Acta Oncologica 27 (1988) Fasc. 5

FROM THE DIVISION OF CLINICAL HAEMATOLOGY AND ONCOLOGY, DEPARTMENT OF MEDICINE, AND THE DEPARTMENT OF CLINICAL IMMUNOLOGY, HUDDINGE HOSPITAL, AND THE DEPARTMENT OF MEDICAL CELL GENETICS, MEDICAL NOBEL INSTITUTE, KAROLINSKA INSTITUTE, STOCKHOLM, SWEDEN.

CHROMOSOMAL ABERRATIONS IN PROGRESSIVE AND INDOLENT CHRONIC B-LYMPHOCYTIC LEUKAEMIA

A longitudinal study

G. JULIUSSON, K. FRIBERG and G. GAHRTON

Abstract

Chromosome analyses of B-cell mitogen-activated cells from 95 patients with chronic B-lymphocytic leukaemia revealed clonal chromosomal aberrations in 50 patients (53%), of which 24 had an extra chromosome 12 with or without other aberrations. Patients with clonal aberrations, especially those with +12, had poorer survival than other patients. Longitudinal studies, with a mean of 3.5 samplings during a median interval of 3.5 years, were performed in 41 patients, of which 24 (59%) had progressive disease. Twenty-nine of the patients in the longitudinal study (71%), 16 with and 13 without clonal aberrations, reatained their karyotype unaltered. In 6 patients a clonal aberration was found only once. Six patients showed minor changes of the karyotype. The karyotype seems to be established at diagnosis, and marks the disease of the individual CLL-patient.

Key words: Leukaemia; B-CLL, chromosomes, longitudinal study.

Clonal chromosomal aberrations are frequent findings in chronic B-lymphocytic leukaemia (CLL) cells, and extra chromosome 12 is the most common karyotypic abnormality (4–6, 9, 11, 16, 17, 20, 23, 25). Recently it was found that the karyotype is a prognostic marker (9, 11–14, 16, 17, 23, 25), independent (11, 13, 17) of other markers such as clinical stage (1, 24). The finding in CLL-cells of clonal chromosomal aberrations (9, 11, 13, 14, 17), complex karyotypes (11, 13, 17), high percentage abnormal metaphases (11, 14), and an extra chromosome 12 (11–13, 16, 17) indicate poor prognosis. However, it has not been clarified if the karyotype marks the malignant transformation of the individual tumour, or if complex karyotypes with multiple aberrations indicate late stages of the leukaemia in which additional chromosome changes have accompanied the evolution of the malignant disease. The latter is true in chronic myelocytic leukemia (CML), since the Philadelphia (Ph) chromosome (21) usually is the only aberration in the chronic phase, whereas additional chromosome abnormalities, such as a second Ph chromosome, an extra chromosome 8 (3), or an isochromosome 17q are acquired at transformation into blastic crisis (26).

We here present an extension of our previously presented cytogenetic results (4–6, 11–14, 16, 17, 25), now comprising 95 patients, with additional chromosome analysis in cells from 41 patients with leukaemic cells obtainable 18 months or more from first cytogenetic study, and survival analyses updated October 15, 1987.

Material and Methods

Patients. Ninety-five patients with CLL (defined as a clonal proliferation of small B-lymphocytes in the peripheral blood to more than 5×10^9/l) were studied; 62 were males and 33 were females, and their ages at diagnosis ranged from 35 to 89 years (median 67.5 years). Diagnosis was made according to the Kiel classification (19), showing 54 patients with CLL 'proper', 35 with leukaemic immunocytoma, 3 with prolymphocytic leukaemia (PLL) (7), and 2 with centrocytic lymphoma; 1 was not subclassified. The cell surface immunoglobulin (SmIg) phenotype was $\mu\kappa$ in 12 patients, $\mu\delta\kappa$ in 35, $\mu\delta a\kappa$ in 1, $\gamma\kappa$ in 7, $\delta\kappa$ in 3, $\mu\lambda$ in 8, $\mu\delta\lambda$ in 11, $\delta\lambda$ in 1, $\mu\delta$ in 1, κ in 1, λ in 1, and 14 were SmIg-negative. Clinical staging at diagnosis according to Rai et al. (24) showed 32 patients at stage 0, 35 at

Presented at ECCO-4, Madrid, November 1–4, 1987.

