1958

Cancer cells and tumor emboli in the blood stream

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CANCER CELLS AND TUMOR EMBOLI IN THE BLOOD STREAM.

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Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

April 1, 1958

Omaha, Nebraska

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I - INTRODUCTION

This thesis will review the literature concerning the presence of cancer cells and tumor thrombi in circulating blood, and the possible significance of manipulation, particularly during operation, in producing hematogenous spread of cancer cells.

The first part of this thesis will consist of a review of the literature extending from the work of Ashworth[1] in 1869 to the work of Sandberg and Moore[2] in 1957. The remaining parts of this paper will be devoted to presenting some of the techniques used for demonstrating tumor cells, the methods used in attempting to obtain controlled studies, and the results of some of these studies. Finally, an attempt will be made to draw logical conclusions from the results of these investigations.

II - HISTORICAL REVIEWS OF THE LITERATURE

Ashworth[1], in 18 69, reported a case of multiple malignant skin tumors, in which blooc, taken from the saphenous veins of one leg unaffected by skin tumors, contained cells of the same size and appearance as those found in the skin tumors.

A microscopic study of 41 cases of advanced malignant disease, but without grossly visible pulmonary metastases, was reported by Schmidt[3] in 1903. In 15 of the 41 cases, he was able to demonstrate tumor emboli in the pulmonary arteries or capillaries. He concluded that the majority of tumor cells reaching the lung perish, but that the surviving cells might reach the venous side of the pulmonary circulation by passing through capillaries and scattering tumor emboli throughout the body without leaving grossly visible pulmonary metastases.

Marcus[4], in 191 , reported "abnormal cells" in the blood taken from the finger, five daya prior to death, of a patient with bronchiogenic carcinoma. Post mortem examination revealed invasion of the wall of the right atrium by tumor.

Pool and Dunlop[5] in 1934 attempted to demonstrate cancer cells in the circulating blood from living patients. Specimens of blood were taken from 40 cases of advanced carcinoma of the breast, colon, stomach, rectum and other sites. Normal blood was used as a control. Of 40 cases, positive cells were found in 17 . These cells were described as large, spherical cells

with round or slightly curved hyperchromatic nuclei.

Brown and Warren[6] in 1938 found the tendency to venous invasion and consequent hematogenous spread to correlate with the grade of tumor differentiation. They noted an incidence of 61% of venous invasion in an autopsy series of 170 patients with carcinoma of the rectum.

Grinnel[7], in his study of 162 specimens removed at operation reported in 1950, found microscopic evidence of invasion of the walls of veins within the primary lesion in 36% of patients with rectal carcinoma and in 33% of patients with colonic carcinoma. He perfused isotonic sodium chloride through the major artery of the colonic segment containing the carcinoma removed by surgery, and collected the perfused fluid from the major vein. He was able to demonstrate malignant cells in one case.

Engell[8] in 1955 presented a detailed study of the occurrence of cancer cells in venous blood coming from the primary tumor, the results of which will be presented in detail later.

In the same year, Fisher and Turnball Jr.[9] published a paper on the demonstration and significance of tumor cells in the mesenteric venous blood of patients with "colo-rectal" carcinoma. This series covered a total of 25 cases.

Sandberg and Moore[2] in 1957 found tumor cells in the peripheral blood of 45 of 105 patients with advanced metastatic adenocarcinoma and in the blood from the regional vein in 25 of 55 patients with adenocarcinoma.

III - TECHNIQUES FOR CYTOLOGICAL STUDY

In their work, reported in 1934, Pool and Dunlop[5] used 5 ml. of venous blood which was withdrawn from the patient and then "oxalated." The specimen was then hemolyzed with 15% acetic acid. The supernatent liquid was poured off and then fixed by overlaying with 10% formalin in 20% alcohol. The specimen was then dehydrated, embedded in paraffin and serial sections made.

In 1954, in his cytologic studies of cell suspensions, Hazard[10] found the best stain for demonstrating metachromatism in tumor cells was Toluidine blue buffered to approximately pH7. The Toluidine blue buffered solution was placed in 20% ethyl alcohol and then added to equal parts of horse serum. He found that supravital staining with neutrol red added little to the details of cell morphology.

