogetic subgroups in the different age groups. The different age groups are shown on the x-axis. (age group 1: 21-30 years; 2: 31-40 years, 3: 41-50 years, 4: 51-60 years, 5: 61-70 years).

otype increased from 39.1% to 48.1% ($p < 0.0001$) from age groups 1 to 5. The proportions of patients with unbalanced aberrations and with complex aberrant karyotype increased from 8.7% to 13.8% and from 5.0% to 16.8%, respectively ($p < 0.0001$). Again, this profile was also found within the subgroups with trisomies, monosomy 7, and deletions of 7q and in the subgroups with deletions of 5q, and with other monosomies.

Population-based incidences of the cytogetic subtypes in AML

Due to the increasing incidence of AML with higher age and due to the decreasing number of persons at risk with higher age we defined the population-based absolute incidence of AML with different cytogetic abnormalities. The age-specific incidences of the cytogetic groups and of the molecular markers were calculated on the basis of the age distribution of the general population of Germany in 1998 (*Statistisches Bundesamt, Fachserie 12, Reihe 4, Bonn, Germany, 1998*). The absolute incidence of cytogetic abnormalities increased with higher age (Figure 3). The cohort with balanced translocations ($n = 549$) showed the smallest increase. It was only 2.0-fold between age groups 1 and 5 (from 0.6 per 100,000 to 1.2 per 100,000). Within this group the smallest change was observed in the cohort with $t(15;17)$ ($n = 136$) with a nearly constant incidence between 21 and 70 years of age. The increase of incidence was only 1.3-fold from 0.15 per 100,000 to 0.20 per 100,000 inhabitants. It was 1.8-fold in both cohorts with 11q23 rearrangements ($n = 81$) and with $inv(16)$ ($n = 102$), 1.9-fold in the cohort with $t(8;21)$ ($n = 106$), and 3.0-fold in the cohort with other balanced translocations ($n = 124$) (Table 4).

In the cohort with normal karyotype ($n = 1132$) the increase of incidence was linear and increased 8.9-fold from 0.5 per 100,000 in age group 1 to 4.1 per

Table 4. Absolute incidences of the different cytogetic subtypes per 100,000 inhabitants in different age groups.

Cytogetic subgroups	n	Age group 1	Age group 2	Age group 3	Age group 4	Age group 5	Ratio of incidence**
$t(8;21)$	106	0.09	0.12	0.16	0.19	0.17	1.9 fold
$inv(16)$	102	0.11	0.14	0.12	0.13	0.20	1.8 fold
$t(15;17)$	136	0.15	0.15	0.18	0.18	0.20	1.3 fold
11q23 rearrangements	181	0.07	0.14	0.19	0.18	0.13	1.8 fold
Other balanced translocations	124	0.11	0.11	0.15	0.19	0.33	3.0 fold
Balanced translocations	549	0.61	0.78	0.95	0.99	1.19	2.0 fold
Normal karyotype	1220	0.46	0.77	1.36	2.21	4.10	8.9 fold
Trisomies	188	0.07	0.05	0.10	0.40	0.82	12.4 fold
-7 / del(7q)	49	0.03	0.02	0.04	0.09	0.19	6.5 fold
del(5q)	31	0	0.005	0.007	0.08	0.49	91.3 fold***
Other monosomies	8	0.10	0.07	0.16	0.59	1.18	11.6 fold
Unbalanced aberrations	276	0.06	0.10	0.23	0.68	1.44	11.6 fold
Complex aberrant	330	0.0	0.03	0.08	0.07	0.24	24.6 -fold***
Other aberrations	180	1.78	2.92	4.77	4.77	8.67	4.9-fold
Total	2555	1.25	1.75	2.84	4.70	8.43	6.7-fold

Age group 1: 21-30 years; 2: 31-40 years, 3: 41-50 years, 4: 51-60 years, 5: 61-70 years. **ratio of the incidence of age group 1/group 5: ***ratio of the incidence of age group 2/group 5.

Table 5. Frequency of *FLT3*-LM, *FLT3*-TKD, *MLL*-PTD, *NRAS*, *KITD816*, and *CEPBA*.

