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Adjuvant immunotherapy with BCG in squamous cell bronchial carcinoma

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ABSTRACT Fifty-four patients with evidence of locally advanced primary squamous cell bronchial carcinoma (SCC), and three patients with adenocarcinoma (AC) had lung resection to remove all the visible tumour. After operation a randomly chosen group of 20 SCC patients received adjuvant BCG immunostimulation by scarifications (BCG-A). An additional group of 14 SCC patients, and three AC patients received initially intrapleural BCG treatment and subsequently scarifications (BCG-B). A control group of 20 SCC patients received no adjuvant treatment. Follow-up studies were done from three to 51 months. Immune reactivity was monitored in vivo with PPD skin tests in 33 treated and in 18 untreated patients. In both the BCG-treated SCC groups recurrence rates decreased statistically significant during follow-up (BCG-A: six to 51 months, $p<0.001$; BCG: 6–9 months, $p<0.01$ and nine to 24 months, $p<0.001$). However, no difference could be demonstrated between systemic and combined systemic and intrapleural treatment. The three BCG-treated AC patients all relapsed within nine months of follow-up. A pronounced increase in skin reactivity to PPD was seen six months after surgery in the BCG-treated patients (BCG-A, $p<0.001$; BCG-B, $p<0.01$), whereas the control patients remained anergic after surgery. This improved immune reactivity went in parallel with a more favourable outcome of the individual patients (BCG-A, $p<0.02$; BCG-B, $p<0.05$). It is concluded that adjuvant BCG immunotherapy used in strongly selected patients with minimal residual squamous cell bronchial carcinoma improves the prognosis. Intrapleural treatment did not improve the prognosis further. A favourable clinical outcome was mirrored by an increase in cellular immune reactivity.

The role of adjuvant immunotherapy in the treatment of cancer is still unclear. Various reports reveal negative or inconclusive data[1] but on the other hand, there are numerous studies done in experimental animal models[2] and in humans[3] providing direct evidence of therapeutic benefit. We have published the preliminary results in a controlled trial of adjuvant immunotherapy with BCG in squamous cell bronchial carcinoma.[4] In a small series of patients with minimal residual bronchial carcinoma after surgery, long-term BCG scarifications improved the prognosis at least temporarily. A favourable clinical outcome was mirrored by an increase in cellular immune reactivity.

In lung cancer the most convincing evidence of the efficacy of adjuvant immunotherapy has come from postoperative intrapleural administration of BCG in patients with localised disease.[5–7] Encouraged by these findings we continued our trial with an additional patient group, initially treated with intrapleural BCG (BCG-RIV-Strain+),[8] and subsequently long-term systemic administration. We aimed to evaluate the benefit, feasibility, and side-effects of this therapy compared with systemic BCG immunostimulation alone. A simple delayed-type skin test for the measurement of cellular immunity was applied to study the immunological effect of BCG in relation to the clinical results.

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Methods

Informed consent was obtained from patients entering the trial. The study included 54 patients with, at operation, evidence of locally advanced primary squamous cell bronchial carcinoma (SCC),

and three patients with adenocarcinoma (AC). According to the criteria of the modified UICC-post-surgical histopathological classification,[9] tumour sizes ranged from T2.N1,2.M0 to T3.N0.M0 and T3.N1,2.M0. These patients were at high risk of recurrence because of residual tumour and appeared to have a very bad prognosis.[10 11]

Lung resections, randomisation after operation, and follow-up were performed as described previously.[4] The observation time of the patients ranged from three to 51 months after operation. After patient 20 entered the control group, no more controls were added and the next patients were all treated.

BCG IMMUNOSTIMULATION

A suspension of BCG vaccine (Strain; RIV, Rijksinstituut voor de Volksgezondheid, Bilthoven, the Netherlands, lot nos 048, 052, and 060) containing 160×10^6 organisms, was administered to scarification sites of 5×5 cm on the volar side of the arms (BCG-A). Only in some cases, when further application on the arms was not feasible, application on the legs was performed once or twice. In addition, in another patient group (BCG-B), treatment was started with a single intrapleural injection of 35×10^6 viable units of the vaccine into the thoracic cavity, by thoracentesis. In these cases, the vaccine was suspended in 2 ml of normal saline, and administered from five to seven days after operation. This dose was chosen after it had been shown that higher doses gave excessive side-effects. In nine of the 14 patients isoniazid (300 mg/day) and pyridoxine (20 mg/day) were started 10 to 30 days after the injection and continued for between four and 12 weeks because of severe illness. In the BCG-A patients, BCG scarifications were started two to three weeks after surgery, repeated at weekly intervals for six weeks and then subsequently twice every three months at a week's interval for 30 months. BCG-B patients, initially treated with intrapleural BCG, received subsequent scarifications monthly for the same period. Patients who refused intrapleural treatment entered the BCG-A group.