Fisher and Turnball Jr.[9], in 1955 took the mesenteric venous blood from surgical specimens of "colo-rectal" carcinoma and emptied it into a 50 ml. centrifuge tube. They then irrigated the venous segment with isotonic saline until the return flow was clear. The blood and perfusate were then combined and centrifuged for 10 minutes. The supernatent fluid was then decanted. A stained cell suspension was prepared from the sediment, using Hazard's technique.[10]

Another technique they used was to fix smears in acetic

alcohol (3% glacial acetic acid in 95% alcohol.) These were then stained using the routine Papanicolaou method (OG6 and EA50.)

A cell block was made using equal parts of heparinized plasma with the remainder of the sediment and adding four drops of thrombin to each cubic milliliter of the total fluid volume. This artificial clot was fixed in Zentter's fluid and processed by paraffin infiltration.

Two direct smears were taken from the surface of the neoplasm for cytological comparison. Using cytologic criteria they found the smears and suspensions to be more satisfactory than the cell block for showing cells morphologically identical with the cells noted in the direct smears, made from the surface of the neoplasm.

Two types of cells were found. One type, with a round nucleus, occurred in groups; and one type, with an ovoid nucleus, occurred singly. The cell type occurring singly appeared only in the suspensions. This is attributed to the fragility of these cells which would have been destroyed in the other procedures. Both types of cells had prominent nucleoli; clumping of the nuclear chromatin, and scanty cytoplasm. The nuclear-cytoplasmic ratio was found to be abnormal.

Endres in 1930 found the most suitable method for hemolyzing erythrocytes was the use of saponin.[11] Saponins are complex steroids which contain sapogenin and a sugar. All the saponins characteristically lower the surface tension of water. They are believed

to dissolve the lipid of the red cells.

Endres[11] obtained complete hemolysis when 20 mgm. of saponin was added to 5 ml. of blood diluted with 5 ml. of plasma.

Engell[8] in his work in 1955 used the Endres principle for hemolyzing the red cells. He took 2-5 ml. of heparinized venous blood and immediately transferred it to a 50 ml. centrifuge tube. The blood was refrigerated if there was to be any delay in completion of the procedure.

The centrifuge tube was filled with isotonic saline, centrifuged for four minutes at 1500 R.P.M. and the supernatent fluid pipetted off. The blood cells were poured into a second 50 ml. centrifuge tube and 45 ml. of isotonic saline was added. The tube was then filled to 50 ml. with 1% saponin in distilled water.

When the fluid had become transparent, i.e., 1-3 minutes for hemolysis, it was again centrifuged at 1500 RP.M. for four minutes and the supernatent fluid was again pipetted off. There remained a slight greyish-white sediment of white blood cells and tumor cells, if any were present. After repeated rinsing with isotonic saline and centrifuging, five drops of citrated plasma and one drop of 10% calcium gluconate are allowed to slide down the side of the glass tube, over the sediment at the bottom of the tube.

After five minutes, the cell block was detached from the bottom of the tube, transferred to 10% formalin for fixation

and then dehydrated and mounted in paraffin for serial sections. The sections were stained with Hansen's hematoxylin and counter-stained with 1% aqueous solution of eosin.

IV - METHODS OF OBTAINING SAMPLES

Fisher and Turnball Jr.[9] studied 25 bowel resections for "colo-rectal" carcinoma. Before handling the tumor the surgeon ligated the arteries and veins at the most proximal portion of the regional mesentery. The venous collaterals were also ligated.

For carcinoma of the rectum and sigmoid, the inferior mesenteric vein and artery were ligated at the inferior portion of the duodenum. For carcinoma of the cecum, the ileo-colic and rightcolic arteries and veins were ligated at their origin at the duodenum. For carcinoma of the transverse colon, the mid-colic vessels with the marginal vessels were ligated.

After primary ligation of the vessels at their proximal portions, the colon was mobilized and a silk tie placed around the mesentery nearest the tumor, thus isolating a column of blood.

The operative specimen was taken to the laboratory, and the isolated venous segment was dissected free. The primary ligature was then removed the the blood emptied into a 50 ml. centrifuge tube. The venous segment was then irrigated with isotonic saline, and the blood and perfusate used for histological preparation.

Engell[8] in 1955 took venous blood samples during the course of operations, before and after manipulation of the tumor, or from the cubital veins in cases of far advanced, inoperable carcinoma with metastases.

In the 31 patients studied with cancer of the colon, blood was obtained from the mesenteric veins during resection. In eight cases of cancer of the stomach samples obtained from a branch of one of the gastro-epiploic veins. For cancer of the lung, blood was taken from the inferior pulmonary vein and from the cubital vein during pneumonectomy or lobectomy. For carcinoma of the breast a small vein, lying underneath the pectoralis major, which ran from the breast to the axilla, was used.

