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Prognostic factors in acute lymphoid leukaemia of childhood. I. Cytogenetic studies

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The results of chromosonal analysis of bone-marrow cells of 30 children with untreated acute lymphoid leukaemia are reported. On the basis of the modal chromosome number found in the cell clone showing the most frequent aberration, the patients could be classified into hypodiploid, pseudodiploid, hyperploid and normal groups. Pseudodiploidy predicted a poor prognosis while the survival rate of patients with normal or hyperploid chromosome counts was favourable.

Since the advent of banding techniques chromosome analysis has increasingly been used for characterisation of malignant cell proliferation. Among the various types of acute leukaemia it was the acute myeloid type (AML) in which correlation between morphological properties and cytogenetic characteristics has been described: e.g. 8; 21 translocation was fairly frequent in type M_2 15; 17 translocation in M_3 (promyelocytic) leukaemia [6]. The importance of cytogenetic studies was further reinforced by the fact that certain translocations involve cellular oncogens or protooncogens and they may have a causative role in activation of the proliferative process. For instance, in 8; 14 translocation characteristic of Burkitt's lymphoma, the protooncogen c-myc is transferred, in Philadelphia positive CML the oncogen c-abl is transferred from chromosome 9 to 22 [5, 12]. In acute lymphoid leukaemia (ALL) the initial attempts to find such correlations have been less successful. In this condition the caryogram is often indistinct, making exact localisation of breaks and translocations nearly impossible. In recent years improved techniques and increasing interest have resulted in a growing importance of cytogenetic studies in ALL [13].

Our attention was turned first to the chromosome analysis of bone-marrow cells of patients afflicted by AML. In recent years we have regularly investigated the spontaneously dividing cells of the bone-marrow specimen obtained for diagnostic purposes. Like other investigators, we have found certain correlations between chromosome aberrations and the type of leukaemia on the one hand and the outcome of the disease on the other hand. Here we offer some results of these observations.

Material and Methods

Patients. 30 patients affected by ALL, admitted to either of our departments during the years 1981—1983, participated in the study. Some of their data are summed up in Table I. The diagnosis was set up by bone-marrow aspiration or biopsy. Criteria of complete remission were as follows: complete clinical recovery, normal peripheral blood picture, at least moderately cell-rich bone-marrow, less than 5% lymphoblasts in the bone-marrow. All bone-marrow or extramedullary exacerbations of the leukaemic process were regarded as a relapse.

Table I

Some haematological parameters of ALL patients

Modal chromosome count in the cell clone with the most frequent chromosomal aberration	Number of patients	Mean age, years	Mean Hb level, mmol/m	Mean initial leucocyte count, G/l	FAB type	Cell surface marker
35—45	5	3	5.3	24	L1 = 2 L2 = 3	T = 2
46—pathological						
a. t(9; 22)	4	5	3.9	14	L1 = 1 L2 = 3	T = 1
b. t(2; 3)	1	4	5.6	8.8	L2	—
c. t(8; 14)	3	10	6.1	3.8	L3 = 3	B = 3
47—60	6	3	5.2	32	L1 = 4 L2 = 2	—
46—normal	11	5	5.3	7.2	L1 = 9 L2 = 2	cALLa + = 7

Cytogenetic methods. Spontaneous division of bone-marrow cells obtained for diagnostic purposes was examined either in direct preparations or after an incubation for some hours. The technique of Rowley and Potter [8] was applied. Karyotyping was carried out in microphotograms obtained after modified ASG-trypsin banding [10]. In evaluation we observed the recommendations of the International System for Human Cytogenetic Nomenclature [7]. The presence of a clonal aberration was stated whenever it occurred in at least two diploid, pseudodiploid or hyperdiploid cells or at least three hypoploid cell divisions.

In order to judge the clinical importance of the cytogenetic finding the aberrations were classified according to the modal chromosome number of the clone showing the most frequent chromosome aberration. 35—45 was classified as hypodiploid, group two contained the pseudodiploid lines carrying translocations, 47—60 was regarded as hyperdiploidy and the fourth group comprised cases with a normal 46 caryotype.

