Efficacy of high-resolution comparative genomic hybridization (HR-CGH) in detection of chromosomal abnormalities in children with acute leukaemia

V. VRANOVA[1], D. MENTZLOVA[2], A. OLTOVA[2], V. LINKOVA[2], D. ZEZULKOVA[2], H. FILKOVA[2], D. MENDELOVA[3], J. STERBA[3], P. KUGLIK[1*]

[1]Department of Genetics and Molecular Biology, Institute of Experimental Biology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37, Brno, Czech Republic, e-mail: kugl@sci.muni.cz; [2]Department of Medical Genetics, University Hospital Brno, Czech Republic; [3]Department of Pediatric Oncology, University Hospital Brno, Czech Republic

Received April 23, 2007

The efficient detection of chromosomal aberrations in childhood acute leukaemias presents a significant component in the diagnostics of this frequent malignant disease. We used comparative genomic hybridization (CGH) and high-resolution comparative genomic hybridization (HR-CGH) to determine the frequency of chromosomal changes in 33 children with acute leukaemia (AL). The yields of chromosomal abnormalities were compared with the results obtained using conventional cytogenetics (G-banding) and fluorescence *in situ* hybridization (FISH). Conventional cytogenetics revealed chromosomal changes in 17 (52 %) of studied patients. The employment of FISH together with G-banding analysis identified chromosomal changes in 27 (82 %) of the AL patients investigated. CGH detected changes in DNA copy numbers in 24 (73 %) patients, 40 losses and 67 gains were found in total. HR-CGH disclosed 98 losses and 97 gains in 26 (79 %) patients. In comparison with CGH, HR-CGH analyses unveiled 88 new chromosomal aberrations: 58 losses and 30 gains. The most commonly gained chromosomes were 21 (22.5 %), X (15 %), 18 (12,5 %) and 17 (10 %). The most common losses involved sub-regions or arms of chromosomes 7 (15 %), 9 (12.5 %), 16, 19 and 1 (10 % each). Cytogenetic and molecular cytogenetic analyses of 33 childhood acute leukaemias revealed chromosomal changes in total 31 (94 %) patients. The evaluation of HR-CGH sensitivity proved that the minimal cell population of malignant cells in which a certain chromosomal change could be found was close to the 20 – 30 % level. Our results confirm the benefits of HR-CGH in detecting chromosomal changes in childhood AL. Supplementing G-banding and FISH with the HR-CGH diagnostic method increases the detection of unbalanced structural chromosomal rearrangements and can reveal small cell clones with gains and losses of whole chromosomes in hyperdiploid AL.

Key words: comparative genomic hybridizadion (CGH), high-resolution comparative genomic hybridization (HR-CGH), chromosomal aberrations, acute myeloid leukaemia, acute lymphoid leukaemia

Acute leukaemia is the most frequent malignant disease in children up to 15 years of age and according to world statistics it represents 31 % of all malignant disease in Caucasians of this age category. It is more frequent in boys than in girls [1]. Acute leukaemia (AL) is classified in two groups depending on the type of hemopoetic tissue affected, i.e. acute myeloid leukaemia (AML) and acute lymphoid leukaemia (ALL). Some 70-90 children are annually diagnosed with ALL and around 15-20 with AML in the Czech Republic [2, 3]. Apart from traditional prognostic factors in patients with AL (like number of white blood cells, age, sex, immunophenotype, DNA index, CNS affection), there are other significant factors – presence of chromosome abnormalities and molecular biological character of leukaemic cells [4–7]. A precise detection of chromosome changes is important both for diagnosis of the disease and for its prognosis, choice of the most suitable therapy and last but not least for a follow-up after the achieved treatment.

The detection of prognostically significant chromosome abnormalities in leukaemic patients has for many years been carried out by means of cytogenetic techniques (namely G-banding and fluorescence *in situ* hybridization – FISH). Due to a poor chromosome morphology and a low mitotic activity of cancer cells, a novel method of comparative genome hybridization (CGH) has been used for this purpose in recent

* Corresponding author

years. This method enables a whole-genome screening of unbalanced chromosome changes and a disclosure of numeric chromosomal changes present in hyperdiploid karyotypes [8–11]. CGH detects changes that are present in 50 % or more of the specimen cells, with the affected region exceeding 5-10 Mb. However, many chromosomal aberrations have a lower incidence than 50 % and this may result in problems with their identification. The recently developed high-resolution CGH (HR-CGH) [12] is primarily suited for the detection and localization of genomic changes down to 3-5 Mb and/or those with a lower prevalence of aberrant clones [13]. HR-CGH was successfully used in the diagnostic and prognostic classification of acute lymphoblastic leukaemia [14].

