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# Cytogenetic abnormalities in Acute Myeloid Leukemia in Sweden

A population based study

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STAMCELLSCENTRUM (SCC) | FACULTY OF MEDICINE | LUND UNIVERSITY 2015





Cytogenetic abnormalities in Acute Myeloid  
Leukemia in Sweden



# Cytogenetic abnormalities in Acute Myeloid Leukemia in Sweden

A population based study

Vladimir Lj Lazarević



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DOCTORAL DISSERTATION

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*Faculty opponent*

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<p>Abstract: The impact of cytogenetic findings in AML was analyzed in the large population-based Swedish AML registry. Karyotypic patterns differed by age: t(8;21), inv(16) and t(11q23) were more common in younger patients, whereas loss of 5q, 7q and 17p, monosomal karyotype (MK) and complex karyotype (CK) were more common in older patients. Patients with <math>\geq 5</math> chromosome abnormalities had worse overall survival than those with fewer abnormalities or normal karyotype in all age groups. Loss of 5q, 7q and/or 17p had, in contrast to MK, a further negative impact on survival. Multivariable analyses on risk factors in patients &lt;80 years with cytogenetic abnormalities and intensive treatment revealed that age and performance status had the most significant impact on survival (both <math>P &lt; 0.001</math>), followed by sex (<math>P = 0.0135</math>) and a karyotype including -7/del(7q) (<math>P = 0.048</math>).</p> <p>We compared outcome of AHD-AML and tAML, i.e., secondary (sAML) with de novo AML. The CR rates were significantly lower but early death rates similar in sAML vs de novo AML. In a multivariable analysis, AHD-AML (HR 1.51; 95% CI 1.26–1.79) and tAML (1.72; 1.38–2.15) were independent risk factors for poor survival. The negative impact of AHD-AML and tAML on survival was highly age dependent with a considerable impact in younger patients, but without independent prognostic value in the elderly.</p> <p>The frequencies of unsuccessful cytogenetics (UC) and unperformed cytogenetics (UPC) were 2.1% and 2.0%, respectively. The early death rates differed between the cytogenetic subgroups (<math>P = 0.006</math>) with the highest rates in patients with UC (14%) and UPC (12%) followed by high-risk (HR) AML, intermediate risk (IR) and standard risk (SR) cases successfully karyotyped (8.6%, 5.9%, and 5.8%, respectively). The CR rate was lower in UC and UPC and HR compared with the other risk groups (<math>P &lt; 0.001</math>). The 5-year OS rates were 25% for UC and 22% for UPC, whereas the corresponding frequencies for SR, IR and HR AML patients without UC and UPC were 64%, 31% and 15%, respectively. Lack of cytogenetic data translates into a poor prognosis.</p> <p>To ascertain the clinical implications of high hyperdiploid (HH; 49–65 chromosomes) and triploid/tetraploid (TT; &gt;65 chromosomes) adult AML diagnosed 1997-2014, and 68 (1.9%) were HH (<math>n = 50</math>)/TT (<math>n = 18</math>). The OS was similar between patients with HH/TT and CK AML (median 0.9 years vs. 0.6 years; <math>P = 0.082</math>), whereas OS was significantly longer (median 1.6 years; <math>P = 0.028</math>) for IR AML. The OS was shorter for cases with HH than with TT (median 0.6 years vs. 1.4 years; <math>P = 0.032</math>) and for HH/TT AMLs with adverse abnormalities (median 0.8 years vs. 1.1 years; <math>P = 0.044</math>). HH/TT AML is associated with a poor outcome, but chromosome numbers &gt;65 and absence of adverse aberrations seem to translate into a more favorable prognosis.</p> <p>Also, among 23 patients (0.4 %) with trisomy 13 with a median age of 72 years (44-84), there was a striking male predominance (80%) with AML-M0 subtype in 37% of patients. Therapy-related AML and MDS/MPN/AML were present in 30% of patients. Median OS time was 9.6 months (95% CI (3.5-13.7)), and 13 months for other patients (95% CI 11.7-14.04), which was almost identical as in previously published studies.</p>		
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A population based study

Vladimir Lj Lazarević



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# Content

Original articles	9
Brief introduction and aims of the PhD thesis	11
Abstract	13
Abbreviations	15
Historical and general aspects of chromosome analysis in AML	17
Cytogenetic risk classification	19
Epidemiology of AML	26
Incidence of chromosomal abnormalities in AML	28
Gender and chromosomal abnormalities in AML	28
Correlation of FAB (morphology) and chromosomal abnormalities in AML	29
Generally accepted cytogenetically high risk AML	29
Not generally accepted cytogenetically high risk AML	31
Registries, population and methods	33
What we have learned from our studies	35
Age dependent incidence of different chromosomal abnormalities	35
Overlap between poor-risk chromosome abnormalities, complex and monosomal karyotypes	36
Unsuccessful cytogenetics	37
Unperformed cytogenetics	38
Hyperdiploidy	38
Secondary leukemia	39
The role of trisomy 13 in the prognosis of AML	39
Brief summary	39
References	41
Acknowledgments	51



# Original articles

This Thesis is based on the following original articles, referred to in the text by their Roman numerals:

- I. **Lazarevic V**, Hörstedt AS, Johansson B, Antunovic P, Billström R, Derolf A, Hulegårdh E, Lehmann S, Möllgård L, Nilsson C, Peterson S, Stockelberg D, Uggla B, Wennström L, Wahlin A, Höglund M, Juliusson G. Incidence and prognostic significance of karyotypic subgroups in older patients with acute myeloid leukemia: the Swedish population-based experience. *Blood Cancer J*. 2014 Feb 28;4: e188.
- II. **Lazarevic V**, Hörstedt AS, Johansson B, Antunovic P, Billström R, Rangert-Derolf Å, Lehmann S, Möllgård L, Peterson S, Stockelberg D, Uggla B, Wennström L, Wahlin A, Höglund M, Juliusson G. Failure matters: unsuccessful cytogenetics and unperformed cytogenetics are associated with a poor prognosis in a population-based series of acute myeloid leukaemia. *Eur J Haematol* 2015 May;94(5):419-23. doi: 10.1111/ejh.12446. Epub 2014 Oct 3.
- III. Hulegårdh E, Nilsson C, **Lazarevic V**, Garelius H, Antunovic P, Rangert Derolf Å, Möllgård L, Uggla B, Wennström L, Wahlin A, Höglund M, Juliusson G, Stockelberg D, Lehmann S. Secondary AML impacts survival in younger but not in older AML patients: the Swedish population-based experience. *Am J Hematol* 2015 Mar;90(3):208-14. doi: 10.1002/ajh.23908. Epub 2015 Jan 16.
- IV. **Lazarevic V**, Rosso A, Juliusson G, Antunovic P, Rangert-Derolf Å, Lehmann S, Möllgård L, Uggla B, Wennström L, Wahlin A, Höglund M, Johansson B. Prognostic significance of high hyperdiploid and triploid/tetraploid adult acute myeloid leukemia. *Am J Hematol* 2015 Sep;90(9):800-5. doi: 10.1002/ajh.24091. Epub 2015 Jul 22.
- V. **Lazarevic V**, Rosso A, Juliusson G. Isolated trisomy 13 in AML from a Swedish population-based perspective. *Blood* 2014, September 9, E-letter; <http://www.bloodjournal.org.ludwig.lub.lu.se/content/124/8/1304.e-letters>



# Brief introduction and aims of the PhD thesis

Population-based registries may provide data complementary to that from the basic science and clinical intervention studies, and are helpful for establishing recommendations for the management of patients in the “real” world. Registries with high coverage of the target population reduce the impact of selection on the outcome and the subsequent problem with extrapolating data to non-studied populations. Therefore, data that can help clinical decision-making in the situations that are not well covered by clinical studies can be provided<sup>1</sup>. We analyzed several aspects of the data from the Swedish AML Registry between 1997-2006 and between 1997-2014 from a population-based perspective in several aspects. The first aim was to describe the incidence and prognostic importance of the known chromosomal abnormalities in AML. Other goals were to test the clinical characteristics and outcome of specific patient groups, such as unsuccessful cytogenetics (UC), unperformed cytogenetics (UPC), high and low hyperdiploidy, as well as isolated trisomy 13. Last aim was to further characterize the prognosis and characteristics of *de novo* AML versus AML with an antecedent hematological disease (AHD-AML) or therapy related AML (t-AML)



# Abstract

The impact of cytogenetic findings in AML was analyzed in the large population-based Swedish AML registry. Karyotypic patterns differed by age: t(8;21), inv(16) and t(11q23) were more common in younger patients, whereas loss of 5q, 7q and 17p, monosomal karyotype (MK) and complex karyotype (CK) were more common in older patients. Patients with  $\geq 5$  chromosome abnormalities had worse overall survival than those with fewer abnormalities or normal karyotype in all age groups. Loss of 5q, 7q and/or 17p had, in contrast to MK, a further negative impact on survival. Multivariable analyses on risk factors in patients  $< 80$  years with cytogenetic abnormalities and intensive treatment revealed that age and performance status had the most significant impact on survival (both  $P < 0.001$ ), followed by sex ( $P = 0.0135$ ) and a karyotype including -7/del(7q) ( $P = 0.048$ ).

