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Tumour Specificity of the SCM Test for Cancer Diagnosis

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Abstract—*Phytohaemagglutinin (PHA), a well-known mitogen, and encephalitogenic factor (EF) are recognized by lymphocytes of patients with different malignant diseases as non-specific antigens. Utilizing these two antigens, the SCM (structuredness of the cytoplasmatic matrix) test offers a means of discrimination between malignant and non-malignant diseases. The SCM test can also be used as a specifity test since lymphocytes from donors with a given malignant disease react exclusively with the tumour-associated antigen (TAA) of that disease. Results from 73 donors (15 healthy patients, 38 patients with different types of malignant disorders and 20 patients with autoimmune diseases) indicate the predictive value of the test. First, the non-specific test was applied in order to establish whether the patients suffered from an active malignant disease. The lymphocytes of those patients which were found to suffer from an active malignant disorder were then exposed to different types of tumour tissues. Twenty-five out of the 38 patients with malignant disorders were found by the SCM test to have an active disease. The lymphocytes of 24 out of these 25 patients showed a positive reaction when exposed to tumour tissue of the same type of cancer of which they were found to suffer by other clinical tests, and displayed no reaction with any other tumour tissues for which they were tested. One patient, with an inconclusive value of the SCM test, showed no reaction with any type of tumour to which he was exposed. The remaining 13 patients, who were diagnosed by the test as non-cancerous, did not show any clinical evidence of malignancy at the time of the test, after their tumours had been excised. Eighteen out of 20 patients with autoimmune diseases showed negative results when tested by the general test and by the various specifity tests.*

INTRODUCTION

WE RECENTLY reported a series of tests [1] which supports the validity of the SCM method for the diagnosis of cancer. This method has been described in a series of articles [2–7]. It consists of measurements of the fluorescence polarization of fluorescein which is introduced into a particular group of lymphocytes after their separation from the blood of the patient. The density of the gradient used to separate the lymphocytes, the conditions of temperature, pH and osmolarity, the excitation wavelength and the wavelength of the fluorescence at which the polarization is measured are all critically important and have been stated by the Cerceks [2]. The test is based on the observation that for healthy persons (and persons suffering from non-malignant diseases) stimulation of the lymphocytes by PHA causes a decrease in the degree of polarization while stimulation by EF does not cause such change, whereas the opposite holds true for lymphocytes from cancer patients (i.e. decrease of the degree of polarization after EF stimulation and no change for stimulation with PHA). Hence, the presence or absence of a malignant disorder can be expressed by the magnitude RR_{SCM}, where

$$RR_{SCM} = \frac{\text{polarization after incubation with EF}}{\text{polarization after incubation with PHA}} \cdot (1)$$

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From the above definition it follows that for healthy persons $RR_{SCM} > 1$, while for cancer patients $RR_{SCM} < 1$.

A different version of the test [8, 9] is based on the fact that when the blood is centrifuged in a Ficoll-Triosyl column in many cases two closely separated layers of lymphocytes appear on the plasma-gradient interface. The upper layer exhibits the properties just described while for patients with malignant diseases stimulation by PHA of the lymphocytes of the lower layer causes a decrease in the degree of polarization.

According to Pritchard *et al.* [8], we define

$$I_{SCM}=\left(\frac{P_0(2)-P_{PHA}(2)}{P_0(2)} - \frac{P_0(1)-P_{PHA}(1)}{P_0(1)}\right)\times 100, \quad (2)$$

where P_0 and P_{PHA} are the polarization value of the control and PHA-stimulated lymphocytes, respectively; argument (1) relates to the first and argument (2) to the second band.

It is seen that for healthy donors I_{SCM} will be negative [since $P_{PHA}(2) \sim P_0(2)$ and $P_{PHA}(1) < P_0(1)$], while for cancer patients I_{SCM} will be positive [since $P_{PHA}(2) < P_0(2)$ and $P_{PHA}(1) \sim P_0(1)$].