The present paper studies the incidence of chromosome abnormalities detected in samples of bone marrow by methods of conventional G-banding, FISH, CGH, and HR-CGH in the group of 33 child patients suffering of acute leukaemia. Our goal was to compare the rate of chromosomal aberrations revealed by various cytogenetic techniques and to verify the sensitivity and diagnostic application of HR-CGH in the detection of unbalanced translocations and numeric chromosomal changes in the children with acute leukaemia.

Patients and methods

Patients. The bone marrow samples from 33 children with acute leukaemia (14 males/19 females; median age 5 years; range 1.5-18 years) were examined at the Department of Medical Genetics at the University Hospital Brno during the years 2003-2005. Twenty-six patients (11 males/15 females; median age 5 years; range 2–18 years) were diagnosed as having ALL (22 patients B-ALL/4 patients T-ALL) and 7 patients (3 males/4 females; median age 10 years; range 1,5-15 years) as having AML. The diagnosis of ALL/AML was based on the FAB classification. Cytogenetic and molecular cytogenetic examinations were performed in 28 patients at the time of diagnosis and in 5 patients during a relapse. Three patients died; two patients during the therapy and one after an early relapse (less than 6 months from the end of therapy). Clinical data are shown in Table 1.

Table 1. Clinical and laboratory data of patients with acute leukemia

Patient no.	Sex	Age	Immunophenotype	WBC ($x10^9$/l)	Number of blasts in BM (%)	Number of blasts in PB (%)	Disposition
1	M	18	pre-B ALL	0.20	85.6	0	remission
2	F	6	com-B ALL	4.3	97.6	39	remission
3	F	5	com-B ALL	6.9	87	5	remission
4	F	4	com-B ALL	2.39	78.6	39	remission
5	F	2	com-B ALL	4.46	86.2	37	remission
6	M	4	pre-B ALL	2.56	70.6	40	remission
7	M	5	com-B ALL	18.8	93	30	LR, remission
8	F	5	pre-B ALL	2.0	97.6	15	remission
9	F	2	com-B ALL	39.3	88.4	62	remission
10	F	5	com-B ALL	2.46	94.8	14	remission
11	F	3	com-B ALL	38.2	90.4	76	remission
12	F	3	pre-B ALL	5.0	79.8	2	remission
13	M	2	pre-B ALL	10.8	58.4	9	LR, remission
14	M	15	T ALL	69.9	84	?	remission
15	M	13	pre-T/My$^+$ ALL	2.8	91.6	49	ER, died
16	M	14	T ALL	338.9	41	31	remission
17	F	10	s-inter.T ALL	6.4	85.8	29	died
18	F	4	com-B ALL	8.6	91.2	36	remission
19	M	6	com-B ALL	2.60	90.6	32	remission
20	F	9	com-B ALL	6.5	84.6	41	remission
21	M	8	com-B ALL	89.3	97	87	ER, remission
22	M	4	com-B ALL	21.9	86.2	58	remission
23	F	9	pro-B ALL	6.8	43.4	5	LR, remission
24	F	4	pre-B ALL	21.30	94.2	82	remission
25	M	3	com-B ALL	29.10	87	73	remission
26	F	3	com-B ALL	44.90	98	64	remission
27	F	2	AML M5a	41.9	69	81	remission
28	M	1,5	AML M5	216	78.4	20	died
29	M	15	AML M2	36.5	14	26	remission
30	M	10	AML M2	38.6	73.4	80	remission
31	F	13	AML M2	5.3	37	0	remission
32	F	7	AML M2	13.5	47.6	40	remission
33	F	14	sAML M2	2.7	69	0	remission

WBC – white blood cells, BM – bone marrow, PM – peripheral blood, LR – late relaps, ER – early relaps, ? – not available

Conventional cytogenetics. Chromosomal analyses were performed on short-term cultured leukaemic cells from the bone marrow according to standard procedures and karyotypes were classified according to the ISCN 1995 nomenclature [15].

Fluorescence in situ hybridization (FISH). Fluorescence *in situ* hybridization was performed with commercially available probes (CEP Chromosome Enumeration DNA Probe, LSI Locus Specific Identifier DNA Probes, WCP Whole Chromosome Paints DNA Probe, ToTel Vysion™ Probe Panel from Abbott-Vysis, Inc., Downers Grove, IL, USA, and Aquarius®, Cytocell, UK) according to the manufacturer's instructions. At least 100 nuclei or 10 metaphases were captured using an Olympus BX61 microscope and a COHU 4910 CCD camera. They were evaluated using the LUCIA G 4.82 – KARYO/FISH/CGH/CGH-Advanced Statistics Software (Laboratory Imaging, Ltd., Prague, Czech Republic).

