Chemoimmunotherapy with Levamisole in Acute Lymphoblastic Leukemia

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Patients with acute lymphoblastic leukemia (ALL) who were in two consecutive protocols and in complete remission (CR) with maintenance therapy, were randomized to receive or not receive levamisole. A total of 15 of 55 low-risk patients of protocol 10-LLA-72 with levamisole had relapses, compared with 25 of 54 not receiving levamisole; 67 and 49%, respectively, remain in CR at 48 months ($P < 0.025$). In protocol 1-LLA-76, 14 of 91 low-risk patients on levamisole and 25 of 93 patients not receiving levamisole had relapses; 78 and 61%, respectively, remain in CR at 36 months ($P < 0.05$). Seventeen of 39 high-risk patients (children with a leukocyte count higher than 50,000 and adults) receiving levamisole had relapses compared with 37 of 61 not on levamisole. The DNCB skin test shows at 18 and 24 months a 74 and 85% positivity in the levamisole group vs. a 38 and 35% positivity in the control group ($P< 0.025$). We conclude that levamisole prolongs the duration of CR and survival in low-risk patients with ALL.

Cancer 48:1500–1507, 1981.

THE DURATION of complete remission and long-term survival in acute lymphoblastic leukemia (ALL) have been substantially improved during the last decade, in spite of the lack of new drugs. One of the major advances has been the prevention of CNS leukemia.[31]

Many groups have obtained a long-term disease-free survival in about 50% of the children with standard risk of contracting ALL.[3,15,26,28,31] Several recent treatment protocols using controlled trials and exploring the usefulness of different intensification or maintenance programs have not discovered new improvements.[3,5,19,20,25–27]

Immunotherapy with BCG in treating ALL has been claimed by Mathe[14,15] to be useful in prolonging complete remission. However, other controlled clinical trials using BCG failed to prove their usefulness.[12,16,18]

Maintenance chemotherapy with 6-mercaptopurine-methotrexate in patients with acute lymphoblastic leukemia produces immunosuppression of both cellular and humoral immunity. Patients with higher levels of immunocompetence had better prognosis with longer duration of complete remission than patients with severe immunosuppression.[10]

Levamisole (an antihelminthic drug) has been reported to restore the immunity in immunodepressed cancer patients.[2,6,13,17,23,24,32–34] Chirigos *et al.*[8,22] demonstrated that levamisole prolonged remission and significantly increased survival when given to mice after their transplanted leukemia had been brought to remission with BCNU.

A clinical trial of levamisole in advanced human breast cancer showed a significant prolongation of the

Presented in part at the 69th Meeting of the AACR, Washington DC, April 1978, and presented at the 16th Meeting of the American Society of Clinical Oncology, San Diego, California, May 1980.

This is a publication of the Grupo Argentino de Tratamiento de la Leucemia Aguda (GATLA) and the Grupo Latinoamericano de Tratamiento de Hemopatías Malignas (GLATHEM).

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The authors thank Daniel Goldar for the statistical analysis, Isabel Kabat for preparing the manuscript, and Estela Koval for her technical assistance in the immunologic tests. Levamisole was furnished by Bagó Laboratories.

Accepted for publication August 20, 1980.

0008-543X/81/1000/1500 $0.90

TABLE 1. Characteristics of the Patients According to Protocol and Immunostimulation

	10-LLA-72		1-LLA-76		Total	
	With LEV	Without LEV	With LEV	Without LEV	With LEV	Without LEV
Total	64	72	121	136	185	208
Age (yr)						
<2	5	1	9	12	14	13
≥2–≤10	47	54	83	92	130	146
>10–≤15	6	8	18	12	24	20
>15–≤20	2	3	2	7	4	10
>20	4	6	9	13	13	19
Leukocyte count						
<50,000	60	60	99	108	159	168
>50,000	4	12	22	28	26	40

LEV = levamisole.

median disease-free interval and survival in the levamisole group compared with the control.[24]

With the aim of exploring the usefulness of levamisole as an adjuvant of maintenance chemotherapy for prolonging complete remission in acute lymphoblastic leukemia, GATLA started a controlled clinical trial in October 1975.[21]

Material and Methods

Two groups of patients with acute lymphoblastic leukemia who were placed on two consecutive protocols, were included in this study.