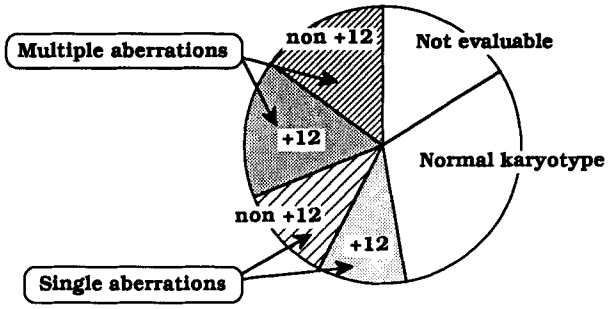

Fig. 1. Karyotypic findings in 95 patients with B-CLL. Not evaluable karyotype, n=18; normal karyotype, n=27; single +12, n=10; single non +12, n=13; complex karyotype including +12, n=14; complex karyotype except +12, n=13.

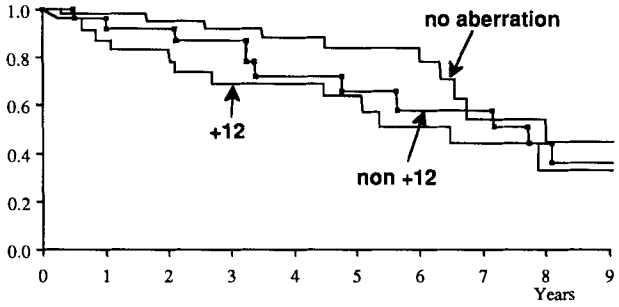

Fig. 2. Probability of survival according to chromosome findings. No aberration: no clonal aberration found, including those who were not evaluable due to insufficient numbers of metaphases, n=45; +12: aberrations including extra chromosome 12, n=24; non +12: clonal aberrations excluding those containing +12, n=26.

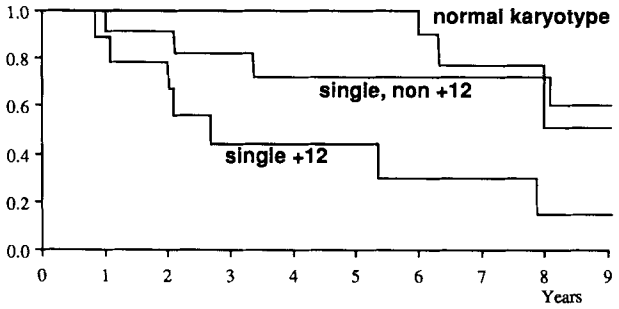

Fig. 3. Probability of survival according to chromosome findings. Single +12: extra chromosome 12 as the sole abnormality, n=10; single, non +12: single aberrations except +12, n=13; normal: no clonal aberration in more than 10 evaluable metaphases, n=27.

stage I, 14 at stage II, 6 at stage III, and 8 at stage IV. According to Binet et al. (1), 65 patients were at stage A, 16 at stage B, and 14 at stage C. Progressive disease was defined as disease progression into a therapy-demanding state, according to previously specified criteria (11, 17).

Cells. Cells were obtained from heparinized blood and also from heparinized bone marrow aspirates, freshly minced lymph nodes, spleens, and pleural effusions. Lymphocytes were isolated by flotation on a Ficoll-Isopaque (Nyegaard & Co A/S, Oslo, Norway) gradient, washed 3 times, and cultured at a concentration of 2×10^6/ml in Eagle's medium containing 10% heat-inactivated human AB serum in humidified air containing 5% CO_2. Four to 6 ml cultures were set up in 50 ml plastic flasks (Falcon 3031, Oxnard, CA, USA). Cells were activated by Epstein-Barr virus (EBV; 1 ml supernatant of the B95-8 cell line/10^7 cells), lipopolysaccharide from E. coli (LPS; O55:B8; 100 μg/ml, tetradecanoyl-phorbol-acetate (TPA; Sigma Chemical Co., St. Louis, MO, USA, 2 μM) and/or cytochalasin B (CB; ICI, England, 1 μg/ml).

Chromosome analysis. Cells were treated with colchicine (0.05 μg/ml) during 90 min before harvesting on day 4, followed by 5 min of hypotonic treatment with a 0.56% solution of potassium chloride, and fixation in methanol-acetic acid (3 : 1). Air dried cells on slides were stained by the Q-banding technique (2). Metaphases were photographed in a Zeiss fluorescence microscope and analysed by conventional karyotyping. A clonal chromosomal abnormality was defined by conventional criteria (10).