CGH/HR-CGH. Phytohemagglutinin-stimulated normal lymphocytes from karyotypically normal males were prepared as metaphase targets for CGH/HR-CGH experiments using standard protocols. CGH/HR-CGH experiments were performed according to the manufacturer's protocol (Abbott-Vysis, Inc., Downers Grove, IL, USA). A test DNA extracted from the patients´ bone marrow using chloroform extraction (Spectrum Green-dUTP-labelled) and a reference DNA from peripheral blood lymphocytes (Spectrum Red-dUTP-labelled) were precipitated in the excess presence of human Cot-1 DNA (Abbott-Vysis) and hybridized to normal metaphases at 37°C for 48-72 hours. The slides were counterstained with DAPI solution and representative images of at least 10 metaphases per case were captured using the Olympus BX61 microscope and the COHU 4910 CCD camera. Chromosomes were karyotyped and ratio profiles were calculated using the LUCIA software as described above.

For the CGH analysis, losses and gains of chromosomal regions were detected when green:red ratio profiles deviated by 20 % from the ratio of 1.0 (<0.80 – losses, >1.20 – gains). The centromeres and acrocentric p-arms were excluded from the CGH analysis.

In our study, HR-CGH modification was developed according to the published methods [12]. The 99.5 % dynamic standard reference interval was based on an average of 17 CGH analyses from healthy donors with normal karyotypes. The interval for each chromosome was then analyzed by the special software – LUCIA G 4.82 Advanced Statistic (Laboratory Imaging). Chromosomal abnormalities were detected by comparing this 99.5 % dynamic standard reference interval to the 99.5 % confidence interval of the mean ratio profile of the test samples. Regions where the two sets of intervals did not overlap were considered aberrant. The centromeres, telomeres and acrocentric p-arms were excluded from the HR-CGH analysis.

Statistical analysis

The spectrum and frequency of chromosomal aberrations were compared between CGH and HR-CGH. Because the experiment was paired and the data had an abnormal distribution, a non-parametric Wilcoxon rank-sum test was used to test for differences between variables. The differences with $p<0.05$ were considered statistically significant.

Results

The results of cytogenetic analyses performed by means of cytogenetic and molecular cytogenetic methods in the cohort of 33 child patients suffering from acute leukaemia are summarized in Table 2.

G-banding and FISH. All 33 bone marrow samples were subjected to a conventional cytogenetic analysis. A pathological karyotype was found in 17 patients (52 %). Another 14 patients (42 %) had normal karyotypes, 2 patients (6 %) manifested no mitoses. The cytogenetic findings were confirmed by FISH. In 10 patients from the normal karyotype/no mitoses group, FISH revealed chromosomal abnormalities too. In total, 27 (82 %) of all patients examined had some chromosomal aberrations detected by G- banding and FISH combined.

ALL. Of 22 patients with B-ALL, 9 children (41 %) were put into a group with favourable prognosis and a highly hyperdiploid karyotype (>52 chromosomes) based on the G-banding and FISH results; a single patient had a slightly hyperdiploid karyotype 47,XY,+21. Besided hyperdiploidia, two of these patients had structural changes disclosed by means of G-banding.

The FISH using the LSI TEL/AML1 ES Dual Color Translocation Probe detected the t(12;21)(p13;q22) translocation in 9 patients (41 %) (patiens no. 18 to 26 in Table 2). The most frequent additional change in these children was the deletion of the second TEL allele (7 patients), 2 children showed two TEL/AML1 fusion signals.

In the group of 4 patients with ALL stemming from T-lymphoblasts, 1 patient had a normal karyotype and 1 patient had a translocation of MLL gene (11q23). Two patients had structural changes on chromosome 7 in their karyotypes. The unfavourable prognosis of this group is confirmed by the fact that 2 of the children with ALL died.

AML. Five of 7 patients with AML (71 %) had clone chromosome aberrations detected, another 2 had a normal karyotype. Of 5 children with AML subtype M2, 2 had a reciprocal translocation t(8;21)(q22;q22) identified by classical G-banding; in 1 patient this was found only by means of FISH. The 2 patients with AML subtype M5 had genetic changes involving the region 11q23 detected both by classical cytogenetics and FISH. In this case, they were translocations t(6;11) and t(9;11).

CGH and HR-CGH. Using the CGH method, we found 107 unbalanced chromosomal abnormalities in 24 patients (73 %) in total; of these, there were 67 gains and 40 losses of DNA sequences (range 0-18, median 2 changes/patient). The gains of genetic material concerned namely surplus of whole chromosomes (40/67 gains). The most frequent hyperploidy

Table 2. Summary of chromosomal changes detected by G-banding, FISH, CGH, and HR-CGH techniques in patients with acute leukaemia. Chromosomal abnormalities classified only by HR-CGH method are in bold.

