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An Evaluation of the SCM Test for the Diagnosis of Cancer of the Breast

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Abstract—*The SCM test (structuredness of the cytoplasmic matrix) consists of measuring the fluorescence polarization of fluorescein which is introduced into a particular sub-group of peripheral lymphocytes. The test has a non-specific part for the general detection of cancer and a specificity procedure which is based on the use of specific cancer extracts. In this article we deal only with the latter in relation to breast cancer. Blood samples from 94 patients have been tested; six of these had mastectomy performed previously; 83 underwent consecutively a surgical procedure and histology was obtained; five were only clinically examined. In 45/49 (92%) patients, correlation between a positive specifity test and tissue malignancy was found. Out of 35 patients with non-malignant proliferative lesions (as found by histology), 25 reacted positively in the test. Two out of five patients with non-malignant, non-proliferative lesions reacted positively in the test. Five patients who were defined as normals by clinical examination reacted negatively in the test. These results indicate the potential of the SCM test for detecting breast malignancy. The clinical implications of the test for cancer diagnosis are discussed.*

INTRODUCTION

THE SCM test was first published by Cercek *et al.* in 1974 [1] and has since been the subject of numerous publications [2–5]. It is essentially a method of observing structural changes which occur when lymphocytes change from the resting phase to the active phase of the cell cycle [6]. These changes can be followed by measuring the degree of polarization of the fluorescence of suitable markers which are introduced into the cells. In the SCM test, fluorescein is used as a marker in a particular group of lymphocytes. The test is based on the fact that the degree of polarization decreases when the lymphocytes from healthy donors or of patients with non-malignant disorders are incubated with phytohemagglutinin (PHA) prior to the measurements, while for cancer patients the polarization remains unchanged. Conversely, after incubation of the lymphocytes with encephalitogenic factor (EF) the degree of polarization decreases for cancer patients while it remains unchanged for healthy individuals and those with non-malignant diseases. Thus a quantitative expression for the presence of a malignant tumor can be given by the RRscm factor [1], which is defined by the ratio of the degree of polarization after incubation with EF and that for incubation with PHA.

Obviously, RRscm is less than unity for cancer patients and greater than unity for all other subjects. In a previous publication we have shown the validity of this test for the identification of cancer patients [5].

The SCM test, however, can also determine the specific nature of the malignant tumor, using tumor tissues [7, 8] or tumor extracts. Thus, when the lymphocytes are incubated with extracts from tumor tissues or cell lines of the same organ type of tumor from which the donor of the lymphocytes suffers, the degree of polarization decreases, while it remains unchanged for patients with different types of malignancy.

In the following, we report the results of the specific SCM test for a group of breast cancer patients, for subjects with benign breast lesions and normals, by application of breast tumor extracts to the specific group of lymphocytes.

MATERIALS AND METHODS

Ninety-four women (ages 17–84) who presented suspicious breast lesions at the Oncology Department, Ichilov Hospital, Tel-Aviv, were examined. Of these, six were suspected to have breast metast-

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ases. All were physically examined and in most of them excisional biopsies were performed with subsequent mastectomy for those found to have a malignant lesion. Final diagnosis was based on histopathological examination of frozen sections of the excised tissue and subsequent examination of paraffin-embedded samples.

From 30 ml of heparinized blood the specific subgroup of lymphocytes was separated on a Ficoll–Triosil gradient solution as outlined by Cercek and Cercek [2] and modified in our laboratory [5]. In cases of intended surgical intervention blood samples were taken before surgery was performed.

Extracts from three different tumor tissues of different histological types and from two different cell lines, all of breast cancer, were prepared for the specificity SCM test.

