

RETICULOENDOTHELIAL ACTIVITY IN CERVICAL CANCER CASES

I. Effect of Radical Surgery and Lymphadenectomy on Reticuloendothelial Activity

YUNG-SHENG CHENG and CHIEN-TIEN HSU

Department of Obstetrics and Gynecology, Taipei Medical College

The significance of the RES activity in the defense mechanism of host against neoplasia has been emphasized by many authors. Excessive surgical stress may impair the RES activity, therefore, an evaluation of the RES by Congo Red test was done in cervical cancer cases, with special regard to the effect of radical panhysterectomy with pelvic lymphadenectomy on it. The mean congo red value was 0.97 for the control group, 0.97 for the cancer cases before operation, and 1.09 for the cancer cases treated without recurrence. A tendency of increased phagocytic activity was noted in stages I and IIa cases while a depression was noted in stages IIb, III and IV cases. A mild depression of the phagocytic activity occurred in nearly half of the patients one week after surgery. Every effort to potentiate the defence capacity of the patient should be attempted to better the prognosis of uterine cervical cancer patients after radical surgery.

Many experimental and clinical data have been published in the last two decades suggesting that the RES plays an important role in the defense mechanism of host against neoplasia. A suppression of activity in the RES during surgical stress, chemotherapy or irradiation has been pointed out by many authors. Our experiences indicate that a rapid recurrence and deterioration may occur sometimes soon after a quite successful operation. Such "adverse effect" may be due to too extensive surgical interference and this possibility should be always kept in mind. It is the aim of the present study to evaluate the RES activity in cervical cancer patients as well as the effect upon the RES of radical surgery with extensive pelvic lymphnode resection; since phagocytic cells are scattered in the connective tissue and lymphoid tissue, an extensive pelvic surgery with lymphadenectomy may result in acute loss of these cells and thus endanger the activity of the RES.

MATERIAL.

Phagocytic activity of the RES was investigated in 5 healthy nurses, 21 women with uterine fibroid, 125 women with cervical cancer and 108 women who had been operated upon for cervical cancer but had no sign of recurrence at the time of the test. Of 125 cases of uterine cervical cancer, 12 were Ca. in situ and 113 were invasive cancer. 10 patients with ca. in situ were operated upon with TeLinde's operation and in 2 other cases, TeLinde's operation with pelvic lymphadenectomy was performed because of questionable early invasion. Of 113 invasive cases, 3 had TeLinde's operation with lymphadenectomy, 94 had Okabayashi's operation with lymphadenectomy and 3 had pelvic exenteration. The rest received irradiation therapy or were discharged without treatment. Patients who had been operated upon were subjected to the test without selection, upon their returning for follow-up.

METHOD

The congo red test was carried out before therapy and one week after operation. If the second test was done at an inappropriate time, the case was excluded from the statistical analysis. The test procedure⁽²⁹⁾ are as follows; (1) withdraw a specimen of fasting blood, (2) inject 1% Congo Red solution, 1 ml per 10 lbs of body weight, but not more than 10 ml, into a vein over in 2 min., (3) exactly 4 and 60 min. after the completion of the injection, withdraw blood from a vein of the opposite arm, (5) pipet 4.0 ml of acetone into 3 tubes, and then to the acetone tubes, add dropwise while shaking 1 ml of each of the sera. Mix vigorously and then centrifuge for 4-5 min., (6) pipet the supernatant into cuvet and measure the absorbance of specimen against the control specimen using a wavelength of 515 to 520 m μ . The calculation of Congo Red Index, i.e. (absorbance of 60 min./absorbance of 4 min.) \times 100, was discarded in the present study, since it can not accurately represent the real phagocytic activity of the RES. Instead, the absolute amount of Congo Red was measured and its clearance expressed as mg of dye per dl per hour. An arbitrary term "Congo Red Value" (abbr. as CRV) is used to indicate this figure in the present study.