We compared outcome of AHD-AML and tAML, i.e., secondary (sAML) with *de novo* AML. The CR rates were significantly lower but early death rates similar in sAML vs *de novo* AML. In a multivariable analysis, AHD-AML (HR 1.51; 95% CI 1.26–1.79) and tAML (1.72; 1.38–2.15) were independent risk factors for poor survival. The negative impact of AHD-AML and tAML on survival was highly age dependent with a considerable impact in younger patients, but without independent prognostic value in the elderly.

The frequencies of unsuccessful cytogenetics (UC) and unperformed cytogenetics (UPC) were 2.1% and 2.0%, respectively. The early death rates differed between the cytogenetic subgroups ( $P = 0.006$ ) with the highest rates in patients with UC (14%) and UPC (12%) followed by high-risk (HR) AML, intermediate risk (IR) and standard risk (SR) cases successfully karyotyped (8.6%, 5.9%, and 5.8%, respectively). The CR rate was lower in UC and UPC and HR compared with the other risk groups ( $P < 0.001$ ). The 5-year OS rates were 25% for UC and 22% for UPC, whereas the corresponding frequencies for SR, IR and HR AML patients without UC and UPC were 64%, 31% and 15%, respectively. Lack of cytogenetic data translates into a poor prognosis.

To ascertain the clinical implications of high hyperdiploid (HH; 49–65 chromosomes) and triploid/tetraploid (TT;  $> 65$  chromosomes) adult AML diagnosed 1997–2014, and 68 (1.9%) were HH ( $n = 50$ )/TT ( $n = 18$ ). The OS was similar between patients with HH/TT and CK AML (median 0.9 years vs. 0.6 years;



P=0.082), whereas OS was significantly longer (median 1.6 years; P=0.028) for IR AML. The OS was shorter for cases with HH than with TT (median 0.6 years vs. 1.4 years; P=0.032) and for HH/TT AMLs with adverse abnormalities (median 0.8 years vs. 1.1 years; P=0.044). HH/TT AML is associated with a poor outcome, but chromosome numbers >65 and absence of adverse aberrations seem to translate into a more favorable prognosis.

Also, among 23 patients (0.4 %) with trisomy 13 with a median age of 72 years (44-84), there was a striking male predominance (80%) with AML-M0 subtype in 37% of patients. Therapy-related AML and MDS/MPN/AML were present in 30% of patients. Median OS time was 9.6 months (95 % CI (3.5-13.7), and 13 months for other patients (95% CI 11.7-14.04), which was almost identical as in previously published studies.

**Key words:** AML, karyotype, population-based studies, prognosis, chromosomes, hyperdiploidy

# Abbreviations

AML	acute myeloid leukemia
sAML	secondary AML
tAML	therapy-related AML
AHD-AML	AML with an antecedent hematological disease
APL	acute promyelocytic leukemia
ATRA	all-trans retinoic acid
CK	complex karyotype
CML	chronic myeloid leukemia
CR	complete remission
DFS	disease free survival
FISH	fluorescent in situ hybridization
MDS	myelodysplastic syndrome
MK	monosomal karyotype
MPN	myeloproliferative neoplasm
NPM1	nucleophosmin 1
PS	performance status
FLT3	fms-like tyrosine kinase 3
CEBPA	CCAAT/enhancer-binding protein alpha
OS	overall survival



# Historical and general aspects of chromosome analysis in AML

A revolution in understanding of chromosome changes in leukemia and in cancer in general started in 1960 with Peter C Nowell and David Hungerford's discovery of a minute chromosome in chronic granulocytic leukemia, subsequently called the "Philadelphia chromosome"<sup>2</sup>. Before them, predecessors paved the ground for this at that time accidental finding. Wilhelm von Waldemeyer coined the term "chromosome" (colored body) in the late 1880s, and in 1890 David P. von Hansemann, described multipolar mitoses and other aberrant mitotic figures in carcinoma samples and suggested that these aberrant cell divisions were responsible for the abnormal chromatin content found in cancer cells<sup>3</sup>. Theodor Boveri proposed that cancer begins within a single cell in which the chromosomal makeup becomes scrambled, permitting cells to proliferate uncontrollably<sup>4</sup>. At the University in Lund Joe Thin Tjio and Albert Levan contributed with the important finding that the normal number of chromosomes in man is 46<sup>5</sup>. By the end of the 1960s, it seemed as if the Philadelphia (Ph) chromosome was an exception and that such specific chromosomal changes would not be characteristic of other malignancies. Moreover, it was not clear at the time if the Ph chromosome was a simple deletion or if there was a translocation of chromosomal material or not. Janet D Rowley used the newly developed specific staining technique to characterize the Philadelphia chromosome as a balanced translocation t(9;22) in chronic myeloid leukemia (CML), and subsequently t(8;21) in acute myeloid leukemia (AML).

These discoveries opened the pathway for further important investigations of cytogenetic changes in hematological malignancies<sup>6,7,8</sup>. After that, an explosion of knowledge about the chromosomal abnormalities, especially in AML, help us achieve a better understanding of the disease, with tailoring of the therapy based on the prognostic and predictive value of the chromosomal changes.

The first major classification of AML was that of the French–American–British (FAB) group, who proposed the criteria for defining AML by morphological subtype<sup>9</sup>. The first prospective study of AML by chromosomal abnormality was the International Workshops on Chromosomes in Leukemia in 1982, from which the Chicago karyotype classification was derived<sup>10</sup>. The long-term survival of patients identified at

this workshop was reported, and multivariate analysis showed that karyotype was an independent predictor of survival for all patients<sup>11</sup>. Cytogenetic abnormalities are identified in 50–60% of newly diagnosed AML of adult patients<sup>12,13,14</sup>. Age and chromosomal abnormalities are the most important prognostic factors in AML<sup>15,16</sup>. Diagnostic karyotype serves as a tool to identify biologically distinct subsets of disease and is widely adopted to provide the framework for risk-adapted treatment approaches<sup>14,16</sup>.

Furthermore, in newly diagnosed AML patients with abnormal karyotype, cytogenetic analysis is also recommended for documenting complete remission (CR)<sup>17</sup>. In fact, several data show that the persistence, after induction chemotherapy, of cytogenetic abnormalities present at diagnosis in leukemic blasts determine a high relapse rate of leukemia and a worse clinical outcome with lower disease-free survival (DFS) rate and overall survival (OS)<sup>18</sup>. Therefore, the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia has introduced into standard response criteria for AML the category of cytogenetic CR defined as the absence of any cytogenetic aberrations in bone marrow leukemic blasts after induction chemotherapy in presence of morphologic CR and complete peripheral hematological recovery<sup>19</sup>.

The presence of some recurrent chromosomal translocation has led to several discoveries relevant for the pathogenesis of leukemia. For example t(8;21)(q22;q22) resulting in the hybrid gene *RUNX1-RUNX1T1*, is considered a favorable cytogenetic abnormality. It results in an in-frame fusion of two genes, leading to a fusion protein of one N-terminal domain from the *AML1* gene and four C-terminal domains from the *ETO* gene. This leads to altered gene transcription, RNA-dependent mechanisms and ribosomal functions, DNA damage and repair, disrupted cytokine-mediated growth regulation, cell-cycle regulation, regulation of apoptosis and stress responses which all contribute to the leukemogenesis. Furthermore, in adults with t(8;21) AML, the presence of the fusion transcript can serve as pre or post transplant PCR-based *RUNX1/RUNX1T1* monitoring of minimal residual disease (MRD)<sup>20,21</sup>. Since the international cytogenetic community strives for consistency in the descriptive and interpretive reporting of both normal and abnormal karyotypes, regardless of technical evaluation method used, an updated edition of the International System for Human Cytogenetic Nomenclature, (ISCN 2013) is recommended<sup>22</sup>. The cytogenetic reports should be written according to ISCN, for example the overall chromosome number should be reported, sex chromosomes, affected chromosomes, type of abnormalities, chromosomal band locations, and in brackets, the number of cells with a given karyotype.