Although the importance of this assay for the early recognition of malignant disorders as well as for the follow-up of patients after removal of the tumor or after therapy is recognized, its ultimate value lies in the specifity of the test. It has been demonstrated [10] that incubation of the particular group of lymphocytes with a piece of tumour tissue or with the extract from such tissue taken from a patient who suffers from the same kind of tumor as does the donor of the lymphocytes causes a decrease in the degree of polarization, while incubation with a different kind of tumor leaves the degree of polarization unchanged. Though convincing in itself, the number of specifity tests published by the Cerceks has been relatively limited. In order to check the validity of the specifity test, and perhaps more so in order to define its limitations as to the stage of the disease, sharpness of discrimination and technical details, we started a programme of such tests. In the following we describe a series of such tests on lymphocytes from patients of suspected or diagnosed carcinoma of breast, colon, uterus, rectum, lung, ovary, stomach and melanoma.

MATERIALS AND METHODS

The separation of lymphocytes was carried out according to the procedure outlined in reference [2] with the additional precautions stated in our recent paper [1]. For the stimulation of the lymphocytes histologically defined biopsies of tumours were used either fresh or after storage in liquid nitrogen [10, 11]. The frozen pieces of tissue were slowly thawed at room temperature and thoroughly washed with PBS. Then pieces of approximately $2 \times 2 \times 2$ mm in size were cut for stimulation. Of the lymphocyte suspension 0.3 ml (5×10^6 cells/ml) was incubated with the piece of tumour tissue for 15–20 min at 37°C. After stimulation, 0.2 ml of the lymphocyte suspension was introduced into 3 ml of the FDA solution for the SCM measurement. The specificity test for cancer patients was carried out with tissues of the following tumours: carcinoma of the breast, lung, uterus, colon, stomach, rectum and melanoma. In many cases biopsies of the same kind of tumour from different donors were used for testing of one given sample of lymphocytes. In a number of cases, in the course of the incubation procedure (in particular with tissues from the intestinal system) a fraction of the cells detached themselves from the tissue and were involuntarily pipetted together with the lymphocytes into the FDA solution. The fluorochromasia in these cells sometimes caused an appreciable fluorescence which constituted a greatly enhanced background. In several cases the tissues were not sterile. The fluorescence background caused by the bacteria was stronger than that of the lymphocytes and thus prevented a meaningful measurement.

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

$$PR = \frac{P_0 - P_T}{P_0}, \quad (3)$$

where P_0 and P_T are the polarization values before and after stimulation with the indicated type of tumour tissue, respectively. In view of the limited accuracy of the test, we arbitrarily define a $PR > 0.1$ as indicating that this patient suffers from the specific malignancy (after the RR_{SCM} value has been found to be < 1.0) while *PR* values > 0.1 (or even negative) indicate that the patient does not suffer from the specified type of cancer.

The clinical observations of the patients were made at Hadassah Hospital, Tel Aviv, Israel. The blood samples were taken from a random population of the Oncological Outpatient Clinic, who were diagnosed before or after surgery. A few blood samples were also provided by the Department of Plastic Surgery, Sheba Medical Center, Tel-Hashomer, Israel. Only after performance of the SCM and the specificity test were the results compared with the clinical records.

RESULTS

The results are shown in Table 1. The first 15 entries in the table refer to healthy donors and serve as control values for the SCM test. The following 38 entries refer to patients with different types of malignancy: 22 out of the 38 are patients with a history of carcinoma of the breast; eight patients with carcinoma of the colon; five patients with melanoma; two with carcinoma of the uterus; and one with carcinoma of the rectum. There were 20 patients with autoimmune diseases; of these, 15 were with rheumatoid arthritis; one with ankylosis spondilosis; one with Behcet; two with systemic lupus erythematosus; and one with scleroderma.

It is seen that for all healthy donors $RR_{SCM} > 1$ and I_{SCM} is negative. Except for one case we also find a correlation for all patients between these values and the clinical picture. In one case only, No. 19, the RR_{SCM} value is 0.92 while I_{SCM} is -1.2.