RESULTS

1. CRV in control cases (Table I),

Table I. Congo Red Value (mg/dl/hour) in Control Women

	No. of cases	CRV (mean)
Healthy nurse	5	1.04 \pm 0.024
Myoma uteri	21	0.96 \pm 0.073
Total	26	0.97 \pm 0.077

Since we have very few volunteer for the test (only 5 nurses were submitted to the

test), cases of uterine fibroid was also used as controls. The mean CRV was 1.04 for nurses and 0.96 for uterine fibroid cases. If these two groups were pooled together, the mean CRV would be 0.97.

2. CRV in cervical cancer cases (Table II).

Table II. Congo Red Value (mg/dl/hour) in Uterine Cervical Cancer

	No. of cases	CRV (mean)
Uterine cervical cancer, before operation	125	0.97 \pm 0.045
Uterine cervical cancer operated without recurrence	108	1.09 \pm 0.097

CRV was measured in 125 cases of uterine cervical cancer before operation and 108 cases of uterine cervical cancer operated in the past, and that without recurrence. The mean CRV for the former is 0.97 and that for the latter is 1.09. No statistical difference could be demonstrated. In the latter group, the interval between the operation and the test ranges from 3 months to 10 years and 10 months. As shown in Table III, the interval, apparently, showed little effect on the CRV. No correlation between the age and

Table III. Congo Red Value in Postoperative Uterine Cancer Cases without recurrence according to the interval between operation and the test

Interval (year)	No. of cases	CRV (mean)
Within 1/2	11	1.12
1/2-1	19	1.04
1-2	27	0.97
2-3	16	1.23
3-4	12	1.18
4-5	7	0.9
5-7	9	1.35
over 7	7	1.0
Total	108	1.09

the CRV could be demonstrated as shown in Table IV. Effect of clinical staging is shown in Table V; in earlier stages, i.e., I & IIa, the mean CRV is rather stimulated whereas it is somewhat suppressed in cases of Stages IIb, III and IV, though there is no statistically significant difference. However, the suppression noted in cases of IIb is significant ($t=2.018$, larger than $t_{0.05}=2.015$), as compared with cervical cancer cases treated without recurrence. The mean age of the patients increases as the clinical staging advances, however, no apparent effect of the age upon the CRV was noted.

Table IV. Congo Red Value in postoperative uterine cancer case without recurrence according to age

Age	No. of cases	CRV (mean)
-35	10	1.03
36-40	13	1.10
41-45	23	1.08
46-50	28	0.99
51-55	17	1.23
56-60	10	0.88
61-65	5	1.68
66-70	2	1.05
Total	108	1.09

Table V. Congo Red Value in uterine cancer cases before operation according to clinical staging

Stage	No. of cases	Age (mean)	CRV (mean)
0	12	42.4	0.86+0.118
I	48	46.1	1.04+0.088
II a	18	50.6	1.08+0.078
II b	30	50.4	0.87+0.085
III	7	54.5	0.93+0.145
IV	10	47.2	0.82+0.192
Total	125		0.97+0.045

3. Effect of radical surgery on CRV

Regardless of the type of operative procedure, a suppression of the RES function occurred in about half of the patients (cf Table VI), however, there is neither significant degree of suppression after the operation (cf Table VII), nor significant difference according to different type of operative procedure (cf Table VIII).

4. Lymphnode metastasis and CRV

Of 113 cases, 100 had pelvic lymphadenectomy. 21% of them showed lymphnode metastasis. The mean CRV for the negative nodes group is 1.01 while it is 0.94 for those cases with positive nodes group, however, no

Table VI. Effect of Radical Surgery on Congo Red Value

Operative procedure	Suppressed		Stimulated		No change	
	No.	%	No.	%	No.	%
S. T.	8	57.1	6	42.9	0	
T.	6	60	4	40	0	
T. & L.	2	40	2	40	1	20
O. & L.	28	45.1	30	48.3	4	6.6
Total	45	49	42	45.6	5	5.4