## Cytogenetic risk classification

Although there are some differences in the classification of cytogenetic risk based on karyotype results among the various cooperative international groups, AML patients are generally classified into three groups: high, intermediate and low risk, also called adverse (unfavorable, poor) risk, intermediate risk and favorable risk<sup>14,23</sup>. It is important to note that the classifications of cytogenetic risk groups in AML patients are based on studies predominantly including younger patients (aged <60 years). Some of these variations are depicted in Table 1<sup>24</sup>.

**Table 1.**

Variation in cytogenetic risk group classification across clinical trial groups, (adapted from Grimwade D, Hills RK. 2009<sup>24</sup>); unrel abn indicates unrelated abnormality; abn, abnormal

	Original MRC	SWOG/ ECOG	CALGB	GIMEMA/A ML10	German AMLCG	HOVON/SA KK	Refined MRC
<b>Low risk</b>	t(15;17) t(8;21) inv(16)/t(16;16)	t(15;17) t(8;21) [lacking del(9q), complex, ie, ≥ 3 unrel abn] inv(16)/t(16;16)/del(16q)	t(15;17) t(8;21) inv(16)/t(16;16)	t(15;17) t(8;21) inv(16)/ t(16;16)	t(15;17) t(8;21) inv(16)/ t(16;16)	t(15;17) t(8;21) alone inv/del(16) and lacking highrisk abn	t(15;17) t(8;21) inv(16)/t(16;16)
<b>Interme diate risk</b>	Normal, Other non-complex	Normal, +6, +8, -Y, del(12p)	Normal, Other non- complex	Normal, -Y	Normal Other non- complex	Normal Other non- complex	Normal, Other non- complex
<b>High risk</b>	abn(3q) -5/del(5q), -7 complex [≥ 5 unrel abn] Excluding those with low risk changes	abn(3q),(9q),(11q),(21q) abn(17p) -5/del(5q), -7/del(7q) t(6;9) t(9;22) complex [≥3 unrel abn]	inv(3)/t(3;3) -7, t(6;9), t(6;11) t(11;19),+8 complex (≥ 3 unrel abn) Excluding those with low risk changes	Other	inv(3)/t(3;3) -5/del(5q) -7/del(7q) abn(11q23) del(12p) abn(17p) complex (≥ 3 unrel abn)	abn(3q), -5/del(5q) -7/del(7q) abn(11q23) t(6;9) t(9;22) complex (≥ 3 unrel abn)	abn(3q), [excl t(3;5)] inv(3)/t(3;3) add(5q)/del(5q)/ -5,-7/add(7q) t(6;11) t(10;11) t(9;22), -17 abn(17p) with other changes Complex (> 3 unrel abn) Excluding those with low risk changes

Large multicenter studies have consistently reported that patients with acute promyelocytic leukemia (APL) with the t(15;17)(q22;q12-21) treated on ATRA- and anthracycline-based protocols together with the core binding factor (CBF) leukemias with t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22) treated with intensive chemotherapy involving cytarabine at a range of doses are characterized by relatively favorable prognoses<sup>24</sup>.

Conversely, adults presenting with AML and abnormalities of 3q [abn(3q)], deletions of 5q [del(5q)], monosomies of chromosome 5 and/or 7 (-5/-7) or complex karyotype have a very poor prognosis with conventional chemotherapy and are therefore considered candidates for allogeneic transplant and experimental treatment approaches<sup>24</sup>. Patients with normal karyotype at diagnosis are generally classified in the intermediate risk group. However, this group of patients is characterized by a notable heterogeneity in clinical outcome, showing a different response to treatment<sup>11</sup>. Although karyotype analysis provides a powerful independent prognostic factor for rates of CR, relapse risk and OS in multivariable analyses, there is still uncertainty present concerning a number of miscellaneous cytogenetic abnormalities that together account for ~10% of AML<sup>14,24</sup>.

The definition of complex karyotype (CK) also varies and is a subject of change. CK comprises the patients with three, four or five chromosomal abnormalities, absence of any of the known recurring balanced abnormalities such as t(8;21), inv(16)/t(16;16), t(15;17), and 11q23/*MLL* (excluding t(9;11) and t(11;19)); loss of at least one of chromosomal regions 5q, 7q, or 17p; and loss of at least one additional area of regions 18q21q22, 12p13, or 16q22q24 or gain of 11q23q25, 1p33p36, 8q22q24, or 21q11q22<sup>25,26</sup>. This definition of CK is problematic because chromosomal analysis is subjective, chromosome morphology is often poor and defining independent abnormalities is sometimes difficult to ascertain<sup>25</sup>.

A study by the HOVON group involving 1975 adults (ages 15-60 years) with AML suggested the existence of a novel adverse-risk group characterized by a presence of an autosomal monosomy in conjunction with at least one other autosomal monosomy or structural abnormality (denoted monosomal karyotype positive, MK+)<sup>27</sup>. It is important to emphasize that karyotypes with t(8;21)(q22;q22), t(9;11)(p21;q23), t(15;17)(q22;q21) or inv(16)(p13q22)/t(16;16)(p13;q22) should not be classified as MK+ AML<sup>27, 28</sup>.

One striking observation is an increasing incidence of adverse versus favorable cytogenetic abnormalities with increasing age. The recent WHO classification reflects the fact that an increasing number of AML can be categorized based upon their underlying cytogenetic or molecular genetic abnormalities, and that these genetic changes form clinico-pathologic-genetic entities<sup>29</sup>. The subgroup “AML with recurrent genetic abnormalities” comprises several primary AML entities. “AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*” and “AML with inv(16)(p13.1q22) or



t(16;16)(p13.1;q22); *CFBF-MYH11*” are considered as AML regardless of bone marrow blast counts. In “APL with t(15;17)(q22;q12); *PML-RARA*,” *RARA* translocations with other partner genes are recognized separately. The former category “AML with 11q23 (*MLL*) abnormalities” was redefined into that “AML with t(9;11)(p22;q23); *MLLT3-MLL*” and is now a unique entity; balanced translocations other than that involving *MLLT3* should be specified in the diagnosis. Three new cytogenetically defined entities were incorporated: “AML with t(6;9)(p23;q34); *DEK-NUP214*”; “AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVII*”; and “AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*,” a rare leukemia most commonly occurring in infants.

Two new provisional entities defined by the presence of gene mutations were added, “AML with mutated *NPM1* [nucleophosmin (nucleolar phosphoprotein B23, numatrin)],” and “AML with mutated *CEBPA* [CCAAT/enhancer binding protein (C/EBP), alpha].” There is a growing evidence that these two gene mutations represent primary genetic lesions (so-called class II mutations) that impair hematopoietic differentiation<sup>30</sup>.

Mutations in the *fms*-related tyrosine kinase 3 (*FLT3*) gene are found in many AML subtypes and are considered class I mutations conferring a proliferation and/or survival advantage. AML with *FLT3* mutations are not considered a distinct entity, although determining the presence of such mutations is recommended by WHO because of their prognostic significance (Table 2).

The former subgroup termed “AML with multilineage dysplasia” is now designated “AML with myelodysplasia-related changes.” Dysplasia in 50% or more of cells, in 2 or more hematopoietic cell lineages, was the diagnostic criterion for the former subset. However, the clinical significance of this morphologic feature has been questioned<sup>31,32</sup>. AMLs are now categorized as “AML with myelodysplasia-related changes” if (1) they have a previous history of myelodysplastic syndrome (MDS) or myelodysplastic/myeloproliferative neoplasm (MDS/MPN) and evolve to AML with a marrow or blood blast count of 20% or more; (2) they have a myelodysplasia-related cytogenetic abnormality (listed in a footnote to Table 2); or (3) if 50% or more of cells in 2 or more myeloid lineages are dysplastic.