PPD SKIN TESTS

Among other previously described immunological studies,[4] delayed-type hypersensitivity (DTH) skin tests with PPD (1 IU tuberculin purified protein derivative in 0·1 ml phosphate buffered saline with 0·005% Tween 80) were performed, two to three weeks before surgical treatment and every three months after the operation. Skin test results were recorded at 48 hours as millimetres of induration. A response with a diameter $\geqslant5$ mm was taken as positive.

STATISTICAL ANALYSIS

Differences between recurrence curves of BCG-treated patients and controls were evaluated by the generalised Wilcoxon test for comparing arbitrarily single-censored samples.[12] Student's t test on paired observations was used to compare the serial results on the same patients in the skin tests. Skin test results in relation to clinical outcome of the disease was related by the χ^2 analysis of two by two tables.

Results

The data of the two patient groups and control patients are given in table 1. No difference can be seen between the 34 BCG-treated patients with SCC and the 20 in the control group. The mean age in the BCG-A group was 57·2 years (range: 25–70, SD: 11·2), in the BCG-B group 57·9 years (range: 45–68, SD: 7·9), and in the control group 62·1 years (range 43–78, SD: 9·0). This difference is not significant. Only one patient was under 40 years. Follow-up times were three to 51 months. Patient 4 from the BCG-A group, and patient 6 from the BCG-B group have been lost to follow-up as a result of myocardial infarction, established by necropsy. In the latter patient however, one small metastasis was found in a mesenteric lymph node. The effect of BCG treatment on the duration of the recurrence-free period in patients with SCC is shown in fig 1. In the event of recurrence, BCG was stopped and the patients were treated with radiotherapy, chemotherapy, and corticosteroids.

Two control patients (10%) relapsed after about three months, 12 out of 20 (60%) relapsed within six months, three out of 20 (15%) relapsed from six to nine months, and two out of 20 (10%) were still recurrence-free after 12 months but relapsed within 15 months. In the BCG-A group, no patients relapsed after six months, eight out of 20 (40%) relapsed six to 12 months and three out of 20 (15%) after 13 to 18 months. One patient relapsed after 23 months. At the time of report, seven out of 20 (35%) patients had no evidence of disease after between six and 51 months (four out of 20=20% greater than two years). In the BCG-B group two out of 14 patients (14%) relapsed within six months, one out of 14 (7%) within nine months, and one out of 14 (7%) within 15 months. At the time of report eight out of 14 (57%) had no evidence of disease after between three and 25 months (two out of 14=14%, greater than two

Table 1 *Lung cancer patients by age, histological type, and differentiation. TNM classification and operation in the various BCG-treated patient groups and in the non-immunotherapy patient group*