Patient no.	G-banding	FISH		CGH	HR-CGH
		results	% aberrant cells		
1	53~55,XY,+X,+4,+C,+14,+17,+18,+21 [cp8]	+21, (+21)	90 %	rev ish enh(4,6p22-pter,10,14,17q,18,21, X,Y) rev ish dim(16q)	rev ish enh(4,6p22-pter,10,14,17q,18,21,X,Y) rev ish dim(**1,2,3,5,6q,7,8,9,11q,12,13,15**,16q)
2	55,XX,+X,der(1),+B,+C,+C,+18,+21,+21,+ 2mar [6]	+X +10 +18 +21,(+21)	80 % 86 % 76 % 85 %	rev ish enh (1q21-q31,4,6,10,14,17, 18,21,X) rev ish dim(1p31- pter,7,11)	rev ish enh (1q21-q31,4,6,10,14,17,18,21,X) rev ish dim(1p31-pter,**2,3,5,**7,**8,9,**11,**12,13, 15,16,19q,20,21**)
3	no mitotic cells	+21, (+21)	80 %	rev ish enh(6,17,21,Xp,Xq11.1-q22)	rev ish enh(**1q22-q32**,6,17,**18q**,21,Xp,Xq11.1-q22) rev ish dim(**3p,16q**)
4	46,XX [12]	+9 +21,(+21)	51 % 32 %	rev ish enh(18,21)	rev ish enh(**6,9q,10q,14,17,**18,21) rev ish dim(**1,2,3,5**)
5	46,XX [9]	+21, (+21), +12p +22	76 % 70 %	rev ish enh(1q25-qter,10p,10q25-qter, 14,17,18,21) rev ish dim(9,11,15,16p,19)	rev ish enh(1q25-ter,**4q,7,**10p,10q25-qter,14,17,18,21) rev ish dim(**1p,2q,3**,9,11,15,16p,19)
6	56~57,XY,+A,+4,+C,+D,+18,+21 [cp9]	+X +10 +18 +21,+12p	100 % 76 % 72 % 85 %	rev ish enh(4,9p21-pter,10q21,12q21-q22, 14q21-22,18q12-qter,21,X) rev ish dim(1p36,7q11-q21,16,19,Y)	rev ish enh(4,**9,10,12,14q,17q,18**,21,X) rev ish dim(**1,7,11p11-15,15**,16,19,Y)
7	47,XY,+21[2] / 46,XY [12]	+21	47 %	NP	NP
8	46,XX [15]	+10 +11 +21 +22	78 % 33 % 50 % 97 %	rev ish enh(21,22)	rev ish enh(**10q23-q26,17q,**21,22) rev ish dim(**1q31-qter**)
9	54,XX,+der(4),+6,+14+17,+18,+19,+21, +mar [cp7]	+21	88 %	rev ish enh(4p15-pter,6p21-pter,6q25-qter, 14q31-qter,17, 18,19,21,22,Xp,Xq25-qter) rev ish dim(1p21-p31,1q25-q31,2q23-q33,3q11-q24,5q11-q23,8q21-q23,13q14-q22)	rev ish enh(4p15-pter,6p21-pter,6q25-qter,14q31-qter,17, 18,19,21, 22,Xp,Xq25-qter) rev ish dim(**1p,1q,2,3,5,7,8,13**)
10	57,XX,+X,+4,+6,+10,+11,+14,+15,+17, +17,+21,+22 [9]	+10	70 %	rev ish enh(X,21)	rev ish enh(**4,6,10,14,17,18q**,21,X) rev ish dim(**2,5,8,9q,13q14- qter,15q15-qter,16q,20**)
11	46,XX [15]	del(12p13)	35 %	NP	NP
12	46,XX,+X,der(9)t(9;20)del(9p),-20[7]	+X t(9;20)	70 %	rev ish enh(X) rev ish dim(9p,20q)	rev ish enh(X) rev ish dim(9p,20q)
13	46,XY,der(1)?,der(4)t(4;11),der(8)?[2]	rearran. MLL gene	50 %	rev ish enh(1q22-qter)	rev ish enh(1q22-qter)
14	46,XY[8]	–	–	NP	NP
15	46,XY,del(7q)[3] / 46,XY [17]	del(7q31)	64 %	rev ish dim(7p21- pter,7q35)	rev ish dim(7p21- pter, 7q35)
16	46,XY,der(19)?,der(20)?[9]	–	–	rev ish enh(19q,19p)	rev ish enh(**1p31-pter;17**,19p,19q) rev ish dim(**1q24-q41,2q23-q34,3q12-q26, 6q11-q24, 13q14-q32**)
17	46,XX,i(7q),der(11)[12] / 46,XX[6]	rearran. MLL gene	47 %	rev ish enh(7q) rev ish dim(7p)	rev ish enh(7q) rev ish dim(7p)
18	46,XX[13]	TEL/AML1 x 2, + AML1, +TEL	75 %	rev ish enh(6p12-p21,21,X)	rev ish enh(**1p31-p36**, 6p12-p21,**9q22-q34,12p12-pter**, 21,X) rev ish dim(**4q,5q11.1-q23**)
19	46,XY[11]	TEL/AML1,del (TEL), +AML1 +16 del(9p21)	59 % 46 % 42 %	rev ish enh(16,21) rev ish dim(9p,20q)	rev ish enh(16,21) rev ish dim(9p,20q)
20	46,XX[10]	TEL/AML1 , + del(TEL)	87 % 34 %	rev ish enh(Xq22-qter)	rev ish enh(Xq22-qter)