“Therapy-related myeloid neoplasms” has remained a distinct entity; however, since most patients have received treatment using both alkylating agents and drugs that target topoisomerase II for prior malignancy, a division according to the type of previous therapy is often not feasible. Therefore, therapy-related myeloid neoplasms are no longer subcategorized. Myeloid proliferations related to Down syndrome are now listed as distinct entities.

**Table 2.**

Acute myeloid leukemia and related precursor neoplasms, and acute leukemias of ambiguous lineage; (adapted from WHO 2008)

Categories
<b>Acute myeloid leukemia with recurrent genetic abnormalities</b>
AML with t(8;21)(q22;q22); <i>RUNX1-RUNX1T1</i>
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); <i>CBFB-MYH11</i>
APL with t(15;17)(q22;q12); <i>PML-RARA*</i>
AML with t(9;11)(p22;q23); <i>MLL3-MLL†</i>
AML with t(6;9)(p23;q34); <i>DEK-NUP214</i>
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); <i>RPN1-EV11</i>
AML (megakaryoblastic) with t(1;22)(p13;q13); <i>RBM15-MKL1</i>
<i>Provisional entity: AML with mutated NPM1</i>
<i>Provisional entity: AML with mutated CEBPA</i>
<b>Acute myeloid leukemia with myelodysplasia-related changes‡</b>
<b>Therapy-related myeloid neoplasms§</b>
<b>Acute myeloid leukemia, not otherwise specified (NOS)</b>
Acute myeloid leukemia with minimal differentiation
Acute myeloid leukemia without maturation
Acute myeloid leukemia with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Acute erythroid leukemia
Pure erythroid leukemia
Erythroleukemia, erythroid/myeloid
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis (syn.: acute myelofibrosis; acute myelosclerosis)
<b>Myeloid sarcoma (syn.: extramedullary myeloid tumor; granulocytic sarcoma; chloroma)</b>
<b>Myeloid proliferations related to Down syndrome</b>
Transient abnormal myelopoiesis (syn.: transient myeloproliferative disorder)
Myeloid leukemia associated with Down syndrome
<b>Blastic plasmacytoid dendritic cell neoplasm</b>
<b>Acute leukemias of ambiguous lineage</b>
Acute undifferentiated leukemia
Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); <i>BCR-ABL1  </i>
Mixed phenotype acute leukemia with t(v;11q23); <i>MLL</i> rearranged
Mixed phenotype acute leukemia, B/myeloid, NOS
Mixed phenotype acute leukemia, T/myeloid, NOS
<i>Provisional entity: Natural killer (NK)-cell lymphoblastic leukemia/lymphoma</i>

For a diagnosis of AML, a marrow blast count of  $\geq 20\%$  is required, except for AML with the recurrent genetic abnormalities  $t(15;17)$ ,  $t(8;21)$ ,  $inv(16)$  or  $t(16;16)$  and some cases of erythroleukemia.

\* Other recurring translocations involving *RARA* should be reported accordingly: for example, AML with  $t(11;17)(q23;q12)$ ; *ZBTB16-RARA*; AML with  $t(11;17)(q13;q12)$ ; *NUMA1-RARA*; AML with  $t(5;17)(q35;q12)$ ; *NPM1-RARA*; or AML with *STAT5B-RARA* (the latter having a normal chromosome 17 on conventional cytogenetic analysis).

† Other translocations involving *MLL* should be reported accordingly: for example, AML with  $t(6;11)(q27;q23)$ ; *MLLT4-MLL*; AML with  $t(11;19)(q23;p13.3)$ ; *MLL-MLLT1*; AML with  $t(11;19)(q23;p13.1)$ ; *MLL-ELL*; AML with  $t(10;11)(p12;q23)$ ; *MLLT10-MLL*.

‡ More than 20% blood or marrow blasts AND any of the following: previous history of myelodysplastic syndrome (MDS), or myelodysplastic/myeloproliferative neoplasm (MDS/MPN); myelodysplasia-related cytogenetic abnormality (see below); multilineage dysplasia; AND absence of both prior cytotoxic therapy for unrelated disease and aforementioned recurring genetic abnormalities; cytogenetic abnormalities sufficient to diagnose AML with myelodysplasia-related changes are:

- Complex karyotype (defined as 3 or more chromosomal abnormalities).
- Unbalanced changes:  $-7$  or  $del(7q)$ ;  $-5$  or  $del(5q)$ ;  $i(17q)$  or  $t(17p)$ ;  $-13$  or  $del(13q)$ ;  $del(11q)$ ;  $del(12p)$  or  $t(12p)$ ;  $del(9q)$ ;  $idic(X)(q13)$ .
- Balanced changes:  $t(11;16)(q23;p13.3)$ ;  $t(3;21)(q26.2;q22.1)$ ;  $t(1;3)(p36.3;q21.1)$ ;  $t(2;11)(p21;q23)$ ;  $t(5;12)(q33;p12)$ ;  $t(5;7)(q33;q11.2)$ ;  $t(5;17)(q33;p13)$ ;  $t(5;10)(q33;q21)$ ;  $t(3;5)(q25;q34)$ .

§ Cytotoxic agents implicated in therapy-related hematologic neoplasms: alkylating agents; ionizing radiation therapy; topoisomerase II inhibitors; others.

*BCR-ABL1*-positive leukemia may present as mixed phenotype acute leukemia, but should be treated as *BCR-ABL1*-positive acute lymphoblastic leukemia.

Genotypes defined by the mutational status of *NPM1*, *FLT3*, *CEBPA*, and *MLL* are associated with the outcome of treatment for patients with cytogenetically normal AML<sup>16,33</sup>. The consequence of these findings is that the benefit of the transplant was limited to the subgroup of patients with the prognostically adverse genotype *FLT3-ITD* or the genotype consisting of wild type *NPM1* and *CEBPA* without *FLT3-ITD*<sup>33</sup>. European LeukemiaNet proposed a new standardized reporting system for correlation of cytogenetic and molecular genetic data with clinical data (APL not shown)<sup>34</sup>(Table 3). There is a bulk of information about the significance of other mutations in AML, for example *DNMT3A*, *TET2*, *IDH1* and *IDH2* that have prognostic significance in AML<sup>35,36</sup>. Some groups propose new classification, which suggests that we do not need to perform karyotype analysis at all at the diagnosis, relying only to specific mutations<sup>37</sup>. Other groups are trying to integrate and refine

the risk-groups including clinical, cytogenetic and molecular markers including microRNA analysis and epigenetics<sup>38-41</sup>.

**Table 3.**

Standardized reporting for correlation of cytogenetic and molecular genetic data in AML with clinical data (adapted from Döhner et al 2010<sup>34</sup>)

Genetic group	Subsets
<b>Favorable</b>	t(8;21)(q22;q22); <i>RUNX1-RUNX1T1</i>
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); <i>CBFB-MYH11</i>
	Mutated <i>NPM1</i> without <i>FLT3</i> -ITD (normal karyotype)
	Mutated <i>CEBPA</i> (normal karyotype)
<b>Intermediate-I*</b>	Mutated <i>NPM1</i> and <i>FLT3</i> -ITD (normal karyotype)
	Wild-type <i>NPM1</i> and <i>FLT3</i> -ITD (normal karyotype)
	Wild-type <i>NPM1</i> without <i>FLT3</i> -ITD (normal karyotype)
<b>Intermediate-II</b>	t(9;11)(p22;q23); <i>MLL3-MLL</i>
	Cytogenetic abnormalities not classified as favorable or adverse†
<b>Adverse</b>	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); <i>RPN1-EV11</i>
	t(6;9)(p23;q34); <i>DEK-NUP214</i>
	t(v;11)(v;q23); <i>MLL</i> rearranged
	-5 or del(5q); -7; abn(17p); complex karyotype‡

\* Includes all AMLs with normal karyotype except for those included in the favorable subgroup; most of these cases are associated with poor prognosis, but they should be reported separately because of the potential different response to treatment.

† For most abnormalities, adequate numbers have not been studied to draw firm conclusions regarding their prognostic significance.

‡ Three or more chromosome abnormalities in the absence of one of the WHO designated recurring translocations or inversions, that is, t(15;17), t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), inv(3) or t(3;3); indicate how many complex karyotype cases have involvement of chromosome arms 5q, 7q, and 17p.

Even though this classification is not thoroughly validated, and based on expert opinion it is just one of the many attempts aiming at simplifying decision making in AML. This area is continually changing and in the USA classifications are pragmatically updated every year<sup>42</sup> (Table 4).