Protocol 10-LLA-72

This protocol[20,23] was opened to include new patients in October 1972 and was closed in December 1975. It included induction with vincristine and prednisone; if by day 29 complete remission was not achieved Daunorubicin was added to the drugs. Patients were randomized to receive or not receive cytosine arabinoside and cyclophosphamide as intensification. All the patients received CNS prevention with ^{60}Co to the cranium and with intrathecal methotrexate. A second randomization divided those on maintenance therapy into two groups: 6-mercaptopurine daily plus methotrexate twice a week vs. 6-mercaptopurine plus methotrexate weekly. Pulses of vincristine and prednisone were done every three months. None of these two schemes show the advantage of prolonging the duration of complete remission.[23] In October 1975, 136 patients who were in their first complete remission or achieved it after that date, were randomized to receive or not receive levamisole. The median duration of complete remission before levamisole was 15 months with a range of 1–34 months.

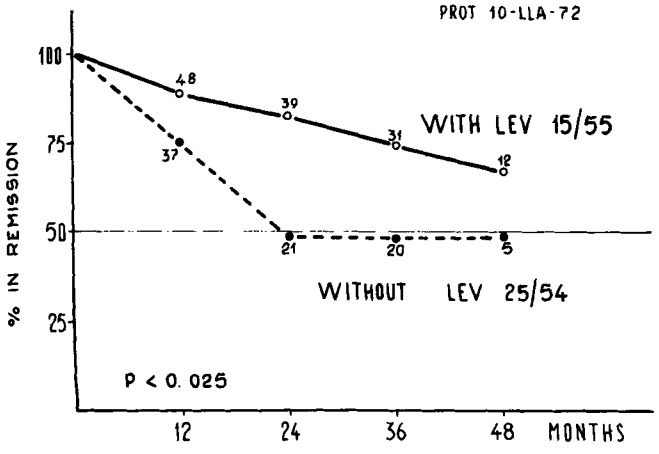

FIG. 1. Duration of complete remission (from time of randomization) in children with ALL and a leukocyte count lower than 50,000 at diagnosis according to immunostimulation with levamisole in protocol 10-LLA-72. The logrank test was used to compare the difference between curves.

The following number of patients entered in the study with or without levamisole at different intervals of time from the moment complete remission was obtained; < 3 months, 10 and 17 patients respectively; 4–6 months, 4 and 10 patients; 7–12 months, 13 and 18 patients; 13–18 months, 15 and 12 patients; and more than 18 months, 22 and 15 patients, respectively. More patients without levamisole had shorter time in complete remission than those with levamisole. However, there was no statistical difference between both groups.

Protocol 1-LLA-76

A second protocol was opened to incorporate new patients in January 1976 and was closed in December 1978.[27] Children with a leukocyte count under 20,000 received, as induction vincristine and prednisone. Children with a leukocyte count over 20,000 and adults received daunorubicin, vincristine, and prednisone. CNS prevention was done with intrathecal methotre-

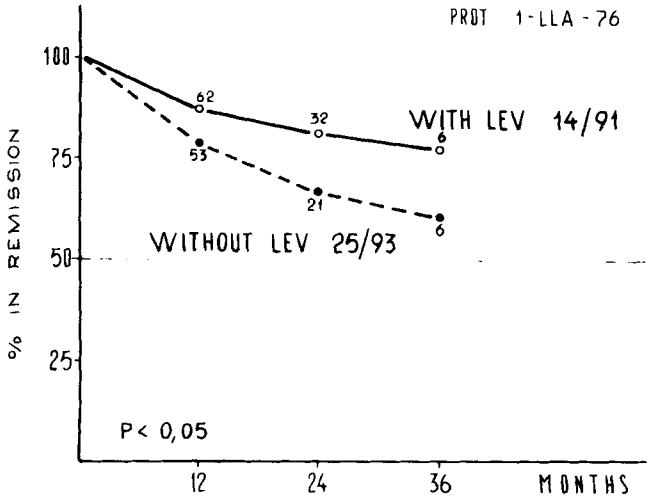

FIG. 2. Duration of complete remission in children with ALL and a leukocyte count lower than 50,000 at diagnosis according to immunostimulation with levamisole in protocol 1-LLA-76.

