Adjuvant Postoperative Radiotherapy, Chemotherapy, and Immunotherapy in Stage III Breast Cancer

II. 5-Year Results and Influence of Levamisole

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One hundred twenty pathologically confirmed operable Stage III breast cancer patients were randomized to receive either postoperative radiotherapy or chemotherapy, or a combination of these, with or without levamisole immunotherapy. Radiotherapy was given to regional lymph nodes and chest wall. Chemotherapy consisted of six cycles of vincristine, doxorubicin, and cyclophosphamide. Radiotherapy provided local and chemotherapy systemic control over the tumor, but the best patient-saving results were achieved with a combination of radiotherapy and chemotherapy. This clinical trial was commenced in 1976, and the first 60 of 120 patients also received oral levamisole, 150 mg/day, on 2 consecutive days weekly as immunotherapy. All patients were followed for at least 5 years. At this stage levamisole seems to increase disease-free and overall survival in all three treatment arms (radiotherapy, chemotherapy, combined treatment). Significance is reached in disease-free survival ($P = 0.035$) and overall survival, adjusted for all other treatment modalities ($P = 0.019$).
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DURING THE LAST DECADES, quite a number of clinical trials have proved early systemic treatment effective in preventing dissemination of malignant neoplasm originating from human breast.[1-3] The value of postoperative radiotherapy remains at local control of the disease with no influence on the risk of metastases or survival.[4,5] Immunocompetence has been shown to improve the prognosis of breast cancer patients.[6,7] Promising results have been achieved with levamisole in breast cancer and colon cancer, but there are controversial reports as well.[8-13] In basic research levamisole has been shown to affect immune functions, which could be supposed to benefit cancer patients.[14-16]

This investigation compares adjuvant chemo(immuno)therapy and postoperative radiotherapy in Stage III breast cancer using levamisole as an immunoadjuvance. After encouraging results in pilot studies and to avoid too many treatment arms, all patients in this trial initially received levamisole and were randomized to three treatment arms: radiotherapy, chemotherapy and radiotherapy + chemotherapy. However, to evaluate the value of levamisole in Stage III breast cancer, the study was continued after the first 60 patients without leva-

misole. The preliminary results were promising in favor of combined radiotherapy and chemotherapy.[17] The results are now, after more than 5 years of follow-up, evaluable also in respect to levamisole.

Patients and Methods

During the years 1976 to 1978, in the first part of the study, 60 consecutive patients with operated Stage III breast cancer were randomized to receive either postoperative radiotherapy or chemotherapy, or a combination of these, plus levamisole. During the years 1978 to 1981, another group of 60 patients with the same criteria were randomized equally, but without levamisole. One patient in the combined treatment group + levamisole did not receive the treatment as designed and was therefore excluded. The first patient in the nonlevamisole radiotherapy group inadvertently received levamisole and was therefore in this study included in the radiotherapy + levamisole group (Table 1).

Surgery was performed in several hospitals. The operation type was modified radical mastectomy. The pectoral fasciae and muscles were left intact. The axillary fat, including lymph nodes, was removed. Chemotherapy and radiotherapy were given at the Department of Radiotherapy and Oncology, Helsinki University Central Hospital.

Patients operated on for Stage III breast cancer were eligible if they (1) were younger then 75 years, (2) had no

TABLE 1.   Patient Characteristics

	Levamisole +			Levamisole −		
	RT	CT	RT + CT	RT	CT	RT + CT
No. of patients	21	20	19	19	20	20
Mean age (range)	49	53	48	56	53	51
	(39–61)	(40–67)	(32–65)	(32–74)	(40–70)	(34–69)
No. of Postmenop	10 (48%)	12 (60%)	8 (42%)	16 (84%)	15 (75%)	10 (50%)
Nodes +	15 (71%)	15 (75%)	13 (68%)	13 (68%)	12 (60%)	11 (55%)
Nodes + >4	10 (48%)	10 (50%)	6 (32%)	9 (47%)	6 (30%)	6 (30%)
Histologic type						
Ductal	20	18	18	18	19	18
Lobular	1	1	1		1	1
Medullary		1		1		1
Tumor size (cm) (mean)	6.1	6.6	6.9	5.9	6.3	6.4
No. of skin involvement	3	3	3	4	1	4
No. of completed 6 courses of CT	—	19	19	—	20	19
No. of reduced doses of CT	—	4	4	—	1	5
Nadirs of leucocyte count (mean) × 10^{9}[1]	3.1	2.75	2.4	3.9	3.2	2.2
Delays in therapy (wk)	10.6	8.3	9.5	9.2	7.2	9.1