**Table 4.**

Prognostic implications of cytogenetic and molecular abnormalities in AML (Adapted from the National Cancer Care Network (NCCN) guidelines<sup>42</sup>)

Risk	Cytogenetics	Molecular
<b>Good</b>	inv(16) or t(16;16) t(8;21) t(15;17)	Normal cytogenetics and: isolated biallelic <i>CEBPA</i> mutation <i>NPM1</i> mutation without <i>FLT3</i> ITD
		<i>KIT</i> mutation in CBF leukemia: inv(16) or t(16;16), t(8;21)
<b>Intermediate</b>	Normal cytogenetics Isolated +8 t(9;11) Other non-good and non-poor changes	
<b>Poor</b>	Complex (> 3 clonal abnormalities) Monosomal karyotype* -5/-5q or -7/-7q 11q23 rearrangements other than t(9;11) inv(3) or t(3;3) t(6;9) t(9;22)	Normal cytogenetics with: <i>FLT3</i> ITD

\*monosomal:  $\geq 2$  monosomies or 1 monosomy and additional 1 or more structural abnormalities (Breems JCO 2008;26:4791) ITD: internal tandem duplication

## Epidemiology of AML

The literature about the incidence of AML varies between countries. It is estimated as 3-4/100.000 people, both in Sweden and USA<sup>43,44</sup>. The incidence has been almost stable over the last years; the incidence gradually increases with age and decreases after the age of 80 years<sup>44,45</sup>. In Sweden there are about 350 new cases per year and in USA about 18.860 new cases of AML (prediction for year 2014; most will be in adults)<sup>46</sup> (Table 4). The median age of AML patients in Sweden is 72 years<sup>15</sup>. Males appear to have higher incidence of AML than women<sup>44</sup>. There is no difference in the incidence between the black and the white ethnicity in USA<sup>46</sup>.

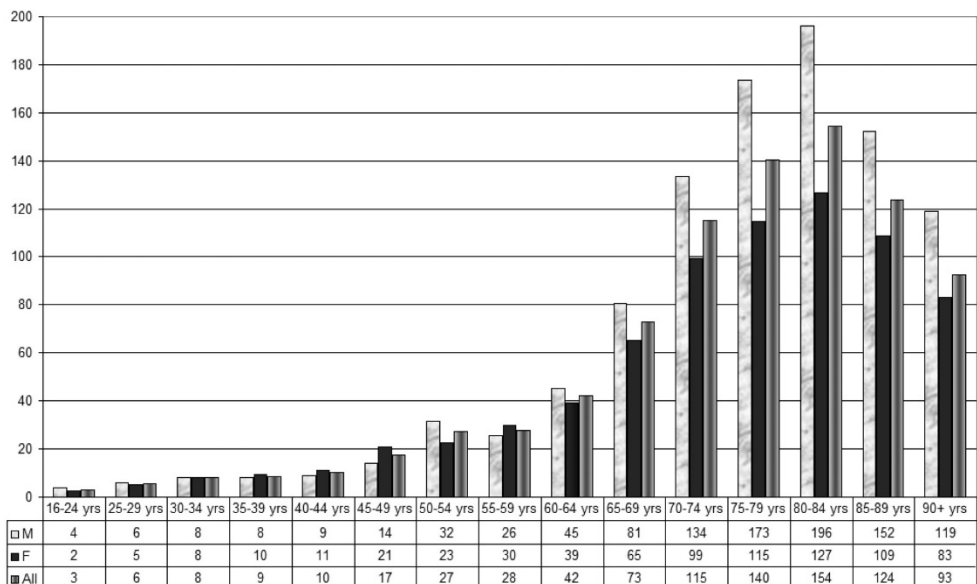
**Table 5.**

Incidence of AML from SEERS database in USA (adapted from Howlader et al.<sup>43</sup>)

	All Races		
	Total	Males	Females
<b>Acute Myeloid Leukemia</b>			
<b>Age at Diagnosis</b>			
<b>Age-Adjusted Rates, 2004-2008</b>			
All ages	3.5	4.3	3.0
Under 65	1.7	1.8	1.6
65 and over	16.1	21.3	12.6
All ages (IARC world std) <sup>b</sup>	2.4	2.8	2.1
<b>Age-Specific Rates, 2004-2008</b>			
<1	1.6	1.6	1.7
1-4	0.9	1.0	0.8
5-9	0.4	0.5	0.4
10-14	0.7	0.7	0.6
15-19	0.8	0.8	0.9
20-24	0.9	0.8	1.0
25-29	1.1	1.1	1.0
30-34	1.3	1.2	1.3
35-39	1.3	1.3	1.3
40-44	1.7	1.8	1.6
45-49	2.3	2.4	2.3
50-54	3.2	3.5	2.9
55-59	4.4	5.0	3.7
60-64	6.2	7.4	5.1
65-69	9.5	11.5	7.8
70-74	14.3	17.8	11.5
75-79	19.0	25.4	14.3
80-84	22.5	31.0	17.1
85+	22.2	31.8	17.7

**Table 6.**

Incidence of AML (non-APL) in 1997 to 2005 (new cases per 100 000 inhabitants, based on the Swedish population in 2005) according to age and sex (from Juliusson et al.<sup>15</sup>)



There are a number of other reports which confirms that in Spain, France and Europe in general, the incidence of AML is similar<sup>47,48,49</sup>. There are sparse data about the incidence of AML in Asia, but at least in some regions of Japan the general incidence of AML is roughly similar to Europe and USA (4.2/100.000 inhabitants)<sup>50</sup>.

## Incidence of chromosomal abnormalities in AML

Clonal chromosomal abnormalities are seen in about 50-60% of patients with AML<sup>51,52,53</sup>. In our study a minority of the patients no karyotype analysis was performed or it was not successful<sup>54</sup>. The presence of normal karyotype was seen in about 40% of patients. Normal karyotype (NK) AML is a very heterogeneous group where additional mutational analyses (*FLT3*-ITD, *NPM1*, *CEBPA*) are nowadays obligatory. There is an association of age and karyotype abnormalities in AML<sup>55,56</sup>. Chromosomal translocations, such as t(8;21), t(15;17), t(16;16) or inv(16) are more common in younger patients whereas deletion of chromosome 5 is more prevalent in patients older than 60 years. It is important however, to note that the incidence of CBF and APL is constant during life<sup>57,58</sup>. Some authors proposed cytogenetic classification based on age and incidence according to the type of abnormalities, i.e. “deletional”, “translocational” or “trisomy” karyotype, but these proposals are not generally accepted<sup>57</sup>.

## Gender and chromosomal abnormalities in AML

There is no proven correlation between chromosomal abnormalities and gender, but there was a higher prevalence in females with t(1;22)(p13;q13), t(4;11)(q21;q23), t(8;16)(p11;p13), t(16;21)(q24;q22) and -X (only women)<sup>28</sup>. More specific for men (younger patients) were der(1;7)(q10;p10), t(6;9)(p22;q34), +13 and -Y (men only). Whether such gender-related differences in frequency reflect a constitutional heterogeneity or a different iatrogenic or environmental exposure is unknown.

## Correlation of FAB (morphology) and chromosomal abnormalities in AML

Most translocations are usually found within one or two French-American-British (FAB) groups for example: t(15;17) in M3, t(8;21) in M2 or M4, inv(16) in M4 or M5, t(11q23) in M4/5; t(6;9) in M2/4; t(9;22) in M1/2 (or biphenotypical), whereas most deletions or trisomies are not associated with any particular FAB group<sup>19</sup>. One of the exceptions is trisomy 13, which is correlated to M0 and spliceosome gene mutations and *RUNX1* mutation with poor prognosis<sup>59</sup>. Another translocation with FAB correlation is t(8;16)(p11;p13) associated with M4/M5a/b, hemophagocytosis and poor prognosis<sup>60</sup>. There are also several recurrent abnormalities, which are not seen often, just to mention t(1;16)(p31;q24) with *NFIA/CBFA2T3* fusion gene in very young children associated with acute erythroleukemia; old FAB M6<sup>61</sup>.