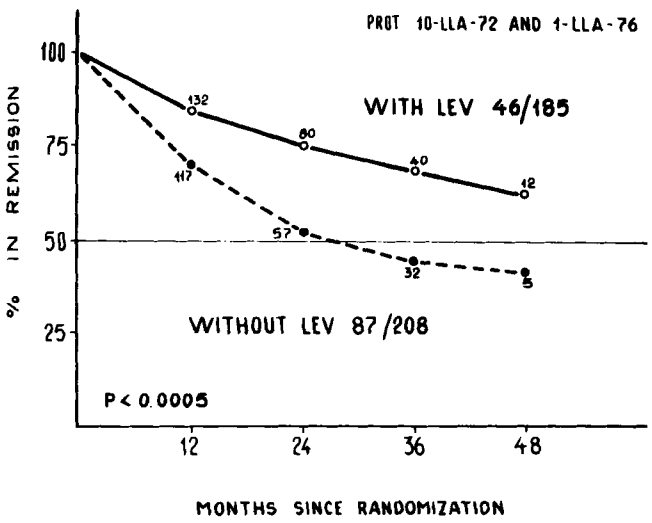

FIG. 4. Duration of complete remission of all the patients in both protocols.

xate only during induction and every three months during maintenance. All the patients received intensification with cytosine arabinoside and cyclophosphamide. During maintenance the patients received 6-mercaptopurine daily and methotrexate twice a week. Group A received courses of vincristine and prednisone every month until the sixth and thereafter every three months. Group B received sequential courses with vincristine–prednisone and the following course with cytosine arabinoside–cyclophosphamide. None of these two groups show at the moment differences in duration of complete remission.

Two-hundred-fifty-seven patients were randomized immediately after achieving complete remission to receive or not receive levamisole. Levamisole was administered daily in capsules of 50 mg or suspension at a dose of 100 mg/m^2.

Both protocols were evaluated in January 1980.

There was no difference among age groups when comparing prognostic factors (Table 1). In protocol 10-LLA-72, fewer patients had a higher leukocyte count in the levamisole group than in the control. However, this difference was not significant.

Statistical analysis was done using the life table method; patients who died in complete remission were considered as lost to follow-up at that time. Only bone marrow relapse was considered as the termination of complete remission. The logrank test was used to compare the difference between curves and the chi-square or student's test, when considered appropriate.

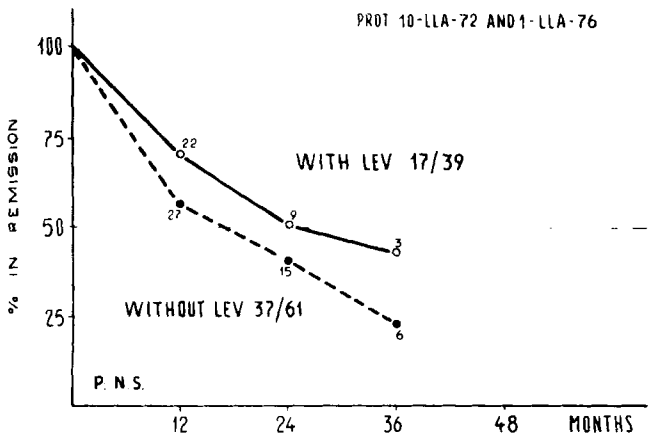

FIG. 3. Duration of complete remission in high-risk ALL (children with a leukocyte count higher than 50,000 and adults) at diagnosis according to immunostimulation with levamisole in protocols 10-LLA-72 and 1-LLA-76.

Results

Duration of complete remission in low-risk patients (children with fewer than 50,000 leukocytes/mm^3).