N. B.: S. T.; Simple panhysterectomy

T.; TeLinde's operation

T. & L.; TeLinde's operation with pelvic lymphadenectomy

O. & L.; Okabayashi's radical panhysterectomy with pelvic lymphadenectomy

Table VII. Effect of Surgery on Congo Red Value

Operative procedure	CRV before operation	CRV after operation	t
S. T.	0.99±0.15	0.89±0.104	0.81
T.	0.81±0.07	0.72±0.11	0.69
T. & L.	0.90±0.12	1.70±0.73	0.98
O. & L.	0.95±0.07	0.98±0.08	0.35

Table VIII. Comparison of CRV among Patients with depressed RES function one week after the op., according to the operative procedures

Operative procedure	No. of cases	CRV mean	N. B. comparison	t
S. T.	8	0.87		
T.	6	0.53	S. T. versus T.	1.40
T. & L.	3	0.9	S. T. versus O. & L.	1.46
O. & L.	28	0.63	T. & L. versus O. & L.	1.17

Table IX. Lymphnode metastasis and CRV

Stage	No metastasis		Positive metastasis		
	No. of cases	CRV	No. of cases	% of metastasis	CRV
I	43	1.10	3	6.5	0.86
II a	10	1.11	5	33.3	0.92
II b	21	0.88	8	27.6	0.90
III & IV	5	0.66	5	50.0	1.14
Total	79	1.01	21	21.0	0.94

statistical significance could be demonstrated between these groups, nor between various stages (cf Table IX).

5. Correlation between RES activity and delayed hypersensitivity

Tuberculin test with Old Tuberculin was performed at the time of the congo red test in 47 cancer patients and 33 patients returned for follow-up examination. No difference could be demonstrated between negative and positive tuberculin test groups, indicating absence of correlation between the RES activity and the tuberculin test (cf Table X).

COMMENT

The significance of the RES in host reac-

tion against neoplasm has been extensively studied in experimental animals as well as human beings (cf Table XI). However, there is still no consensus of opinions as to the change of activity of the the RES in the tumor bearing hosts. Absence of an absolutely accurate test for the RES activity could account for the lack of unanimity of the results obtained in the literature. In experimental animals most authors^(6,7,20,21) demonstrated an enhancement of the RES function in terms of the rate of colloidal carbon clearance. But, in human cancer patients, the results are rather confusing and contradictory. Earlier studies, with the congo red test indicated a depression of the RES func-

Table X. Delayed Hyersensitivity to Old Tuberculin and Congo Red Value

	O. T.(-)		O. T.(+)	
	No. of cases	CRV	No. of cases	CRV
Uterine cervical cancer	16	0.74	31(65.9%)	0.97
Uterine cervical cancer treated without recurrence	9	0.94	24(72.7%)	0.89

tion: Stern⁽²⁸⁾ found a depression in 86% of the patients. Omori⁽²²⁾ reported that a hypofunction was found in 80% of 139 gastric cancer cases, and that cases with increased function of the RES showed less extensive spreading and a better 5 year survival. On the contrary, Salky *et al*⁽²⁷⁾, by using RE test lipid emulsion, found a profound enhancement of phagocytic activity in 91% of 53 patients bearing primary cancers of various organs and emphasized that the enhancement was noted even in patients of terminal stage. Magarey *et al*⁽¹⁸⁾ have investigated the RES function with aggregated human serum albumin labeled with I¹³¹ in 200 patients and concluded that no significant difference was found between patients with localized cancers and patients without malignant disease, but there was significantly greater phagocytic activity in patients with metastatic cancers. A tendency of early stimulation followed by depression is suggestive in the present study, however, no statistically significant difference could be proved.

A certain relationship between the RES and development of the delayed type hypersensitivity has been suggested⁽¹⁴⁾. But, no definite correlation between the CRV and the tuberculin test could be demonstrated in the present study.

At present, it is quite impossible to determine whether these contradictory results are derived from a real difference in host reaction or are solely derived from a difference in methods of measuring the phagocytic activity. Moreover, the phagocytic activity is merely one facet of its protean function.