V - CRITERIA FOR IDENTIFYING TUMOR CELLS IN BLOOD SAMPLES

Fisher and Turnball Jr.,[9] used as criteria for tumor cells, those cells with both prominent nucleoli and clumps of nuclear chromatin, scanty cytoplasma and an abnormally high nuclear-cytoplasmic ratio.

Engell[8] identified tumor cells by the characteristics of the nuclei, i.e., the nuclei were hyperchromatic and varied in size, shape and location in the cell. The chromatin was irregular and coarse and there were one or more nucleoli. He also made a direct measurement of the nucleolus - nucleus ratio.

Sandberg and Moore[2] in their series described tumor cells as cells with slate-blue, foamy cytoplasm, occasionally containing fine azurophilic granules and large nuclei often containing several large, light blue nucleoli.

VI - RESULTS

Sandberg and Moore found tumor cells in the peripheral blood of 45 of 105 patients[2] and in the blood from a regional vein in 22 of 55 patients with advanced metastatic carcinoma.

Fisher and Turnball Jr. studied a series of 25 patients, 15 with rectal carcinomas, 4 with sigmoid carcinomas, 3 with carcinomas of the transverse colon and 1 with carcinoma of the descending colon.

All tumors were classified according to Duke's classification. i.e., (A) invasion of muscle only; (B) invasion to the serosa; (C) invasion through the serosa and involvement of the regional lymph nodes.

For cytological study they used cell suspensions and smears, as has been mentioned previously. They obtained consistent results in the identification of tumor cells in the suspension and smears. In only one instance was the suspension positive for tumor cells and the smear negative for tumor cells. In no instance was a smear positive for tumor cells and the suspension negative for tumor cells.

Tumor cells were found in 8 cases, (32%.) In the seven cases that had histopathologic evidence of venous extension 4 cases (22.2%) had tumor cells in the perfusates. In the 18 lesions not demonstrating venous extension 4 cases (22.2%) were positive for tumor cells by cytologic examination.

TABLE I: ANALYSIS OF CASES STUDIED BY FISHER AND TURNBALL, JR. FOR HISTOPATHOLOGIC AND CYTOLOGIC EVIDENCE OF CANCER CELLS.

	Number of Case	Number of Cases with Venous Exten ion	
		Histopathologic	Cytologic
A	5	6	1
B	15	5	6
C	5	2	1
Total	25	7 (8%)	8 (32%)

Engell[8] in his study of the 76 patients with rectal carcinoma, found eight cases (38%) positive for tumor cells in 21 cases classified as group A according to Duke's classification.

In 19 cases classified as group B, 10 cases were positive for tumor cells. The remaining 36 cases were classified as group C according to Duke's classification. Of these, 23 cases (64%) were positive for tumor cells.

TABLE II: ANALYSIS OF CASES OF CARCINOMA OF THE RECTUM STUDIED BY ENGELL FOR THE PRESENCE OF TUMOR CELLS.

	Number of Cases	Cases Positive for Tumor Cells	
		Number	Percent
A	21	8	38
B	19	10	53
C	36	23	64
Total	76	41	54%

In further studying the series of cases classified as group C, 4 died and upon post mortem examination were found to have hepatic metastases. Of these, 4, none had gross evidence of hepatic metastases at the time of operation. In none were tumor cells found in the blood samples taken prior to operation. In only 1 case were tumor cells demonstrated after operation.

In his series of rectal carcinoma, Engell[18] collected blood samples prior to manipulation and after manipulation. In analysing the 23 class C cases positive for tumor cells, only 9 are found to be positive for tumor cells after manipulation and negative prior to manipulation. In 3 cases blood positive after manipulation had not been checked before manipulation. In 2 cases blood before manipulation was positive and that withdrawn after manipulation was negative; and in 9 of the cases blood prior to manipulation

was positive but was not checked after manipulation. In one case blood withdrawn before and after manipulation was positive.

Whan one studies group A lesions and analyzes the 8 cases positive for tumor cells, the results are even less definite. In only one case of the eight was the blood after manipulation positive and before manipulation negative. In two cases blood withdrawn after manipulation was positive but had not been checked before manipulation. In one case blood withdrawn before manipulation was positive for tumor cells and blood withdrawn after manipulation was negative. In two cases blood before manipulation was positive but was not checked after manipulation. In two cases blood drawn before and after manipulation were positive.