Protocol 10-LLA-72

Of 55 children, 15 (<15 years old) treated with levamisole had bone marrow relapse compared with 25 of 54 children not treated with levamisole (Fig. 1). Sixty-seven and 49%, respectively, remain in complete remission at 48 months from randomization, with or without levamisole. The difference is statistically significative ($P < 0.025$). In protocol 1-LLA-76 (Fig. 2) 14 of 91 children treated with levamisole and 25 of 93 children not receiving levamisole had bone marrow relapse. Seventy-eight and 61%, respectively, remain in complete remission at 36 months ($P < 0.05$). Seventeen patients treated with levamisole and nine without

levamisole of both groups had CNS relapse without bone marrow relapse; one patient not treated with levamisole had isolated testes relapse.

Duration of complete remission in high-risk patients (children with more than 50,000 leukocytes/mm^3 and adults).

Seventeen of 39 patients treated with levamisole had bone marrow relapse, compared with 37 of 61 relapses in patients without levamisole (Fig. 3). This difference was not significant. Three CNS relapses without bone marrow relapse were observed in the group not receiving levamisole and one in the group treated with levamisole. None had isolated testes relapse.

Overall Duration of Complete Remission

Figure 4 shows the duration of complete remission in all the patients included in this study, 46 of 185 patients treated with levamisole and 87 of 208 patients treated without levamisole had relapses. Sixty-three and 42%, respectively, remain in complete remission at 48 months ($P < 0.0005$).

In Figure 5 we can see the time that elapsed from complete remission to the first relapse in either CNS, marrow, or testes, or death in remission in all the patients of both groups. In the group treated with levamisole, 74 of 185 had at least one event compared with 121 of 208 in the group without levamisole, remaining 47 and 33%, respectively, free of disease and alive at 48 months ($P < 0.0005$). Comparing only the low-risk patients with first event of relapse or death, at 48 months 49% of the group treated with levamisole and 38% of the group without levamisole remain free of disease ($P < 0.005$). In high-risk patients, 41 and 21%, respectively, have not had a relapse or died at 48 months. Because of the low number of patients, this difference is not statistically significant.

Duration of Complete Remission According to the Time of Randomization after Achieving Complete Remission

Some of the patients of protocol 10-LLA-72 were in complete remission for several months before randomization with or without levamisole. Table 2 shows the percentage of patients remaining in complete remission at 36 months after randomization. In all the periods, the group treated with levamisole shows a higher percent of complete remissions. The difference was significant in the period of less than four months ($P < 0.001$) and more than 18 months ($P < 0.05$).

Death in Complete Remission

Table 3 shows that 3% of patients of protocol 10-LLA-72 who received levamisole died in complete remission compared with 10% in the group not receiving levamisole. Likewise, in protocol 1-LLA-76 fewer patients died in complete remission in the group treated with levamisole (5 vs. 10%). When comparing the overall percent of patients who died in complete remission, it is observed that the difference was statistically significant ($P < 0.05$). The causes of death in complete remission in the levamisole group were: gastrointestinal infection with severe diarrhea, two patients; hypertensive pneumothorax, one; liver failure associated to viral hepatitis, one; chicken pox, one; neumopathy, three.

In those patients not receiving levamisole the causes of death were: liver failure associated to viral hepatitis,

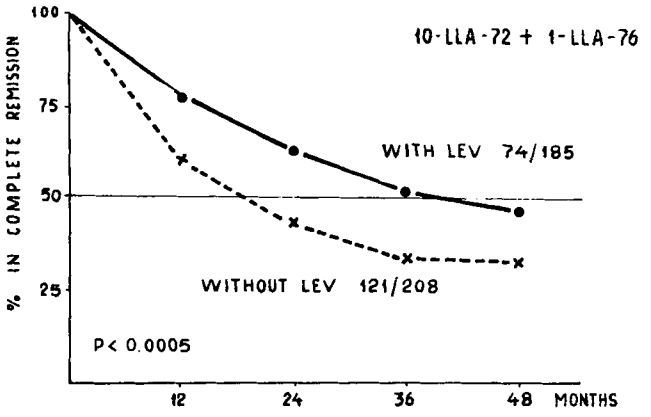

FIG. 5. Months from complete remission to first event of relapse. CNS, marrow, or testicular relapse or death in complete remission in all the patients of both protocols.

