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Cancer Cells in the Circulating Blood of Patients with Larynx Cancer. Part III

Krążące komórki nowotworowe we krwi żylnej chorych z rakiem krtani. Cz. III

Свободно циркулирующие клетки злокачественной опухоли в венозной крови больных раком гортани. Ч. III

Discussion

The clinical course of a neoplastic disease in man is chiefly effected by the degree of spreading dynamics of the local tumour and its biological malignancy and the patients general immunity which plays a significant role in his further survival. The last factor is connected with the still unexplained problem of the latent life of cancer cells in the human organism and their appearance in the form of local recurrence or metastatic foci in several years or even decades after primary treatment. A very interesting and also unelucidated problem concerning general resistance of the carrier's organism in the course of a neoplastic disease can be a considerable regression or even idiopathic regression of a malignant neoplastic process (14). Among many pathomechanisms of the spreading of neoplastic disease that of the formation of distant hematogenic metastases, in which four periods can be distingnished, is particularly significant.

1) The penetration of cancer cells into the system of blood vessels through the infiltrated wall of a vein or lymphatic vessels directly connected with the venous system. It is also possible that cancer cells get into the blood vessels by intravasation through a wall defect or under the influence of increased perivascular pressure.

2) The translocation of cancer cells with the blood stream to various distant regions of the organism.

3) The localization of cancer cells in precapillary arterioles or capillary vessels.

4) The formation of metastatic foci by cancer cells or their necrosis.

Such a pathomechanism of the hematogenic distribution of cancer was confirmed by experimental studies on animals and clinical observations of some authors (R u d o w s k i, 1964), as well as by studies concerning the presence of free cancer cells in the peripheral blood of patients with cancer of different organs (1, 2, 3, 4, 5, 6, 7, 12, 15, 16, 17). Though in a vast majority of cases cancer cells including blood vessels undergo necrosis and distruction due to the action of resistant mechanisms of the carrier, part of them live under certain conditions and form an active metastatic focus. The essence

of immunological reactions of the organism which very often prevent the development of the neoplastic process, is still uknown in spite of a frequently constant dissemination of f.t.c. into the circulating venous blood. The experimental studies of S a i t o (16) on the behaviour of cancer cells injected into the portal vein and on the formation of metastatic foci showed that most cells are retained in the liver, and, although a certain number of them pass through the "filtering" barrier — as proved by the author in his studies — f.t.c. decrease in their number in the circulating blood to the lowest level as early as 5—7 days after the inoculation.

The studies and observations of many authors on the presence of f.t.c. in venous blood also showed that stress reactions of the organisms to an operation trauma (1, 10, 12, 15, 16), belong to the known and considerably frequent causes. They increase the distribution of cancer cells by the circulating blood, which in turn decrease the general resistance of the organism connected with biochemical disturbances (retention of sodium, chlorine, water; loss of potassium, phosphorus, nitrogen and calcium; decreased protein anabolism), disorders of hypophyso-adreneal and enzymatic systems and other less known organic processes, which increase the inclination of cancer cells to split off by weakening the systems binding them in oxydation of the tumour. The following belong to other known factors affecting the immunity decrease in neoplastic disease: disorders of the liver function, general anaeesthesia, artificial hypothermia, starvation of the organism and heavy psychic traumas.

These factors, beside the mechanical trauma evoked by operation, affect — as reported by R u d o w s k i (14) — the known fact of cancer cell dissemination after an operation, even in clinically early cases. In those patients in whom the criteria of technical and biological radicalness were followed during operation, the neoplastic process after the operation outside the regional lymphatic system was observed to develop by the direct dissemination of f.t.c. from the tumour into venous blood stream or through lymphatic vessels connected with the venous blood system. The influence of a mechanical trauma (an essential component of operation trauma) on an increased dissemination of f.t.c. into the circulating blood was observed by many authors (1, 12, 13, 15, 16); K u s a m a *et al.* (quot. after S a i t o, 1961) showed in experimental studies on animals that even a delicate massage of the tumour region considerably increased the number of cancer cells in peripheral blood. The studies of M a r s h and M o o r e (quot. after 1) also proved more frequent hematogenic metastases in the experimental animals after massaging neoplastic tumours. R o b e r t s and C o l e (quot. after 16) observed "a shower of cancer cells" in peripheral blood during the operation performed on tissues in the neoplastic region. The results of the studies carried out as well as clinical observations indicate that, mechanical traumas of a tumour or even its region cause cancer cells to spit off from the tissue of the tumour, and then they are transported by the circulating blood or lymph. The results of these studies account for the necessity — if possible — to ligate the vessels nourishing and draining the tumour site immediately after its being localized and before its surgical removal.