RT: radiotherapy; CT: chemotherapy; Postmenop: postmenopausal.

history of other malignancies, (3) were cooperative, (4) had no serious heart disease, (5) had no rheumatoid arthritis (because of the risk of agranulocytosis[18] due to levamisole), and (6) were able to take care of themselves (Karnofsky >80%).[6] Staging of the disease was based on the International Union Against Cancer (UICC) criteria of clinical Stage III breast cancer. In the current report, Stage N+ cases included only pathologically confirmed palpable and nonpalpable nodes with tumor infiltration. Thorough reexamination of the pathologic involvement of the nodes gave rise to minor shifts in patient numbers in the subgroups compared to the previous publication.[17]

Radiotherapy

Postoperative radiotherapy was delivered from a cobalt-60 unit 9 to 10 weeks after surgery in average. The fields covered the supraclavicular and infraclavicular regions and the axilla, the parasternal regions, and the chest wall. The dose given to each region was 45 Gy over a period of 3 weeks in 15 fractions. The supraclavicular fossa, second interspace, and the anterior axilla were covered by a direct portal. The rest of the parasternal chain was covered by a direct portal, 6 cm laterally from the midline of sternum. There was a gap of 0.5 cm on the skin between the two portals. The posterior axillary portal was irradiated with 30 Gy over a period of 2 weeks in 10 fractions. The midline dose in the axilla was about 50 Gy in 5 weeks. The total treatment time was 5 weeks.

Chemotherapy

Chemotherapy consisted of six cycles of vincristine, Adriamycin (doxorubicin), and cyclophosphamide (VAC). The original schedule was designed to include intravenous vincristine 1.2 mg/m^2 and doxorubicin 45 mg/m^2 on day 1, and oral cyclophosphamide 200 mg/m^2/day on days 2 through 6. The cycle was repeated every 4 weeks. Because of the risk of myelotoxicity the dose of cyclophosphamide was reduced to 150 mg/m^2/day.

The delay from surgery to chemotherapy averaged 7 to 8 weeks. Irradiation prolonged onset of chemotherapy by another 6 to 7 weeks. The doses were adjusted according to blood test values as follows: leucocytes 2 to 3 × 10^6 or thrombocytes 100 to 150 × 10^6, reduction by 50%, leucocytes less than 2 × 10^6 or thrombocytes less than 100, a new cycle after recovery to 3.0 × 10^6 or 150 × 10^6, respectively.

Levamisole Immunotherapy

Levamisole, 150 mg/day on 2 consecutive days each week except on the weeks chemotherapy was given, was started simultaneously with radiotherapy or chemotherapy. The treatment was continued for 1 year unless toxic side effects or recurrences appeared.

Follow-Up

The patients were seen by the doctor every 3 months during the first 2 years and thereafter every 4 to 6
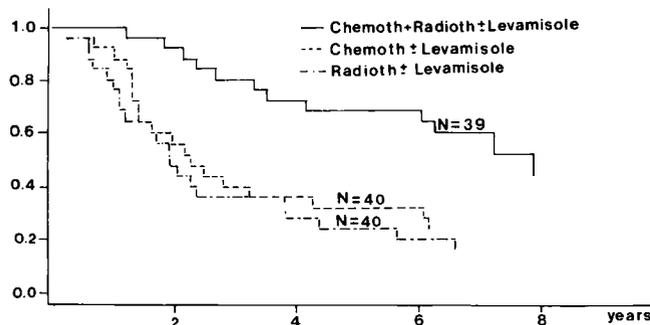
FIG. 1. Disease-free survival of patients given combined treatment, radiotherapy and chemotherapy with or without levamisole.

months for up to 5 years and once a year for up to 10 years. Routine evaluations included physical examination, chest radiography, erythrocyte sedimentation rate, leucocyte count, 5-nucleotidase and alkaline phosphatase determinations. Bone and liver scans were taken before treatment and after 1 year, unless symptoms required more frequent check-ups.

