Prognostic Factors in Adult Acute Lymphoblastic Leukemia

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Introduction

The introduction of three and four drug combination chemotherapy into the treatment of acute lymphoblastic leukemia (ALL) in adults has resulted in complete remission being achieved in approximately 70% of cases (Willemze, Hartgrink-Groeneveld, 1975; Jacquillat and Weil, 1973; Gee and Haghbin, 1976; Muriel and Pavlovsky, 1974; Atkinson and Wells, 1974; Einhorn and Meyer, 1975; Rodriguez and Hart, 1973; Whitecar and Bodey, 1972; Spiers and Roberts, 1975). In spite of the use of early central nervous system prophylaxis and continuous maintenance chemotherapy, however, the duration of complete remission remains considerably shorter than in childhood ALL. It is well documented that certain presentation features influence the prognosis in childhood (Henderson, 1969; Simone and Holland, 1972). We have, therefore, analysed the data from 42 adults in whom complete remission was achieved to determine which presentation features influence the prognosis in adults.

Materials and Methods

A. Patients

Between November 1972 and December 1976, 62 consecutive previously untreated adults with ALL were treated with combination chemotherapy at St. Bartholomew's Hospital. All patients received adriamycin, vincristine, prednisolone and L-asparaginase, as previously reported (Lister, Whitehouse, 1978), and complete remission was achieved in 43 (69%). One patient returned to India without maintenance therapy and subsequently relapsed. The remaining 42 cases form the basis of this analysis. All recieved early central nervous system therapy and continuous maintenance chemotherapy until relapse or for three years, whichever was shorter.

B. Diagnostic Criteria

The diagnosis of ALL was based upon conventional morphological criteria (Bennett and Catovsky, 1976) for May-Grunwald-Giemsa stained bone

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marrow smears, which showed at least 30% infiltration by agranular, Sudan Black negative blast cells. The periodic-acid-Schiff (PAS) stain was performed in all cases and considered positive if more than 5% of the blasts exhibited block or coarse granular activity. Cases with an occasional fine granular or negative reaction were also negative for napthol-As-Acetate esterase activity. Cytogenetic analysis showed that all patients were negative for the Philadelphia chromosome.

C. Treatment Programme

The treatment programme included three main elements: the induction and consolidation of remission, treatment of central nervous system (CNS) or CNS prophylaxis, and maintenance treatment.

1. Induction and Consolidation of Remission

At the start of the study we planned to give doxorubicin (adriamycin) and vincristine every week for a minimum of four courses regardless of the peripheral blood count. But the incidence of pancytopenia after the 2nd injection was so high that the schedule was modified and the 2nd course of doxorubicin and vincristine was given at least 14 days after the first. The interval between the later courses of doxorubicin and vincristine depended on the bone marrow findings.

Table 1. Treatment given for inducing and consolidation remission (OPAL[a])

Drug	Administration	Dose	Intervals of Treatment
Doxorubicin	Intravenous	30 mg/m^2	Days 0, 14, 28, 42[b]
Vincristine	Intravenous	1.4 mg/m^2 (max 2 mg)	Days 0, 14, 28, 42[b]
Prednisolone	By mouth	40 mg	Daily
Colaspase	Intravenous	10,000 IU/m^2	Days 0–14
Allopurinol	By mouth	200 mg three times a day	Daily until blasts cleared from blood

[a] Oncovin (vincristine), prednisolone, adriamycin (doxorubicin), and L-asparaginase (colaspase).

[b] Bone marrow was assessed on day 49; if leukaemic infiltration persisted two further injections of doxorubicin and vincristine were given about 14 days apart.

2. CNS Prophylaxis and Treatment

In the early part of the study lumbar puncture for CSF cytology was not performed until clinical and haematological remission had been achieved. Patients with no evidence of infiltration then proceeded to CNS prophylaxis. This consisted of cranial irradiation (2400 rads) given in 15 fractions over three weeks with concomitant intrathecal methotrexate 12,5 mg twice weekly for five doses during the same period. Analysis of the CSF findings in the first 28 patients who achieved complete remission indicated a high incidence of asymptomatic leukemia disease (Lister and Whitehouse, 1977). The first injection of intrathecal methotrexate was therefore introduced

during the induction of remission, when the platelet count reached $50 \times 10^9/l$ in the absence of circulating blast cells. The total number of doses of methotrexate was also increased to seven. Patients with proven CNS disease who were in clinical and haematological remission received more intensive radiotherapy and intrathecal chemotherapy. Cranio-spinal irradiation (2400 rads) was given in 20 fractions together with 5 doses of intrathecal methotrexate 12,5 mg followed by five doses of intrathecal cytarabine 50 mg given over four weeks.

