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Cell Kinetics, Growth Rate and the Natural History of Breast Cancer. The Heuson Memorial Lecture

MAURICE TUBIANA and SERGE KOSCIELNY
Institut Gustave-Roussy, 94800 *Villejuif, France*

BREAST CANCERS have widely different clinical behaviour ranging from that of a highly aggressive neoplasm to a virtually chronic disease. Anatomical staging and histopathological grading provide useful prognostic information; however, there remain significant heterogeneities within groups of patients with tumours of the same size and microscopic morphology. This emphasizes the need for better prognostic indicators.

CLINICAL ASSESSMENT OF GROWTH TEMPO

In previous decades, considerable interest was given to the influence of tumour growth rate on the course of the disease. The growth tempo of tumours was assessed by questioning the patients and a lower survival for patients with a rapid growth rate was reported [1–3]. However these assessments of growth tempo were exposed to the subjectivity of both the patient and the physician. Attempts were made to reduce the errors associated with the inaccuracies in the answers of the patients regarding the duration of the symptoms and the time course of tumour size [4–6]. It was found that 5 years after initial treatment, categories of growth rates remained associated with significant differences in survival after adjustment for differences in lymph node involvement [6].

At Villejuif from 1963 to 1980, the growth tempo of breast tumours was recorded prospectively in all patients with breast cancer prior to the initial treatment. The patients were classified according to two criteria: the increase in size of the breast tumour as assessed by the questioning of the patients and the presence or absence of inflammatory reactions. The presence of inflammatory reactions has a strong prognostic impact [7]. Multivariate analyses showed that, ten years after treatment, the prognostic significance of inflammatory reactions remained significant, independently of all other prognostic indicators such as tumour size or histopathological grading. On the other hand, whereas rapid growth rate without inflammatory reactions has a significant prognostic influence in univariate analyses, its impact is much lower when, in multivariate analyses, other prognostic indicators (in particular the size of the tumour and the histologic grade) are included in the analysis (Fontaine *et al.*, in preparation). This lack of significance might partly be due to the imprecision inherent in the clinical assessment of growth tempo.

DOUBLING TIME

In the sixties, the introduction of the concept of tumour doubling time (DT) enabled a quantitative estimation of tumour growth rate. However the measurement of DT necessitated sequential mammographies over several months and it was felt unethical to submit patients routinely to such investigation. Nevertheless a few studies were performed on relatively small numbers of patients [8]. Gershon-Cohen *et al.* [9] and Heuser *et al.* [10] found a higher incidence of positive axillary nodes in patients with a rapid growth rate than in those with a slow growth rate. Kusama *et al.* [11] found in a retrospective analysis that the long-term survival after surgical treatment was higher in the subgroup of patients in which the long-term survival was identical despite considerable spread of DT. Moreover at Villejuif, Malaise *et al.* were able to show that the DT is correlated with the time interval between initial treatment and relapse as well as with the duration of survival after relapse [12].

LABELING INDEX

Thus these preliminary studies, despite their methodological limitations, underlined the clinical interest of the assessment of growth rate. In the early 1970s it became apparent that the percentage of tumour cells in S phase, as determined by thymidine labeling index (LI) is highly correlated with the proportion of tumour cells engaged in a proliferative cycle. This is due to the fact that the ratio of the duration of the S phase over the duration of the cell cycle is relatively constant among human tumour cells [13]. Moreover the studies carried out on experimental and human tumours showed a good consistency between LI measured *in vivo* and *in vitro* after incubation with tritiated thymidine [14]. Hence in a first approximation, *in vitro* LI is proportional to the potential DT.

The actual DT is always much longer than the potential DT which takes no account of the rate of cell loss which may be in some human tumours in excess of 90% of the rate of cell production [15]. However, despite this theoretical problem, studies have shown that there is good correlation between LI and actual DT, for two reasons: firstly the variations in the rate of tumour cell loss are relatively limited within a group of tumours of a given histologic type; secondly the rate of cell loss does not vary at random but is positively correlated with the growth fraction, hence although actual DT is not proportional to potential DT, there is a highly significant correlation between LI and actual DT [15].