Disease-free survival and survival were calculated from the date of operation. Survival curves (uncorrected) were constructed according to the life-table method using the computer program (with log-rank test) designed by Peto *et al.*[19,19a] A log-rank analysis also was carried out stratifying the patients according to treatment (radiotherapy, chemotherapy or combined radiotherapy and chemotherapy) and analyzing for an effect of levamisole, adjusting for the other treatments.[19] The Cox life-table regression analysis (program 2L of BMDP-81) was used in a forward stepwise mode to assess the simultaneous effects of age, node involvement, tumor size and various treatments. The differences in the tables were analyzed by the chi-square test, Fisher's exact test, and analysis of variance.

Patient Characteristics

The study included 119 evaluable patients with characteristics which are given in Table 1. The treatment
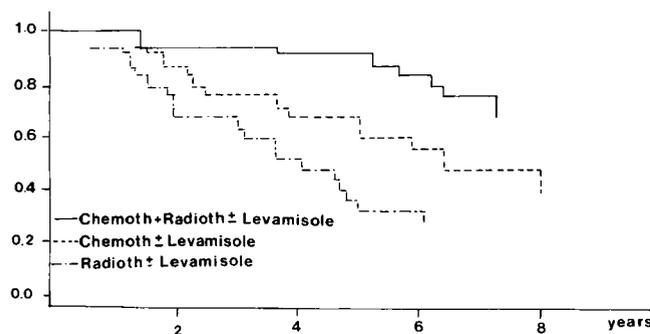

FIG. 2. Survival of patients given combined treatment, radiotherapy and chemotherapy with or without levamisole.

groups were comparable. The mean age and number of postmenopausal patients were slightly higher in the nonlevamisole group. Nodal involvement was encountered slightly more often in the levamisole group. The tumors were slightly but not statistically significantly larger in the levamisole groups. The nadirs of leucocyte count were similar in the various groups. Only one patient discontinued after four completed courses of chemotherapy because of nausea. The means of nadirs of drug doses in the various groups were comparable: doxorubicin 50 mg, vincristine 1 mg, and cyclophosphamide 150 mg × 5 as total doses in the levamisole groups and the combined nonlevamisole group, and doxorubicin 60 mg, cyclophosphamide 200 mg × 5 and vincristine 1 mg in the nonlevamisole chemotherapy group. All these features more likely favored the nonlevamisole patients. The incidence of estrogen and progesterone receptor-positive patients was not calculated, because receptors were not routinely measured in all hospitals.

Results

Failure Pattern and Survival

Patients treated by combined radiotherapy and chemotherapy exhibited fewer recurrences and increased survival. As has been reported previously,[17] irrespective of levamisole treatment disease-free survival in the combined treatment group was highly significantly increased ($P < 0.001$) compared to the groups receiving either chemotherapy or radiotherapy alone (Fig. 1). Also the difference in survival (Fig. 2) in favor of the combined treatment group was highly significant compared to patients receiving radiotherapy alone ($P < 0.001$), and significantly increased compared to the chemotherapy group ($P < 0.01$). The difference in survival between the chemotherapy and radiotherapy groups seems to be related to the different failure pattern in these two treatment groups. The first site of recurrence was predominantly local in patients given chemotherapy ± levamisole, but metastatic in those receiving radiotherapy ± levamisole (Table 2).

This 5-year analysis implies a beneficial effect of levamisole in all treatment groups. The difference in favor of levamisole reaches significance in terms of disease-free survival ($P = 0.035$) between combined treatment groups, and is close to significance in survival (Fig. 3, $P = 0.062$). However, when an adjusted log-rank analysis[19] was applied, stratifying the patients according to treatment (radiation, chemotherapy, or combined treatment) and analyzing for an effect of levamisole, adjust-