Results

Clonal chromosomal aberrations were found in the cells of 50 patients (53%) (Fig. 1); 10 of them had an extra chromosome 12 as the only aberration and another 14 had +12 together with various other aberrations (Table 1). Twenty-six patients had clonal aberrations except +12; 13 of them were found as single aberrations whereas another 13 had complex karyotypes. The single chromosomal aberrations mostly involved chromosomes 6, 11, 12, and 13 (Tables 1, 2).

Table 1

Clonal chromosomal aberrations with +12
(indicates previously reported karyotype)*

+12* (10 patients)
+12/+del(12)(q22)*
+12, del(2)(q31)*
+12, del(14)(q22)
+12, del(14)(q24)*
+12, −17*/−17, +t(12; 17)(q13; p13)
+12/−19
+12, −20*
+12, −22*
+12, −X*
+12, −4, −13, −21, −22, del(11)(q22)*
+12, t(7; 14)(q22; q32), del(9)(p12)*
+12, t(18; 22)(q21; q22)
+12, del(6)(q24), del(15)(q23), t(2; 14)(p22; q32), t(1; 18)(p35; q21)*
+12, t(5; ?)(p15; ?), t(17; ?)(q25; ?), iso(17q), del(6)(q23), del(9)(q12) dup(12)(q13−q22), t(10; 10)(p15; q24)*

Survival analyses showed poor survival for patients with clonal aberrations (Fig. 2), especially those with extra chromosome 12, with or without (Fig. 3) additional karyotypic abnormalities.

In the serial cytogenetic study (Table 1) (15) 16 patients

Table 2

Clonal aberrations except +12
(indicates previously reported karyotype)*

Single	Complex
−10*	+8, +13*
+11	−21/+22
−19	t(14; ?)(q32; ?), t(14; ?)(q32; ?)
iso(2p)*	t(3; 17)(q29; q24), t(8; 14)(q24; q32)*
del(6)(p12)*	+18, −21, +del(3)(p13)*
del(6)(q15)*	+3, +18, del(21)(q21)
del(11)(q14)*	+3, del(17)(p12), t(11; 14)(q13; q32), +t(7; ?)(q36; ?)
del(11)(q21)	−17, +del(3)(p13), t(14; ?), del(22)(q12)*
del(11)(q22)*	−18/t(10; 15)(q23; p12), t(11; 15)(q25; q22)
t(11; 14)(q13; q32)*	−8/−18, +t(18; ?)(p11; ?)
t(6; 13)(q21; q34)	t(1; 5)(q21; p15), t(6; 6)(q23; q27), t(11; 14)(q13; q32)
t(13; 13)(q31; q34)*	t(Y; 9)(q12; q13), t(4; 6)(p16, q15), t(12; 14)(q15; q32),
int del(13)(q13, q21)	del(1)(p22p32), del(6)(q15)*

Table 3

Serial chromosome analyses

	Disease status Progressive	Indolent	Total
Number of patients	24	17	41
Mean number of samplings	3.2	4.0	3.5
Mean interval between samplings	3.8 yrs	4.4 yrs	4.1 yrs
Median interval	3.5 yrs	4.1 yrs	3.5 yrs
Range	1.5–7.4 yrs	2.2–8.6 yrs	
Chromosome results			
No change			
No aberration	3 13 %	10 59 %	13 32 %
Unaltered clonal	12 50 %	4 24 %	16 39 %
Total	15 63 %	14 82 %	29 71 %
Change			
Clonal change	3 13 %	3 18 %	6 15 %
Aberration once	6 25 %	0 0 %	6 15 %
Total	9 38 %	3 18 %	12 29 %

Number of patients and percentage within disease status group.

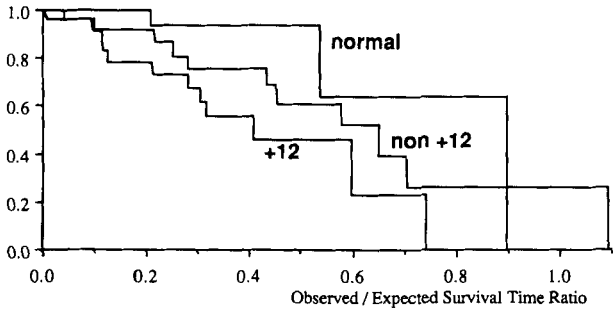

Fig. 4. Age-corrected survival (i.e., observed/expected survival time ratio) for patients according to chromosome findings. +12: aberrations including extra chromosome 12 with or without other aberrations, n=24; non +12: clonal aberrations excluding those with +12, n=26; normal: no clonal aberration found in more than 10 evaluable metaphases, n=27.

with clonal chromosomal aberrations displayed the same karyotype in more than one sample, with an interval of 1.5 to 8.6 years (mean 3.9 years). Twelve of these patients (75%) had had a progressive disease, and 8 (50%) had been treated with cytostatic drugs between the samplings.