The congo red test was originally devised by Adler & Reimann⁽¹⁾ and the phagocytic activity is usually expressed by a congo red index, i. e., the percentage of the dye retained in one hour. During interpretation of the result, several factors should be taken into consideration. Since the liver possesses a large collection of Kupffer cell⁽¹⁴⁾, hepatic function may affect the result of the test. In animal experiments, Aoki & Kudo⁽²⁾ noted a correlation between indian ink index, congo red index and BSP test, however, Zaitzu⁽²⁰⁾ could not demonstrate a definite correlation between congo red index and BSP test in gastric cancer patients. In the present study all of the subjects were submitted to routine liver function tests and the results were all within the normal limit. Benacerraf *et al*⁽³⁾ emphasize that a sufficient dose of the colloid has to be injected where $K \times D = \text{constant}$, (K; rate of clearance, D; dose); below this dose the method is only blood flow (mainly of hepatic flow) dependent. The congo red test is also utilized in the diagnosis of amyloidosis where a large amount of the dye is absorbed by amyloid materials, resulting in a marked decrease of congo red index. Therefore, the presence of amyloidosis should be ruled out before the test. In the case of urinary loss of the dye (e. g. nephrosis) or presence of plus 2 albuminuria, a false decrease of congo red index (or an increase of CRV) may occur⁽²⁹⁾. Popper & Schaffner⁽²⁸⁾ doubt that congo red is a valid RES test substance. Nowadays, instead of congo red, more ideal substances, such as, colloidal carbon^(4,5), aggregated human serum albumin^(3,4,15,26),

Table XI. Review of the literature

Authors	Years	Species	Tumor	Method	RES activity
Biozzi <i>et al</i>	1958	Rat	Guerin carcinoma	Carbon	Increased
Old <i>et al</i>	1960	Mouse	Isotransplanted sarcoma	ditto	Stimulation after an initial depression
			Isotransplanted	ditto	Increased
			MC induced sarcoma		
			Spontaneous mammary tumor	ditto	Mild stimulation
Old <i>et al</i>	1961	Mouse	Sarcoma 180	ditto	Isolate a transmissible agent which can stimulate the RES
Omori	1964	Rat	AH-130 tumor	Congo red	Early appearance and rapid growth in the RES blocked group
			Walker tumor	ditto	Earlier and more extensive hematogenic metastasis in the RES blocked group
Edwards	1966	Mouse DBA/2J	Adenocarcinoma of breast (Ca. D ₂)	Histology	Thymus atrophy. Spleen & lymphnodes, initial hypertrophy, ultimate exahusion
Blamey	1969	Rat Wister	MC induced sarcoma	Carbon	Marked increase
Stern	1941	Human	Cancers	Congo red	Depression (86%)
Omori	1964	Human	Gastric Ca. 139 cases	Congo red	Depression (80%); Cases with increased RES function showed less extensive spreading and better 5 year survival.
Groch <i>et al</i>	1965	Human	Acute leukemia	I ¹³¹ human serum albumin	Depression
Zaitsu	1967	Human	Gastric cancer 68 cases	Congo red	Depression (80.8%), related to stage
Salky <i>et al</i>	1967	Human	Primary cancer 53 cases	Lipid emulsion	Enhancement (91%) even in terminal stage
Magarey <i>et al</i>	1968	Human	Localized ca. Metastatic ca.	I ¹³¹ human serum albumin	No change Increase

I¹³¹ labeled lipid emulsion^(12,15,25,26,27,31), are in use. Since these substances are not available to us and for the sake of safety for the patients, we used the congo red test in the present study. The traditional calculation of congo red index was discarded, since the index, as discussed by Katsuya⁽¹⁶⁾, is not a good indicator of the phagocytic activity. Hence, the absolute amount of the dye cleared away by the RES cells per dl of serum per hour is measured.