The search for f.t.c. in the venous blood in patients with carcinoma, and particularly in patients with cancer of various organs has become the subject of many authors' studies. In oncological diseases in which it is easy to dia-

gnose clinical neoplastic changes being relatively readily confirmed by cytological examinations of smears or histological examination of oligobiopsy, the phenomenon of the f.t.c. occurrence in the venous or draining blood of such patients can be considered as a biological symptom of one of intermediate growth stages and cancer spreading in the carrier's organism. Although the fact itself of finding f.t.c. in the blood of patients with cancer, living without clinicals symptoms of hematogenic metastases does not explain the complex immunological mechanisms existing in these patients, the search, however for a correlation between the observed state of cancer cells disseminated into the blood and the clinical condition of the patient and biological condition of the tumour brings a very significant supplementary clinical material of cancer of the given organ.

Cancer of the larynx is particularly suitable for searching for possible correlations between the occurrence of f.t.c. in the venous blood stream and the dynamics of the development of neoplastic process. This results from the fact that the pathomorphology and local character of spreading of the primary focus (feature T, as well as histological malignancy) can be determined very accurately by visual, radiological and histological examinations in the earliest clinical period of this disease. Moreover, this organ is almost constantly under a "mechanical trauma" resulting from speaking, coughing and swallowing food as well as from physical strains (support of the diaphragm in movements of the glottis). The studies of the patients with laryngeal cancer were carried out on specially selected clinical groups. The completion of the particular groups of patients based on definite and uniform selection criteria in accordance with their clinical condition and therapeutic procedure was found indispensable to prepare the ground for searching for correlations between dissemination of f.t.c. and the clinical condition of the patients. Such a selection of patients and persons after treatment was only possible because the evaluation of the results of treatment and the efficiency of therapeutic methods was not the aim of the author's studies. The groups of the cancer patients studied were also selected with regard to the stage of clinical advancement of the cancer by applying a division corresponding to the final results (survival percentage) obtained by the therapeutic methods used at present. Only such a division of the material studied, the clinical classification of which is a true analogue of morphological pathomechanisms of cancer spreading (which affect hematogenic dessemination of cancer and consequently the occurrence of f.t.c. in the circulating blood), could be useful in the clinical search for a correlation between the features studied in the patients. The more so, because feature T of the tumour, whose tissual and cellular pathomorphology affects a constant or periodical but adequately frequent (under the influence of glottis movements) dissemination of f.t.c. into the blood stream, can be and usually is accurately defined; this saves the feature T from the clinical arbitrary interpretation which so often happens in its evaluation in other oncological diseases. Therefore, for the above mentioned reasons, the author did not use the European classification (version CIECL) in these studies but the American (AJC) modification of UICC classification.

The clinical groups studied also had to be divided with regard to the periods of the examinations carried out. However, those periods should involve patients at least with similar chances of survival with the therapeutic method applied, and excluding those patients whose qualification after

treatment did not correspond to that preceding the treatment (in operated groups B and C), because this could make the interpretation of the results difficult. Such a selection of the material was possible because the results of the studies carried out were compared only with the clinical condition of the patients examined (of the given group), and the efficiency of the individual therapeutic methods was not estimated. In the patients with laryngeal cancer, slowly circulating cancer cells in the venous blood stream were found. In the group of 78 patients with laryngeal cancer (group A) circulating cancer cells in peripheral venous blood were found in 61,5% of those tested before primary treatment of the tumour, in 54,8% of the patients qualified for radical treatment and in 88% treated palliatively. The statistical analysis of these results carried out showed: a) a significant relationship between the increase in dissimination of f.t.c. and the increase in the number of patients with a more malignant histological form of the tumour (II and III stage malignancy); b) a significantly more frequent dissimination of f.t.c. in cases of laryngeal cancers with initial supraglottic localization of the tumour, c) a highly significant dependence of the increase in dissemination on the stage of clinical advancement of cancer according to AJC, d) a highly significant dependence of the increase in dissimination of f.t.c. on the stage of advancement of the feature the observations carried out in this group of patients do not account for prognostic significance of f.t.c. present in the blood of patients with laryngeal cancer before primary radical therapy of the cancer (9).