Twenty-four out of 25 patients who according to their RR_{SCM} and I_{SCM} values were diseased also showed a decrease in the respective P values when their lymphocytes were stimulated with pieces of tissue of the same kind of tumour from which these patients were suffering. With other types of tumour tissue no decrease in the degree of polarization was observed. These differences are clearly demonstrated by the respective PR values in columns 6-12. The lymphocytes of patient No. 19 (with inconclusive RR_{SCM} and I_{SCM} values) were not stimulated by any of the tissue samples.

The RR_{SCM} and I_{SCM} values of patients Nos 27, 28 and 29 are those of a healthy donor, though the patients had widely disseminated metastasis. Also, the specifity test failed for those patients in that the lymphocytes reacted positively with tissues of different types of tumour. Similar phenomena have been observed with the leukocyte adherence inhibition test when leukocytes from patients of advanced metastasis reacted with extracts from different tumours [12, 13].

Three of the patients, Nos 21, 30 and 37, who were diagnosed by the test as diseased but at the time of the test did not show any clinical evidence of metastasis, later presented metastasis. Patient No. 21, whom we tested in February 1982, showed single metastases in March 1983; patient No. 30, who was tested in May 1982 showed a local recidive in November 1982; and patient No. 37, who was tested in June 1982, showed liver metastasis in January 1983. Also, patient No. 19, who was tested in December 1981, showed metastasis in March 1983. Patients Nos 60 and 64, with autoimmune diseases, showed a positive SCM test. Patient No. 60 did not react with any of the applied specifity tests, while patient No. 64 reacted with cancer of the ovary.

DISCUSSION

The results show a good correlation between the RR_{SCM}, I_{SCM} tests and clinicopathological diagnosis. All 15 healthy donors appear as such according to the SCM test as well as according to the I_{SCM} test. Eighteen out of 20 patients with autoimmune disease showed negative results with the general SCM test as well as with all the applied specific tests. Two patients showed positive reaction with the general SCM test; one of them did not react with any of the applied specifity tests, while the other showed cancer of the ovary, although we have at the moment no clinical evidence of a malignant diesease of these patients.

Of the 38 patients, 11 patients who have been free from clinical symptoms for at least 1 yr after surgery had RR_{SCM} and I_{SCM} values of healthy donors. Of the 25 patients who reacted positively to tissue samples, 12 reacted to tissues of breast cancer, six reacted to samples of colon cancer, five reacted to melanoma, one reacted to cancer of the uterus and one to cancer of the rectum. Two patients were not tested for technical reasons. One patient (No. 19), with conflicting RR_{SCM} and I_{SCM} values, showed no reaction with any of the tissues. All the breast cancer patients who responded positively were suffering from active disease, or were clinically defined as stage II to IV. These data emphasize the capability of the SCM test not only in identifying cancer patients, but also in identifying the type of tumour. Also, the fact that high-risk patients who were clinically free from disease but responded positively to the test later developed metastases may serve as an indication for the capability of the test in detecting the disease at a much earlier stage than commonly used clinical tests can do.

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Table 1. *Tumor type specific test*