Molecular marker	n	%
<i>FLT3</i> -LM	416/1799	23.1
<i>FLT3</i> -TKD	101/1565	6.5
<i>MLL</i> -PTD	110/1881	5.8
<i>NRAS</i>	212/2029	10.4
<i>KITD816</i>	24/1379	1.7
<i>CEPBA</i>	52/295	17.6

100,000 in age group 5. In the cohort with complex aberrant karyotype (n=330) and in the cohort with other unbalanced aberrations (n=276) the incidences increased dramatically with higher age. In the cohort with unbalanced aberrations the increase was 11.6-fold from 0.1 per 100,000 in age group 1 to 1.8 per 100,000 in age group 5. Considering cohorts 3 to 5, the sharpness of increase was similar to that of cases with complex aberrant karyotypes. In the cohort with complex aberrant karyotype the increase was 24.6-fold, from 0.06 per 100,000 in age group 1 to 1.44 per 100,000 in age group 5 (Table 4).

The comparison of the different unbalanced aberrations showed a 6.5-fold increase in the cohort with monosomy 7 and deletions of 7q (n=49). The cohort with trisomies (n=188) showed a 12.4-fold increase. The subgroup with single deletions of 5q (n=31) showed the steepest increase (91.3-fold) from 0.0005 per 100,000 to 48.6 per 100,000 from age group 2 to age group 5.

Absolute incidences of complex typical and complex untypical karyotypes

We separated the patients with complex aberrant karyotypes (n=330) into those with complex typical karyotypes (n=263) and those with complex untypical karyotypes (n=67). Complex typical karyotype was defined as showing a deletion of at least one of the following genomic regions: 5q, 7q or 17p, in addition to at least one further of the following lesions: loss of 5q, 7q, 17, 12p, 16q, 18q or gain of 1p, 8q, 11q, or 21q; a complex untypical karyotype was defined by not fulfilling the definition of *typical*.³⁹

The absolute incidence of complex typical karyotypes showed a steeper 39.6-fold increase from 0.0292 per 100,000 to 1.16 per 100,000 than that of complex untypical karyotypes (from 0.0292 per 100,000 to 0.281 per 100,000; 9.6-fold).

Incidence of molecular markers in the different age groups

Table 5 shows the incidence of the molecular markers. The most frequent molecular lesion was

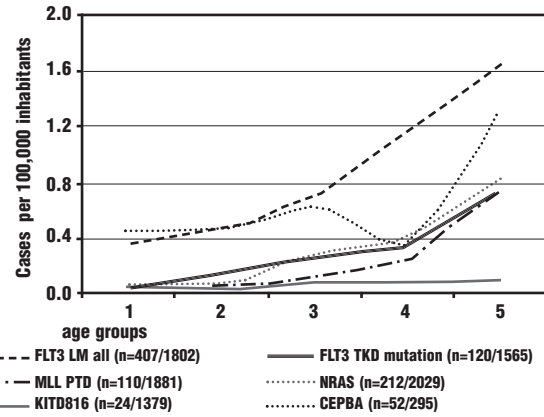


Figure 4. Population-based incidences of the molecular markers *FLT3*-LM, *FLT3*-TKD, *MLL*-PTD and *NRAS* in the different age groups. The absolute incidence of the different molecular markers in AML on the basis of German population data is shown. The y-axis shows the absolute incidence of the respective molecular marker in the specific age group. The different age groups are shown on the x-axis (age group 1: 21-30 years; 2: 31-40 years, 3: 41-50 years, 4: 51-60 years, 5: 61-70 years).

Table 6. Absolute incidences of *FLT3*-LM, *FLT3*-TKD, *MLL*-PTD, *NRAS*, *KITD816*, and *CEPBA* per 100,000 inhabitants in the different age groups.