In the group of 45 patients with laryngeal cancer (group B) treated surgically by total laryngectomy, circulating cancer cells were found in the peripheral venous blood in 62% of the tested patients before the operation, in the venous blood draining from the tumour — in 93% of the patients tested in the course of surgical treatment and in peripheral venous blood in 22% of the tested patients after the operation. The statistical analysis of these results carried out showed: a) a highly significant effect of the post-operative trauma on dissemination of f.t.c. into the blood draining from the tumour during the operation, b) a significant influence of the post-operative treatment on elimination of f.t.c. in peripheral venous blood on the 5th — 7th day after operation (after total laryngectomy). The observations in this group do not account for a prognostic significance of the increased dissemination of f.t.c. during the operation in the group of patients treated or laryngeal cancer by radical surgery.

In the control group of 40 persons (group C) in the period from 1 to 5 years after the radical treatment of laryngeal cancer (total laryngectomy) cancer cells circulating in peripheral venous blood were found in 2,5% of the patients tested and persons living without clinical symptoms of recurrence, and in 100% of the patients tested with clinical symptoms of recurrence after surgical treatment. The statistical analysis of these results carried out showed a highly significant relationship between clinical symptoms of cancer recurrence after its surgical treatment and the presence of f.t.c. in peripheral venous blood. It was found that f.t.c. present in the peripheral venous blood of the patients after radical surgical treatment of laryngeal cancer has a statistically significant prognostic value for further fate of the patients. Some authors also report that a more frequent dissemination of f.t.c. into the blood stream of patients can be influenced by such factors as the stage of clinical

advancement of cancer, its histological malignancy, and even its localization (1, 4, 5, 16, 17).

As it appears from the studies carried out, the occurrence of f.t.c. in the venous blood draining from the cancer during radical surgical treatment of laryngeal cancer had no prognostic significance for direct (post-operative) results of the treatment. The efficiently acting general immunological mechanisms rapidly eliminated circulating cancer cells from the blood stream to 22% of positive determinations on the 5th — 7th day after operation. With regard to the selection criteria of the patients in this group cases of total removal of the larynx were not taken into consideration, which during the operation appeared technically non-radical in relation to the feature T. In those patients — because of the possibility of a weakening general immunity due to the advanced tumour and operation stress as well — the possibility of applying prophylactic chemotherapy during operation should be taken into consideration, or higher portions of fresh blood should be transfused (above 11), which, as observations showed, effects the degeneration of circulating cancer cells by weakening their mitotic activity.

However, the statistical analysis of the results of tests for the presence of f.t.c. in the group of persons controlled from 1 year to 5 years after a radical operation of laryngeal cancer already showed a highly significant relationship between clinical symptoms and f.t.c. present in the peripheral venous blood of these patients. The observations on the occurrence of f.t.c. in the peripheral blood in 50% of the tested patients and in 59% of them in the appeared with or prior to symptoms of clinical recurrence. The author could not compare the results of his own studies on the occurrence of circulating cancer cells in the blood of the patients with laryngeal tumour with those of other authors because their publications were unavailable.

