Clinical Relevance of Complex Chromosomal Aberrations in Bone Marrow Cells of 107 Children with *ETV6/RUNX1* Positive Acute Lymphoblastic Leukemia (ALL).

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INTRODUCTION

Cryptic translocation **t(12;21)(p13;q22)** which give origin to the hybrid gene *ETV6/RUNX1* can be found by I-FISH in approximately 20-25% of children with B precursor ALL as the most frequent specific aberration. Very small chromosomal segments are involved in this translocation and therefore classical cytogenetic techniques are not sensitive enough for its identification.

This translocation is generally associated with good outcome. Despite of its favorable prognostic value, late relapses may occur within this group of patients. One of the reasons could be the high instability of the genome of leukemic cells, which is manifested at the chromosomal level by additional aberrations and/or complex chromosomal rearrangements. Additional chromosomal abberations were proved in about 50-70% of patients with *ETV6/RUNX1* positive ALL. Genetic changes that are most frequently associated with t(12;21) are the deletion of the wild type *ETV6* allele, trisomy of chromosome 21 and/or duplication of the *ETV6/RUNX1* fusion gene. Also non-specific structural and/or complex chromosomal rearrangements could be found.

The significance of complex chromosomal aberrations in *ETV6/RUNX1* positive cells is not clear. However, complex karyotypes in bone marrow cells mean adverse prognostic effect, as it was already proved in adult patients with acute myeloid leukemia or other hematologic malignancies, where secondary changes appear during progression of the malignancy.

The aim of the study was to evaluate the incidence and clinical significance of complex chromosomal aberrations for prognosis of children with *ETV6/RUNX1* positive ALL.

MATERIALS & METHODS

Patients

- ✓ 107 children with *ETV6/RUNX1* positive ALL (46 girls; 61 boys)
- ✓ diagnosed between 1995-2006 in 7 haematological centers in Czech Republic
- ✓ clinical data are summarized in the table

Treatment protocols:

BFM 90 (12x), BFM 95 (62x), ALLIC 02 (31x), FRALLE 93 (1x), pr.0491 (1x)

Conventional cytogenetics

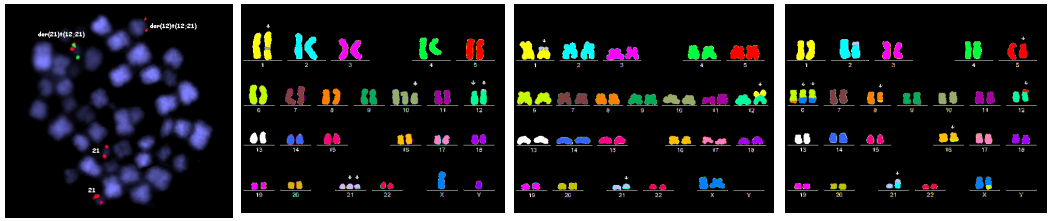
Chromosomal preparations were made from 24-hours cultivated bone marrow cells without stimulation. The slides were prepared by conventional technique. Cytogenetic examinations were performed on G-banded slides stained by Wright's/Giemsa stain or G-banded with trypsin according to the routine laboratory protocols and at least 20 metaphases were analyzed for each patient. Chromosomal abnormalities were described according to the ISCN nomenclature.

Fluorescence in situ hybridization (FISH)

For the assessment of *ETV6/RUNX1* fusion gene RT-PCR and/or double target interphase FISH with locus-specific probe (Abbott-Vysis™) were used (200 interphase nuclei analysed, cut-off level 2.5% tested on controls, standard deviation ≥0.5%).

Structural and/or complex chromosomal aberration were proved by FISH with whole chromosome painting probes (Cambio™, Cambridge, UK) and/or by mFISH with the "24*X*Cyte" probe kit (MetaSystems™ GmbH, Altlussheim, Germany). Chromosomes were counterstained by DAPI (4,6-diamidino-2-phenylindole; blue color).