TABLE 2. Recurrence Rate and Site of First Recurrence According to Nodal Status at 5 Years

| | Recurrent disease | | | |
| | T3N0 patients | | T3N1–2 patients | |
Treatment	Local	Distant	Local	Distant
Levamisole +				
RT	—/6	2/6	1(+5)/15	12/15
CT	1/5	—/5	10(+3)/15	3/15
RT + CT	—/6	1/6	1/13	2/13
All	1/17	3/17	12/43	17/43
Levamisole −				
RT	—/6	3/6	2/13	11/13
CT	4/8	2/8	3(+2)/12	5/12
RT + CT	−(+2)/9	4/9	1(+1)/11	4/11
All	4/23	9/23	6/36	20/36

No. of local recurrences found simultaneously with metastases at distant site is given in parentheses.
RT: radiotherapy; CT: chemotherapy.

ing for other treatment groups survival was significantly better in the levamisole than in the nonlevamisole group ($P = 0.019$). The multifactorial analysis also confirmed the positive effect of levamisole on survival. In the regression analysis, chemotherapy emerged as the most important variable, followed by radiotherapy. These two variables included in the model, levamisole still significantly improved survival (improvement $P = 0.0238$, regression coefficient −0.6240, standard error 0.2786). Node involvement further significantly affected survival, but tumor size and age of the patient did not enter the model.

The disease-free survival seems to be somewhat increased in patients treated by radiotherapy + levamisole compared to radiotherapy alone (NS) (Fig. 4). The overall prognosis in patients not receiving adjuvant chemotherapy was, however, poor. Only 2/28 (7%) node-positive patients, both treated with levamisole, were alive and asymptomatic at 5 years.

Disease-free survival in respect to levamisole treatment is similar in the chemotherapy groups (Fig. 5). Table 2 shows that the site of first recurrence is predominantly local (chest wall or lymph node) in patients treated by chemotherapy alone. Patients given levamisole developed even more frequently local recurrences (14/20, 70%) than did patients given chemotherapy without levamisole (9/20, 45%). On the other hand, fewer metastases at distant sites (bone, liver, lung or brain) as a first sign of recurrence were encountered in patients receiving chemotherapy + levamisole (3/20, 15%) than in patients treated only with adjuvant chemotherapy (7/20, 35%). The difference does not, however,
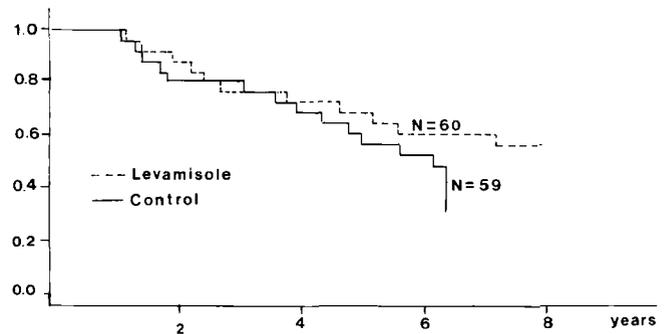
FIG. 3. Survival of patients treated with or without levamisole.

reach statistical significance. Local recurrence was often followed by death from fulminant spread of the disease.

In the current study, the best results were achieved when radiotherapy was combined with chemotherapy. A difference in favor of levamisole treatment seems to appear only after 3 years, the difference ($P = 0.035$) being statistically significant in disease-free survival (Fig. 6). Only 4/19 patients (21%) in the combined treatment + levamisole group but as many as 9/20 patients (45%) in the combined treatment–levamisole group had relapsed at 5 years (Table 2). The respective number in the radiotherapy ± levamisole group was 31/40 (78%), which shows the significance of adjuvant chemo (immuno) therapy.

