Operative Wound Seeding with Tumor Cells: *

Its Role in Recurrences of Head and Neck Cancer

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IN 1906, Crile noted [3] that only one per cent of a series of 4,500 autopsies on patients with cancer of the head and neck demonstrated metastases below the clavicle. More recent reviews [2, 4, 7] of autopsy material have shown that such spread is not a rare occurrence. These later reports indicate that up to 50 per cent of all such patients can be expected to exhibit distant spread of their cancer. However, this leaves a significant number of treatment failures due to local regrowth of cancer in the operative wound following operation. In addition, it is the exception rather than the rule in head and neck cancer to find widespread metastases in the absence of local disease. On occasions, patients are seen with multiple "seeded" type of discrete implants scattered throughout a wound, consistent with the take of malignant cells seeded in the wound at the time of operation. A situation similar to this has been seen in animal tumors in this laboratory where it has been possible to transplant a tumor from one animal to another using suspensions of malignant cells which will produce multiple local growths at the site of inoculation or disseminated metastases if given intravascularly. Washings taken from operative sites after removal of all gross evidence of cancer have revealed the presence of individual as well as clumps of tumor cells.[8]

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The purpose of this study was to determine the frequency of occurrence of these cells in wound washings in cases of cancer of the head and neck and to investigate the role of this "wound seeding" in relation to the development of local recurrence.

Methods and Materials

The basis of this study is 69 patients with epidermoid carcinoma. The locations of their tumors are shown in Table 1. Fourteen patients with nonepidermoid tumors are included (Table 2), for comparison of recovery of tumor cells. The number comprising this latter group is too small for extensive treatment of their data.

These 83 patients underwent 107 operative procedures. In all instances, the patients had cancer limited to the areas of the primary growth and regional node or both, as determined by clinical and laboratory examination. The operation performed was designed to remove all cancer present, palliative procedures being excluded from the study. In general, most cases would be considered locally advanced but still operable.

Following removal of the specimen, the operative site was sprayed with normal saline which was collected by aspiration. The fluid was then centrifuged and the sediment smeared onto slides with a portion embedded in paraffin for sectioning. Staining was then performed according to the Papanicolaou technic.[6] Recently a new method [5] has been employed which uses

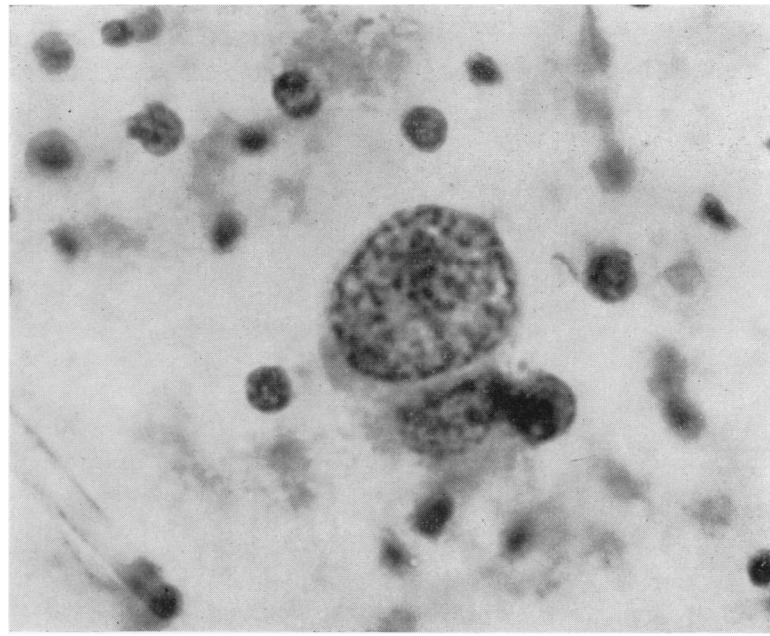

FIG. 1. Positive wound washing. Note the large size of the central cell and the clumping of the nuclear chromatin. Multiple nuclei are also seen in the smaller of the two cells (× 1360).

