Acute lymphoblastic leukaemia: cyclical chemotherapy with three combinations of four drugs (COAP-POMP-CART regimen)

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Summary

Forty-two adults and children with previously untreated acute lymphoblastic leukaemia (ALL) were entered into a programme of chemotherapy in which three combinations, each of four drugs, were administered in a predetermined cyclical rotation together with cranial irradiation and intrathecal injections of methotrexate. Forty-one patients (98%) entered remission and no patient developed neuroleukaemia. Relapse of ALL occurred in 10 patients, and three patients died during remission, while eight patients stopped treatment after two and a half years and have remained in remission for two to 26 months. Comparison of remission and survival experience in this mixed group of children and adults with the experience of children treated at Memphis and in the Medical Research Council's UKALL-I trial showed no significant differences. On the other hand, analysis by prognostic factors showed that neither age nor blast cell count at presentation had any adverse effect in patients treated in this study. No relapses occurred in nine patients with blast cell counts greater than $20 \times 10^9/l$ at presentation. This regimen is effective treatment for ALL and may be of special value in patients with poor prognoses. The regimen has not as yet proved superior for the treatment of children with ALL who do not have adverse prognostic features.

Introduction

New regimens of multiple-drug chemotherapy coupled with irradiation of the nervous system and the intrathecal adminis-

tration of methotrexate have greatly improved the prognosis for children with acute lymphoblastic leukaemia (ALL).¹⁻⁴ The length of remission and survival have increased and the incidence of overt central nervous system (CNS) involvement by ALL has decreased dramatically.⁴ Many children have discontinued all treatment for periods approaching eight years without evidence of relapse.⁵ Between February 1970 and September 1974 we treated both children and adults with ALL using a regimen which incorporates three combinations, each of four drugs, in a predetermined cyclical rotation, together with prophylactic treatment of the CNS.^{6,7} We report here the results obtained at 31 March 1975.

Patients and methods

Forty-two previously untreated patients with an apparently unequivocal diagnosis of ALL or acute undifferentiated leukaemia were included in the study and no exclusions were made either initially or retrospectively. There were 28 male and 14 female patients and their ages ranged from 2 to 43 years (mean 8 years, median 5 years); 31 patients were Europeans and 11 were of Indian origin (table I). ALL was diagnosed by the examination of Romanowsky-stained films of peripheral blood and bone-marrow; cytochemical stains (PAS, Sudan Black, myeloperoxidase) were performed on some but not all patients. Electron microscopical examination of leukaemic cells and tests for markers of T- or B-lymphocytic origin⁸ were not performed when most of these patients were diagnosed, but these tests were carried out on some of the patients who later relapsed. Demonstration of T-lymphocytic markers was not used as a basis for exclusion of patients since most patients remained in remission and their leukaemic cells could not therefore be tested. The first patient was admitted to the study on 2 February 1970 and the 42nd patient on 30 September 1974; the maximum and minimum follow-up times were therefore 62 and 6 months respectively.

TABLE I—Details of 42 patients with untreated ALL included in study

	No of patients		No of patients
Age (years):		Total leucocytes ($\times 10^9/l$):	
2-4	19	0-4.9	15
5-9	10	5-19.9	16
10-14	4	≥ 20	11
15-19	5	Blast cells ($\times 10^9/l$):	
20-43	4	0-0.499	14
Haemoglobin (g/dl):		0.5-19.9	19
0-5.9	17	≥ 20	9
6-8.9	14	Neutrophils ($\times 10^9/l$):	
≥ 9	11	0-0.199	13
Platelets ($\times 10^9/l$):		0.2-0.999	16
0-29.9	10	≥ 1.0	13
30-49.9	13		
≥ 50	19		

Conversion: SI to traditional units—Blood counts: $1 \times 10^9/l = 1000/mm^3$.