## Generally accepted cytogenetically high risk AML

### Complex karyotype

Definition of complex karyotype differs from group to group<sup>14,28,29</sup>. It is defined by the presence of  $\geq 3$ ,  $\geq 4$ ,  $\geq 5$  cytogenetic abnormalities in bone marrow not including inv(16), t(16;16), t(8;21), t(15;17) and t(9;11). The incidence of complex karyotype increases with age, especially over the age of 60 and is more common in secondary AML. It confers poor prognosis with lower CR rate, and shorter DFS and OS. Complex karyotype is mostly based on the presence of deletions of chromosome 5 and 7 combined with other abnormalities<sup>62</sup>. There is a strong association between complex karyotype and mutation of the *TP53* gene<sup>63</sup>.

### inv(3) and t(3;3)

Rearrangements of the long arm of chromosome 3 as the paracentric inversion of chromosome 3 [inv(3)(q21;q26)] and the translocation between the long arms of both homologous chromosomes 3 [t(3;3)(q21;q26)], are found in 1.0-2.5 % of AML<sup>14,17,23,28, 62</sup>. The inversion is more common than translocation, but they do not differ clinically<sup>28</sup>. It is a recognized WHO entity involving *RPN1-EVII* genes (*EVII* gene now called *MECOM*). It is associated with slightly younger age, normal or high platelet count, previous MDS or present dysplasia, especially dysmegakaryopoiesis, and bone marrow fibrosis ("dry tap") and a poor prognosis, even with allogeneic stem cell transplantation. Deletion of chromosome 7 is often an additional chromosome abnormality, like complex and monosomal karyotypes<sup>63</sup>. Data support consideration



of MDS with  $\text{inv}(3)(\text{q}21\text{q}26.2)/\text{t}(3;3)(\text{q}21;\text{q}26.2)$  as an AML with recurrent genetic abnormalities, irrespective of blast percentage<sup>64</sup>.

## Monosomy and deletion of chromosome 7

Monosomy of chromosome 7 can be isolated or found in the context of complex karyotype. Monosomy 7 and deletion of 7q are present as a single chromosomal alteration only in 35% and 33% respectively of all AML cases with chromosome 7 aberrations<sup>17, 65</sup>. Although, the majority of the large leukemia study groups consider isolated deletion of chromosome 7q to be a bad prognostic factor, some groups (CALGB) think that it belongs to intermediate-risk AML<sup>62</sup> (Table 1). Recurrent somatic mutations in *CUX1*, *LUC7L2* and *EZH2* genes are recurrent in  $-7/\text{del}(7\text{q})$ <sup>66</sup>. Monosomy 7 is often present in the context of monosomal karyotype<sup>27</sup>. AML patients with chromosome 7 aberrations are characterized by frequent multilineage dysplasia in bone marrow cells and poor clinical course with low rate of CR (20–30%) and low DFS and OS, particularly in AML patients with  $-7$  or patients with 7q- in the context of a complex karyotype<sup>17</sup>.

## MLL

Translocations (also, but less frequent, inversions and deletions) involving  $11\text{q}23/MLL$  are present in 5% of *de novo* AML and 10% of tAML (mostly previously treated with topoisomerase-II inhibitors). A number of translocations has been described, mostly in AML M4/M5. The expression of *NG2* (chondroitin sulphate proteoglycan molecule) is relatively specific for AML and ALL<sup>67</sup>. There is an abundance of gene partners for *MLL*, more than 65 described to date<sup>28</sup>. The most common translocations are  $\text{t}(9;11)(\text{p}21;\text{q}23)$ ,  $\text{t}(11;19)(\text{q}23;\text{p}13)$ ,  $10\text{p}12/11\text{q}23-16$  rearrangements, and  $\text{t}(6;11)(\text{q}27;\text{q}23)$ . A number of studies has shown a relatively favorable prognosis for  $\text{t}(9;11)(\text{p}21;\text{q}23)$  while other  $11\text{q}23/MLL$  translocations have been associated with unfavorable prognosis. Different  $11\text{q}23/MLL$  rearrangements can be difficult to identify by cytogenetics and often is needed FISH, Southern blot and/or RT-PCR to identify various rearrangements. Secondary abnormalities differ between various translocations. The most prevalent secondary abnormalities (20–60%) are trisomy 8 although there is no strong evidence that these secondary abnormalities have prognostic significance<sup>28</sup>. Recent studies show that *MLL*-PTD (partial tandem duplications) does not have a prognostic impact in CN-AML patients treated with intensive therapy. Many patients still succumb to the disease and the course of disease for patients not eligible for intensive chemotherapy is even more dismal<sup>68,69,70</sup>. New and effective drugs targeting *MLL* are entering into the early phase of clinical trials<sup>71,72</sup>.

## Monosomy or deletion of chromosome 5

Among newly diagnosed AML patients, monosomy of chromosome 5 (-5) and deletion of the long arm of the chromosome 5 (5q-) represents approximately 6–9% of all the chromosomal abnormalities<sup>6,23,62</sup>. It occurs more often in the patients older than 60 years and is rarely described as an isolated abnormality in AML<sup>28</sup>. However, del(5q) is, in contrast to monosomy 5, relatively often the sole anomaly; more than 200 such AML cases have been reported. Similarly to the aberrations of chromosome 7, these chromosomal alterations are frequently observed in patients previously exposed to alkylating agent or to other leukemogenic factor favouring multilineage dysplasia in bone marrow cells followed by MDS and finally by a secondary AML<sup>28</sup>. Similarly with -7 the presence of -5 in the context of a MK confers very poor prognosis (4-year OS: 0%) in newly diagnosed AML patients<sup>27</sup>.

### t(6;9)(p22;q34)

This is a very rare chromosomal abnormality (<1%), but significant as a recognized abnormality in the 2008 WHO classification, involving *DEK* and *NUP214* genes (known also as *CAN*). These patients are often children or young men (median 23-30 years) and the disease is presented as *de novo* AML or MDS. It is characterized by basophilia, Auer rods and even multilineage dysplasia, and found in all FAB types. It predicts short survival and these patients are candidates for allogeneic stem cell transplantation<sup>28</sup>.

## Not generally accepted cytogenetically high risk AML

### 17p abnormalities

These abnormalities are accepted as a marker for high-risk AML in SWOG/ECOG, AMLSG and the revised MRC classification and the ELN classification (Table 1 and Table 3). It is described as 17p deletion or add17p, and is often a part of complex karyotype together with abnormalities of chromosomes 5 and 7<sup>73</sup>. They indicate a resistant disease with short survival and often involvement of tumor suppressor gene *TP53*<sup>74</sup>. Another abnormality that also leads to deletion of 17p is isochromosome 17q which occurs as the sole change almost always in *de novo* AML, but similar to other 17p deletions, i(17q) is associated with a poor prognosis<sup>75</sup>.

### t(9;22)

The well-known Philadelphia translocation is seen in <1% of AML, mainly FAB types M1 or M2 (often acute leukemia with ambiguous lineage). Both p190 och p210

*BCR/ABL1*-fusion transcript is described in AML, as well as in t(9;22)-positive ALL, whereas p210 is typical for CML. It is difficult to establish whether or not a t(9;22) AML is a *de novo* AML or if it is a blastic phase of a precedent and unknown CML. Cytogenetic classifications of cooperative groups in USA do not always include t(9;22) in high risk AML, neither ELN; however HOVON/SAKK and refined MRC consider AML with this chromosomal aberration a high risk abnormality (Table 1). Philadelphia positive AML cases have to be considered as *de novo* AML<sup>76,77</sup>. Despite that, data in the literature are scarce. The patients' clinical features (no history of abnormal blood counts, lack of an argument for a previous chronic phase and lack of basophilia or splenomegaly), cytogenetic abnormalities (chromosome 7 monosomy, chromosome 16 inversions and chromosome 10 deletions), molecular features (*NPM1* mutation and p190 prevalence) and genome signature are different from those with CML<sup>78</sup>. The t(9;22) allows the use of target therapy (TKI) in association with conventional chemotherapy<sup>79</sup>.