TABLE 2. Percentages of Complete Remission at 24 Months Since Randomization According to Immunostimulation

Time from CR to randomization (mo)	LEV	No. of patients	Relapses	% in CR at 36 months since randomization	P
0–3	Yes	106	20	76	<0.001
	No	132	50	52	
4–6	Yes	23	7	63	N.S.
	No	31	13	38	
7–12	Yes	19	9	36	N.S.
	No	18	11	22	
13–18	Yes	15	5	66	N.S.
	No	12	6	45	
>18	Yes	22	4	89	<0.05
	No	15	7	53	

LEV = levamisole.

TABLE 3. Percentage of Patients Who Died in Complete Remission According to Immunostimulation with Levamisole

	With LEV		Without LEV			Total	
Protocol	Dead/total	%	Dead/total	%	P	Dead/total	%
10-LLA-72	2/64	3	7/72	10	N.S.	9/136	7
1-LLA-76	6/121	5	14/136	10	N.S.	20/257	8
TOTAL	8/185	4	21/208	10	<0.05	29/393	7

LEV = levamisole.

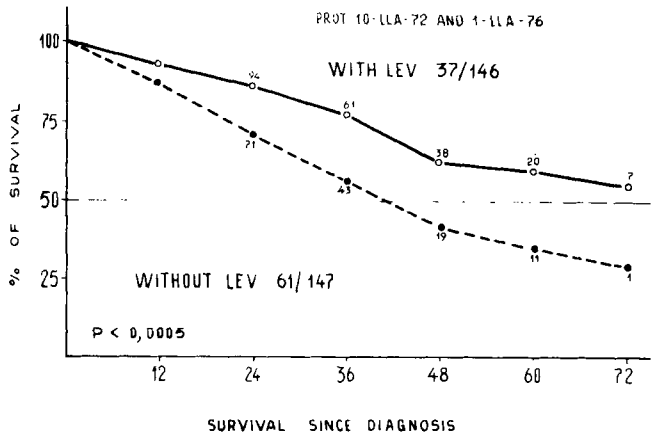

FIG. 6. Survival of children with ALL and a leukocyte count lower than 50,000 at diagnosis according to immunostimulation with levamisole (Protocol 10-LLA-72 and 1-LLA-76).

one; chicken pox, four; meningitis, one; sepsis, seven; pneumopathy, six; unknown, two patients.

Toxicity

The only toxic reactions observed were mild gastrointestinal problems (nausea and occasionally vomiting) that obliged to reduce the doses in less than 10% of the patients. No cases of severe agranulocytosis related to levamisole were reported. As all the patients received concomitant maintenance therapy with 6-mercaptopurine and methotrexate, most showed leukopenia around 3000/mm^3.

Survival

The survival of children with a leukocyte count lower than 50,000 at diagnosis was determined in both protocols (Fig. 6). Thirty-seven of 146 patients in the levamisole group died compared with 61 of 147 in the control group. Sixty and 35%, respectively, remain alive at 60 months from diagnosis ($P < 0.0005$). In those patients at high risk, 16 of 39 patients treated with levamisole died compared with 37 of 61 in the control group. Of these patients, thirty-four and 33%, respectively, remain alive at 48 months from diagnosis. This difference is not statistically significant (Fig. 7).