Results

In 30 cases, 65% of all ALL cases diagnosed during the three years, we were able to obtain appropriately banded metaphases of good quality, 10—30 in each patient. Comparison of the patients' clinical, haematological and cytogenetic data resulted in

TABLE II

Relationship between cytogenetic finding and clinical course in ALL

Modal chromosome count in the cell clone with the most frequent chromosomal aberration	Number of patients	Remission rate	Duration of remission, months	Survival time, months	Death rate	
35—45	5	3/5	13	15	3/5	
46—abnormal						
a. t(9; 22)	4	4/4	10	13	2/4	6/8
b. t(2; 3)	1	1/1	10	11	1/1	
c. t(8; 14)	3	1/3	1	1	3/3	
47—60	6	6/6	13	14	2/6	
46—normal	11	9/11	7	8	2/11	

some characteristic relationships (Table II). The mean age of patients exhibiting 8; 14 translocation was higher than that of the three other groups and all three patients' cells exhibited L_3 morphology. Surface marker studies revealed in all three patients cells of type B. The majority of cases with a normal 46 karyotype showed type L_1 and a positive reaction with cALLa antigen.

In evaluating the clinical course, usual survival curves could not be constructed because of the shortness of follow-up periods. The poorest remission rate and clinical course were seen in B-cell leukaemia exhibiting 8: 14 translocation. All three patients died soon after onset of the disease. The best clinical results were achieved in the group with 46 chromosomes and in the group characterised by hyperploidy (Table II). The overwhelming majority of these patients 15 among 17, attained remission and 13 of them are still in remission at the time of preparing this manuscript. Two of the four patients with 9: 22 translocation relapsed and died. The girl with 2: 3 translocation died of alveolar proteinosis during complete remission. Similarly, infection was the cause of death in one patient with hypoploidy being in remission at the time of death. Hyperploidy was encountered in 6 patients; 2 of them died, 4 are still in complete remission. The best remission rate was seen in this group.

DISCUSSION

Improved chromosome banding technique allowed us to perform cytogenetic analysis of bone-marrow blast cells in 30 children with recently diagnosed ALL admitted in the period 1981 to 1983. The 65% success rate approximated the international mean but lagged behind the results achieved by the great centers with extensive experience. Yunis et al. [15] obtained evaluable karyotypes by bone-marrow cultures and methotrexate banding in 90—95% of all cases examined. Their

results have not yet been confirmed by many investigators, but their achievement is still remarkable for the high success rate and resolving power. The possibility of identifying several thousands of bands foretells a new era of even more refined analysis of breaks.

Our patients were classified according to the modal chromosome count of the clone exhibiting the most frequent chromosome aberration. Five patients showed hypoploidy. According to the morphological FAB classification [2] two of these patients were classified as L_1 and three as L_3; in two patients surface marker tests revealed T-cell properties (Table I). In spite of the short follow-up, two patients have already relapsed and another patient died during complete remission.

There is no unanimous opinion about the chances of remission in the literature. Some authors regard hypoploidy as a sign of poor prognosis [4], in others' opinion these patients face an outcome more favourable than the average [3, 9, 13]. In our experience the rate of hypoploidy is influenced by the technique of preparation, therefore only clonal aberrations must be used for prognostic analysis.

Nearly all investigators agree in that the worst prognosis is encountered in the group with 46 plus translocation, i.e. pseudodiploidy [11, 13, 14]. Our findings confirm this opinion, as these patients posed the most severe clinical problems, although their age or blast mass alone would not have classified them into a group of poor prognosis. Their remission rate did not significantly differ from that of the other groups; still, these patients had the lowest survival rate. Especially cases with B-type cells and 8; 14 translocation showed a poor response to therapy: only one patient attained transitory remission and all three patients died soon after diagnosis (Table II). This type of translocation results in rapid, agressive proliferation and promotes the development of chemotherapy-resistent cell clones. Recent observations, however, suggest that therapy with new types of cytostatic drugs capable of destroying cells with a short cell cycle can be successful. Two Philadelphia positive (9: 22 translocation) cases out of four have died but the two survivors have been in remission for 10 and 19 months. The death of the child with 2: 3 translocation in complete remission could be ascribed to chance.