Two patients developed a prolymphocytic transformation (7). One chlorambucil-treated patient retained her [+12,2q−] karyotype unaltered at transformation. The other patient that underwent PLL-transformation was untreated, and acquired a new marker chromosome in addition to the [+3,17p−,t(11,14)] abnormalities that were found at diagnosis. This marker was a long chromosome due to an unidentified translocation to the long arm of chromosome 7.

In 6 patients clonal chromosomal aberrations were found at one study only. In 5 of these cases the aberration was found at the first study. In one patient the aberration was lost following CHOP treatment (i.e. cyclophosphamide, doxorubicin, vincristine, prednisone). Another 4 patients lost clonal aberrations prior to treatment, whereas a trisomy 11 appeared following splenectomy and several years of intermittent CHOP treatment in a patient with severe CLL-associated thrombocytopenia.

Cells from 6 patients showed changes in the repertoire of clonal aberrations, including one of the patients with PLL transformation. A second patient with +12,11q−, and multiple monosomies at all samplings lost a minor subclone with a 1;18−translocation together with the trisomy 12, and acquired a clone with an additional monosomy 4. Another 3 patients lost their major clone, whereas initially less frequent abnormalities later were found in all aberrant metaphases. Two of these patients had single monosomies at first analysis (−8 and −19 respectively), whereas the third had +12,−17. The surviving clones contained a chromosome 18-translocation in the first case, a trisomy 12 in the second, and a marker chromosome

consisting of a translocation between chromosomes 12 and 17 in the third patient. The sixth patient with changes in the karyotype had cells with various non-clonal chromosomal aberrations, including 47,XX,+12 and 45,XX,−17,6p− initially. Two years later a single clone with 47,XX+12,5p−,6p−,9q−,iso(17q) was found.

Five patients had two clones with unrelated clonal chromosomal aberrations. Three of them are described above. Two patients had 45,XY,−21/47,XY,+22, and 45,XY,−18/46,XY,t(10,15),t(11,15) respectively, and we were not able to study these patients further.

Mitogen-activated CLL-cells from 13 patients did not reveal clonal chromosomal aberrations in any study. Cells from several of these patients had a very low mitotic rate with sparse numbers of evaluable metaphases despite numerous cultures with different mitogens and combinations of mitogens at multiple samplings.

Discussion

The present report confirms previous findings by us and by others that extra chromosome 12 is by far the most common chromosomal aberration in CLL. Half of all single karyotypic abnormalities as well as half of all multiple aberrations included trisomy 12 (Fig. 1). Other chromosomes were only occasionally involved in numerical aberrations, whereas the structural aberrations were most commonly found in chromosomes 6, 11, and 14 and less frequently in chromosomes 3, 12, 13, and 17.

Despite numerous studies utilizing different B-cell mitogens and combinations of mitogens we still failed to detect clonal chromosomal aberrations in almost half of the studied cases. We and others have shown that clonal chromosomal aberrations are more frequently found in more advanced stages and therapy-demanding or treated cases (6, 11, 17, 25, 27–28). This study was strictly consecutive, and there was a great dominance of stage 0 patients. It seems not achievable with the present technique to increase the relative number of patients with detectable clonal aberrations when including all indolent stage 0 cases.

Our longitudinal study showed that CLL-cell clones in general have a great stability of the karyotype. Clonal aberrations only rarely appeared in subsequent samplings from patients without chromosome abnormalities at diagnosis. Patients with clonal chromosomal aberrations mostly retained the same karyotype for several years, also during progressive disease and cytotoxic therapy. Multiple clonal aberrations were found in early disease, and did not superimpose single aberrations during the study period. The incidence of clonal aberrations was not associated to the time elapsed from diagnosis, and it was not associated with the blood lymphocyte count.

In 5 patients we found metaphases with clonal aberrations at diagnosis, but only cytogenetically normal cells at repeated study. Three of them had single aberrations that were found in a low frequency, and none had an extra chromosome 12. It might have been a real loss of these specific clones due to poor viability in vivo. However, the loss might also be due to our difficulties in the detection of such clones.