If one does not consider the five patients who died immediately post operatively, this leaves a series of 71 patients. At the end of the follow up period, ranging from 4 to 46 months, 49 of the 71 patients or 69% were living without known metastases or cancer tissue left behind in the primary site. If the survival of the patient were entirely dependent upon the finding of tumor cells, then only the 26 patients with negative cytological findings should have survived. This would be a 36% survival, but 49 patients lived (69%.)

In the study o cancer of the rectum and colon there was a total of 107 cases. Of the 107 cases tumor cells were observed in 63 cases (59%.) In the four months to four years follow-up, thirty-two patients had died of cancer or were known to have

metastases or recurrences. Of these 32 patients, 23 (72%) had had tumor cells in the blood. Seventy patients were surviving without known metastases or recurrences. Of these, 38 patients (54%) had shown tumor cells. This meant that the finding of tumor cells was approximately the same in patients who had survived and in those who had died of cancer.

TABLE III: ANALYSIS OF CASES OF CARCINOMA OF THE RECTUM AND COLON STUDIED BY ENGELL FOR THE PRESENCE OF TUMOR CELLS.

	Number of Cases	Cases Positive for Tumor Cells	
		Number	Percent
Patients dead with metastases	2	23	72
Patients who are livine without metastases	,0	38	54
Patients dying post operatively	5		
Total patients	107	63	59%

As one studies this series, it will be found that 43 patients had negative cytological findings. (Five patients included in the series of 107 patients who had positive cytological findings, died post operatively and are excluded.)

If the finding of tumor cells were definitely related to the development of subsequent metastases, then approximately 43 patients (42%) should have survived. However, 70 of the 102 patients (69%) were surviving without known metastases at the end of the follow up period. In evaluating these figures, however, one should remember that a 5 year follow up period might well alter the figures markedly.

Engell's studies[8] of carcinoma at different sites are not really adequate for evaluation. For example, of the six cases of carcinoma of the breast, there were metastases in the axillary lymph nodes of all but two.

VII - SUMMARY

The purpose of this thesis was to review the liaterature concerning the presence of cancer cells and tumor thrombi in the circulating blood, and the possible significance of manipulation during operation in producing hematogeneous spread of cancer cells.

Ashworth[1] in 1869 first reported a case of tumor cells in saphenous blood. Schmidt[3] in 1903, from a study of autopsy cases on patients dying of metastatic carcinoma concluded that tumor cells reaching the lung might reach the venous side of the circulation and scatter tumor emboli throughout the body. Pool and Dunlop[5] in 1934 tried to demonstrate cancer cells in the circulating blood of living patients. Brown and Warren[6] in 1938 correlated hematogenous spread of tumor with the grade of tumor differentiation. In 1955, Engell[8] and Fisher and Turnball Jr.[9] published detailed studies on tumor cells in blood.

The techniques used by the various authors are quite similar in principle. Blood and the fluid obtained by perfusing isotonic saline through the isolated venous segments, if the specimen studied were an operative specimen, was centrifuged to obtain a cell sediment. This sediment was then treated with various hemolyzing agents. This sediment was then examined cytologically.

The methods of obtaining blood samples ranged from taking blood samples from the regional veins before and after manipulation

to carefully ligating the arterial supply proximally and the venous supply distally, thus isolating a column of blood.

In the identification of tumor cells, the authors used similar criteria: scanty, foamy cytoplasm with an abnormally high nuclear-cytoplasmic ratio, larger hyperchromatic nuclei with one or more nucleoli. Engell[8] also measured the nucleus/nucleolus ratio which has been found to be high in cancer cases.

Fisher and Turnball Jr. in 1955, in a series of 25 patients with "colo-rectal" carcinoma, found circulating tumor cells in 8 cases (32%.)

Sandberg and Moore[2] in their series of patients found tumor cells in the peripheral blood of 45 of 105 patients and in the regional blood in 22 of 55 patients with advanced adenocarcinoma with metastases.

VIII - CONCLUSION

From a review of the literature and the study of the more detailed series, it has been found that there is a definite relationship between the occurrence of circulating tumor cells and the grade of differentiation of the tumor.

In an inadequate series, it appears that operative manipulation has no definitive influence on the presence of tumor cells in blood. One might, then, and probably erroneously, infer that the incidence of hematogenous metastases would not be reduced if all the veins from the tumor were ligated prior to manipulation.

There appears to be, at present, no clear relationship between the presence of tumor cells in blood and the clinical course. However, more extensive controlled investigation must be done before it can be really established that circulating tumor cells have no prognostic significance.

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