Table 2 shows the patterns of recurrence at 5 years according to nodal status and treatment groups. The axillary status is generally known to be a major prognostic factor, which was confirmed also in this study. Of the patients with nonmetastatic axillary lymph nodes treated with levamisole, 4/17 (24%) relapsed as compared to 29/43 (67%) patients with initially metastatic regional lymph nodes. The respective numbers in patients treated without levamisole were 13/23 (57%) with nonmestatic and 26/36 (72%) with metastatic lymph
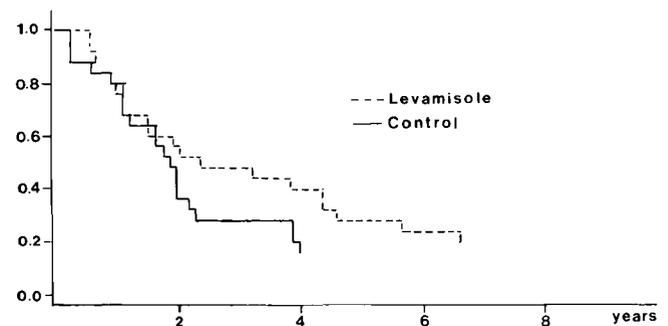
FIG. 4. Disease-free survival of patients treated with or without levamisole in addition to radiotherapy.
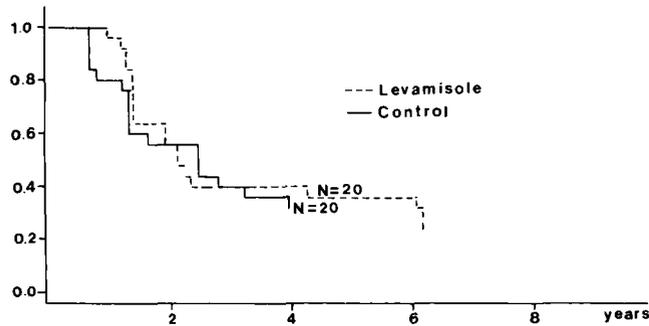
FIG. 5. Disease-free survival of patients treated with or without levamisole in addition to chemotherapy.

nodes. Levamisole thus seems to benefit particularly node-negative patients. The natural course of the disease in node-positive patients is more aggressive, and the benefit of levamisole cannot be evaluated only by these numbers. The number of distant metastases as a first sign of recurrence is lower in node-positive patients given levamisole (17/43, 40%) than in those not given levamisole (20/36, 56%). The difference does not, however, reach statistical significance.

Side Effects

As usual, radiotherapy caused some malaise, nausea, fatigue and leukopenia, which were at times difficult to distinguish from the side effects of levamisole. Most patients given radiotherapy developed fibrosis in the lung within 3 months. Severe pneumonitis was encountered slightly more frequently in the levamisole group.

In the chemotherapy group, most patients experienced nausea, vomiting, and reversible alopecia. Three patients exhibited nonlethal transient arrhythmias and one skin rash. The dosage of doxorubicin had to be reduced in four patients.
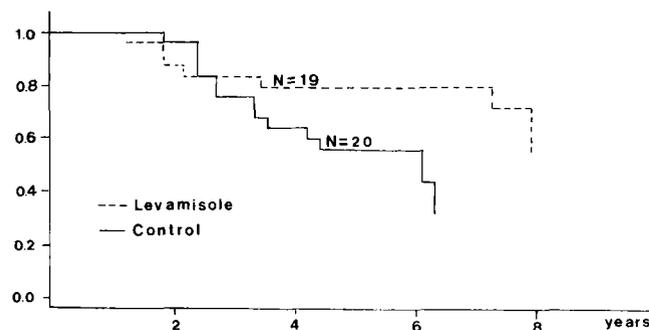

FIG. 6. Disease-free survival of patients treated with or without levamisole in addition to combined treatment ($P = 0.035$).

The frequency of side effects of levamisole (nausea, abdominal pain, fever, headache [19/60], allergic reactions [2/60], and hematologic disturbances [14/60]) was unexpectedly high. Only 9/20 patients in the radiotherapy group, 13/20 in the chemotherapy group and 11/19 in the combined treatment group continued with levamisole for 1 year. Three patients in the radiotherapy group developed agranulocytosis after 2 to 7 months of treatment. In the chemotherapy group, agranulocytosis due to levamisole was found in one patient after 11 months of treatment and granulocytopenia in three patients. In the combined treatment group, 7/19 patients developed agranulocytosis, one patient during irradiation, six patients after 5 weeks to 11 months of treatment. The development of agranulocytosis has an immunologic basis as levamisole has not been shown bone marrow toxic.[20] After withdrawal of levamisole, granulocytopoiesis was reversed in 2 weeks. In the absence of complications no treatment (antibiotics, *etc.*) was given. Agranulocytosis was not treated with corticosteroids.