In 1972, a prospective study was undertaken at Villejuif in which the LI was measured consecutively in 128 patients with mammary carcinomas. The preliminary results, reported in 1975, showed that the LI was higher in patients with relapse [16]. The data were updated in 1981 [17], 1984 [18] and recently. As we shall see below, the results confirm the high prognostic significance of the LI determination.

Several other groups have independently studied the prognostic significance of LI; two of these studies, those of Meyer *et al.* [20–22] and of Silvestrini *et al.* [22, 23], are of particular importance in view of the large number of patients included and of a follow-up ranging from 4 to 6 years. The conclusions of these three studies are consistent (Table 1) and we shall briefly summarize them.

In the three studies the value of LI is not correlated with the presence or absence of invasion of the axillary nodes nor with the clinical stage or the size of the primary breast tumour. However in the series of Meyer *et al.* [21], there is a slight increase in LI with increasing tumour size and in our series the LI is slightly higher in patients with a large number of involved axillary nodes [17], but in both cases the differences are relatively small. The LI is higher in patients with carcinoma with severe inflammatory reactions [17, 21], with undifferentiated histologic type, with necrosis. It is significantly correlated with histopathological grading [17, 21]. In the series of Meyer *et al.* which comprises 757 patients, mean LI showed a stepwise decrease with increasing age.

The main point which emerges from these three studies is the high independent prognostic significance of LI. Despite its correlation with the histopathological grade, LI has in our series a significant impact on relapse and death, independent of all the other prognostic indicators. In the three studies, multivariate analyses have shown that the prognostic impact of LI is independent of stage, size of the tumour, status of the axillary nodes and histologic grade; it remains significant within the group of patients without involvement of axillary nodes. This was recently confirmed by a study performed by Silvestrini *et al.* [23] on a series of 258 patients with operable breast cancer without nodal involvement. The 6-year relapse free survival was 80% in patients with low LI tumours vs. 60% in patients with high LI tumours. Moreover the prognostic significance of LI was observed both in premenopausal and post-menopausal patients and there are in the low LI subgroup patients with large tumours or with involved axillary nodes.

When it became apparent that the rate of early relapse was higher in patients with a high LI, the main question which arose from this observation was whether this higher incidence was due to a higher likelihood of distant dissemination or simply to a more rapid growth rate of occult metastases [16, 17, 20]. The follow-up in our series is longer than 15 years and it has become possible to answer this question. The time interval between initial treatment and detection of relapse is much shorter in patients from the high LI group (1 year) than in the intermediate group (3 years), however after a follow-up of 15 years the incidence of relapse is identical in the two groups (15-year relapse-free survival is equal to 30% in both subgroups) (unpublished data). Conversely it now clearly appears that the low incidence of relapse in the low LI subgroup (15-year relapse-free survival equal to 75%) cannot be explained only by differences in growth rates and therefore appears to be due at least partly to differences in the metastasizing probability of the neoplastic cells. From a practical point of view, this means that LI can help one to define the population of breast carcinoma patients most likely to benefit from adjuvant chemotherapy, even among patients without axillary node involvement.

Table 1. *Thymidine labeling index (LI) as predictor of relapse after radical therapy of breast carcinoma*

Author	Stage	Interval (years)	LI	Probability of relapse
Tubiana *et al.* [42]	(T_1–T_3)	15	Low	0.25
			Intermediate	0.7
			High	0.7
Meyer *et al.* [20]	I	4	Low	0.1
			High	0.5
	II		Low	0.3
			High	0.5
Silvestrini *et al.* [23]	I, II (N−)	6	Low	0.20
			High	0.40