Age group	1	2	3	4	5	Ratio of incidence**
<i>FLT3</i> -LM (n=416)	0.35	0.47	0.71	1.15	1.64	4.7 fold
<i>FLT3</i> -TKD (n=101)	0.02	0.14	0.27	0.33	0.73	29.4 fold
<i>MLL</i> -PTD (n=110)	0.04	0.05	0.12	0.25	0.74	17.8 fold
<i>NRAS</i> (n=212)	0.04	0.04	0.28	0.39	0.82	19.7 fold
<i>KITD816</i> (n=24)	0.04	0.03	0.09	0.07	0.10	2.3 fold
<i>CEPBA</i> (n=52)	0.44	0.46	0.62	0.38	0.13	2.9 fold

Age group 1: 21-30 years; 2: 31-40 years, 3: 41-50 years, 4: 51-60 years, 5: 61-70 years; **ratio of the incidence of age group 1/group 5.

represented by *FLT3*-LM (416/1799; 23.1%). *CEPBA* mutations occurred in 17.6% (52/295), *NRAS* mutations in 10.4% (212/2029). *FLT3*-TKD were identified in 6.5% of all patients (101/1565), *MLL*-PTD in 5.8% (110/1881) and *KITD816* in 1.7% (24/1379). The absolute population-based incidences for all analyzed molecular markers increased from age group 1 to 5.

The smallest increase was observed in the cohorts with *KITD816* (2.3-fold) and *CEPBA* (2.9-fold). In the cohort with *FLT3*-LM the incidence was constant over the decades (*FLT3*-LM: 4.7-fold).

The highest increases were observed in the cohort with *FLT3*-TKD mutations (29.4-fold), in the cohort with *NRAS* (19.7-fold), and in the cohort with *MLL*-PTD (17.8-fold), (age group 1: 0.3 per 100,000; age group 5: 73 per 100,000) (Figure 4, Table 6).

Discussion

The overall incidence of AML is 2.4 cases per 100,000 inhabitants per year in the USA⁴⁰ and rises from 0.8 in the first decade of life to >15 per 100,000 by the age of 75 years old.⁴¹ Indeed, 6.1% of all AML cases occur in the first decade, whereas 33% of all cases are found in patients over 75 years of age (*SEER Cancer Statistics Review, 1975-2001*, <http://seer.cancer.gov/csr/1975-2001/>). In our study the incidence of AML increased constantly from 2.5 per 100,000 in age group 1 (21-30 years) to 8.7 per 100,000 in age group 5 (61-70 years). This was comparable with the SEER data from the USA. There the absolute incidence of AML increased from 1.2 per 100,000 inhabitants in the age group 20-24 years to 10.9 in the age group 60-64 years (*SEER Cancer Statistics Review, 1975-2001*, <http://seer.cancer.gov/csr/1975-2001/>). The distribution of patients with AML and balanced aberrations is relatively even over all age groups. Moorman *et al.* found 23% of all patients with this subtype were aged 20-30 years old and 17% were aged 70-80 years.¹³

The proportion of patients with balanced translocations in relation to all cases in a certain age group decreases with higher age. In a study by Mauritzson *et al.* the percentage of AML patients with balanced translocations decreased from 21% in the age group 20-49 years to 4% in the age group >75 years.⁴² In a study by Preiss *et al.* the frequencies of the subgroups with t(8;21), t(15;17), inv(16), and 11q23 rearrangements were between 4.2%-8.3% in the age group 15-34 years and between only 0.9% and 2.6% in elderly patients.⁴³ The median age of patients with acute promyelocytic leukemia was 42 years in contrast to the median age of 63 years for AML in general in the study by Mauritzson *et al.*,⁴² and 49 years in contrast to 67 years in all patients in the study by Preiss *et al.*⁴³

In contrast, unbalanced aberrations occur at a higher relative frequency with increasing age.^{9,13} The sharpest increase was reported in the subgroup with 5q deletions.⁴² Our results with respect to the relative frequency of cytogenetic subtypes confirm these results.

While it is well known that younger adults with AML more frequently have balanced aberrations and elderly patients more frequently have unbalanced abnormalities, large studies focusing on the population-based incidence of specific karyotype abnormalities are lacking. We evaluated the population-based age-specific incidences of the different cytogenetic subgroups in 2555 patients with *de novo* AML and secondary AML following myelodysplastic syndromes. Patients with therapy-related AML were not included because of the influence of the drug regimen applied for the primary tumor on the type of genetic

abnormality. Furthermore, the age at diagnosis is influenced by different latency periods after different drugs and by the age distribution of the primary tumors.

The data from this study clearly indicate that the age-dependent increase in incidence of AML substantially differs between cases with balanced, normal karyotypes, and unbalanced karyotype and suggest that mechanisms of leukemogenesis are different and more or less age-dependent.