BCG-A (SCC)							*Control*						
Patient	*Age (yr)*	*Histological type*	*Differentiation*	*Stage*	*Operation*		*Patient*	*Age (yr)*	*Histological type*	*Differentiation*	*Stage*	*Operation*	
1	25	SCC	moderately	T2N1M0	bilob	ect	1	58	SCC	well	T3N0M0	pn	ect
2	57	SCC	well	T2N1M0	pn	ect	2	68	SCC	well	T3N1M0	pn	ect
3	67	SCC	moderately	T3N1M0	pn	ect	3	57	SCC	moderately	T3N1M0	pn	ect
4	55	SCC	moderately	T3N0M0	pn	ect	4	73	SCC	moderately	T2N1M0	lob	ect
5	49	SCC	moderately	T2N1M0	pn	ect	5	67	SCC	well	T3N0M0	pn	ect
6	64	SCC	moderately	T3N0M0	pn	ect	6	64	SCC	moderately	T3N0M0	pn	ect
7	45	SCC	moderately	T2N1M0	pn	ect	7	52	SCC	un	T3N0M0	pn	ect
8	64	SCC	moderately	T3N0M0	pn	ect	8	69	SCC	moderately	T2N1M0	bilob	ect
9	69	SCC	un	T2N1M0	pn	ect	9	68	SCC	un	T3N1M0	pn	ect
10	40	SCC	moderately	T2N1M0	pn	ect	10	56	SCC	un	T2N1M0	pn	ect
11	53	SCC	moderately	T3N1M0	pn	ect	11	43	SCC	moderately	T2N1M0	pn	ect
12	53	SCC	moderately	T3N1M0	pn	ect	12	66	SCC	moderately	T3N0M0	pn	ect
13	60	SCC	moderately	T2N1M0	pn	ect	13	53	SCC	moderately	T2N1M0	pn	ect
14	65	SCC	moderately	T3N1M0	pn	ect	14	47	SCC	well	T3N1M0	pn	ect
15	70	SCC	moderately	T3N0M0	lob	ect	15	71	SCC	moderately	T2N1M0	pn	ect
16	69	SCC	moderately	T3N0M0	pn	ect	16	67	SCC	moderately	T3N1M0	pn	ect
17	61	SCC	moderately	T3N1M0	pn	ect	17	64	SCC	moderately	T3N0M0	pn	ect
18	64	SCC	moderately	T2N1M0	pn	ect	18	61	SCC	moderately	T2N1M0	lob	ect
19	61	SCC	moderately	T3N1M0	pn	ect	19	57	SCC	moderately	T3N0M0	pn	ect
20	53	SCC	moderately	T2N1M0	lob	ect	20	78	SCC	moderately	T3N0M0	bilob	ect
BCG-B (SCC)							*BCG-B (AC)*						
1	47	SCC	moderately	T3N1M0	pn	ect	1	53	AC	well	T1N2M0	pn	ect
2	53	SCC	moderately	T2N1M0	pn	ect	2	44	AC	un	T1N2M0	pn	ect
3	57	SCC	moderately	T3N1M0	pn	ect	3	61	AC	well	T2N1M0	pn	ect
4	59	SCC	moderately	T3N1M0	pn	ect							
5	63	SCC	moderately	T2N1M0	pn	ect							
6	65	SCC	moderately	T3N2M0	pn	ect							
7	67	SCC	un	T3N2M0	pn	ect							
8	68	SCC	moderately	T3N1M0	pn	ect							
9	50	SCC	un	T3N2M0	pn	ect							
10	60	SCC	un	T3N2M0	pn	ect							
11	45	SCC	well	T3N0M0	pn	ect							
12	68	SCC	moderately	T3N1M0	pn	ect							
13	55	SCC	moderately	T3N1M0	pn	ect							
14	54	SCC	moderately	T3N1M0	pn	ect							

years, four out of 14=28%, greater than 1·5 year, and eight out of 14=57%, greater than one year).

Figure 2 shows the "cumulative" percentage of tumour recurrence-free patients of the different BCG-treated groups and of the control patients, during the follow-up studies, and using the life-table method. Survival rates without evidence of disease were significantly better in the BCG-A group (six to 51 months, $p<0·001$), and in the BCG-B group (six to nine months, $p<0·01$; and nine to 24 months, $p<0·001$) than in the control patients, but there was no difference between BCG-A and BCG-B. The three BCG-treated patients with primary, locally advanced, adenocarcinoma of the bronchus (AC) all relapsed within six to nine months after the operation.

PPD SKIN TESTS

The results of the PPD skin tests are shown in table 2. Before operation, the number of positive tests (PPD $\geqslant$5 mm) was the same in the two BCG-treated groups as in the control group. When re-tested six months after operation, significantly increased skin reactivity was found in the BCG-treated patients (BCG-A, $p<0·001$; BCG-B, $p<0·01$). whereas the control patients remained anergic after surgery. In addition, patients who had no evidence of recurrence for longer than 12 months, or as long as the follow-up at the time of report, showed statistically significant stronger PPD reactivity compared with patients with recurrence of the disease (BCG-A, $p<0·02$; BCG-B, $p<0·05$).

SIDE-EFFECTS OF TREATMENT

BCG scarifications were tolerated well. In general, only slight side-effects were noted.[4] After intrapleural BCG injections, however, we saw strong immediate reactions with high fever (38·5°C–40·5°C), continuing for seven to 14 days. Although this fever abated in all the patients spontaneously or after starting isoniazid treatment, six patients had to be readmitted to hospital because of recurrence of fever, fatigue, nausea, weight loss, and