Streptolysin 0 to remove red blood cells and segmented leucocytes leaving only tumor cells and lymphocytes. This method utilizes filtration with direct staining and examination of the filter for malignant cells. No differences have been noted between the former and present technics in the recovery of tumor cells. Following staining, the slides were interpreted by experienced cytologists and classified as positive, negative, or suspicious according to whether malignant cells were identified. Figure 1 is an example of a positive wound washing from a patient with squamous cell carcinoma of the floor of the mouth. The suspicious category is an indeterminate one in which the cells recovered are not normal but fail to meet all the necessary criteria in order to be called positive. Such factors as inflammation in the area or previous irradiation could account for some of the changes noted. For this reason, they are followed separately and not grouped with either the positive or negative washings.

TABLE 1. *Epidermoid Carcinoma*

Sites	No. of Patients
1. Tongue	12
2. Extrinsic larynx	11
3. Floor of mouth	9
4. Lip	6
5. Paranasal sinuses	5
6. Palate	5
7. Tonsillar area	4
8. Pyriform sinus	4
9. Alveolar ridge (mandible)	4
10. Buccal mucosa	3
11. Pharyngeal wall	3
12. Skin	2
13. Cervical nodes—undeter. primary	1
	69

Results

Table 3 shows the results of the washings from 107 operations. Twenty-six per cent of the operative wounds in the epidermoid group yielded positive smears, four of 19, or 22 per cent, were positive in the nonepidermoid cases.

Table 4 shows results in relation to types of operations performed. The ratio of positive to negative is about 50 : 50 when only a wide local excision of the tumor was per-

TABLE 2. *Nonepidermoid Tumors*

Types	No. of Patients
1. Malignant melanoma	4
2. Basal cell carcinoma	4
3. Salivary gland tumors	
a. Malignant mixed tumor	2
b. Mixed tumor	1
4. Fibrosarcoma	
a. Maxilla	1
b. Nasopharynx	1
5. Rhabdomyosarcoma	1
	14

formed. This ratio decreases when an *en bloc* excision of the lymph drainage area is included with the primary tumor, and finally, when a dissection of the lymph node area is performed alone, no positive cases are noted.

Attempts were made to determine the factors responsible for the presence of malignant cells recovered in the washings. A multitude of factors such as location of the tumor, duration prior to operation, and previous treatment were examined and failed to show any significant difference between positive and negative groups. It was further noted that it was impossible to define any rigid criteria within which the positive cases would fall, and which would have totally distinguished the groups. Two features were noted, however, which did appear to be significant:

1. There was a higher percentage of positive smears in the presence of an ulcerated tumor surface. Failure to recover cells in radical neck dissections where no exposed tumor was present is thought to be accounted for, at least partially, by this factor.

2. Table 5 shows results in relation to surgical margins. The data include both the surgeon's and pathologist's impression concerning presence of tumor at the margin of the specimen. "Gross" refers to the surgeon's evaluation formed at the operating table and "micro" refers to the pathologist's impression as determined by histologic study. The distinction is made because of difficulty in a small number of cases in determining actual lines of resection after removal of the specimen. The important fact here is the greater number of cases in the positive group being associated with malignant cells identified at the cut margin. That this is not an absolute distinction is also seen.

TABLE 3. *Wound Washings (107 Operative Procedures)*

	Epidermoid	Nonepidermoid
Negative	54 (61%)	13 (68%)
Suspicious	11 (13%)	2 (10%)
Positive	23 (26%)	4 (22%)
	88	19

TABLE 4. *Wound Washings—Relation to Operation*

Epidermoid Carcinoma	No. of Operations with Washings		
	Neg.	Susp.	Pos.
Resection of primary or local tumor	13	5	14
En bloc dissection of tumor with lymph node area	25	5	9
Radical neck dissection	16	1	0

In the final analysis, however, the major importance of the study was to determine whether or not groups which differed in the recovery of malignant cells behaved differently in relation to local recurrence. Table 6 reveals that the number of operative procedures followed by locally-recurrent tumor is almost identical for both positive and negative washing groups. In this chart cases are grouped solely on the basis of presence or absence of local recurrence. When these data are studied further, according to the Life Table Method,[4] to take into account variation in duration of follow up and to allow for withdrawals

TABLE 5. *Surgical Margins*

Washings	No. of Specimens		
	Gross Clear Micr. Clear	Gross Clear Micr. Tumor	Gross Tumor Micr. Tumor
Negative (54)	51 (94%)	2	1
Suspicious (11)	11 (100%)	0	0
Positive (23)	14 (61%)	3	6

from follow up at any particular time owing to death of metastatic disease, the results are shown in Figure 2. Here, the probability of developing local recurrence is plotted in a cumulative manner for each group. There are no significant differences in the rate of local recurrence, time of development of the recurrent cancer, or even total numbers with recurrences in any group.