In another 6 patients we were able to detect significant but mostly minor changes in the karyotype. In one there was a loss of a small subclone and an acquisition of an additional monosomy; both these differences could be explained by the limited number of metaphases available. In 3 other patients with clonal changes there was a displacement of the major clone by a minor clone, which was found also initially. It thus seems that these latter clones were more viable and had a greater proliferative potential than the clones that were more frequent in the first sample. In 2 untreated patients the displaced clones were single monosomies, whereas in another patient a clone including trisomy 12 was displaced following treatment by a clone with a marker chromosome that had a partial trisomy of chromosome 12 distal to band q13. When carrying a clone with full trisomy 12 this patient had a progressive disease, but since the change to the clone with the +t(12; 17) marker chromosome, i.e. a partial trisomy 12, he has had 3.5 years with an asymptomatic Rai stage 0 disease without therapy or disease progression. It is thus possible that the clone with a full trisomy 12 was the cause of the disease progression. The CLL-cells of the fifth patient showing clonal changes acquired a marker chromosome in addition to her previous aberrations when transforming into PLL. Another patient did also show a PLL transformation, but in that case the karyotype consistently showed +12,2q− abnormalities.

In 2 previous reports (8, 22) on a total of 19 CLL patients (mean and median study interval 1.7 years; range 0.1–3.4 years), two patients developed clonal aberrations in addition to the original single +12 anomaly, one patient developed multiple clones with different trisomies, whereas 3 patients developed very complex karyotypes with several unrelated minor clones; all these changes occurred during cytostatic treatment. In another study one patient with a 47,XY,+12 karyotype developed an additional extra chromosome 21 (18). These, as well as other results showing that +12 is the most frequent aberration in CLL, have been interpreted as +12 being the primary chromosomal aberration, whereas other aberrations are secondary. The present results demonstrating a mostly stable karyotype throughout the course of the disease indicate that although +12 might be the primary chromosomal aberration in most CLL-patients, other aberrations may also play a primary role in CLL-cell clones of other patients. It appears that the original aetiologic factor in patients who develop CLL rapidly results in chromosome abnormalities which do not by themselves make the cells prone to further abnormalities. This is also mirrored by the clinical course of CLL, which generally lacks the drastic changes seen in CML, where new chromosomal

aberrations accompany the blastic transformation with a rapid downhill course.

The present study indicates that the karyotype marks cellular characteristics of the individual CLL-cell clone; karyotype evolution seems rare, and the clonal aberrations did not simply indicate a chromosomal fragility of the malignant cells. Occasionally, clonal changes can be associated with clinical changes of the disease. Abnormal clones with a low frequency at diagnosis may be lost during the course of the disease. Karyotyping can be performed at any time during the course of the disease, but studies at diagnosis should be preferred to achieve the best prognosis prediction. Again it was found that patients with clonal aberrations, especially those with +12, seemed to have poor survival compared to those with normal karyotypes or clonal aberrations except +12.

Request for reprints: Dr Gunnar Juliusson, Department of Medicine, Huddinge University Hospital, S-141 86 Huddinge, Sweden.