Continued

Patient no.	G-banding	FISH		CGH	HR-CGH
		results	% aberrant cells		
21	46,XY,del(6q),del(12p), del(13q)[3]	TEL/AML1 + del(TEL)	90 %	rev ish dim(6q15-q22,12p12)	rev ish dim(6q15-q22,12p12)
22	46,XY[11]	TEL/AML1, + del(TEL)	72 % 67 %	rev ish dim(12,17)	rev ish dim(12,17)
23	46,XX[12]	TEL/AML1 x 2 + del(TEL), +AML1	31 %	NP	rev ish dim(**1p31-p35,8p12-pter**)
24	no mitotic cells	TEL/AML1	80 %	rev ish dim(X)	rev ish enh(**1p31-p36,14q11-q23**) rev ish dim(X)
25	46,XY[17]	TEL/AML1, + del(TEL)	95 % 12 %	rev ish enh(Xq25-qter) rev ish dim(12p12-pter)	rev ish enh(Xq25-qter) rev ish dim(12p,**18p,Y**)
26	46,XX[17]	TEL/AML1, + del(TEL)	95 % 51 %	NP	NP
27	46,XX,der(11)t(9;11)del(11p)[8]	rearran. MLL gene	11 %	NP	NP
28	52,XY,+3,+der(6)t(6;11),+8,+8,inv(9),+13,+19[5] / 46,XY,inv(9)[2]	rearran. MLL gene	58 %	NP	rev ish dim(**11p11.2-p14**)
29	46,XY,t(8;21),del(9q)[4]/ 46,XY[8]	AML1/ETO	90 %	rev ish dim(9q11.1-q22,16q,19q)	rev ish enh(**4q21-q26**) rev ish dim(9q11.1-q22,16q,19q)
30	46,XY[6]	AML1/ETO	98 %	rev ish dim (7,9q21-q22)	rev ish enh(**1p31-p36**) rev ish dim (7,9q21-q22)
31	46,XX [31]	–	–	NP	NP
32	46,XX,t(8;21) [15]	AML1/ETO	80 %	NP	NP
33	46,XX,der(18)t(18;19),del(19q), -21,+mar [13]	+ 21	31 %	rev ish dim(19q13.1-13.4)	rev ish enh(**4p,8p**) rev ish dim(19q13.1-13.4)

– no alterations found, NP = normal profile, rearran. MLL gene = rearrangement of MLL gene

affected chromosomes 21 (22.5 %), X (15 %), 18 (12.5 %) and 17 (10 %). Losses of genetic material impacted more frequently on specific chromosomal regions than on whole chromosomes. The most frequently deleted regions were identified on chromosomes 7 (15 %), 9 (12.5 %), 1, 16, 19 (each by 10 %).

HR-CGH, that is more sensitive in comparison to CGH, disclosed even 195 aberrations in 26 patients (79 %) of our study cohort (range 0-24, median 3 changes/patient) ($p = 0.01^*$); of these, there were 97 gains and 98 losses of DNA sequences. HR-CGH increased the number of detected aberrations by 88 new changes (30 gains and 58 losses of DNA sequence); it revealed further 32 missing chromosomes (64 %) not detected by CGH. By means of HR-CGH, we could find brand new chromosomal changes in 20 patients with AL (61 %) that were not identifiable either by conventional cytogenetic analysis or by FISH and CGH (Table 2).

The combined application of G-banding, FISH, CGH and HR-CGH revealed chromosomal aberrations in 31 (94 %) patients with AL in total.