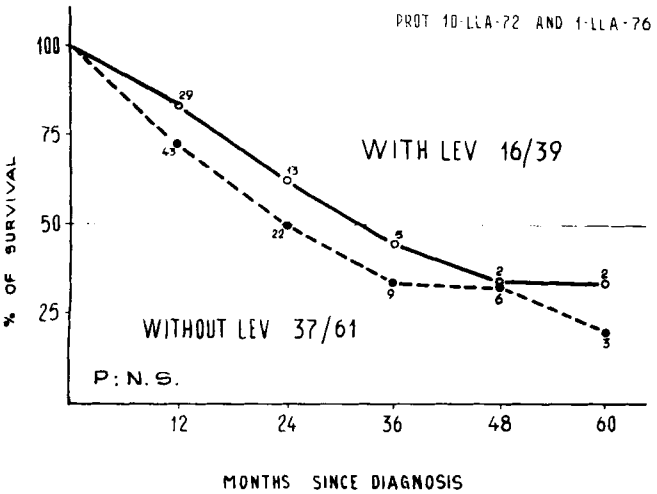

FIG. 7. Survival of high-risk patients (<15 years with >50,000 leukocyte count and adults) according to immunostimulation with levamisole (Protocol 10-LLA-72 and 1-LLA-76).

Results of the Immunologic Studies

Skin tests: (Table 4). No difference was observed in the positive reaction of PPD and Candidine delayed skin tests compared with the group of patients not treated with levamisole or following the period of administration of levamisole. This test was interrupted in October 1977.

The DNCB skin test (Table 5) shows at 18 and 24 months 74 and 85% positivity in the levamisole group vs. 38 and 35% positivity in the control group ($P < 0.025$). In the levamisole group the percent of positivity increased from 25% at the beginning to 69% at 12 months and 85% at 24 months. There is a statistatically significant difference between 0 vs. 3 months ($P < 0.005$); 0 vs. 12, 18, or 24 months ($P < 0.0005$). Of 44 patients who underwent DNCB skin test and were included in the group not treated with levamisole, 33 had negative skin tests. Eight of them became positive during maintenance treatment. Of 56 patients who underwent this test in the group treated with levamisole, 42 were negative before immunostimulation. Of these, 29 became positive. The difference was statistically significant ($P < 0.0005$). Of the 11 initially positive DNCB skin tests, three became negative, compared with one out of 14 of the group treated with levamisole.

T and B lymphocytes: No statistical difference was observed between groups, or during the period of administration in the group treated with levamisole. Thirty-two of 34 patients not treated with levamisole showed abnormal T lymphocyte count at the beginning and five achieved the normal level. Thirty-nine of 44 patients of the group treated with levamisole had T lymphocyte count below the normal range and 13 increased their value to normal levels. This difference was not significant. The studies of T and B lymphocytes were interrupted in October 1977.

Discussion

The progressive improvement in the prognosis of ALL has been the result of two major developments: the more efficient use of chemotherapeutic agents and the prevention with irradiation of CNS relapse.[25,31] At

TABLE 4. Positivity of Skin Tests According to Immunostimulation

	Initial		3 months		6 months		9 months		12 months	
Skin test Lev	Pos/T	%	Pos/T	%	Pos/T	%	Pos/T	%	Pos/T	%
PPD										
With	4/34	12	5/28	18	6/25	24	5/17	29	4/15	27
Without	5/25	20	3/19	16	2/12	16	2/12	16	2/9	22
Candidine										
With	21/34	62	18/28	64	17/25	68	11/17	64	9/15	60
Without	13/25	52	9/19	47	6/12	50	6/12	50	6/9	66

LEV = levamisole.

present, approximately 50% of the children with standard risk may enjoy a long-term leukemia-free survival.[3,5,26–28] However, during the past few years several protocols exploring different intensification schemes or maintenance therapy using two, three, or four drugs (continuous vs. intermittent therapy) failed to demonstrate the advantage of one method over the other in prolonging complete remission.[3,5,19,20,25–27]

Levamisole has been widely used as an antihelminthic in animals. Several studies in animals and in man show that levamisole can increase the antibody formation to different antigens, increase blood levels of total hemolitic complement in cancer patients, increase delayed hypersensitivity, stimulate the blastogenic response, and increase the number of macrophages showing phagocytosis.[2,6,13,17,23,24,32–34]

Levamisole has been used in clinical cancer therapy of solid tumors. Rojas *et al.*[24] showed that levamisole given in a controlled prospective study of irradiated breast cancer patients prolongs the disease-free survival. The median duration of remission was nine months for the control group and 25 months for the levamisole group. Likewise, a significant difference was found in survival: 90% of the levamisole-treated patients were still alive 30 months after irradiation treatment as opposed to only 35% of the control group.