The patients tolerated agranulocytosis amazingly well. In fact, these patients exhibited a better prognosis. In the radiotherapy group two of three patients are still alive, one with no signs of disease and another patient with bone metastases which appeared after 6 years from the start of the treatment. The patient in the chemotherapy group had local lymph node recurrence which was then irradiated. She has thereafter exhibited no signs of disease. In the combined treatment group, one of seven patients developing agranulocytosis has died from lung metastases, and one patient developed bone metastasis after 7 years. The remaining five patients are still alive with no signs of disease, although four of them had initial lymph node involvement.

Discussion

The inadequacy of local treatment of breast cancer is obvious in node-positive patients. Even when surgery leaves no evident tumor behind, 65% of the women with axillary nodes relapse within 5 years and postoperative radiotherapy does not increase survival of breast cancer patients.[21]

The aim of early systemic treatment is to prevent dissemination of the disease either by directly attacking the cancer cell or by modulation of different biological mechanisms. Cancer chemotherapy, although immunosuppressive, has been shown to reverse pretreatment immunosuppression by inhibiting elevated suppressor function.[22] On the other hand, a long treatment suppresses natural killer cells in breast cancer patients treated with melphalan and methotrexate, the degree of suppression correlating with higher recurrence rates.[23]

The Milan group reported better results with 6 months of chemotherapy than with 12 months.[24]

Postoperative irradiation combined with adjuvant chemotherapy has given variable results. Cooper *et al.* have reported adverse effects,[25] whereas Allen *et al.* found irradiation in addition to doxorubicin and cyclophosphamide beneficial in patients with 1 to 3 positive nodes, but deleterious in patients with four positive nodes.[26] In a study on radioimmunotherapy and chemoimmunotherapy conducted in Kuopio by Klefström *et al.*, the relapse rate increased when radiotherapy was delayed and given after completed courses of chemotherapy. Irradiation after chemotherapy produced more pronounced immunosuppression (consequently higher recurrence rates) than before chemotherapy.[27] It was concluded that heavier and longer treatments provide no further benefit, rather than contrary, disease-free survival decreasing due to immunosuppression. It is on this assumption that the beneficial effect of antianergic treatment is based on.

In the current study, the results of adjuvant chemotherapy based on doxorubicin suggest a beneficial effect in Stage III breast cancer patients. Adjuvant chemotherapy prolonged disease-free survival, but local control was insufficient without irradiation, resulting in most cases in fulminant spread of the disease. Combined radiotherapy and chemotherapy increased both disease-free and overall survival. According to the multifactorial analysis levamisole treatment did improve survival.

The results of studies on levamisole in breast cancer have been controversial. In primary breast cancer levamisole more likely benefits patients older than 50 years.[9,11] Also, the study of Rojas *et al.*[8] included mainly postmenopausal patients. In the Danish study levamisole was deleterious in premenopausal patients when the tumor was over 5 cm (Stage III), well differentiated and more than four nodes were involved (all features which are likely to predict inefficacy of radiotherapy alone). In the Auckland study, on the other hand, levamisole combined with melphalan benefited more advanced cases with four or more positive nodes. The conflicting reports of levamisole partly reflect differences in patients, modes of analysis and treatments. Immunomodulators may have different and opposing effects, depending on the dosage and duration of the treatment in addition to primary immunocompetence and age of the patient as well as tumor load.

In our study, levamisole did not inhibit local recurrence. Distant metastases were encountered less frequently in the levamisole groups. Earlier basic and clinical research has also shown levamisole to prevent mainly metastatic spread.[28] Levamisole did not prove a harmless drug. Agranulocytosis has been a common side effect in all our studies of levamisole. Oddly enough, patients developing agranulocytosis tended to exhibit a better prognosis.

If several years of disease-free survival can be achieved at the cost of a short period of chemotherapy, adjuvant therapy is worthwhile. However, to show that women with subclinical metastatic cancer have been cured, improved long-term survival with a true plateau on the relapse curve must be demonstrated. The number of patients in this study is too small to make real curves evident. However, there is a promising trend in the disease-free survival curve in the radiotherapy + chemoimmunotherapy group. On the basis of this study there seems to be cancer patients who benefit of levamisole treatment. To develop better tolerated treatment regimens and to achieve better results, further research also on this biological response modifier is warranted.

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