The preparation of the calibrated extracts was as follows: pieces of tumor tissue (1–3 g) or pellets of tumor cell lines were thoroughly rinsed several times in PBS, suspended in 5 volumes (v/v) of 3 M KCl solution (pH = 7.4) and homogenized. The homogenate was stirred overnight at 4°C, then centrifuged at 20,000 ***g*** for 30 min at 4°C. The supernatant was collected and the KCl was removed by gel filtration through a Sephadex G-25 column; the eluted solution was precipitated with 2 M (final concentration) ammonium sulfate for 1 h at 4°C and centrifuged for 30 min at 20,000 ***g*** and 4°C. The precipitate was collected and suspended in double distilled water. To remove the ammonium sulfate, the suspension was gel-filtrated through a Sephadex G-25 column and the eluted solution was lyophilized. For practical reasons the lyophilized powder was diluted in PBS at a concentration of 10 mg/ml and stored at −90°C. The optimal concentrations of each of the stimulating extract solutions were found empirically (manuscript in preparation). The results did not show any of the extracts to be clearly superior to the others.

Aliquots of 0.2 ml of separated lymphocytes suspended in PBS at a concentration of 5×10^6 cells/ml were incubated with the optimal concentration of one of the breast tumor extracts for 15–20 min at 37°C. The stimulated cells were introduced into 3.5 ml of a FDA solution (which leads to the development of fluorescein as a result of fluorochromasia). Shortly afterwards the fluorescence polarization of the fluorescein was measured in a Perkin–Elmer (MPF-44) spectrofluorimeter [5].

RESULTS

The results of the specificity tests are expressed in polarization ratio (PR) units. The PR is defined as:

$$PR = \frac{PO - PT}{PO}$$

where PO and PT are the polarization values before and after stimulation with the indicated type of tumor extract, respectively. Random changes in the degree of polarization, however, occur due to various effects which influence the measurements. A PR > 0.1 was found empirically as indicative for the presence of the malignancy.

The PR values for all patients are given together with the clinicopathological diagnosis in Tables 1 and 2. Table 1 shows the results for donors with breast malignancies. Table 2a shows the results for donors with proliferative lesions of the breast. Table 2b shows the results for donors with non-proliferative disorders. Table 2c shows the results for clinically normal donors. Table 3 summarizes the results by a comparison between the SCM test and the clinicopathological findings.

DISCUSSION

Ninety-four women attending the breast clinic, most of them because of suspicious breast lesions and some for follow-up, were tested by the SCM test. Out of those, 83 have been tested by the SCM test prior to any surgical intervention, and were later found by pathology to suffer from malignant or benign tumors. In our previous publication [8] we reported on the specificity SCM tests which were performed for the recognition of active post-operative malignancy on follow-up patients with diagnosed carcinoma. Correlation was sought between a positive SCM test and clinical symptoms of tumor recurrence after surgery.

In the present study the blood samples were tested using calibrated breast tumor extracts for stimulation of the lymphocytes instead of incubation with tumor tissues. The use of calibrated extracts renders the test procedure more standardized and increases reproducibility. A good correlation between positive SCM test and clinicopathological diagnosis of breast cancer was found, particularly in stages I and II of the disease. Two of the cancer patients who reacted negatively in the test were metastatic stage III or IV cases. In widely disseminated metastasis the specifity test may fail or the lymphocytes may lose the ability to react with tumor antigens altogether. Similar effects have been reported for other cancer tests [9, 10]. The other two cancer patients who reacted negatively in the test had a few foci of avascular (non-infiltrating) lobular neoplasia, which may escape the interaction with the peripheral immune system.