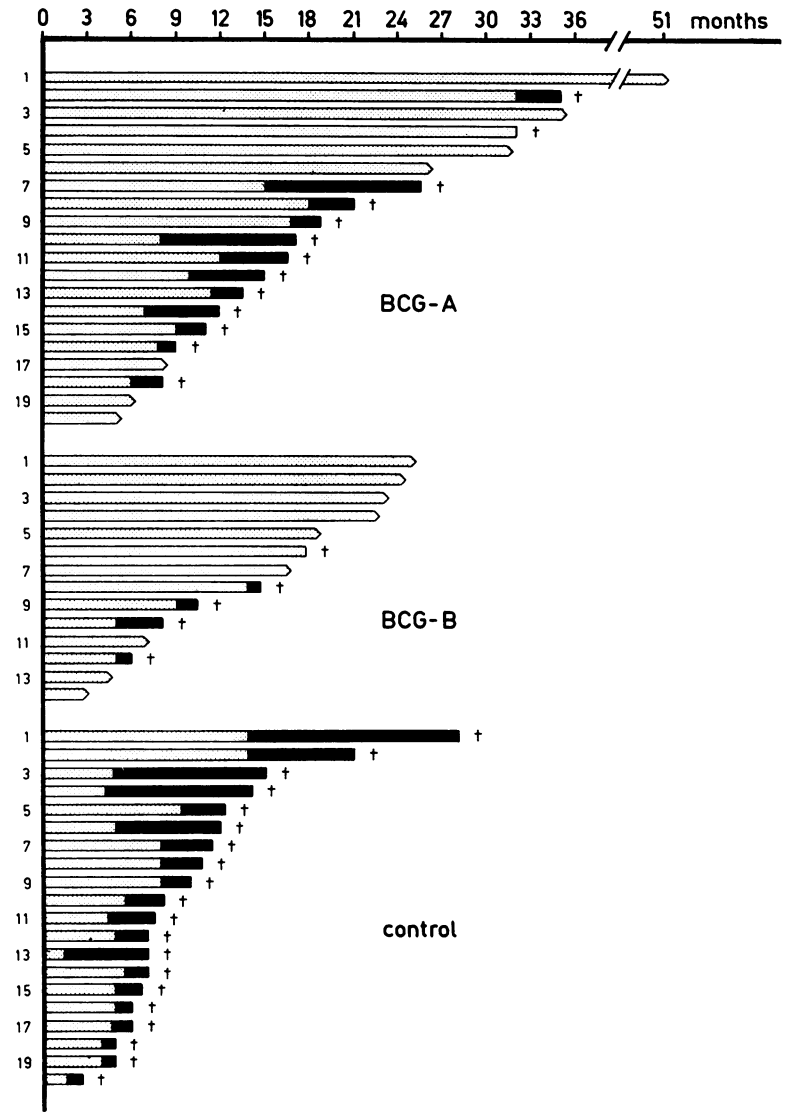

Fig 1 *Effect of BCG treatment on duration of period, free of recurrence, or metastasis, after surgical resection of squamous-cell lung cancer in patients with locally advanced disease: BCG-A=systemic treatment; BCG-B=initially intrapleural treatment and subsequently systemic. ——— =free of recurrence or metastasis ▬▬ =recurrence or metastasis: †=death.*

in some cases anaemia. Extensive investigation of these patients showed no evidence of generalised BCG infections. Liver biopsy specimens taken in three patients with abnormal liver function tests, and without tuberculostatic drug treatment at that time, showed non-specific liver cell damage. In one patient (BCG-B patient 6) there was transient proteinuria. All cases recovered after several weeks. Only BCG-B patient 12 developed a specific BCG-induced, culture-proven empyema shortly before abdominal metastases occurred. Rechallenging the patients initially treated intrapleurally with BCG scarifications did show in general more reactivity at the local site than has been described in patients who have received only systemic treatment.[4] Transient regional lymph node swelling appeared in some cases after excessive local reactions. No correlation between the extent of local reactions and clinical outcome of the disease could be demonstrated.

Discussion

The follow-up results, using BCG as a long-term adjuvant immunotherapy, confirmed earlier data.[4] A statistically significant prolongation of the re-

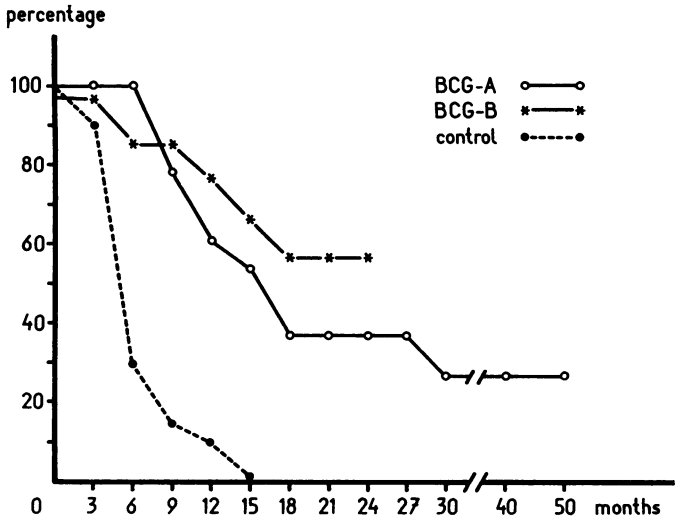

Fig 2 *Cumulative percentages of squamous-cell lung cancer patients free of recurrence or metastasis on different moments after surgery in BCG-treated groups, compared with non-immunotherapy control group. BCG-A= systemic treatment. BCG-B=initially intrapleural treatment and subsequently systemic.*