REFERENCES

1. Binet J. L., Auquier A., Dighiero G. et al.: A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 48 (1981), 198.
2. Caspersson T., Lomakka G. and Zech L.: The 24 fluorescence patterns of the human metaphase chromosome—distinguishing characters and variability. Hereditas 67 (1971), 89.
3. Gahrton G., Lindsten J. and Zech L.: Involvement of chromosomes 8, 9, 19, and 22 in Ph^1 positive and Ph^1 negative chronic myelocytic leukemia in the chronic and blastic stage. Acta Med. Scand. 196 (1974), 355.
4. — Robèrt K.-H.: Chromosomal aberrations in chronic B-cell lymphocytic leukemia. Cancer Genet. Cytogenet. 6 (1982), 171.
5. — — Friberg K., Zech L. and Bird A. G.: Extra chromosome 12 in chronic lymphocytic leukaemia. (Letter.) Lancet I (1980), 146.
6. — — — — — Nonrandom chromosomal aberrations in chronic lymphocytic leukemia revealed by polyclonal B-cell-mitogen stimulation. Blood 56 (1980), 640.
7. Galton D. A. G., Goldman J. M., Wiltshaw E., Catovsky D., Henry K. and Goldenberg G. J.: Prolymphocytic leukemia. Br. J. Haematol. 27 (1974), 7.
8. Han T., Ohtaki K., Sadamori N. et al.: Cytogenetic evidence for clonal evolution in B-cell chronic lymphocytic leukemia. Cancer Genet. Cytogenet. 23 (1986), 321.
9. — Ozer H., Sadamori N. et al.: Prognostic importance of cytogenetic abnormalities in patients with chronic lymphocytic leukemia. N. Engl. J. Med. 310 (1984), 288.
10. ISCN. An international system for human cytogenetic nomenclature. Report of the Standing Committee of human cytogenetic nomenclature. Birth Defects 21 (1985), 1.
11. Juliusson G.: Leukaemic B-lymphocytic malignancy. A clinical, immunological and cytogenetic study, utilizing mitogens to activate and differentiate malignant B-lymphocytes in vitro. Stockholm. Karolinska Institute (dissertation), Stockholm 1985.
12. — Immunological and cytogenetic studies improve prognosis prediction in chronic B-lymphocytic leukemia: a multivariate analysis of 24 variables. Cancer 58 (1986), 688.
13. — and Gahrton G.: Prognostic implication of trisomy 12 and non-trisomy 12 karyotypes in B cell chronic lymphocytic leukemia. Blood 66 (1985), 470.
14. — — Abnormal/normal metaphase ratio and prognosis in chronic B-lymphocytic leukemia. Cancer Genet. Cytogenet. 18 (1985), 307.
15. — Friberg K. and Gahrton G.: Consistency of chromosomal aberrations in chronic B-lymphocytic leukemia. A longitudinal cytogenetic study of 41 patients. Cancer 62 (1988), 500.
16. — Robèrt K.-H. and Gahrton G.: Cytogenetic abnormalities in chronic lymphocytic leukemia. (Letter.) N. Engl. J. Med. 311 (1984), 123.
17. — — Öst Å. et al.: Prognostic information from cytogenetic analysis in chronic B-lymphocytic leukemia and leukemic immunocytoma. Blood 65 (1985), 134.
18. Lee C. F., Fernandez L. A., Lee S. H. S. and Ghose T.: Karyotypic evolution of chronic lymphocytic leukemia B cells. Cancer Genet. Cytogenet. 23 (1986), 329.
19. Lennert K.: Malignant lymphomas other than Hodgkin's disease. Handbuch der speziellen patologischen Anatomie und Histologie. 1. Band, p. 111. Teil 3B. Springer, New York 1978.
20. Morita M., Minowada J. and Sandberg A. A.: Chromosomes and causation of human cancer and leukemia. XLV. Chromosome patterns in stimulated lymphocytes of chronic lymphocytic leukemia. Cancer Genet. Cytogenet. 3 (1981), 293.
21. Nowell P. C. and Hungerford D. A.: A minute chromosome in human chronic granulocytic leukemia. Science 132 (1960), 1497.
22. Ohtaki K., Han T. and Sandberg A. A.: Sequential chromosome abnormalities in B cell chronic lymphocytic leukemia: a study of 13 cases. Cancer Genet. Cytogenet. 20 (1986), 73.
23. Pittman S. and Catovsky D.: Prognostic significance of chromosome abnormalities in chronic lymphocytic leukemia. Br. J. Haematol. 58 (1984), 649.
24. Rai K. R., Sawitsky A., Cronkite E. P., Chanana A. D., Levy R. N. and Pasternack B. S.: Clinical staging of chronic lymphocytic leukemia. Blood 46 (1975), 219.
25. Robèrt K.-H., Gahrton G., Friberg K., Zech L. and Nilsson B.: Extra chromosome 12 and prognosis in chronic lymphocytic leukaemia. Scand. J. Haematol. 28 (1982), 163.
26. Rowley J. D.: Ph1 positive leukaemia including chronic myelogeneous leukaemia. Clin. Haematol. 9 (1980), 55.
27. Sadamori N., Han T., Minowada J. and Sandberg A. A.: Clinical significance of cytogenetic findings in untreated patients with B-cell chronic lymphocytic leukemia. Cancer Genet. Cytogenet. 11 (1984), 45.
28. — — — — Chromosomes and causation of human cancer and leukemia. LII. Chromosome findings in treated patients with B-cell chronic lymphocytic leukemia. Cancer Genet. Cytogenet. 11 (1984), 161.