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The scheme of treatment is illustrated in fig 1 and the details of the drug treatment are summarised in table II. Treatment to induce remission was with the COAP combination in the first 15 patients. This exacerbated neutropenia, and subsequently induction with vincristine and prednisolone was used. Vincristine (1 mg for children and 2 mg for adults) was administered on days 1, 8, and 15 and prednisolone (50 mg/day for children and 100 mg/day for adults) was given from day 1 to day 15 inclusive. Three patients received colaspase (asparaginase) 8000 U m^{-2} day⁻¹ as part of their induction treatment

TABLE II—Details of quadruple drug combinations used in treatment regimen

	Days administered	Route
Modified COAP¹⁰ combination		
Cyclophosphamide 100 mg m ⁻² day ⁻¹	Days 1-5*	Orally
Oncovin (vincristine) 1 mg (2 mg for adults)	Day 1	IV
Ara-C (cytarabine) 100 mg m ⁻² day ⁻¹	Days 1-5*	IV or IM
Prednisolone 100 mg (200 mg for adults)	Days 1-5	Orally
Modified POMP¹¹ combination		
Prednisolone 100 mg (200 mg for adults)	Days 1-5	Orally
Oncovin (vincristine) 1 mg (2 mg for adults)	Day 1	IV
Methotrexate 7.5 mg m ⁻² day ⁻¹	Days 1-5*	IV or IM
Purinethol (mercaptopurine) 300 mg m ⁻² day ⁻¹	Days 1-5*†	Orally
CART⁶ combination		
Cytarabine 100 mg m ⁻² day ⁻¹	Days 1-5‡	IV or IM
Asparaginase 30 000 IU m ⁻² day ⁻¹	Days 1, 4, 7, 10, 13	IM or SC
Rubidomycin (daunorubicin) 40 mg/m ²	Day 1	IV
Thioguanine 100 mg m ⁻² day ⁻¹	Days 1-5	Orally

*May be given for only 3 days on first course.

†Mercaptopurine should be given on one only of each pair of POMP courses.

‡May be given for less than 5 days if pancytopenia occurs.

IV = Intravenously. IM = Intramuscularly. SC = Subcutaneously.

but this practice was discontinued when the myelosuppressive effects of colaspase during remission induction were shown.⁹ Consolidation and maintenance treatment was by intermittent pulses of treatment, using the combinations cyclophosphamide, vincristine, cytarabine, and prednisolone (COAP)¹⁰; prednisolone, vincristine, methotrexate, and mercaptopurine (POMP)¹¹; and cytarabine, colaspase, daunorubicin, thioguanine (CART).⁶ These combinations exploit known synergies between various antileukaemic drugs, avoid excessive overlap of drug toxicities, and ensure that all the most effective drugs are administered repeatedly. Treatment was given in five-day courses with nine-day treatment-free intervals. Most patients were admitted to hospital for induction therapy and thereafter attended the outpatient clinic once every 14 days. Drugs requiring intravenous administration (vincristine, daunorubicin) were given at the clinic, but intramuscular injections (cytarabine, methotrexate, colaspase) were commonly given at home by the family doctor, district nurse, or relatives. The doses of antileukaemic drugs other than vincristine, prednisolone, and colaspase could often be increased during maintenance treatment. For patients who became recurrently pancytopenic the treatment-free interval was lengthened from 9 to 16 days but full doses were given. During the maintenance phase the children continued to attend school and the adults returned to work.

Subcutaneous cerebrospinal-fluid (CSF) reservoirs were inserted as a means of administering extended intrathecal prophylactic treatment^{6 7 12} in eight cases but when the efficacy of conventional CNS prophylaxis was established⁴ the prophylactic use of reservoirs was discontinued.¹³ A radiation dose of 2400 rads (linear accelerator or cobalt machine) was administered to the skull through laterally opposed fields in 12 fractions. The first lumbar puncture was performed immediately after the induction of remission (fig 1), CSF was obtained for cytological examination, and methotrexate (7.5 mg/m²) was instilled. Five more doses of methotrexate were administered by the intrathecal route during the period of cranial irradiation (fig 1) and when necessary the doses of cyclophosphamide and cytarabine given in the COAP combination, which was being administered concurrently, were reduced to permit the completion of this intrathecal treatment.

Cyclic maintenance treatment was continued (fig 1) until relapse occurred, or until 130 weeks (2½ years) after bone-marrow remission had first been shown. During maintenance treatment the bone marrow was examined at least every six weeks. At the end of the 130th week the bone marrow was examined once more and a lumbar puncture was carried out, with sampling of fluid for cytological examination and the instillation of methotrexate (7.5 mg/m²). Thereafter blood counts were performed every four weeks and bone marrow examinations every eight weeks for one year, every six and 12 weeks respectively in the second year, and every 12 and 24 weeks in the third year.

Ten additional patients treated by this regimen were not included in the study because they had received previous treatment: six had had relapses of their disease—three in the bone marrow, one in the CNS, and two in both. The results of treatment in these 10 patients are included for comparison with those in the untreated patients.