Table 2 *Skin test responses to PPD in BCG-treated SCCpatients and in non-immunotherapy control patients before and three or six months after operation. Relation with clinical outcome of the disease. Favourable clinical outcome=no evidence of disease (NED)>12 months or as long as the follow-up at the time of report but at least six months after surgery. Unfavourable clinical outcome=evidence of disease <12 months or within the follow-up at the time of report*

	Control		*BCG A*		*BCG-B*	
	Before operation	*Three months after operation*	*Before operation*	*Six months after operation*	*Before operation*	*Six months after operation*
PPD≥5 mm	6/18 (33·3)	5/18 (27·8)	4/19 (21·1)	14/17 (82·4)	4/14 (28·6)	10/12 (83·3)
PPD≥5 mm in favourable clinical outcome				11/17 (64·7)		9/12 (75·0)
PPD≥5 mm in unfavourable clinical outcome				3/17 (17·6)		1/12 (8·3)

currence-free period after surgery was shown in the BCG-treated groups compared with the non-immunotherapy group. In addition, in the individuals who were initially treated with intrapleural BCG, the same improvement of prognosis was found. However, no difference could be demonstrated between the results obtained in patients treated only systematically compared with those receiving initial intrapleural treatment.

In locally advanced disease, after removal of most of the tumour load, McKneally *et al*[5] did not find increased survival after treating patients once with intrapleural BCG. The reason for this may be that, especially in this patient group, who appeared to be more immunosuppressed than patients with localised disease,[13 14] only long-term immunostimulation may tilt the balance in favour of immunological eradication of residual tumour cells. Our findings that the restoration of immune reactivity appeared only after several months of treatment, even in patients with a better prognosis,[4] are in favour of this hypothesis. However, comparison of results of different immunotherapy trials is difficult because of major variations in technique, BCG dosage, schedule, and BCG strain used.[3 15] The RIV-BCG strains used in this trial have shown various, well-determined, immunological and anti-tumour effects, under experimental circumstances.[8]

The development of in vitro measurements of immunological changes related to immunotherapy in man is important. However, at this moment, no test has been identified that can show a correlation of such immunological changes with the clinical outcome of the disease in the individual patient. Our previously described correlation of in vitro lymphocyte reactivity with BCG therapy[4] became apparent, as was already mentioned, only after several months of treatment. In this study, the cellular immune reactivity, as measured in vivo

by simple PPD skin testing, showed a somewhat better correlation with the individual outcome of the disease. Therefore, this test may be, at present, more useful as a monitoring device.

A second problem in comparing different clinical trials is variation in the selection of patients. Especially in lung cancer trials careful selection of patient groups, taking account of histological type and differentiation, is needed. This may be because patients with various histological types of bronchial carcinoma appear to show differences in immune status[14] and various lung tumours show differences in immunological anti-tumour reactivity at the tumour site.[16] Furthermore, lung cancers show large differences in clinical behaviour.

The present study revealed that in squamous cell carcinoma, BCG treatment was successful. In the small group of patients with adenocarcinoma, however, this adjuvant therapy showed no effectiveness. Other studies have shown that the effect of immunotherapy in small cell undifferentiated lung cancer is also small.[17 18] In patients who are at high risk of recurrence because of residual tumour after surgery, certain side-effects should be accepted as an accompaniment of adjuvant therapy that improves the prognosis. In various studies, unpleasant side-effects have occurred.[19] BCG administration by scarifications, however, appeared to be safe and excessive reactions after this treatment have been rare both in this trial and in others.[20 21] Intrapleural injection of BCG however, showed strong side-effects, and consequently the dose of injected viable organisms had to be restricted. No significant improvement of results could be shown as an effect of this modified initial treatment when compared with the results in patients treated by scarifications only. Therefore a question remains as to whether this initial treatment is worth while. This question can be answered by increasing the number of patients studied in the two different therapy groups, and when better immunological monitoring of the therapy in the individual patient can be performed. Nevertheless, the results obtained in this follow-up study confirm our previous preliminary findings, and show the existence of a correlation between adjuvant BCG treatment, favourable clinical outcome, and improved immune reactivity in patients with squamous cell bronchial carcinoma.

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