As can be seen from the results, patients with malignant tumors could be differentiated from healthy donors. (All five clinically normals reacted negatively.) In the group of 40 patients with benign breast diseases a positive reaction with the specificity SCM test was found with rather high frequency: 71% of the 35 women with proliferative (hyperplasia) and 40% of the five non-proliferative dis-

Table 1. *Breast cancer disease*

Patient No.	Age	PR units	Pathology	Stage
1	54	0.19	Infiltrating duct carcinoma IDC	IV
2	47	0.29	Infiltrating lobular carcinoma	II
3	50	0	A few foci of lobular neoplasia	I
4	59	0.25	IDC	II
5	78	0.22	IDC	I
6	69	0.16	IDC	II
7	38	0.21	IDC	I
8	44	0.12	Invasive small cell lobular carcinoma	II
9	69	0.31	IDC	I
10	58	−0.01	IDC	IV
11	48	0.21	Comedo carcinoma	II
12	40	0.20	Intraductal papilloma	I
13	51	0.11	IDC	III
14	36	0.24	IDC	II
15	73	0.18	Infiltrating lobular carcinoma	III
16	64	0.13	IDC	III
17	78	0.16	IDC	IV
18	62	0.19	Carcinoma cells	IV
19	74	0.22	IDC	IV
20	57	0.18	IDC	II
21	36	0.29	IDC	IV
22	44	0.17	IDC—Comedo type	III
23	40	0.18	IDC	II
24	58	0.23	Intraductal carcinoma	IV
25	70	0.14	IDC	II
26	84	0.23	Intraductal carcinoma (nipple)	I
27	74	0.15	Lobular duct carcinoma *in situ*	I
28	31	0.16	Infiltrating lobular carcinoma, Comedo carcinoma	III
29	73	00.25	Papillary and apocrine carcinoma	
30	56	0.19	Metastatic carcinoma	IV
31	80	0.19	IDC	II
32	68	0.13	IDC	I
33	60	0.15	IDC	I
34	52	−0.01	IDC	III
35	50	0.01	Lobular neoplasia (carcinoma *in situ*)	I
36	78	0.22	Infiltrating carcinoma, foci of lobular carcinoma	II
37	73	0.13	IDC	III
38	65	0.21	IDC, Comedo carcinoma	I
39	52	0.18	IDC	II
40	49	0.11	IDC	
41	66	0.18	IDC	I
42	67	0.30	Intraductal carcinoma	I
43	74	0.19	Intraductal carcinoma, Comedo type	II
44	50	0.18	IDC	I
45	60	0.10	Infiltrating mucoid carcinoma	II
46		0.18	Adenocarcinoma	
47	80	0.18	Duct and papillary carcinoma	II
48	60	0.19	IDC	
49	49	0.20	Metastatic large cell carcinoma	IV

orders reacted positively in the test. It is now well recognized that benign breast disorders comprise a wide spectrum of histologic lesions with progressive changes towards carcinoma. Numerous publications deal with the higher risk of women with such benign diseases, in particular those with epithetial hyperplasia and atypia, to develop breast cancer [11–13].

Thompson *et al.* [14] and Sonner *et al.* [15] showed that patients with benign breast diseases showed

Table 2a. Benign proliferative breast disease

Patient No.	Age	PR units	Pathology
1	62	0.03	Stromal fibrosis
2	34	0.19	Fibrocystic disease and stromal fibrosis
3	32	0.16	Fibrocystic disease and stromal fibrosis
4	70	0.06	Fibroadenoma
5	24	0.04	Fibroadenoma
6	39	0.19	Fibroadenoma
7	50	−0.02	Fibrosis (embedded in fatty tissue)
8	44	−0.02	Extensive fibrosis
9	26	0.19	Stromal fibrosis
10	42	0	Microcystic blunt duct adenosis
11	85	0.19	Fibrosis, microcystic blunt duct adenosis
12	77	0	Sclerosing adenosis, fibrosis
13	50	0.23	Papillomatosis, stromal fibrosis intraductal epitheliosis
14	20	0.22	Fibroadenoma
15	64	0.35	Stromal fibrosis
16	53	−0.05	Stromal fibrosis, duct epitheliosis
17	54	0.17	Epithelial proliferation, fibrosis
18	48	0.22	Intraductal epitheliosis
19	62	0.19	Stromal fibrosis
20	17	0.20	Fibrocystic mastopathy, stromal fibrosis
21	34	0.21	Tubular adenoma
22	50	0.29	Severe epitheliosis, duct papillomatosis, stromal fibrosis
23	24	0.22	Stromal fibrosis, fibroadenoma
24	26	0.25	Stromal fibrosis, intraductal papillomatosis
25	26	0.15	Fibroadenoma
26	42	0.10	Fibrocystic disease
27	65	0.19	Sclerosing adenosis, fibrosis
28	44	0.19	Stromal fibrosis, papillomatosis
29	51	0.12	Fibrocystic disease, intraductal papilloma
30	19	0.02	Fibroadenoma
31	55	0.22	Fibrocystic adenosis
32	69	0.19	Fibrocystic intraductal papillomatosis
33	36	0.30	Stromal fibrosis
34	47	0.13	Fibrocystic disease
35	20	0.02	Fibroadenoma