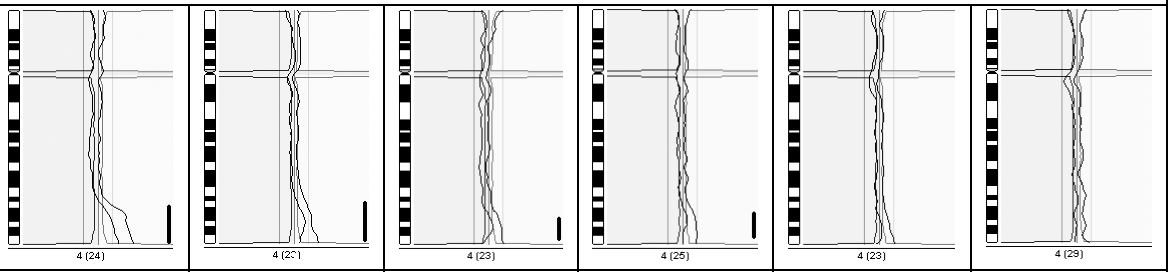
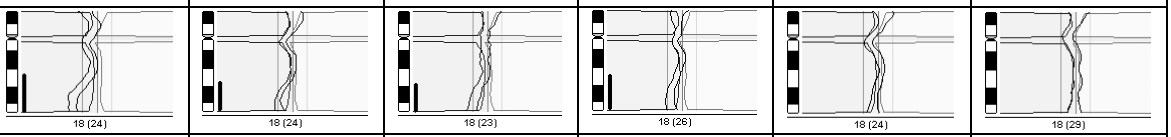
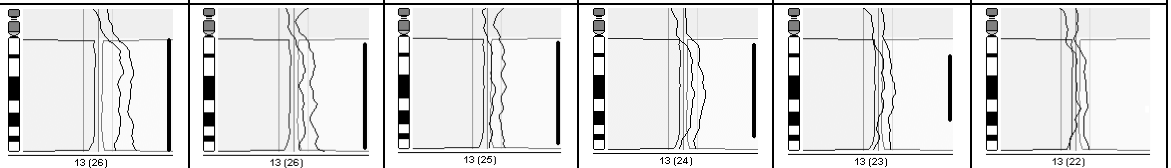
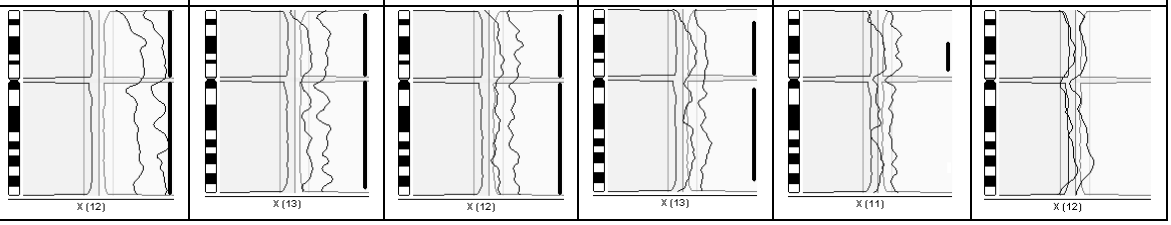
Evaluation of HR-CGH. In order to test a HR-CGH sensitivity, we analyzed patients with microdeletion syndromes. In our experiments, HR-CGH detected the microdeletion of 4 Mb at 15q11-13 (Prader-Willi/Angelman), however it was not able to detect the microdeletion of 2 Mb at 22q11.2 (VCFS/DiGeorge syndrome) (data not shown). Thus, the resolution of HR-CGH system was established at the interval of 4-5 Mb.

To determine a clone prevalence that can be identified by HR-CGH, series of tests with DNA of two patients with cytogenetically and CGH-confirmed congenital chromosomal abnormalities were carried out.

For structural rearrangements, a boy with the congenital duplication of 4q32-qter and the deletion of 18q21-qter was chosen. To determine the clone prevalence, the patient´s DNA was mixed with an increasing concentration of normal DNA from a karyotypically normal man prior to DNA labelling (SpectrumGreen-dUTP) and hybridized against a control DNA (a male DNA labelled by SpectrumRed-dUTP). With the increasing proportion of contaminating normal DNA in the hybridization, the magnitude of the colour ratio changes became smaller and the system ability to detect the changes decreased. In these experiments, HR-CGH was not able to detect any structural changes at 70 % contamination by the normal DNA (Table 3).

For numerical rearrangements, a girl with the congenital trisomy of chromosome 13 was chosen. The principle was the same as described above, only the hybridization was carried out using a female DNA (the patient) against a male DNA

Table 3. The dependence of HR-CGH sensitivity (the ability to detect chromosomal aberration) on the proportion of aberrant DNA in a test sample.

Normal DNA	0 %	50 %	60 %	70 %	80 %	90 %
Test DNA	100 %	50 %	40 %	30 %	20 %	10 %
dup(4q32-qter)						
del(18q22-qter)						
dup(13)						
dup(X) (XX vs. XY)						

Legend: CGH profiles of four cases with chromosomal rearrangements with different ratios of contamination of patient DNA with normal DNA. Aberrations are detected by LUCIA G 4.82 – Advanced Statistic software if the lines on the left side of each picture (deletion) or on the right side (duplication) are present (more information in the text).

(the control). Due to this, not only the ratio of changes of chromosome 13, but also of chromosome X could be detected. The HR-CGH system was not able to detect chromosome aberrations at 70 % contamination by normal DNA for chromosome X and 80 % contamination for chromosome 13 (Table 3).