FIG 1—Scheme of treatment protocol. After induction of remission with 15 days' prednisolone and injections of vincristine (VCR) on days 1, 8, and 15 there is a week's pause followed by treatment every 14 days with the three combinations COAP, POMP, or CART. Each course lasts five days with a nine-day rest period. Cyclic part of the treatment is repeated seven times before treatment is concluded. LP = Lumbar puncture I/T MTX = Intrathecal methotrexate.

Results

Remission induction—One boy of 16 died four days after treatment began: necropsy disclosed massive liver necrosis but failed to show its cause. The remaining 41 patients (98%) entered complete haematological remission. None was found to have CNS involvement on lumbar puncture and all completed the planned course of prophylactic cranial irradiation and intrathecal injections of methotrexate.

Prevention of CNS leukaemia—No patient developed symptoms or clinical signs of CNS leukaemia, and lumbar punctures performed on patients whose treatment was concluded showed normal cerebrospinal fluid.

Duration of remission and survival—Ten patients from 2-16 years old suffered a bone marrow relapse: there was no preponderance of a particular age group. One had been changed to a different treatment regimen after 11 months because of problems with intravenous injections, and in two cases the bone marrow appearance at relapse suggested acute undifferentiated leukaemia¹⁴ rather than true ALL. These three patients were, nevertheless, included in all the analyses. The acid phosphatase reactivity in the blast cells¹⁵ of another patient favoured the diagnosis of T-cell ALL. Four of the 10 relapsed patients achieved a second remission and a fifth underwent marrow transplantation. More than half the 41 patients who achieved complete remission were alive and in remission at the time of writing. Three patients died in complete remission: one from pneumococcal septicaemia, one from viral pneumonia, and one from a possibly anaphylactoid reaction after an intravenous injection of colaspase. Taking into account both deaths and relapses, the percentages of patients who were alive and in remission at 6, 12, 18, 24, and 30 months (2½ years) were 93%, 82%, 76%, 69%, and 56% respectively. The probability of a patient being alive and in remission at various time intervals is shown in fig 2 and compared with the results of the UKALL-I trial.⁴

FIG 2—Probability of a patient remaining alive and in remission during maintenance phase of present study (41 patients), compared with experience in UKALL-I trial (157 patients). Apparent advantage of the present study was not statistically significant but study included adult patients whereas UKALL-I included only children.

The difference between the two curves favoured our series but was not statistically significant. Eight patients aged 3-18 years completed two and a half years' treatment during complete remission and received no treatment for 2 to 26 months. None relapsed.

Features of possible prognostic significance—The results were analysed with respect to features of definite¹⁶ or possible prognostic significance: age, sex, race (Indian or European), haemoglobin concentration, platelet count, total leucocyte count, neutrophil count, and blast cell count. None of these features had any relation to the rapidity of remission induction or the occurrence of relapse, death, or relapse and death combined. In particular, no relapses occurred in the nine patients who had more than 20×10^9 blast cells per litre at presentation, and high blast cell counts showed no correlation with poor prognosis ($\chi^2 = 0.00$; not significant). Increasing age had no adverse effect on prognosis; others^{17, 18} have also found that adults with undoubted ALL who are treated intensively fare no worse than children.

Haematological effects of treatment—Blast cell counts fell rapidly during induction treatment. During the phase of intrathecal methotrexate administration and cranial irradiation it was usually necessary to omit cytarabine from the concurrent COAP; occasionally cyclophosphamide was omitted or its dose reduced. In about a third of the cases five-day courses were administered every three weeks during the maintenance phase instead of every fortnight to permit bone-marrow recovery. A syndrome of anaemia, reticulocytosis, thrombocytosis, neutropenia with cells resembling Pelger neutrophils, and absolute monocytosis occurred often and was attributed to the treatment.¹⁹ The syndrome may be misinterpreted as indicating early relapse of the leukaemia, and because severe neutropenia often exists in the presence of a normal total leucocyte count it is unwise to begin a new course of treatment until the blood film has been inspected. Haematological values always returned to normal rapidly; the simultaneous occurrence of neutropenia and monocytosis was reminiscent of idiopathic cyclical neutropenia.²⁰