### Trisomy 8

The most common trisomy in AML; about 10% of all AML patients bear this abnormality, isolated trisomy 8 is seen in 5% of cases<sup>28</sup>. Trisomy 8 is considered by all the international cooperative groups as an intermediate cytogenetic-risk alteration; except in the CALGB 8461 study +8 as an isolated chromosomal aberration was classified in the high-risk category<sup>12,13,17</sup> (Table 1). It is frequent in all ages, but prevalence is higher in older age<sup>28</sup>. Several evidences indicate that +8 occurs in association with other cytogenetic aberrations does not modify the prognosis of the associated alteration; the favorable prognostic impact of t(8;21), inv(16) and t(15;17) is not altered by the presence of an additional +8<sup>17</sup>. There is abundant evidence that trisomy 8 is not sufficient for leukemogenesis. Although individuals with a constitutional +8 mosaicism have an increased risk of AML, only a minority develops this disease, and that after a long latency period<sup>80</sup>. Secondly, there does not seem to be an increased risk of AML in CML patients with trisomy 8-positive/t(9;22)-negative clones emerging after treatment with imatinib<sup>81</sup>. Thirdly, the discriminating gene expression pattern of AML with isolated +8 does not depend on the upregulation of chromosome 8 genes alone, concluding that additional genetic changes may be present. In fact, array-based analyses have revealed several cryptic chromosome changes in AML with +8 as a seemingly sole change and mutations of the *ASXL1*, *JAK2*, and *TET2* genes have been shown to be common<sup>82,83,84,85,86</sup>.

# Registries, population and methods

Swedish population registries were introduced in 1686 for taxation and military purposes, with the first report on survival in 1746. Since 1947, all Swedish citizens have a unique personal identification code, which is the same for all registrations, such as taxation, level of education, and medical purposes including causes of death. Thus, all Swedish patients and their medical history are possible to track even after migration within the country or after return from staying abroad. The Swedish Cancer Registry is a compulsory dual-report system developed in 1958. Follow-up of vital status is therefore complete with a minimal loss, and it is possible to perform socioeconomic groupings based on national registries. First, all pathology specimens indicating malignancy are reported by the pathologist to the Regional Tumor Registry; and second, all patients with a newly diagnosed cancer are reported by the clinic; missing data are actively requested. The Swedish Adult Acute Leukemia Registry was founded in 1997 by the Swedish Society of Hematology. It is supported by the Swedish Board for Health and Welfare and run in collaboration with the Regional Tumor Registry in each of the six Swedish healthcare regions, covering a population ranging from 0.9 to 1.9 million people, in total 9 million. Each region has 1 or 2 university hospitals and 3 to 8 county hospitals treating leukemia, and patients are not referred for treatment outside the home region. No patients have been treated at private hospitals. Pediatric patients (<18 years) are reported to the Nordic Society of Pediatric Hematology and Oncology (NOPHO) database, and are not included. Reporting of data on all newly diagnosed patients with acute leukemia, *de novo* or secondary (blastic phase of chronic myeloid leukemia excluded), has thus been compulsory since 1997. Almost all patients have 3 separate registrations (pathology, clinical report to national cancer registry, and report to leukemia registry), although the reports to the leukemia registry is mostly given retrospectively. The initial registration form for the leukemia registry included patient identification, use of diagnostic procedures, and French-American-British type. Furthermore, the physician was requested to report whether the patient at diagnosis was eligible for intensive combination chemotherapy or not. This decision was based on clinical data and local routine, but not on karyotype, because cytogenetic reports were usually not available when treatment should be initiated. Remission induction always consisted of an anthracycline plus cytosine arabinoside (Ara-C), according to regional protocols and estimated patient status, in general TAD, 3 plus 7, or similar, with possible dose reductions for the elderly. Patients in remission subsequently received consolidation with 1 to 3 courses of combination chemotherapy, usually including Ara-C at more than or equal to 1 g/m<sup>2</sup> per dose. Allogeneic stem cell transplantation was also reported. Chemotherapy used with a palliative intent such as single-drug, low-dose

Ara-C, hydroxyurea, or thioguanin, was not regarded as a remission induction, despite the potential for myelosuppression and the achievement of remission.

From 2007 the registry has become web-based and modified. Karyotype, mutation analysis and more details on lab data at diagnosis and specifics on primary therapy and transplantation procedures were included. All patients have a yearly follow up until death, with reporting of relapse, relapse treatment and the outcome. The electronic reporting system INCA (informationsnätverk för cancervården; a national IT-platform for managing registering of cancer patients for clinical care and research) is common to all cancer registries in the Swedish National Cancer Programme (<http://www.kvalitetsregister.se/sekundarnavigering/inenglish.132.html>). Registries are supported by SKL (Sveriges Kommun och Landsting; the Swedish Association of Local Authorities and Regions), and data are monitored by RCC (Regional Cancer Centrum; Comprehensive Regional Cancer Center) in the South region. Since patients diagnosed during the period 1997-2006 did not have reported data on specific karyotype and genetic risk, the Swedish AML group (<http://www.sfhem.se/aml-gruppen-1>) decided to retrospectively supplement the registry with cytogenetic data.

### Specific tasks and background

The first issue was to gather the original data in the paper form from all university regions for all available patients, including the data not done, missing or lost. All the karyotype reports from the different genetic laboratories were sent in paper form. The karyotypes were analyzed and classified or re-classified according to ISCN criteria. For example, karyotypes were first classified as normal karyotype, or more than 30 recurrent abnormalities. Mutations in *FLT3*, *NPM1* or *CEBPA* were not included since these abnormalities were only sporadically performed during this period.

The second task was to systematize this data in the new computerized system. This was done in collaboration with statistician and IT-technician from RCC South.

Randomized controlled trials (RCT) are regarded as the gold standard in evidence-based medicine and are considered the highest grade of evidence<sup>87</sup>. Randomization is an effective measure to balance for confounding factors. However, even RCT has limitations and have a selection bias. For example, it is an open question how representative the patients included in the study are, and thus the potential to deduce the results and conclusions of the trial to the general population<sup>88</sup>. Indeed, studies show under-representation of patients >65 years of age and evidence for race and sex disparities in randomized clinical cancer trials<sup>89,90,91</sup>. Data from the Swedish Acute Leukemia Registry confirm the value of population-based studies in AML that have the potential to deliver reliable epidemiological data. Registries are also useful as a complement to clinical studies to support decisions about individual patient management, although in retrospect<sup>92,93</sup>.

# What we have learned from our studies

## Age dependent incidence of different chromosomal abnormalities

Our study has shown that the median age of patients with *inv(16)* or *t(16;16)* was 48 years, and with *t(8;21)* 55 years. Altogether the patients with CBF leukemia were younger than the average AML patient (median age 71 years). The incidence of CBF shows rather insignificant increase during the lifetime (range 1.3-2.4/million/year;  $p=0.16$ ). Likewise, the patients with *inv(3)(q21q26)/t(3;3)(q21;q26)*, *t(6;9)(p22;q34)*, and *11q23* rearrangements; not *t(9;11)* were more prevalent in younger patients (median age 58, 36, and 54 years, respectively). In contrast, the incidence of AML with *5q*, *7q* and/or *17p* increases with older age (range 0.9-43/million/years;  $p<0.0001$ ), as well as patients with complex karyotype. This most likely illustrates different pathophysiologic mechanisms involved in leukemogenesis in different AML groups. The data from one population-based study clearly indicates that the age-dependent increase in incidence of AML substantially differs between the cases with balanced, with normal, and with unbalanced karyotypes, and suggest that mechanisms of leukemogenesis are different and more or less age-dependent<sup>94</sup>. The results of our study and of others 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<sup>57,94</sup>. At least a proportion of, if not all, balanced translocations of pediatric leukemias already develops in the prenatal period. This was demonstrated by the observation of twins developing acute leukemias with reciprocal gene fusions, e.g. *cALL* with *TEL-AML1*, after a latency of up to 14 years<sup>95</sup>. 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*<sup>95</sup>. 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, unbalanced

distribution of the chromosomes to the daughter cells, or incorrect repair of DNA double strand breaks<sup>57,96,97</sup>. 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. 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, unbalanced distribution of the chromosomes to the daughter cells, or incorrect repair of DNA double strand breaks<sup>97,98,99</sup>. The age-specific distribution of the molecular markers might be due not only to different mutational mechanisms in dependence on age but also due to age-specific changes in hematopoiesis and to changes in the available pools of hematopoietic precursors as targets for leukemogenesis. 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<sup>94</sup>. WHO classification from 2008 roughly separates AML in three categories: *de novo* AML, therapy-related AML and secondary AML (with antecedent MDS or MPN). These categories seem to have a different ontogenesis and age-distribution<sup>100</sup>. There is accumulating data about the time sequence of events which lead to overt leukemia, where specific gene mutations and even *CBFH/MYH* translocation in *inv(16)* occur in preleukemic stem cells. It supports a model in which mutations in "landscaping" genes, involved in global chromatin changes such as DNA methylation, histone modification, and chromatin looping, occur early in the evolution of AML, whereas mutations in "proliferative" genes occur late<sup>101</sup>. These findings indicate that preleukemic HSCs can survive induction chemotherapy, identifying these cells as a reservoir for the reevolution of relapsed disease. Cytogenetic data from the Swedish Acute Leukemia Registry can also provide insight into the clonal origin and evolution, especially in the cases with complex karyotype<sup>102, 103, 104</sup>.