Discussion

Recent studies prove that childhood AL are characterized by many recurrent structural aberrations such as t(8;21), t(12;21), der(11q23), t(9;22), t(8;14), t(15;17), t(6;9), inv(16), t(3;5), t(1;19) and also by frequent numeric chromosomal abnormalities, e.g. monosomy 7, trisomy 8, 21, 4, 10, 17 etc. Our work consisted of the bone marrow samples investigation from 33 child patients with acute leukaemia with the aim to establish and compare the detection frequency of chromosomal changes by means of G-banding, FISH, CGH, and finally to evaluate the importance and yield of HR-CGH. It has already been proved that the application of classical CGH in leukaemic patients substantially increases the efficiency of cytogenetic analyses in comparison to conventional cytogenetic testing, namely as a result of the identification of hyperploid karyotypes and prognostically significant deletions [16–17]. Existing results indicate that a minimum length of DNA sequence, the deletion or addition of which can be detected by classical CGH, is approximately 5-10 Mb with at least 50 % incidence of aberrant clones in the studied sample [18].

When detecting chromosome abnormalities by HR-CGH, a dynamic reference interval is used instead of fixed limits. The interval is based on the application of control profile created by a statistical processing of 17 normal CGH analyses.

This is why the HR-CGH technique is more sensitive as it is able to detect minor deletions or additions of sequences of 3-5 Mb size. The example here is the detection of microdeletions and microduplications in karyotypes of patients with mental disorders where no karyotypic changes could be found by means of the classical cytogenetic testing [19–20].

The only study dealing with HR-CGH and its benefit for the detection of unbalanced chromosome abnormalities in child patients with acute leukaemia was published by the Danish team of Kristensen in 2003 [14]. They subjected 92 bone marrow samples from child patients with acute lymphoid leukaemia (ALL) to the cytogenetic testing by G-banding and HR-CGH. G-banding enabled the detection of pathological finding in 68 % patients whereas HR-CGH in 87 % patients. Our study yielded similar results – unbalanced cytogenetic abnormalities were proved by HR-CGH in 79 % patients with AL. Moreover, it provided information on additional chromosomal changes in 61 % patients (80 % in the Danish study). HR-CGH increased namely the number of whole chromosome losses by 64 % as compared to CGH. The most frequent hyperdiploid chromosomes detected by means of CGH and HR-CGH were chromosomes number 21, X, 17, 18 and 14. Comparable results were published also by Jarošová et al. [16] in 65 patients with ALL or Schulz et al. [17] in 71 child patients with ALL. They reported that most frequent hyperdiploid chromosomes were numbers 21, X, 10, 18, 6, 17, 4 and 14. The chromosomes most often affected by losses of genetic material were numbers 7, 9, 1, 16 and 19.

The higher sensitivity of HR-CGH over classical CGH can be explained by its ability to detect not only minor unbalanced structural chromosomal aberrations but also clones with structural and numeric changes of chromosomes present in a cancerous tissue even in a lower percentage. When verifying the resolution ability in our experiments, we confirmed that the detection limit of HR-CGH for microdeletions is around 4 Mb and that by means of this technique, unbalanced structural changes or numeric aberrations can be detected at mere 20–30 % incidence of aberrant clones in samples. A similar sensitivity of HR-CGH had earlier been proved in our studies on re-evaluation of results by means of interphase FISH [21].

Current oncology puts emphasis on as precise patient stratification according to cytogenetic and molecular cytogenetic findings as possible. This is why demands on the sensitivity of routinely used diagnostic methods grow. The efficient detection of chromosomal aberrations presents a significant component in the cytogenetic diagnostics of child patients with AL. The prognostic importance of trisomy 4, 10 and 17 [22] or of small clones with hyperdiploid karyotype in children with ALL [23] has been proved only recently. Prognostically significant unbalanced structural aberrations (12p, +21) are often additional to chromosome aberrations t(12;21) [24] in ALL patients.

We assume that our results achieved with CGH and HR-CGH are comparable to those published earlier despite a smaller number of patients involved. The HR-CGH is indeed more sensitive than the classical CGH and this is why it complements well the basic G-banding when accompanied by FISH. Its application as a modern diagnostic method shall be efficient not only in cases of unsuccessful cultivation or inadequate number of mitoses but in all child patients with AL and normal karyotype.

This work was supported by the grant of The Ministry of Education, Youth and Sports of the Czech Republic (MSM0021622415).

References

[1] VORLÍČEK J, KRAHULCOVÁ E, PENKA M, et al..: Clinical Oncology – Special Part. Brno: Masarykova univerzita, 1999 (in Czech).

[2] MAYER J, STARÝ J, ADAM Z, et al.: Leukemie. Praha: Grada Publishing, 2002.

[3] MIHÁL V. Evaluation of diagnostics and therapy of acute lymphoblastic leukaemia in childhood. Clinical Oncology 2004; (Supplement): 92–96 (in Czech).