Several studies have indicated a relation

ship between operative stress and cancer growth^(10,17). Robert *et al*⁽²⁴⁾ have reported that there is a rapid elevation of the blood concentration of malignant cells immediately after surgical manipulation of cancer patients. By blocking the RES with zymosan or typhoid vaccine, Omori⁽²²⁾ has noted that there was an earlier appearance, a rapid growth rate and a more extensive metastasis of AH 130-, or Walker tumor cells in rats. Our experiences indicate, occasionally, lung metastasis appearing immediately after the operation

for early uterine cancer⁽¹³⁾. It is a well known fact that surgical stress⁽⁶⁾, intensive chemotherapy or radiotherapy⁽¹⁰⁾ may suppress the RES activity. Saba & Luzio⁽²⁵⁾ investigated the phagocytic activity by RE test lipid emulsion in male Holtzman rats in which a surgical stress was created by making a midline laparotomy to expose the peritoneal cavity. They found a significant but transient impairment of phagocytosis and that the depression was associated primarily with impairment of the liver phagocytosis. Donovan⁽⁹⁾ has studied the effect of surgery in 9 patients who had cholecystectomy, resection of the sigmoid colon, radical groin dissection, subtotal gastrectomy, low thigh amputation with radical groin dissection, inguinal hernia repair and cholecystomy with common duct exploration. In 8 out of 9 cases, the RES function decreased. The degree of impairment correlated with duration of operation and the existence of decreased systolic blood pressure. It should be pointed out that the decrease occurred within the first 24 hours and then returned to preoperative level in one week. In radical surgery for cervical cancer, a large amount of parametrial tissues as well as pelvic lymph nodes with areolar tissue is resected. Such a sudden and massive removal of lymphatic and/or connective tissue may produce a profound suppression of the RES, since these tissues are believed to be provided with a large amount of phagocytic cells⁽¹⁴⁾. The results of the present study, against our expectation, showed no significant difference in the CRV before and after the operation, though a mild suppression was noted in half of the cases. The possible explanation is that: (1) the method is inaccurate, (2) the time of measurement is inappropriate and (3) there is no suppression at all. Further study is mandatory to clarify these questions. Of particular concern is the ultimate prognosis of those patients who display a depres-

sion during the early postoperative period. Follow-up study of these patients is very important.

CONCLUSION

Because of lack of appropriate controls, and what is more, due to the aforementioned *a priori* defect in the test method, the results obtained by the present study are contraictory and irrelevant. Suffice it to say that there is a tendency of early stimulation followed by depression in the phagocytic activity in cervical cancer cases. Seemingly, an extensive pelvic surgery with lymphadenectomy does not bring a marked variation to the RES function, however, a mild suppression occurred in nearly half of the patients. Every effort to potentiate the defense capacity of the patients should be done during the early postoperative period. One of our greatest concerns is the ultimate fate of those patients who showed depressed phagocytic activity, but it should await the results of further follow-up.

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子宮頸癌病人之網內系機能

(I) 廣泛性子宮切除及淋巴節切除之影響

鄭永盛 徐千田

臺北醫學院婦產科

過去有很多臨床以及動物實驗證實，生體對癌瘤的發生，生長之防禦作用與網內系機能，頗有密切的關連。有很多學者提出警告，外科侵襲甚大，或化學療法或放射線療法過強時，有時會危害網內系機能，癌瘤之增殖反而增強或在短時間內招致再發；頸癌之根治手術的侵襲甚大，在短時間內會切除大量的骨盆內結締組織及淋巴腺組織，而這些組織內却富於網內系吞食細胞，因此對病人的網內系機能可能有影響，為明瞭其影響的程度，我們以 Congo Red 試驗測定病人開刀前後的網內系吞食機能而獲得結果如下：

1. 健康年青護士的 CRV 為 1.04 子宮肌瘤病人為 0.96 子宮頸癌開刀前為 0.97，開刀後而無再發症狀者為 1.09。
2. 早期頸癌 (I, IIa) 之吞食機能有增加，晚期者 (IIb, III, IV) 有減弱的趨勢。
3. 頸癌病人的吞食機能在開刀前後雖無統計學上有意之差，但大約有一半之病人，發生吞食機能之減弱，雖然目前我們不清楚這些病人之五年生存率究竟如何，但我們應該致力於改善病人的網內系機能，增加其防禦能力以期最佳的治療效果。