Patient No.	Clinical findings	1 P_o	2 P_{EF}	3 P_{PHA}	4 RR_{SCM}	5 I_{SCM}	6 PR Ca of ovary	7 PR Ca of lung	8 PR Ca of breast	9 PR Ca of rectum	10 PR Ca of stomach	11 PR Ca of colon	12 PR Melanoma	13 PR Ca of uterus	Clinical status
1	Healthy		0.188	0.152	1.19	-15.4									
2	Healthy		0.194	0.160	1.21	-20.0									
3	Healthy		0.194	0.167	1.16	-17.2									
4	Healthy		0.192	0.156	1.23	-20.0									
5	Healthy		0.190	0.149	1.28	-37.0									
6	Healthy	0.191	0.189	0.146	1.30	-33.0					0.03	-0.05	0.02		
7	Healthy	0.182	0.179	0.144	1.24	-17.1			-0.10			-0.09	0.04		
8	Healthy	0.179	0.211	0.162	1.30	-5.6	-0.03		-0.08			-0.10			
9	Healthy	0.196	0.204	0.154	1.32	-22.5	0.05						0.00	0.02	
10	Healthy	0.179	0.182	0.142	1.28	-30.0			-0.02						
11	Healthy	0.175	0.173	0.159	1.09	-9.0			-0.04			-0.10			
12	Healthy	0.187	0.180	0.165	1.09	-8.0			-0.03			0.02			
13	Healthy	0.181	0.192	0.164	1.17	-17.0			-0.04			-0.02			
14	Healthy	0.180	0.181	0.161	1.12	-16.0			-0.08			-0.02			
15	Healthy	0.204	0.190	0.132	1.44	-			0.07			0.02			
16	Ca of breast stage IV	0.211	0.161	0.227	0.71				+0.237		+0.04	-0.08			evidence of metastasis
17	Ca of breast stage III	0.182	0.159	0.197	0.76				+0.291		+0.00	-0.121			no evidence of metastasis
18	Ca of breast stage II	0.206	0.142	0.185	0.77				+0.223		+0.09	+0.09			no evidence of metastasis
19	Ca of breast stage II	0.186	0.165	0.179	0.92	-1.2			-0.108			-0.134	-0.03		no evidence of metastasis
20	Ca of breast stage II	0.194	0.194	0.163	0.18	-14.0									no evidence of metastasis
21	Ca of breast stage II	0.191	0.151	0.189	0.80	+22.0		+0.07	+0.204		+0.005	+0.03			no evidence of metastasis
22	Ca of breast stage IV	0.187	0.152	0.186	0.82	+24.5			+0.112		-0.08	+0.03	-0.04		evidence of metastasis
23	Ca of breast stage IV	0.189	0.152	0.175	0.85	+17.0			+2.201		-0.02		+0.03		evidence of metastasis
24	Ca of breast stage II	0.193	0.184	0.165	1.15	-11.5									no evidence of metastasis
25	Ca of breast stage IV	0.191	0.146	0.195	0.75	+13.0			+0.236		+0.04	-0.04	+0.02	+0.04	evidence of metastasis
26	Ca of breast stage IV	0.189	0.167	0.190	0.88	+14.5			+0.116		+0.03	-0.07	+0.03	-0.02	evidence of metastasis
27	Ca of breast stage IV	0.186	0.177	0.157	1.13	-13.4		+0.145	+0.167			+0.151			evidence of metastasis
28	Ca of breast stage IV	0.182	0.179	0.162	1.10	-			+0.176			+0.291			evidence of metastasis
29	Ca of breast stage IV	0.206	0.195	0.158	1.28	-27.0			+0.238			+0.300			evidence of metastasis
30	Ca of breast stage II	0.192	0.148	0.203	0.73	+29.0			+0.250				+0.03		no evidence of metastasis
31	Ca of breast stage II	0.183	0.179	0.157	1.14	-16.0									no evidence of metastasis
32	Ca of breast stage II	0.205	0.164	0.190	0.86	+22.0			N.D.*						no evidence of metastasis
33	Ca of breast stage I	0.185	0.177	0.155	1.17	-22.0									no evidence of metastasis
34	Ca of breast stage II	0.184	0.192	0.159	1.20	-14.0									no evidence of metastasis
35	Ca of breast stage II	0.193	0.202	0.165	1.20	-14.0									no evidence of metastasis
36	Ca of breast stage II	0.200	0.204	0.174	1.17	-20.0									no evidence of metastasis
37	Ca of breast stage II	0.202	0.200	0.205	0.95	+10.5			N.D.						no evidence of metastasis
38	Ca of colon Duke's D	0.200	0.140	0.177	0.80						+0.08	+0.275			
39	Ca of colon Duke's D	0.232	0.171	0.222	0.77				+0.01		+0.01	+0.625			
40	Ca of colon Duke's D	0.213	0.153	0.215	0.71			+0.01	-0.02		+0.01	+0.380	+0.03		
41	Ca of colon Duke's D	0.203	0.158	0.197	0.80	+8.0						+0.365	+0.03		
42	Ca of colon Duke's B	0.173	0.173	0.155	1.12	-23.0									no evidence of metastasis
43	Ca of colon Duke's B	0.187	0.190	0.166	1.14	-16.0									no evidence of metastasis
44	Ca of colon Duke's D	0.194	0.153	0.189	0.81	+5.0				+0.067	+0.072	+0.474			
45	Ca of colon Duke's D	0.194	0.172	0.185	0.93						0.00	+0.278			