The best results were achieved in the groups with hyperploidy and normal karyotype. Fifteen out of seventeen patients belonging to either group attained full remission and 13 of them are still in remission. This is in agreement with other observations of investigators [9, 13].

Chromosome analysis of the bone-marrow at the time of diagnosis is not only helpful in characterising the leukaemic process but also presents important prognostic information. Our findings have corroborated previous observations in that certain types of chromosome aberration, especially pseudodiploidy predict relative re-

sistance to usual therapy. By help of Cox's multivariance analysis the participants of the Third International Workshop on Chromosomes in Leukaemia stated that the prognostic value of chromosomal aberrations is independent of the prognostic factors, like age, sex and initial leucocyte count [13].

Further advance in preparation techniques will increase the value of cytogenetic findings in the diagnosis and therapy planning of leukaemia. By the rapid progress in molecular genetics it can be hoped that certain DNA technologies will soon be used in the exact diagnosis of leukaemia; e.g. rearrangement of the immunoglobulin chain genes in B-cell lymphoma and leukaemia already proves the presence of monoclonal cell proliferation at an early stage [1].

References

1. Arnold A, Cossman J, Bakhshi A, Jaffe ES, Waldmann TA, Korsmeyer SJ: Immunoglobulin-gene rearrangements as unique clonal markers in human lymphoid neoplasms. N Engl J Med 309: 1593, 1983
2. Bennett, JN: Proposals for the classifications of the acute leukaemias. Br J Haemat 33: 451, 1976
3. Bloomfield CD, Lindquist LL, Arthur D, McKenna RW, LeBien TW, Peterson BA, Nesbitt ME: Chromosam abnormalities in acute lymphoblastic leukaemia. Cancer Res 41: 4838, 1981
4. Brodeur GM, Williams DL, Look AT, Browman WP, Kalwinsky DK: Near-haploid acute lymphoblastic leukemia: a unique subgroup with a poor prognosis? Blood 58: 14, 1981
5. de Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeiyer A, Bootsma D, Spurr NK, Heisterkamp N, Groffen J, Stephenson JR: A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature 300: 765, 1982
6. Fourth International Workshop on Chromosomes in Leukemia (FIWCL). Cancer Genet. Cytogenet 11: 332, 1984
7. International System for Human Cytogenetic Nomenclature. Cytogenet Cell Genet 21: 309, 1978
8. Rowley JD, Potter D: Chromosomal banding patterns in acute lymphocytic leukemia. Blood 47: 705, 1976
9. Secker-Walker LM: The prognostic implications of chromosomal findings in acute lymphoblastic leukemia. Cancer Genet Cytogenet 11: 233, 1984
10. Summer AT, Evans HJ, Buckland RA: New technique for distinguishing between human chromosomes. Nature New Biol 232: 31, 1971
11. Swansbury GJ, Secker-Walker LM, Lawler SD, Hardisty RM, Sallan SE, Carson OM, Sakurai M: Chromosomal findings in acute lymphoblastic leukaemia of childhood: an independent prognostic factor. Lancet 2: 249, 1981
12. Taub R, Kirsch I, Morton C, Lenoir C, Swan D, Tronick S, Aaronson S, Leder P: Translocation of the c-myc gene into the immune globulin heavy chain locus in human Burkitt lymphoma and murine plasmocytoma cells. Proc Natl Acad Sci USA 79: 7937, 1982
13. Third International Workshop on Chromosomes in Leukemia (TIWCL). Cancer Genet Cytogenet 4: 111, 1980
14. Williams DL, Tsiatis A, Brodeur GM, Look AT, Melvin SE, Bowman WP, Kalwinsky DK, Rivera G, Dahl GV: Prognostic importance of chromosome number in 136 untreated children with acute lymphoblastic leukemia. Blood 60: 864, 1982
15. Yunis JJ: Recurrent chromosomal defects are found in most patients with acute nonlymphocytic leukemia. Cancer Genet Cytogenet 11: 125, 1984

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