3. Maintenance Treatment
This consisted of oral 6-mercaptopurine 75 mg daily, starting when complete remission had been achieved and always after allopurinol had been stopped. Once CNS therapy had finished oral cyclophosphamide 300 mg weekly and oral methotrexate 30 mg weekly were started together. The doses of all the drugs were adjusted to maintain the total white cell count at $3 \times 10^9/l$ and treatment was continued for three years and then stopped.

D. Cell Surface Marker Studies

The panel of membrane markers used included spontaneous sheep red blood cell rosette formation (for T cells), reactivity with anti-human immunoglobulin (for B cells) and with anti-ALL serum (for "common ALL" cells). Ficol-Triosil density gradient separation of peripheral blood or bone marrow samples was used to separate blasts and mononuclear cells.

The sheep red blood cell rosette (E-rosette) tests was performed by addition of a suspension of 1×10^6 test cells in 50 µl of medium with 50 µl of foetal calf serum (absorbed with sheep red blood cells) to 100 µl of a 2% suspension of sheep red blood cells which were neuraminidase treated (15 U/ml at 37°C for 30 minutes). The cells were centrifuged at 400 g for 5 minutes and left undisturbed at room temperature for 1 hour before gentle resuspension and counting in an haemocytometer.

The anti-immunoglobulin serum was a fluoresceinated $F(ab^1)_2$ preparation of sheep antibody to human IgG (courtesy of Dr. I. Chantler, Wellcome Research). It was used in a direct immunofluorescence technique by incubating 1×10^6 test cells in 50 µl of medium containing 0,02% sodium azide, with the anti-immunoglobulin at a 1 in 10 final dilution for 30 minutes at 4°C, washing cells 3 times and counting in suspension on a slide with a Zeiss Standard 16 phase contrast microscope with epifluorescence and narrow band FITC filters.

The anti-ALL serum has been previously described in detail (Brown and Capellaro, 1975). It was raised in rabbits against non-T, non-B ALL cells coated with antilymphocyte serum. After extensive absorption with normal haemopoietic cells, lymphocytes and acute myeloid leukemia cells, it was functionally specific for the majority of cases of non-T, non-B ALL and some cases of chronic myeloid leukemia in "lymphoid" blast crisis (Roberts and Greaves, 1978). This antiserum was used in an indirect immunofluorescence technique by incubation for 30 minutes at 4°C with 1×10^6 viable cells in suspension, washing cells twice and then incubation

for 30 minutes at 4°C with a goat anti-rabbit immunoglobulin antiserum which was fluorescein labelled. The cells were then washed 2 times before counting in suspension as above.

E. Statistical Analysis

Remission duration curves and graphic presentations were developed by standard life table formulae (Armitage 1971) and statistical significance was determined by the Log Rank analysis method (Peto and Pike, 1977). The significance of clinicopathological correlations was determined by the Mann Whitney U test.

Results

A. Overall Duration of Remission

The data from 42 patients are evaluable. Twenty two have relapsed. One elected to stop maintenance after 8 months and relapsed shortly thereafter. He has been analysed as not having relapsed, but as being in continuous complete remission for 8 months. One patient died at home during an influenza epidemic whilst in complete remission. The remainder continue in complete remission between 7 and 64 months. The median duration of complete remission was 21 months. Seven patients have already been in continuous remission more than 3 years.

B. Influence of Presentation Features on Remission Duration

1. Age

The age of the patients at presentation did not influence the duration of remission. The number of older patients is small, so statistical analysis would be unwise. However, only 2 patients out of 9 over the age of 40 have relapsed, both at 4 months: the remainder continue in remission between 4 and 46 months.

2. Bulk of Disease at presentation

I. Hepatosplenomegaly (Fig. 1)

Both the liver and spleen were clinically enlarged in 20 patients. Only 6 of these patients remained in complete remission, compared with 15 out of 22 patients in whom there was not hepatosplenomegaly. The duration of complete remission was significantly shorter for patients with hepatosplenomegaly ($p = < .001$).

II. Blast count at presentation

All 4 patients in whom the presentation blast cell count was greater than $100 \times 10^9/l$ had relapsed by six months. However, comparison of the duration of remission for patients with blast cell counts above and below $10 \times 10^9/l$ reveals no statistically significant difference.