General adverse effects of treatment—Cranial irradiation always caused severe alopecia. The hair regrew despite the maintenance chemotherapy and alopecia did not recur unless the treatment was changed because of relapse. Nausea and vomiting were frequent during the CART schedule, less common with COAP, and rare with POMP. Severe neuropathy from vincristine, and definite features of Cushing's syndrome were never observed. Oral ulceration attributable to the methotrexate in POMP was uncommon and mild. Pancytopenia, pronounced on the original POMP combination, was less severe when mercaptopurine was omitted from one of each pair of courses (fig 1). Signs suggestive of a cerebrovascular accident were attributed to methotrexate in three cases: one severe incident followed intrathecal administration, and two mild ones followed systemic administration. Both types of complication have been observed before.^{21, 22} Three deaths occurred during complete remission: two from infection, and one after the administration of colapsase. Deaths during remission are unacceptable, but have occurred in other series also.³

Criticisms of the protocol—While all the participants and patients found the protocol acceptable and no child was withdrawn from treatment by the parents some practical criticisms must be made. The supervision and administration of the injection schedules was more demanding of medical time and more unpleasant for young patients than in many other protocols. Problems with peripheral veins necessitated an alteration of the protocol in two patients, one of whom later relapsed. Recurrent episodes of neutropenia were disquieting although usually not attended by morbidity. Identifying fevers or rashes as effects of individual drugs was difficult because quadruple combinations were used. It was not usually possible to increase the doses of drugs in line with the growth of the children, and increased spacing of treatment (see above) was sometimes necessary. On the other hand, the treatment was generally well-tolerated and very little time was spent in hospital, while interference with holidays and schooling was minimal. The treatment-free intervals seemed to be beneficial psychologically as well as haematologically.

Additional patients treated with the regimen—The four girls and five boys excluded from the study because they had been treated before were aged from 3 to 7 years, and a 33-year-old man was also excluded for the same reason. Five patients had had bone-marrow relapses; two, who had not received cranial irradiation, had also relapsed in the CNS. Four of these five patients achieved haematological remission but only two—neither of whom had CNS involvement—remained in remission for over 24 months. These two patients stopped treatment and had not relapsed up to the time of writing. Four patients in remission were changed to the new regimen because their treatment was considered inadequate.^{3, 4} One patient remained in remission and has been off treatment for eight months, two relapsed 23 and 28 months after beginning the regimen, and one died in complete

remission from subacute measles encephalitis. The 10th patient had received no cranial irradiation and had relapsed in the CNS alone. He was treated with intrathecal methotrexate and cytarabine plus cranial irradiation and received the new regimen for 25 months. After nine months off treatment he remained in haematological and CNS remission.

Discussion

Treatment for ALL is assessed, firstly, by its capacity to induce and maintain haematological and CNS remission and by the length of survival during and after treatment; secondly, by the hazards of the treatment weighed against its efficacy; and finally, by the expense, demands on medical and laboratory man-hours, and convenience for the patients and their relatives, which vary according to social, economic, and geographic factors.

The induction of remission in 41 out of 42 patients (98%) is gratifying but in a larger series a lower rate might be expected. We compared the results of remission maintenance treatment with those in the UKALL-I trial⁴ and in studies V-VII of the Memphis group.³ Most of the statistical comparisons were made at two years, because at two and a half years the number of patients in our series was small and the survival curve was beginning to be unstable ($SE \pm 10\%$). At two years, comparing the numbers of patients who had neither relapsed nor died in these three series, there was no statistically significant difference. The small difference found favoured our current regimen (fig 2). At two and a half years a few more deaths and slightly fewer relapses had occurred in our series than in UKALL-I. At first sight, therefore, the COAP/POMP/CART regimen seems to possess no significant advantage, while it is slightly more unpleasant for the patients, more troublesome for medical staff, and significantly more costly than treatment by either the UKALL-I or the Memphis regimens. A third of our patients, however, were aged 10 years or more and a quarter were over 15 years old. Age above 10 years is usually considered to be a prognostically unfavourable feature, and when this is taken into account the possible advantage suggested in fig 2 may be real.

In our series neither age nor the peripheral blood blast cell count at diagnosis, both of prognostic significance in some series, had any significant effect, which suggested that combination chemotherapy of the intensity administered in this study may override the effects of factors that are of prognostic importance when patients receive less aggressive treatment. The COAP/POMP/CART regimen does not seem to have improved the prognosis for good-risk patients below the age of 10 with a low blast cell count; but further evaluation of the regimen in such patients is justified. The apparently beneficial effect of this intensive treatment on patients with a poor prognosis certainly merits further study. We are unable to comment on the possible value of the regimen for patients with the T-lymphoblastic variant of ALL. Evidence of T-cell origin was obtained in one patient who relapsed, but we do not know how many cases of T-cell leukaemia exist among the patients who have not relapsed. The same restriction, of course, applies to other therapeutic trials which began before lymphoblasts were regularly typed.