RESULTS



ETV6/RUNX1+, +21, del(ETV6)

48,XY,der(1)ins(1;10)(q21;p?),+der(10)del(10)(p?),del(12)(p13),t(12;21)(p13;q22),+21

46,XX,t(1;12;21)(p12;p13;q22)

47,XX,t(X;6)(q22;q21),+der(6)t(X;6)(q22;q21),t(2;5;12;21)(p14;q32;p13;q22),t(6;8;16)(q24;q23;q22)

Clinical data and actual clinical conditions of patients:

		Age (years)	WBC (x10⁹/l)	Blasts (%)	EFS (months)	Follow-up (months)
N	valid	107	104	93	107	107
	missing	0	3	14	0	0
Mean		**5.18**	**20.9**	**35.42**	**52.584**	**69.33**
Median		**4.20**	**7.75**	**30.00**	**47.000**	**70.50**
Std. deviation		3.17	34.36	30.45	38.34	41.57
Minimum		1.20	0.70	0.00	3.00	3.00
Maximum		16.9	226.00	98.00	178.00	178.00

- Most of the patients are living in the first or second complete remission.
- Relapse appeared in 17 children (17,9%).
- Three patients died (two because of relapse and one for treatment complications).

Molecular-cytogenetic findings:

In 64 children (59.8%) we found besides t(12;21)(p13;q22) additional numerical and/or structural chromosomal aberrations. The most frequent of them were:

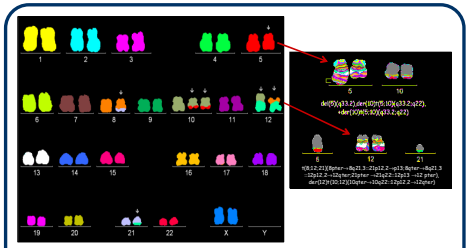
- trisomy/tetrasomy of chromosome 21 (20 cases)
- deletion of nontranslocated *ETV6* allele (24 cases)
- deletion of 6q (7 cases)
- rearrangements of the long arm of chromosome X (6 cases)

Variant translocations of chromosome 12 and 21 with other partner chromosome were found in 12 children.

Complex karyotypes (more than three chromosomal breaks) were identified in 38 children (35.3%).

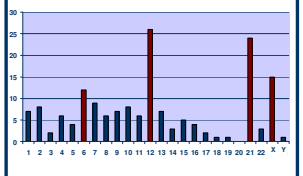
Variant *ETV6/RUNX1* translocations:

t(1;12;21)(p12;p13;q22)
t(1;12;21)(p36.1;p13;q22)
t(4;12;21)(p15;p13;q22)
t(7;12;21)(p21;p13;q22)
t(8;12;21)(q21.3;p13;q22)
t(8;12;21)(q24;p13;q22)
t(10;12;21)(qter;p13;q22)
t(12;13;21)(p13;q12;q22)
t(12;15;21)(p13;q?;q22)
t(2;5;12;21)(p14;q32;p13;q22)
2x t(12;21;?)(p13;q22;?)

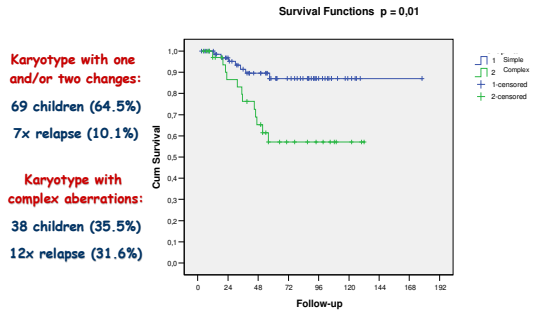


47,XX,del(5)(q33.2),t(8;12;21)(q21.3;p13;q22),der(10)t(5;10)(q33.2;q22),+der(10)t(5;10)(q33.2;q22),der(12)t(10;12)(q22;p12.2)

Representation of different chromosomes in additional aberrations:



Analysis of Event Free Survival (EFS)



Analysis of **event-free survival** (EFS) revealed **significantly shorter survival** in patients with additional structural and/or complex aberrations in *ETV6/RUNX1* positive cells (p=0,01).

CONCLUSIONS

- Cryptic translocation t(12;21)(p13;q22) can be associated with additional chromosomal abnormalities (deletions, translocations, insertions).
- In our cohort of patients with *ETV6/RUNX1* positive ALL complex karyotypes indicated poor prognosis.
- Finding of complex chromosomal aberrations in leukemic cells is accompanied by higher risk of relapse even in those cases where the prognostically positive aberration is primarily present.

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Statistical analysis: A. Dohnalova, Dept. of Biostatistics of 1st Medical Faculty, Charles University, Prague

DISCLOSURE

No relevant conflicts of interest to declare.

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