## Overlap between poor-risk chromosome abnormalities, complex and monosomal karyotypes

We found a strong overlap between some chromosome abnormalities, especially the changes of chromosomes 5, 7 and 17. This not only indicates that they identify the same subgroup of patients characterized by poor prognosis, but also that they cooperate in the leukemogenic process and/or have similar mechanisms behind their occurrence. Furthermore, the prognostic impact of a complex karyotype or MK was clearly influenced by the presence of 5q, 7q and 17p losses. Adding MK into the risk stratification of Swedish AML patients did not improve survival prediction. We hence conclude that the negative impact of MK seems to be mostly carried out by

abnormalities of chromosomes 5, 7 and 17. This is opposed to other studies<sup>27, 105</sup>, but is in line with the data from the Spanish and German MDS/AML registries<sup>106,107</sup>. In our series of complex karyotype, monosomal karyotype and changes of chromosomes 5/7/17 overlap in more than 80% of cases. Therefore, we believe that because of the overlap, monosomal karyotype is more “accumulative” and “statistical” than a true “biological entity”. There is now a huge body of evidence on the clear correlation between *TP53* mutation and complex karyotype (and most likely monosomal karyotype)<sup>63,105,108</sup>. Since among 234 complex karyotype AML cases analyzed, *TP53* mutations determined by DNA sequencing were more frequent (141/234 cases, i.e., 60%) than *TP53* losses determined by array-CGH analysis (94/234 cases, i.e., 40%), it might be concluded that *TP53* loss of function indeed causes chromosomal instability (CIN) with subsequent development of complex karyotype alterations, rather than being a consequence of CIN<sup>63,100,104</sup>.

## Unsuccessful cytogenetics

Unsuccessful (UC) and unperformed cytogenetics (UPC) are often reported together as a “not determined karyotype”<sup>109</sup>. We separated the UC and UPC groups assuming that their causes and prognostic impact may differ. The incidence of UC of 2.1% is lower than in previously published studies. These patients are >60 years of age (median 66 years) which partly explains a dismal prognosis for these patients. We confirmed the findings of a group from USA with lower CR rate and poor prognosis for AML patients with UC, but we also found a higher early death (ED) rate<sup>54</sup>. The definition of unsuccessful cytogenetic karyotype (UC) is a lack of analyzable metaphasis. There are several possible explanations of this phenomenon. Some cases with UC are undoubtedly due to insufficient number of cells in the bone marrow aspirates sent for cytogenetic analysis. Furthermore, human errors in taking the bone marrow aspirates cannot be excluded, such as too small volumes or diluting the bone marrow cells with peripheral blood, and technical problems in the laboratory. Finally, there may well be some biological explanations for UC, representing the intrinsic properties of the leukemic clone, such as inability to divide *in vitro*. In fact, UC is not specific for AML. There are reports of dismal prognosis of ALL cases with UC<sup>110,111</sup> as well as of myelodysplastic syndromes with UC<sup>112</sup>, with the latter suggesting that UC is a property of dysfunctional stem cells. However, the underlying reasons for UC are most likely manifold and heterogeneous, and hence next to impossible to ascertain in a retrospective, registry-based study of this type.



## Unperformed cytogenetics

The issue of unperformed karyotype is even more controversial than unsuccessful karyotype. This group is almost invisible in the literature, since karyotype is usually mandatory in clinical trials. UPC is often lumped together with unsuccessful karyotype or just classified as not done, not available or not determined<sup>109</sup>. The population-based AML Registry is an excellent source to identify such a patient group. It is impossible in retrospect to know the reasons for why the karyotype was performed or not from case to case, but it is likely that many were not fit for intensive treatment. We found 364 patients in the Registry, mostly older than 60 years of age, and their outcome was very similar to those with high risk AML, with increased ED rate, decreased CR rate and poor OS. The presence of UPC emphasizes the need for proper genetic analyses of all patients for whom treatment with curative intent is planned, and even in the cases where therapy is not planned in order to have data for future analysis.

## Hyperdiploidy

Our report again emphasizes the value of population-based studies, because it is the first population-based study of hyperdiploid AML. We found a male preponderance (71%), and dominance of AML types M2, M4, M5. This is in contrast to childhood AML, where the FAB type M7 was predominant. Furthermore the majority of secondary hyperdiploid AML originated from previous MDS. Tri/tetraploidy AML and high hyperdiploidy had different outcome, since tri/tetraploid AML (>65 chromosomes) had a better survival showing the possibility of different leukemogenic pathways and possibly to be regarded a separate entities. Surprisingly, none of the high hyperdiploidy/tri and tetraploidy AML cases was secondary to a myeloproliferative neoplasm (MPN). The outcome was also different when the hyperdiploidy included chromosome abnormalities with bad prognosis, such as del 5, del 7 or del 17. It is very probable that different diseases with hyperdiploid cytogenetics, have different pathogenetic effect induced by different chromosomes or other mutations<sup>113, 114, 115</sup>. Our results are in line with the previous studies showing that this group is more heterogeneous than previously described and that the overall survival of this group is a bit closer to HR than IR AML<sup>116</sup>.

## Secondary leukemia

The incidence of secondary AML was 26.4%, which is comparable to other population-based studies<sup>117</sup>. The median latency period between MDS and AML was 1 year, indicating that most MDS patients who progress to AML do so within a short time frame. Median latency times between MPN and AML were between 7 and 8 years, whereas the median latency between the malignancy and tAML was slightly longer compared to most of the previous studies with 5.8 years<sup>118,119,120</sup>. Median latency between a nonmalignant disease and tAML is seldom reported, but was shown to be 14.3 years in our cohort. CR rates were lower than *de novo* AML, but not depending on the ED rate. We confirmed the negative prognostic importance of sAML. This was highly significant for younger patients, but did not add to the prognostic information in elderly AML patients. The reason for this difference in younger but not in older patients could potentially be due to the fact that sAML biologically and genetically is more similar to AML in general in older patients. The reason for the poor outcome in secondary AML remains somewhat elusive. Although an increased frequency of high-risk cytogenetics explains some of the treatment resistance, there must clearly be some additional factors conferring the poor prognosis.

## The role of trisomy 13 in the prognosis of AML

We specifically analysed the prognosis of patients with trisomy 13 in our AML Registry, including patients diagnosed between 1997-2014. We found that all patients had died, including younger patients as well as two patients who underwent allogeneic stem cell transplantation. The results corresponded to the data from the German group, to which we provided a comment<sup>59</sup>. This is an obvious example of shortcomings of AML classification. Trisomies are generally regarded as indicators of the intermediate risk AML, but our analysis confirmed that this entity is consistent with a high risk<sup>59</sup>.

## Brief summary

Data from the Swedish AML Registry show the higher incidence of loss of 5q, 7q and 17p, MK and CK in patients >60 years. The patients with  $\geq 5$  chromosome abnormalities had very short OS even after intensive chemotherapy. The most significant prognostic factors were age, PS, followed by sex and karyotype (deletion of

chromosome 7q). Overall survival of patients with UC and UPC AML were in between IR and HR AML. Triploid/near tetraploid AML ( $\geq 65$  chromosomes) had a better outcome than high hyperdiploidy ( $< 65$  chromosomes). Isolated trisomy 13 had a similar outcome as HR AML. Therefore, hyperdiploid AML, UC, UPC and isolated trisomy 13 should be regarded as HR AML. Secondary AML had an impact on survival in patients  $< 60$  years, but not in patients  $> 60$  years of age.

New diagnostic techniques have come into the everyday practice of the physicians treating AML, including molecular changes identified through Next Generation Sequencing<sup>121,122</sup>. Nevertheless, karyotype analysis is still the gold standard for diagnosing AML. By utilizing the population-based perspective we could further describe some of the heterogeneous groups and subgroups of AML and thus humbly contribute to the “old world” of cytogenetics.

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