A clinical, randomized, placebo-controlled double-blind study was conducted[1] in resectable lung cancer patients using levamisole. A dose of 50 mg of levamisole was given three times daily on three consecutive days every fortnight for two years after surgery. The disease-free survival in the patients weighing 70 kg or less was 91% with levamisole and 80% with a placebo, six months after the operation. At 12 and 24 months, 83 and 71%, respectively, treated with levamisole and 64 and 49% treated with a placebo remain disease-free.

Another randomized trial[35] of levamisole in patients with squamous cancer of head and neck shows a disease-free survival rate at 12 months of 34% for those treated with a placebo and 82% for the levamisole group ($P < 0.025$). These preliminary results suggest the benefit of levamisole as an adjuvant treatment in patients with primary operable cancer of the head and neck.

Chirigos *et al.*[8,22] demonstrated that levamisole prolonged the remission and significantly increased the survival when given to mice after their transplanted leukemia had been brought to remission with BCNU.

Two studies used levamisole as an adjuvant of chemotherapy during maintenance therapy in acute myeloblastic leukemia in complete remission.[4,7] Brincker *et al.*[4] report a median duration of remission of 16 months with levamisole and ten months with a placebo. This difference was not significant in view of the limited number of patients. In other similar study, Chang *et al.*[7] reported a median duration of 174 days for the control group and 163 days for those treated with levamisole.

Salmon *et al.*[29] from the Southwest Oncology Group have recently reported a randomized trial with chemoimmunotherapy in treating multiple myeloma. Patients achieving a 75% tumor regression were randomized to maintenance chemotherapy with vincristine, melphalan, cyclophosphamide, and prednisone (VMCP) or VMCP plus levamisole. Of 55 patients on VMCP there were 14 relapses and 11 deaths. Of 58

TABLE 5. Percentage of Positivity to DNCB According to Immunostimulation with Levamisole

	With LEV		Without LEV		
Months	No./positive	%	No./positive	%	*P*
0	14/56	25	11/44	25	N.S.
3	24/43	56	6/24	25	<0.025
6	20/38	53	6/28	21	<0.025
12	22/32	69	8/23	35	<0.025
18	23/31	74	8/21	38	<0.025
24	17/20	85	6/17	35	<0.005
30	9/13	69	5/12	42	N.S.
36	8/8	100	5/12	42	<0.01
42	7/8	87	3/8	37	<0.05

Difference in the group with Levamisole (months): 0 vs. 3 = $P < 0.005$; 0 vs. 6 = $P < 0.01$; 0 vs. 12, 18, or 24 = $P < 0.0005$; 0 vs. 30 = $P < 0.005$; 3 vs. 24 = $P < 0.025$; and 6 vs. 24 = $P < 0.025$.
LEV = levamisole.

patients on VMCP plus levamisole there were seven relapses and six deaths. The remission duration curves differ significantly ($P < 0.002$).

This study evaluated the use of levamisole in two consecutive protocols in a randomized way in acute lymphoblastic leukemia, in complete remission, and as an adjuvant of maintenance chemotherapy.[21,26,27] In the first study most of the patients started therapy in October 1975 after several months of complete remission. In the second study all the patients were randomized immediately after achieving complete remission.

In both protocols the low-risk group of patients (less than 16 years and a leukocyte count lower than 20,000) treated with levamisole in combination with maintenance chemotherapy shows a statistically significant longer duration of hematologic, complete remission and survival than patients not treated with levamisole ($P < 0.005$). In those patients at high risk, the group treated with levamisole also shows longer duration of complete remission and survival. However the difference is not statistically significative.