Recent figures from Memphis²³ suggest that if treatment is ended after two to three years in continuous (haematological and CNS) remission about 10% of patients will relapse, usually in the first 12 months. Relapse was much more frequent in patients who had not received CNS irradiation (six out of eight cases). We have not observed any relapses after stopping treatment.

It has been suggested that the repeated use of colapsase in courses separated by several weeks might cause severe allergic manifestations. One of our patients developed urticaria after his first and only injection, and another died suddenly after an injection of colapsase. Neither bronchospasm nor skin whealing occurred but an anaphylactoid reaction could not be ruled out. Allergic manifestations were not observed in the other 39 patients who entered remission and had repeated courses of colapsase. Possibly in this regimen the schedule of administration of the

accompanying immunosuppressive drugs prevents the development of allergy. A few patients experienced transient confusion, nausea, and subjective difficulty in breathing after the first injection only of colaspase in every course of CART treatment. These reactions are unpleasant and potentially dangerous, but they did not occur when the initial injection was administered subcutaneously. They are unlikely to be allergic but might be due to the rapid deamination of a normal plasma content of asparagine with a transient rise in blood ammonia when the colaspase is administered intravenously.

Our results suggest that the COAP/POMP/CART regimen merits further study, particularly in adults with ALL and in patients of any age with high blast cell counts at presentation. Its value in cases of documented T-cell ALL is as yet uncertain. It is not significantly superior when given to children with ALL who have no adverse prognostic features, though our data do not exclude the possibility that such superiority may exist.

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Current "corrected" calcium concept challenged

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Summary

There is wide individual variation in the number of millimoles of calcium bound per gram of albumin in the serum. Individual regression coefficients for serum calcium concentration on serum albumin concentration have been determined in 62 people (25 of our own patients and 37 reported by others). The 95 percentile range was 0.007-0.053 mmol/g, with a median value of 0.025 mmol/g. Accordingly, it is not valid to "correct" a person's measured serum total calcium concentration for variations in serum albumin concentration using an average regression coefficient. Rather, the individual's own regression coefficient must be used. A tourniquet test seems to be the simplest technique for determining this value. Even then, precise interpretation of an individual's corrected serum calcium concentration is possible only when an appropriate reference range for corrected serum calcium concentration has been established. Such an appropriate reference range must be determined from an adequate number of normal people using the individual's own correction factor in each case.

Introduction

Most routine laboratories can estimate the serum total calcium concentration very accurately, but since total calcium is the sum of ionised (50%), protein bound (40%), and complexed (10%) calcium fractions, and it is only the ionised fraction that is physiologically important, the estimation falls short of clinical requirements. Since albumin is the main (or sole) protein to which calcium is bound changes in serum albumin concentration will change the serum total calcium concentration. Hence numerous correction factors, relating measured serum calcium concentration to specific gravity, total protein, and serum albumin concentrations, have been developed.¹⁻⁵

We report here the wide individual variation in the regression coefficients of serum calcium concentration on serum albumin concentration found in 25 hospital inpatients and in a further 37 patients reported by others. We consequently question the validity of "correcting" an individual's measured serum calcium concentration by an average correction factor. Alternative approaches to the problem are discussed.

Patients and methods

Concentrations of both serum total calcium and serum albumin were measured by dye-binding methods on the Technicon SMA 12/60. Serum total calcium was measured using the standard Technicon cresolphthalein complexone method with a typical between-batch coefficient of variation of 1.8%. Serum albumin estimations were performed by a modified bromocresol green method with increased linearity⁶ and with a typical between-batch coefficient of variation of 2.0%. Purified human albumin (Dade) was used as a standard.

Mean regression coefficient of calcium on albumin—Blood was collected without stasis from 163 fasting healthy laboratory personnel aged from 17 to 65 years. These ambulant subjects were not on any medication and blood was collected without stasis and separated within two hours. Data were also collected from 163 hospital inpatients with a variety of medical and surgical conditions but without renal impairment (serum creatinine concentration <150 µmol/l

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