RELATIONSHIP WITH DNA CONTENT AND OTHER BIOLOGICAL CHARACTERISTICS

The information available from LI assays is therefore remarkable; however, the method has two disadvantages: (i) the measurement is laborious; (ii) it requires at least 10 days for processing. This is why its use remained limited till these technical problems were overcome during the past few years. New techniques have been introduced for the estimation of the proportion of proliferating cells. The assay of the DNA content of tumour cell nuclei, either by flow cytophotometry or by image cytometry, enables the measurement of the proportion of cells in S phase in diploid tumours; it yields results which are similar to the LI [24]. In aneuploid tumours or in tumours in which several subclones are present, the determination of the proportion of cells in S phase is less accurate. However, a good correlation was reported between thymidine LI and percentage of S-phase cells determined by cytometry [24, 25]. Moreover, bromodeoxyuridine (BrdU) can be used as a substitute for tritiated thymidine and labeled cells can be detected by use of an antibody to BrdU. The combination of an assay of cell DNA by flow cytophotometry and of BrdU labeling gives information on the proportion of cells engaged in a cell cycle, the duration of the cell cycle and the ploidy of the tumour cell population [26].

The data clearly show that the mean LI of aneuploid breast tumours is significantly higher than that of diploid tumours [26, 27]. For example, in the study on 1000 tumours by Dressler *et al.* [28], the median proportion of cells in S phase was 2.6% in diploid tumours vs. 10.4% in aneuploid tumours ($P < 0.0001$). In some tumour types aneuploidy appears to be associated with poor prognosis [29–31]. Thus it remains to be examined in breast tumours whether the unfavourable prognostic variable is aneuploidy or a high proportion of S-phase cells. In a few types of tumours, the data suggest that poor prognosis is linked to the higher growth fraction rather than to aneuploidy but in breast tumours no data are yet conclusive [32]. It is noteworthy that pretreatment ploidy of the primary tumour as well as the pretreatment LI [23] have very little influence on survival after relapse.

Breast tumours with diploid DNA content tend to be of low histologic grade and oestrogen receptor rich, whereas those with higher ploidy are more anaplastic and poorer in oestrogen receptor proteins [25, 33–35]. It has been reported [27] that the proportion of cells in S phase was highest in hypodiploid tumours which is also the group with the lowest levels of oestrogen and progesterone receptors. This emphasizes the strong correlation which exists between these various biological characteristics. However, despite the existence of a significant negative correlation between LI and oestrogen or progesterone receptor content [21, 23, 32], LI is a prognostic indicator independent of steroid receptor content. Thus the simultaneous measurement of the hormonal receptors, ploidy and LI or the percentage of S-phase cells is not redundant and may help one to identify the subset of poor risk patients, in particular among axillary node negative patients [28].

Studies are currently investigating EGF receptors in breast cancer biopsies [36–38]. The data have shown a striking relationship between EGF receptor level and/or EGF binding affinity and the proliferative rate [36, 38], whereas there is much less correlation between ploidy and EGF receptor detection [36].

Currently, one of the main avenues for research is the study of correlation between proliferative rate and oncogene expression or amplification, in particular that of those oncogenes which are assoc-

iated with growth factors or receptors for growth factors.

BREAST CANCER NATURAL HISTORY

Another avenue for research is the analysis of the influence of growth rate on breast cancer natural history, in particular the size of the primary tumour at metastatic dissemination. The natural history of human breast cancer is a topic which has been much discussed but for which quantitative data were scanty till recently.

During the past two decades, numerous investigations have shown that the growth rate of human breast cancers is throughout its clinical course either constant or progressively decreasing [39]. Moreover when the growth rates of the primary tumour and of its distant metastases were measured, it was found that they are related but the latter are more rapid [39]. On this basis, we developed a model of the natural history of breast cancer in order to extract from the analysis of a series of 4000 patients treated at Villejuif, information which otherwise could not have been obtained [40, 41].