TABLE 6. *Local Recurrence—Epidermoid Carcinoma*

Wound Washings		Recurred	Did Not Recur
Negative	(54)	19 (35%)	35 (65%)
Suspicious	(10)	6 (60%)	4 (40%)
Positive	(21)	8 (38%)	13 (62%)
	85	34 (40%)	51 (60%)

Discussion

Tumor cells have been noted to occur in the washings from approximately 25 per cent of a series of operative cases of cancer of the head and neck. With reference to local recurrence, however, there appears to be no difference between this group and the group in which the cells were not present. If the only factor in local recurrence was implantation of free cells, then it would be expected that all cases with positive wound washings would recur. According to Barrett,[1] failure of these tumor "autografts" to uniformly take and grow may indicate departures from the usual concepts of immunity and graft rejection when applied to tumor tissue. Thus, both the rejection of autografted cells and the difficulties in demonstrating antibodies, even in homologous systems, provide further stimuli for evaluating the entire sphere of host-tumor relationships. This poorly-defined term must be used to account not only for the positive cases which failed to recur, but also for many instances of the regrowth of cancer in the negative group.

The failure to achieve local permanent cure of cancer is merely the end result of many factors, of which wound seeding is only one. Among others must be included 1) failure to excise completely the primary growth as shown by the observation of tumor cells at the margins of resection; 2) development of second and multiple primaries; and 3) local vascular invasion both through extension beyond the margins of operative specimens and through release of malignant cells when the vessels are cut across. This is one obvious source for cells

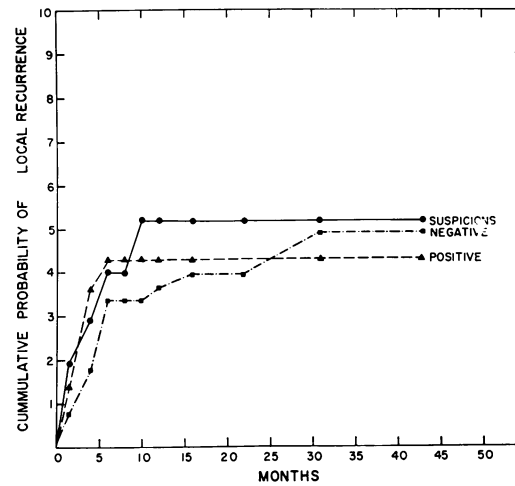

FIG. 2. Local recurrence data plotted according to Life Table Method with probability of local recurrence charted cumulatively against time in months.

recovered in washings. A further interesting note concerning vessel invasion with malignant cells is that although the positive and negative cases were almost identical in the numbers showing this feature, almost all of the local recurrences in the negative group were seen to come from cases in which vascular involvement with tumor cells could be demonstrated histologically.

Underlying this entire study was the premise that by demonstrating the role of wound seeding with tumor cells, a basis for the use of local adjunctive chemotherapy could be established, for it is against such free cells that this form of therapy can be expected to be most effective. The fact that the role of these cells cannot be defined clearly does not preclude the possible value of such agents. It does indicate that studies involving use in patients must be carefully and cautiously interpreted. It is possible that a threshold number of cells must be present before regrowth of cancer, suggesting that a quantitative approach to the data presented might be informative. At present, we have no way of knowing how many cells must be present in a wound before they can be detected by the methods used in this study.

Seeded recurrence is certainly seen clinically, and perhaps future studies will further define the mechanisms involved and suggest methods for prevention.

Summary

1. Tumor cells have been demonstrated in washings from operations on a series of patients with cancer of the head and neck.

2. Positive and negative groups behave similarly in relation to development of local recurrences of cancer.

3. Other mechanisms for local recurrence are discussed along with future lines of study of the problem.

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