3. Cytochemistry

The PAS reaction was positive in 20 cases and negative in 22. There was no

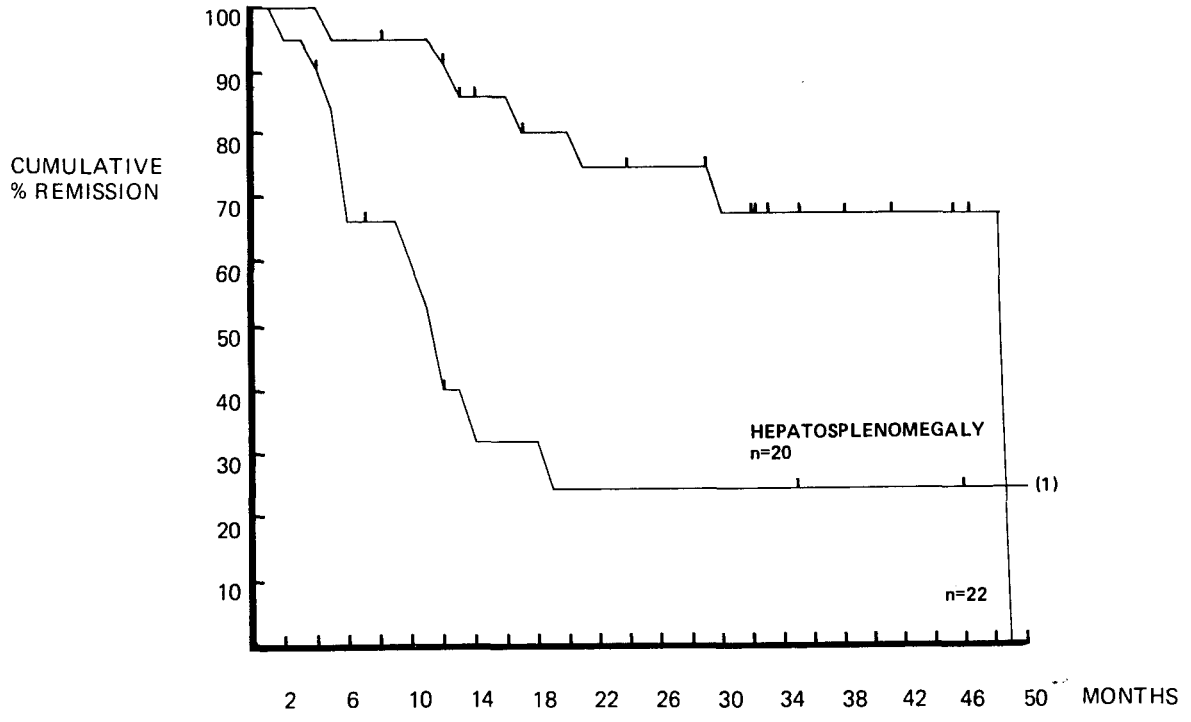

Fig. 1. Duration of complete remission in acute lymphoblastic leukemia. Influence of reactivity with anti-ALL serum

difference in the duration of complete remission between the 2 groups. Ten out of 20 patients in whom the reaction was positive have relapsed, the remainder being in complete remission between 12 and 64 months. Eleven out of 22 patients in whom it was negative have relapsed, the remainder being in complete remission 2 and 25 months.

4. Cell Surface Marker Studies

These were performed in only 29 cases in whom complete remission was achieved. The remaining 13 cases were not studied because they were treated before the techniques necessary were in routine use in our laboratory and not enough viable cells were stored to allow frozen samples to be tested. Thus the duration of follow-up of these cases is shorter than that of the whole study and the median duration of complete remission has not yet been reached.

Complete remission was achieved in only 3 out of 5 cases of Thy-ALL. Two have relapsed at 5 and 10 months, and the third continues in complete remission at 35 months.

The blasts from 16 of the remaining cases of unclassified or null ALL reacted positively with the anti-ALL serum. The duration of remission was significantly longer than that of the 10 cases of which did not react with the antiserum. Only 3 out of 16 anti-ALL positive common ALL cases have relapsed. The remainder continues between 7 and 46 months. Six out of the 10 anti-ALL, non-T, non-B cases have relapsed and only 4 remained in remission between 8 and 41 months (Fig. 2).