The study of the relationship between the size of the breast tumour and the dissemination probability was made without any assumption as to the pattern of tumour growth. The study of experimental tumours has shown that a threshold volume at which the first metastasis is initiated exists for each tumour type. By analogy the increase in the incidence of metastasis as a function of the clinical diameter of the tumour in a series of patients can be interpreted as the increasing proportion of tumours which are larger than their threshold volume [40].

We therefore subdivided the population of patients into eight classes according to the volume of the tumour at surgery and plotted for each class the actuarial cumulated proportion of patients with metastases as a function of time after treatment up to the 25th year. This proportion at 25 years is assumed equal to the dissemination probability before initial treatment in this class of patients. The volume at the time of detection (in logarithmic coordinates) and the metastasis initiation probability (in probit coordinates) shows a remarkable linear relationship. Thus, the threshold distribution is lognormal, with a median (termed V_{50}) of 23.6 ml (dia. = 3.56 cm) and a 95% confidence range of individual values from 0.14 to 4000 ml [40].

In a given patient, the time interval between the treatment of the primary tumour and the detection of a distant metastasis depends upon four variables: the size of the tumour at the time of metastatic dissemination, the size of the tumour at treatment, the doubling times of the tumour and of the metastases [39]. In a subgroup of patients, the mean size of the tumour at the time of treatment is known, the size of the tumour at the time of metastatic dissemination can be calculated, moreover the ratio of the doubling times of the primary tumour and of the metastases is known [39, 41]. Therefore in the various subgroups of patients, the duration of the doubling times can be assessed through the computer analysis of the metastasis appearance curve as a function of time after initial treatment.

Thus it becomes possible to compare the natural history of patients with different values of the primary tumour DT. The results of the calculations are remarkably consistent with the results of our study on the prognostic significance of the LI. They make it possible to prolong the curve till the 25th year and to foresee that even after this long follow-up, there will be a markedly lower incidence of relapse in patients with a slow growth rate than in the other two groups.

Our previous studies on the mean tumour volume at distant spread (V_{50}) have shown that two main prognostic variables have a strong impact on this volume: the histologic grade and the number of involved axillary nodes [40]. However, we have recently shown that the significance of the number of involved nodes is not the same for a small or a large tumour [42]. In order to assess the propensity of a tumour to invade lymph nodes, it was therefore necessary to combine the two pieces of information (size of the tumour and number of involved lymph nodes) in one figure. We have developed a method for assessing the tumour size at the involvement of the axillary lymph nodes and have shown that it is possible, in each subgroup of patients, to compute the mean tumour size V_1 at the time at which the first axillary node was invaded (Koscielny *et al.*, unpublished data). The results show that on the average during tumour progression, the capacity for lymphatic spread is acquired much earlier than the capacity for haematogenous spread. There is a highly significant correlation between V_{50} and V_1. Three subsets of tumours were compared: with a small, intermediate or high value of V_1. The differences in the mean volumes of the tumour at initiation of distant metastatic spread and axillary node involvement are considerable between the three subsets. The value of DT in the three subsets of patients ranges from 3.5 in the subgroup with the small V_1 to 7 months in the intermediate subgroup and 14 months in the most favourable subgroup. In the subset of patients in whom the tumour is rapidly growing, first axillary lymph node invasion occurs when the tumor diameter is approx. 2–3 mm and metastatic dissemination is initiated when the tumor is relatively small (less than 1.5 cm dia.). On the contrary, for tumours with a slow growth rate, the first axillary lymph node is invaded when the tumour diameter is approximately equal to 4 cm and on average the first distant metastasis is initiated only when the tumour reaches 6 cm in

diameter.

However, the present analysis is not consistent with the concept that breast cancer is a conglomerate of different diseases with widely different natural histories. The unimodal distribution of the parameters rather suggests that there is a continuum from slow growing disease with late axillary involvement and distant dissemination to the most aggressive, rapidly growing and early metastasizing subtype.

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