The results of our study and of others^{10,13,42,44} illustrate two different age profiles in AML from the cytogenetic point of view. The first one is characterized by a rather constant incidence over lifetime and is represented by balanced translocations. In contrast, unbalanced aberrations and especially complex aberrant karyotype show a sharp increase of incidence in older age. This is suggestive of different mechanisms in the underlying pathogenesis of AML.

It was also demonstrated that the absolute incidence of complex typical karyotypes increases more sharply than that of complex untypical karyotypes. Thus, different mechanisms can be hypothesized for these subtypes too.

Balanced aberrations comprise translocations and inversions.^{3,12,45} At least a proportion of, if not all, balanced translocations of pediatric leukemias already develop in the prenatal period. This was demonstrated by the observation of twins developing acute leukemias with reciprocal gene fusions, e.g. c-ALL with *TEL-AML1*, after a latency of up to 14 years.^{2,46-48} The retrospective polymerase chain reaction analyses of Guthrie cards of children with AML with t(8;21), t(15;17), and inv(16), who had developed leukemia with a latency of up to 12 years led to the detection of clonotypic sequences of the respective gene fusions *AML1-ETO*, *PML-RARA*, and *CBFB-MYH11*.^{48,49}

On the other hand, unbalanced aberrations lead to genomic imbalances and may occur due to a variety of mechanisms, such as sister chromatid exchange of ring chromosomes,⁵⁰ unbalanced distribution of the chromosomes to the daughter cells,⁵¹ or incorrect repair of DNA double strand breaks.⁵² These genetic alterations seem to occur more frequently in aging cells as aging cells are more likely to acquire such abnormalities due to shortening of telomeres and less efficient DNA repair capacity.

The different biological background of AML with balanced translocations or with complex aberrant karyotypes is also reflected in the number of clonal aberrations found in both subtypes: AML with a complex aberrant karyotype is characterized by a median number of 10 chromosomal abnormalities (range 3-30).³⁹ In contrast, the median number of clonal changes in the cohort with balanced translocations

tions in this study was only one (range 1-11). This can be compared to non-hematologic entities: some tumors occurring in neonates and children, such as retinoblastoma, require only two mutations, whereas tumors of elderly patients, such as carcinoma of the prostate, are thought to require 12 mutations.^{4,6} The transformation from adenoma to carcinoma of the colon is accompanied by the acquisition of increasing numbers of genetic alterations.⁵³ In contrast, in AML with balanced translocations two genetic events could be sufficient for leukemogenesis. This finding is in keeping with the hypothesis model that at least two *hits* from different types of mutation are needed to induce AML. Type I mutations encode tyrosine kinases and increase proliferation. Type II mutations encode transcription factors and block differentiation.²¹

We also found different age profiles for molecular markers. The incidence of *CEPBA* and *KITD816* was nearly constant between different age groups. A linear increase, comparable to that for all AML or for AML with a normal karyotype, was found for *FLT3-LM*. In contrast, a sharp increase comparable to that for complex aberrant karyotype was found in *FLT3-TKD*, *MLL-PTD*, and *NRAS*. Libura *et al.* discussed similar molecular pathways in the pathogenesis of *FLT3* and *MLL* mutations.⁵⁴ The age-specific distribution of the molecular markers might be due not only

to different mutational mechanisms in dependence on age but also to age-specific changes in hematopoiesis and to changes in the available pools of hematopoietic precursors as targets for leukemogenesis.

Our results with respect to *FLT3-LM* correspond to those of Thiede *et al.* who found a constant frequency independent of age.¹⁸ Neubauer *et al.* were also not able to define an influence of age on the frequency of *RAS* mutations in AML.³² Döhner *et al.* did not find a correlation between the frequency of *MLL-PTD* and age, but focused only on patients up to 60 years.³⁵ In our study the incidence of *MLL-PTD* increased in relation to age from 0.04 per 100,000 in age group 1 to 0.74 per 100,000 in age group 5.

In conclusion, the different age profiles of the cytogenetic subtypes and of the recurrent molecular markers indicate different mechanisms of the pathogenesis of AML and point to the need to develop different targeted therapeutic strategies for the different subtypes.

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