Children at low risk in this study who were treated with levamisole have a better survival than those in the control group, 60 vs. 35%, respectively, at 60 months. Recently other groups have reported survival above 50% in children with acute lymphoblastic leukemia. Sallan *et al.*[28] reported a disease-free survival of 70% at 36 months in 129 children who were treated between 1973–1977 and obtained complete remission. The Children Cancer Study Group had 724 children treated in the years 1972 and 1974 with different CNS intensification therapies whose estimated survival from the time of diagnosis at five years is 61%. This study includes all the acute lymphoblastic leukemia patients treated during this period and it probably represents the highest rate of survival in a large group of patients (M. E. Nesbit, personal communication). Only 4% of the patients treated with levamisole died in complete remission compared with 10% in the control group.

In our previous study,[26] 23 (6%) of 357 patients died in complete remission. However, the duration of time of risk was shorter; the median duration of complete remission was 25 months in low-risk children, ten months in high-risk children and 24 months in adults. At 36 months, 39, 25, and 39%, respectively, remain in complete remission.

Simone *et al.*[30] reported in the period 1968–69, 14 of 162 children (8.6%) who died in complete remission. In the period 1962–67, ten of 218 children (4.6%) died in complete remission. The authors argued that the increased frequency coincides with the addition of more aggresive combination therapy, which resulted in longer duration of complete remission and survival and a larger population of patients at risk. In a more recent publication of the same institution, Aur *et al.*[3] report that during the period 1972–1975, 14 of 268 children (5.2%) died in complete remission. In this period no further increase in duration of complete remission or survival was obtained compared with the period 1968–69. However, much knowledge has been acquired in treating infection complications in children in remission in the last decade.

Other authors[15,18] report fewer deceases in complete remission in the group treated with immunotherapy (BCG and irradiated cells) than in the group with chemotherapy. These studies used immunotherapy only. In the present study both groups received the same maintenance chemotherapy and it is likely that levamisole protects the deleterious effect in the immunocompetence of the chemotherapy. As the patients who died in complete remission were considered as lost to follow-up, the difference in the duration of complete remission of the levamisole and control groups are not influenced by the different percents of patients who died in complete remission.

In our study, several immunologic tests were performed to measure immunocompetence at the beginning and during the study. The skin tests PPD and Candida and T- and B-lymphocyte counts have shown no difference when comparing patients treated with levamisole and those not treated with levamisole during the course of the study. DNCB shows a constant increase in positivity during the study. At 12 and 24 months, 69 and 85% of the patients treated with levamisole had a positive skin test compared with 35 and 35% in the group not receiving levamisole ($P < 0.025$). In the group treated with levamisole, the percent of positivity was 25% at the beginning and increased after 24 months to 85% ($P < 0.0005$). Other authors have also reported the ability of levamisole to increase the positivity of DNCB.[2,6,24,32,34]

Two other immunologic tests performed in a small number of the same group of patients show positive results. The blast transformation of peripheral lymphocytes was significantly increased in those patients who received levamisole.[11] Also, the capacity of B lymphocytes to differentiate in plasma cells induced by pokeweed mitogen and evaluated by direct immunofluorescence are increased statistically (mean 3.28 ± 0.59) compared with the same group of patients not receiving without levamisole (0.27 ± 0.19) ($P < 0.0005$). Both groups of leukemia patients show decrease in plasma cells differentiation compared with normal (6.77 ± 1.09) ($P < 0.0005$).[9]

We obviously do not know whether the effect in the duration of remission and survival in acute lymphoblastic leukemia that appears to result from daily levamisole therapy is due to its immunopotentiating effects against infections or affects the body's immune

response against leukemia or some other biochemical effect of this agent.

The mechanism by which levamisole might act in acute leukemia remains speculative. Levamisole is known to stimulate immune response in an immunocompromised host. In our patients, levamisole significantly increased the delayed reactivity to DNCB, the blast transformation of peripheral lymphocytes (both functions of T lymphocytes), and increased the capacity of B lymphocytes to differentiate in plasma cells (a B lymphocyte function).

Agranulocytosis has been associated with levamisole.[2] The current experience with a large number of leukemia patients in association with chemotherapy did not suggest significant toxicity.

We conclude that levamisole used as an adjuvant of maintenance chemotherapy prolongs the duration of complete remission and survival in children with ALL and a leukocyte count under 50,000.

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