[4] PUI CH, SCHRAPPE M, RIBEIRO RC, et al.: Adolescent Lymphoid and Myeloid Leukemia. Hematology 2004; 1: 118–145.

[5] WEINBLATT ME. Acute Myelocytic Leukemia online. June 23 2004: http://www.emedicine.com/ped/topic1301.htm

[6] RUBNITZ JE. Acute Lymphoblastic Leukemia on line. Feb 25 2005: http://www.emedicine.com/ped/topic2587.htm

[7] CARROLL WL, BHOJWANI D, MIN DJ, et al.: Pediatric Acute Lymphoblastic Leukemia. Hematology 2003; 1: 102–131.

[8] KARHU R, SIITONEN S, TANNER M, et al.: Genetic aberrations in pediatric acute lymhoblastic leukemia by comparative genomic hybridization. Cancer Genet Cytogenet 1997; 95: 123–129.

[9] WONG N, CHEN SJ, CAO Q, et al.: Detection of chromosome over-and underrepresentations in hyperdiploid acute lymphoblasic leukemia by comparative genomic hybridization. Cancer Genet Cytogenet 1998; 103: 20–24.

[10] HASS O, HENN T, ROMANAKIS K, et al.: Comparative genomic hybridization as part a new diagnostic strategy in childhood hyperdiploid acute lymphoblastic leukemia. Leukemia 1998; 12: 474–481.

[11] RICE M, BREEN CJ, O´MEARA A, et al.: Comparative genomic hybridization in pediatric acute lymphoblastic leukemia. Pediatr Hematol Oncol 2000; 17: 141–147.

[12] KIRCHHOFF M, GERDES T, ROSE H, et al.: Detection of chromosomal gains and looses in comparative genomic hybridization analysis based on standard reference intervals. Cytometry 1998; 31: 163–173.

[13] KIRCHHOFF M, GERDES T, MAAHR J, et al.: Deletions below 10 megabasepairs are detected in comparative genomic hybridization by standard reference intervals. Genes Chrom Cancer 25; 410–413: 1999.

[14] KRISTENSEN TD, WESENBERG F, JONSSON OG, et al.: High-resolution comparative genomic hybridisation yields a high

detection rate of chromosomal aberrations in childhood acute lymfoblastic leukaemia. Eur J Hematology 2003; 70: 363–372.

[15] SHAFFER LG, TOMMERUP N, editors. ISCN (2005): An International System for Human Cytogenetic Nomenclature. Basel: S. Karger, 2005.

[16] JAROŠOVÁ M, HOLZEROVÁ M, JEDLIČKOVÁ K, et al.: Importance of using comparative genomic hybridization to improve detection of chromosomal changes in childhood acute lymphoblastic leukemia. Cancer Genet Cytogenet 2000; 123: 114–122.

[17] SCHOLZ I, POPP S, GRANZOW M, et al.: Comparative genomic hybridization in acute lymphoblastic leukemia: correlation with interphase cytogenetics and loss of heterozygosity analysis. Cancer Genet Cytogenet 2001; 124: 89–97.

[18] KALLIONEMI,OP, KALLIONIEMI A, PIPER J, et al.: Optimizing comparative genomic hybridization for analysis of DNA sequences copy number changes in solid tumors. Genes Chrom Cancer 1994; 10: 231–234.

[19] KIRCHHOFF T, ROSE H, MAAHR J, et al.: High resolution comparative genomic hybridization analysis reveals imbalances in dyschromosomal patients with normal or apparently balanced conventional karyotypes. Eur J Hum Genet 2000; 8: 661–668.

[20] KIRCHHOFF M, ROSE H, GERDES T, et al.: Screening for submicroscopic chromosomal imbalances by comparative genomic hyridization. E.C.A. Newsletter 2001; 8: 3–6.

[21] VRANOVÁ, V., NEČESALOVÁ E., KUGLÍK P, et al.: Screening of genomic imbalances in glioblastoma multiforme using high-resolution comparative genomic hybridization. Oncology Reports 2007; 17: 457–464.

[22] SUTTCLIFFE MJ, SHUSTER JJ, SATHER HN, et al.: High concordance from independent studies by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI Standard-Risk B-precursor Acute Lymphoblastic Leukemia: a Children's Oncology (COG) iniciative. Leukemia 2005; 19: 734–740.

[23] ZEMANOVA Z, MICHALOVA K, SINDELAROVA L, et al.: Prognostic value of structural chromosomal rearrangements and small cell clones with high hyperdiploidy in children with acute lymphoblastic leukemia. Leuk Res 2005; 29: 273–281.

[24] ATTARBASCHI A., MANN G, KONIG M, et al.: Incidence and relevance of secondary chromosome abnormalities in childhood TEL/AML+ acute lymphoblastic leukemia: an interphase FISH analysis. Leukemia 2004; 18: 1611–1616.