Table 2b. Non-proliferative disease

Patient No.	Age	PR units	Pathology
1	38	0.21	Cyst
2	32	−0.15	Fatty tissue in lymph node
3	71	−0.05	Cyst
4	80	−0.07	Fibrotic tissue
5	50	0.19	Cystic disease

Table 2c. Clinically normal

Patient No.	Age	PR units	Clinical examination
1	60	−0.02	Mass in breast—normal
2	48	−0.04	Mass in breast—normal
3	19	−0.03	Mass in breast—normal
4	59	0.03	Mass in breast—normal
5	20	−0.02	Mass in breast—normal

Table 3. Correlation between specifity SCM test and clinicopathologic diagnosis

Clinicopathology	SCM test	
	Positive	Negative
Clinically normal	0/5	5/5
Benign non-proliferative diseases	2/5	3/5
Benign proliferative diseases	25/35	10/35
Breast malignancy	45/49	4/49

leukocyte adherence inhibition (LAI) activity to extracts of breast cancer and suggested that some of these lesions acquire cell surface tumor specific antigens before the appearance of morphological signs of *in situ* cancer.

Avis *et al.* [16] reported that lymphocytes from patients with fibroadenoma alone or combined with fibrocystic disease of the breast were specifically cytotoxic to breast adenocarcinoma cells, which implies extensive antigenic cross-reactivity between benign and malignant hyperplastic disease.

Naturally, the significance of the results presented here depends on the eventual clinical application of the test. We believe the sample to be too limited to permit a statistical evaluation. Still, it may be in place to express the predictive value of even these limited results in more commonly accepted quantitative terms. From Table 3 it can be readily seen that the predictive value of a positive result (PVP = percentage of total positives which are correct) is 62.5%. This is the percentage of those found to have cancer by the histopathological examination out of those found positive by the test. If, however, the test aspires also to the diagnosis of benign proliferative breast disease this value rises to 97.2%. For the diagnosis of breast tumors at large the PVP value is 100%. A similar calculation can of course be included for the PVN value, i.e. the percentage of negative values which are correct. This turns out to be a rather low value for the general breast disease. In the present case, this value is, however, meaningless. The 'normal' patients included in Table 3 were not part of the population who turned to the clinic with symptoms or complaints but were individuals chosen deliberately out of the general population. They were therefore not part of the target population of the test. Instead of five of these individuals one could have tested many more with equal results which would have enlarged the PVN value to any desired extent. The most important point in this context is perhaps the following. From those patients who have been sent home without biopsy or operation it was very difficult to obtain blood for the execution of the test. It is reasonable to assume that these patients would have yielded mostly normal values. Their inclusion in the evaluation of the test would, of course, have caused a greatly increased PVN value.

Finally, we have seen that the confinement of the results to those cases which have been identified as malignant by histopathology reduces the PVP to 62.5%. This outcome can be interpreted in several ways. We believe that the results shall be considered in view of what is known about the level of risk of these patients to develop cancer. Seen this way, we think the test is highly sensitive for the identification of individuals of high risk to develop malignant disease.

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