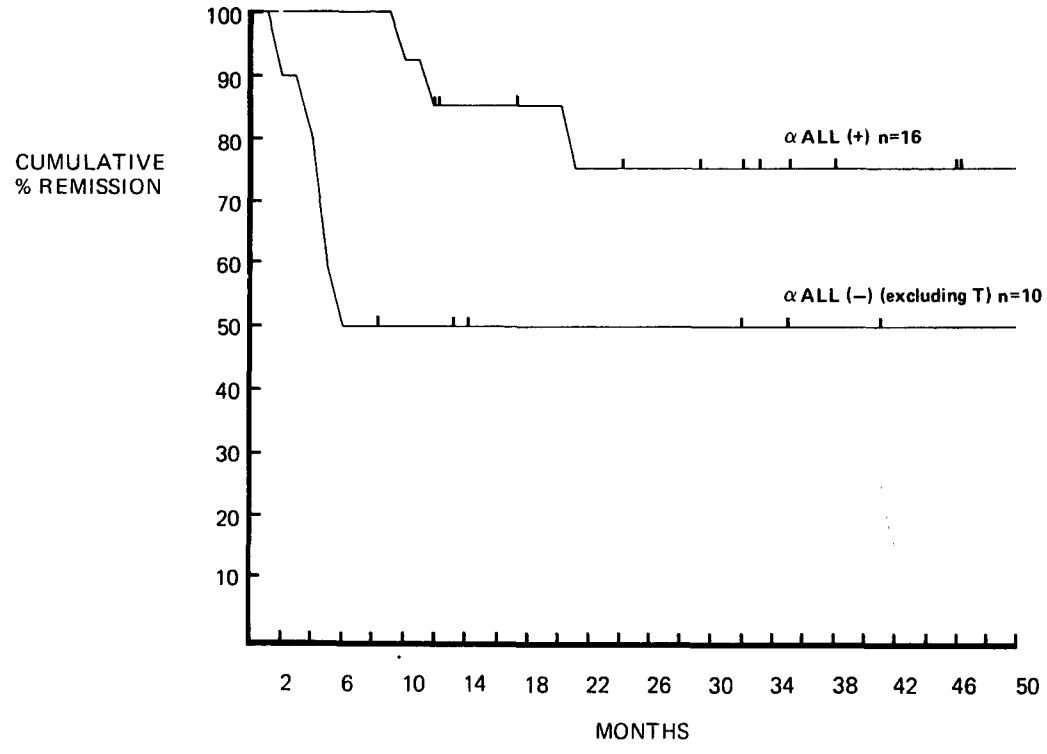

Fig. 2. Duration of complete remission in acute lymphoblastic leukemia. Influence of hepatosplenomegaly

Discussion

These results support the contention that the prognosis in ALL in adults is influenced by the extent of disease at presentation. The presence of hepatosplenomegaly was associated with a very short duration of remission. All patients with a very high blast count (greater than $100 \times 10^9/l$) had relapsed within six months, even though the previously significant adverse influence of a presentation blast count above $10 \times 10^9/l$ has not been confirmed.

The cell surface marker studies demonstrate a significant advantage for patients whose blast cells reacted with the anti-ALL serum. The number of patients was small and the findings should be interpreted with caution. However, the fact that the results are identical with those reported in childhood ALL reported by Chessels et al. Chessels and Hardisty (1977) suggests that our observations are valid.

The response to the initial therapy remains the most important prognostic factor, with survival being very significantly longer for patients in whom complete remission was achieved than for those in whom it is not. The recognition that the same prognostic factors apply to both childhood and adult lymphoblastic leukemia makes it possible to develop treatment programmes for adults on the basis of data obtained from childhood studies. This is most important since the number of adults with lymphoblastic leukemia is small and data are hard to obtain. The recognition that presentation features influence the prognosis must lead to the intensification of therapy for patients with adverse prognostic factors and also the avoidance of intensification of therapy for those patients in whom adverse prognostic factors are not found.