The numerical data concerning the percentage of f.t.c. observed in the venous blood stream in patients with cancer of various organs vary considerably in the papers of different authors. Engell (4) found f.t.c. in the peripheral blood in 50% of the tested patients and in 59% of them in the draining blood, as reported in his monography on the occurrence of f.t.c. in the blood of patients with cancer of various organs. Other authors give the following numbers determining the percentage of f.t.c. occurrence in the venous and draining blood stream in the studies they carried out in definite groups of cancer patients: Cousineau *et al.* (1961) — 39%, Saito (1961) — from 26,7% in peripheral blood to 100% in draining blood in various groups of patients, Roberts (1962) — 48%, Blichowski (1963) — 90% (Mammal cancer), Gerkowicz (1964) — from 56% to 93%, Nagy (1965) — 3,8% Waga (1966) — from 12,5% to 44,4%, Panecka (1969) — from 15,8% to 40%, Pimenta de Mello (quot. after 11) found f.t.c. in circulating blood in 96,5% of patients tested. Nagy (1965) reported that this percentage in various papers ranges from 1% to 96,5%. However, in a comparative interpretation of the occurrence of f.t.c. in the blood stream in general, and their levels in the reports of various authors in particular, in onocologically heterogeneous groups of patients (composed of various units), not only the frequently dissimilar efficiency of the methods used for concentration of cancer cells but variation of additional local external factors as well should be taken into consideration. These factors, which are characteristic for the given localization of cancer may affect the various dissemination of cancer cells of the given

tumour into the blood stream. Therefore, the author found it proper to search for a correlation between the number and level of f.t.c. occurrence in the venous blood stream and the clinical condition and the dynamics of the pathologic process within the same oncological unit and the same group of patients studied (Klonowski 1972). Although in vast majority of cases cancer cells including blood vessels undergo necrosis and destruction due to the action of immunological mechanisms of the carrier, part of them survive under certain conditions and form an active metastatic focus. The essence of immunological reactions of the organism is still unknown, which after result in generalization of the neoplastic process in spite of frequently constant dissemination of f.t.c. into the venous blood stream.

The carried out studies on the occurrence of f.t.c. in patients with laryngeal cancer in connection with clinical observations of these patients showed that the prognostic value of this test depends on efficient action or breakdown of immunological mechanisms of the carrier and — as can be assumed — on the „critical mass" of the circulating cancer cells. As the authors report (8) transplantation of 10^8 cancer cells to man frequently results in acceptance of the transplant. It should be assumed that such a number of cancer cells can overwhelm the efficiency of the mechanisms of immunological control in man with a neoplastic disease. Though further studies are needed to learn about the correlation between general immunity of the organism in case of neoplastic disease and the occurrence and distribution of free cancer cells, the results however, obtained in these studies indirectly elucidate some phenomena of clinical immunology in laryngeal cancer.

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STRESZCZENIE

Omówiono wyniki badań na obecność w.k.n. w krwi żylnej u chorych na raka krtani w badanych grupach. W grupie 78 chorych — grupa A — stwierdzono obecność krążących komórek raka w krwi żylnej obwodowej u 61,5 badanych przed pierwszym leczeniem nowotworu, w tym u 54,8% chorych kwalifikujących się do leczenia radykalnego i u 88% leczonych paliatywnie. Przeprowadzona analiza statystyczna tych wyników wykazała: a) istotną zależność między wzrostem wysiewalności w.k.n. a wzrostem liczby leczonych z bardziej złośliwą postacią histologiczną guza (2° i 3° hp), b) istotnie częstszą wysiewalność w.k.n. w rakach krtani o nadgłośniowym umiejscowieniu wyjściowym guza, c) wysoce istotną zależność wzrostu wysiewalności w.k.n. od stopnia klinicznego zaawansowania raka wg AJC, d) wysoce istotną zależność wzrostu wysiewalności w.k.n. od stanu zaawansowania cechy T guza nowotworowego. Poczynione obserwacje w tej grupie badanych nie przemawiają za znaczeniem prognostycznym obecności w.k.n. w krwi chorych na raka krtani przed pierwszym radykalnym leczeniem nowotworu. W grupie 45 chorych — grupa B — leczonych radykalnie operacyjnie całkowitym wycięciem krtani, stwierdzono obecność krążących komórek raka w krwi żylnej obwodowej u 62% badanych przed leczeniem operacyjnym, obecność w.k.n. w krwi żylnej drenującej u 93% badanych w czasie leczenia operacyjnego, oraz obecność w.k.n. w krwi żylnej obwodowej u 22% badanych po operacji. Przeprowadzona analiza statystyczna tych wyników wykazała: a) wysoce istotny wpływ urazu operacyjnego na wysiewalność w.k.n. do krwi drenującej z guza w czasie zabiegu, b) istotny wpływ leczenia operacyjnego na zanikanie w.k.n. w krwi żylnej obwodowej w 5—7 dobie od operacji (po całkowitym wycięciu krtani). Poczynione obserwacje w tej grupie badanych nie przemawiają za znaczeniem prognostycznym zwiększonej wysiewalności w.k.n. w czasie zabiegu w grupie radykalnie leczonych operacyjnie z powodu raka krtani.