Table 1. *Cont'd*

46	Melanoma	0.198	0.173	0.198	0.87	+10.0		-0.04	+0.04		+0.03		+0.146		no evidence of metastasis 4 days after surgery
47	Melanoma	0.191	0.167	0.194	0.88						-0.06	+0.136		no evidence of metastasis	
48	Melanoma	0.189	0.166	0.183	0.81			-0.159	+0.03		-0.02		+0.265		evidence of metastasis
49	Melanoma	0.217	0.173	0.205	0.84	+26.0					+0.10		+0.379		evidence of metastasis
50	Melanoma	0.206	0.141	0.176	0.80	+9.0			+0.02				+0.360	+0.01	evidence of metastasis
51	Ca of uterus	0.214	0.192	0.140	1.37	-44.0									no evidence of metastasis
52	Ca of uterus	0.191	0.168	0.184	0.92	+22.0				+0.04		+0.04		+0.104	no evidence of metastasis
53	Ca of rectum Duke's B[2]	0.179	0.158	0.182	0.87	+16.0				+0.198					no evidence of metastasis
54	Rheumatoid arthritis	0.185	0.171	0.130	1.32	-28.0									
55	Rheumatoid arthritis	0.171	0.174	0.140	1.25	-20.0									
56	Rheumatoid arthritis	0.180	0.191	0.163	1.17	-						-0.05			
57	Rheumatoid arthritis	0.194	0.193	0.161	1.20	-10.0						0.05			
58	Rheumatoid arthritis	0.201	0.188	0.166	1.13	-	0.04						0.06		
59	Rheumatoid arthritis	0.182	0.178	0.131	1.36	-29.7	0.04				0.01			0.01	
60	Rheumatoid arthritis	0.164	0.165	0.136	1.21		-0.10					-0.10			
61	Rheumatoid arthritis	0.173	0.181	0.153	1.18	-7.0	0.00				-0.05		0.03		
62	Rheumatoid arthritis	0.196	0.156	0.180	0.87	+6.0	0.08		-0.04						
63	Rheumatoid arthritis	0.187	0.187	0.165	1.13	-12.0	0.02		-0.10		-0.03				
64	Rheumatoid arthritis	0.180	0.203	0.165	1.23	-8.0	-0.17				-0.10	-0.07			
65	Rheumatoid arthritis	0.200	0.197	0.164	1.18	-25.0			0.04				0.06	0.07	
66	Rheumatoid arthritis	0.198	0.164	0.200	0.82	+24.6	+0.21		0.05				-0.08	0.08	
67	Rheumatoid arthritis	0.215	0.200	0.171	1.17	-15.0	0.08		0.09				0.08		
68	Rheumatoid arthritis	0.184	0.181	0.144	1.26	-24.0	0.06		-0.03				0.03		
69	Ankylosis spondilosis	0.159	0.169	0.146	1.16										
70	Behcet	0.197	0.213	0.157	1.35	-10.0					0.01	0.02			
71	S.L.E.†	0.183	0.177	0.146	1.21	-19.3	0.04					0.03	0.07	0.04	
72	S.L.E.	0.192	0.184	0.141	1.3	-15.0		0.04						0.08	
73	Scleroderma	0.188	0.194	0.158	1.23	-16.0	0.02	0.07						0.07	

Column 1 gives the control value, i.e. the degree of polarization without any stimulation of the lymphocytes (P_0).
Column 2 gives the polarization values for EF-stimulated lymphocytes (P_{EF}).
Column 3 gives the polarization values for PHA-stimulated lymphocytes (P_{PHA}).
Column 4 gives the RR_{SCM} value, i.e. P_{EF}/P_{PHA}.
Column 5 gives the I_{SCM} values as defined by Equation (2).
Columns 6-12 give polarization ratios (PR) for stimulation of the lymphocytes by the various cancer tissues as defined by Equation (3).
*N.D., not done.
†S.L.E., systemic lupus erythematosus.

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