References

1. Armitage, P.: Statistical Methods in Medical Research. London: Halstead Press 1971
2. Atkinson, K., Wells, D.G., Clinik, H., Kay, H.E.M., Powles, R., McElwain, T.J.: Adult acute leukemia. Br. J. Cancer **30,** 272–278 (1974)
3. Bennett, J.M., Catovsky, D., Daniel, M.-T., Flandrin, G., Gralnick, H.R., Sulton, C.: Proposals for the classification of acute leukemias. Br. J. Haematol. **33,** 451–458 (1976)
4. Brown, G., Capellaro, D., Greaves, M.F.: Leukemia-associated antigens in man. J. Natl. Cancer Inst. **55,** 1281–1289 (1975)
5. Chessells, J.M., Hardisty, R.M., Rapson, N.T., Greaves, M.F.: Acute lymphoblastic leukemia in children: Classification and prognosis. Lancet **1977 II,** 1307–1309
6. Einhorn, L.H., Meyer, S., Bond, W.H., Rohn, R.J.: Results of therapy in adult acute lymphocytic leukemia. Oncology **32,** 214–220 (1975)
7. Gee, T.S., Haghbin, M., Dowling, M.D., Cunningham, I., Middleman, M.P., Clarkson, B.: Acute lymphoblastic leukemia in adults and children. Cancer **37,** 1256–1264 (1976)
8. Henderson, E.S.: Treatment of acute leukemia. Semin. Hematol. **6,** 271–319 (1969)
9. Jacquillat, C., Weil, M., Gemon, M.F., Auclerc, G., Loisel, J.P., Delobel, J., Flandrin, G., Schaison, G., Izrael, V., Busel, A., Dresch, C., Weisgerber, C., Rain, D., Tanzer, J., Najean, Y., Seligmann, M., Boiron, M., Bernard, J.: Combination therapy in 130 patients with acute lymphoblastic leukemia (Protocol 06 LA 66-Paris). Cancer Res. **33,** 3278–3284 (1973)
10. Lister, T.A., Whitehouse, J.M.A., Beard, M.E.J., Brearley, R.L., Brown, L., Wrigley, P.F.M., Crowther, D.: Early central nervous system involvement in adults with acute non-myelogenous leukemia. Br. J. Cancer **35,** 479–483 (1977)
11. Lister, T.A., Whitehouse, J.M.A., Beard, M.E.J., Brearley, R.L., Wrigley, P.F.M., Oliver, R.T.D., Freeman, J.E., Woodruff, R.K., Malpas, J.S., Paxton, A.M., Crowther, D.: Combination chemotherapy for acute lymphoblastic leukemia in adults. Br. Med. J. **1,** 199–203 (1978)
12. Muriel, F.S., Pavlovsky, S., Penalver, J.M., Hidalgo, G., Benesan, A.C., Eppinger-Helft, D., De Macchi, G.H., Pavlovsky, A.: Evaluation of induction of remission, intensification and central nervous system prophylactic treatment in acute lymphoblastic leukemia. Cancer **34,** 418–426 (1974)
13. Peto, R., Pike, M.C., Armitage, P., Breslow, N.E., Cox, D.R., Howard, S.V., Mantel, S.V., McPherson, K., Peto, J., Smith, P.G.: Design and analysis of randomised clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br. J. Cancer **35,** 1–39 (1977)
14. Roberts, M.M., Greaves, M.F., Janossy, G., Sutherland, R., Pain, C.: Acute Lymphoblastic Leukemia (ALL) Associated Antigen – I. Expression in Different Haemotopoietic Malignancies. Leukemia Research, **2** (no. 1) 105–114 (1978)
15. Rodriguez, V., Hart, J.S., Freireich, E.J., Bodey, G.P., McCredie, K.B., Whitecar, J.P., Coltman, C.A.: POMP combination chemotherapy of adult acute leukemia. Cancer **32,** 69–75 (1973)
16. Simone, J.V., Verzosa, M.S., Rudy, J.A.: Initial features and prognosis in 363 children with acute lymphocytic leukemia. Cancer **36,** 2099–2108 (1975)
17. Spiers, A.S.D., Roberts, P.D., Marsh, G.W., Paretch, S.J., Franklin, A.J., Galton, D.A.G., Szur, Z.L., Paul, E.A., Husband, P., Wiltshaw, E.: Acute lymphoblastic leukemia: Cyclical chemotherapy with 3 combinations of 4 drugs (COAP-POMP-CART regimen). Br. Med. J. **4,** 614–617 (1975)
18. Whitecar, J.P., Bodey, G.P., Freireich, E.J., McCredie, K.B., Hart, J.S.: Cyclophosphamide (NSC-26271), Vincristine (NSC-67574), Cytosine Arabinoside (NSC-63878), and Predisone (NSC-10023) (COAP) combination chemotherapy for acute leukemia in adults. Cancer Chemother. Rep. **56,** 543–550 (1972)
19. Willemze, R., Hillen, H., Hartgrink-Groeneveld, C.A., Haanen, C.: Treatment of acute lymphoblastic leukemia in adolescents and adults: A retrospective study of 41 patients (1970–1973). Blood **46,** 823–834 (1975)