W grupie 40 osób — grupa C — kontrolowanych w okresie od 1 roku do 5 lat po radykalnym leczeniu raka krtani (całkowite wycięcie krtani) stwierdzono obecność krążących komórek raka w krwi żylnej obwodowej u 2,5% badanych i żyjących bez klinicznych objawów wznowy raka, oraz u 100% badanych z klinicznymi objawami wznowy po leczeniu operacyjnym. Przeprowadzona analiza statystyczna tych wyników wykazała wysoce istotną zależność między klinicznymi objawami wznowy raka po jego leczeniu operacyjnym a obecnością w.k.n. w krwi żylnej obwodowej. Stwierdzono, że obecność w.k.n. w krwi żylnej, obwodowej osób po radykalnym leczeniu operacyjnym raka krtani, ma statystycznie istotną wartość prognostyczną dla dalszych losów chorego.

РЕЗЮМЕ

В группе из 78 больных раком гортани я обнаружил присутствие циркулирующих клеток рака в венозной периферической крови — у 61,5% исследовавшихся перед началом лечения вообще злокачественной опухоли в том числе у 54,8% больных, заквалифицированных на радикальное лечение, и у 88% из тех, кого лечили паллиативно. Статистический анализ полученных результатов показал: а) существенную зависимость между увеличением рассева с.к.о. и увеличением количества лечившихся больных с более злокачественной гистологической формой опухоли. (2° и 3° гп); б) существенно более частый рассев с.к.о. рака гортани с набсвязочной выходной локализацией опухоли; ц) высокосущественная зависимость увеличения рассева с.к.с. от степени клинического распространения рака, согласно „American Joint Committee on Cancer staging"; д) высокосущественная зависимость увеличения рассева с.к.о. от

степени развития свойства „T" опухолевидного новообразования. Наблюдения, проводившиеся в этой группе исследуемых не подтверждают прогностического значения факта наличия с.к.о. в крови больных раком гортани перед началом радикального лечения опухоли. В группе из 45 больных раком гортани, подвергшихся радикальному лечению, хирургическому, с полным удалением гортани, мною обнаружено наличие циркулирующих раковых клеток в венозной периферической крови у 62% исследованных перед операционным вмешательством; наличие с.к.о. в венозной дренирующей крови у 93% исследуемых во время операционного вмешательства, а также наличие с.к.о. в венозной периферической крови у 22% больных, исследовавшихся после операции. Статистический анализ этих результатов показал: а) высокосущественное влияние операционной травмы на рассев с.к.о. в крови, дренирующую из опухоли во время операции; б) существенное влияние на исчезновение с.к.о. в венозной периферической крови на 5—7 сутки после операции (при полном удалении гортани). Наблюдения над этой группой исследуемых не подтверждают прогностического значения факта увеличения рассева с.к.о. во время операции в группе больных, леченных по случаю рака гортани радикально, хирургически. В группе из 45 человек, контролируемых в течение 1 года — 5 лет после радикального лечения рака гортани (полное удаление гортани) мною обнаружено присутствие циркулирующих раковых клеток в венозной периферической крови у 2,5% исследуемых людей, у которых не наблюдались признаки возобновления рака, и у 100% исследованных с клиническими признаками возобновления рака после операционного вмешательства. Статистический анализ этих результатов показал высокосущественную зависимость между клиническими признаками возобновления рака после его хирургического лечения и наличием с.к.о. в венозной периферической крови. Выяснено, что наличие с.к.о. в венозной периферической крови у пациентов после радикального лечения рака гортани имеет статистически существенное прогностическое значение для дальнейшей судьбы больного.