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Markers and Prognostic Factors in Breast Cancer Disease; Workshop Report

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INTRODUCTION

THIS report is based on 31 posters from the 3rd EORTC Breast Cancer Working Conference and the literature evaluated in the light of a common surgical pathologist's and a surgeon's view.

Markers and prognostic factors may be divided into four main groups: the patient herself (e.g. age, race, age at menarche, first birth, menopause, etc.), biochemical factors, histopathological factors, and growth rate, the borderline between the groups not being too definite.

In the workshop the abstracts submitted were divided into the following groups: age as a prognostic factor, the histopathological factors, the biochemical factors, steroid hormone receptors, and DNA and growth rate problems.

To include all these topics in a 3-hr workshop was really a challenge to the audience and to the chairmen. We naturally understood that a lot of common problems existed: first and foremost, selection problems, tumour inhomogeneity problems, and problems with methods and measurements.

From the beginning we realized that breast cancer disease is a diffuse transformation of glandular tissue, including both breasts. Furthermore, we had a growing understanding that the prognosis is mostly determined by the first clinically appearing cancer, probably reflecting the fastest growth rate in a particular case. We accepted and discussed the very essential development stages in the breast cancer disease, i.e. the first stage represented by females with *in situ* carcinoma, the next stage by the development from *in situ* to invasive carcinoma, and the last stage by recurrences and deaths. We emphasized that probably about 25% of all women are included in the risk group for breast cancer harbouring *in situ* carcinoma and that about one-third of all these women at risk develop invasive breast carcinoma. In our research today we are mostly dealing with invasive carcinomas, leaving the patients at risk to the future.

AGE AS A PROGNOSTIC FACTOR

This is really an interesting and controversial problem. We had two posters attacking the problem in two different ways, just as we meet the problem in literature. Muscolino *et al.* [1] evaluated a consecutive series of 135 patients aged less than 35 yr. The patients were stages in node-negative and -positive groups. The 5-yr survival was 91.1 and 48.8% respectively; the same for patients of all ages. They emphasized that the ratio between node-negative and node-positive patients among these young women was 1:2, compared to 1:1 in all ages.

In this way of evaluating the problem it must be understood that we pick up about 1% or less of all breast cancer cases and, therefore, in rather small studies, we may obtain contradictory results by chance. Table 1 shows one large, older series of patients and some series from the last 10 yr, all approaching the problem in the same way as Muscolino. Some of the studies show no difference in survival comparing younger women with all ages or older women and some show a poor survival, especially in node-positive patients. It must be emphasized that the follow-up periods in the papers stating no difference are only 5 yr, as opposed to papers with poorer prognosis, where the follow-up periods seem to be longer.

Høst *et al.* [7] attacked the problem by evaluating the age distribution in the total Norwegian breast cancer material from 1953 to 1978, including more than 28,000 cases. The 5-yr age-adjusted relative survival rate appears in Table 2. Both younger and older women seem to have a lower survival rate. The differences could not be explained by differences in staging or, as regards younger women, differences in therapy.

Table 1. *Survival of breast cancer in younger women compared to all ages or older women*

Reference	No.	Age (≤) (yr)	Follow-up (yr)	Survival
[2]	549	35	5	no difference
[3]	58	30	5	no difference
[4]	111	30	10	no difference (node-negative) poorer (node-positive)
[5]	85	35	10–20	
[6]	803	40	5–15	poorer
[1]	135	35	5	no difference

Table 2. 5 yr *relative survival rate in various age groups*

	Age			
	≤34 yr	35–49 yr	50–74 yr	≥75 yr
Survival rate (%)	59	72	63	53

Table 3. *Histopathological factors*

	certain	*in situ* carcinoma (DCIS, LCIS)
Markers	uncertain	atypical hyperplasia cyst multiple papilloma radial scar juvenile papillomatosis
Prognostic factors	'certain'	node involvement tumour size tumour type tumour grading invasion of blood vessels
	uncertain	invasion of skin, deep fascia, lymphatics, nerves tumour growth patterns tumour necrosis stromal factors immunological factors, etc.

No information about differences in autopsy rates and death certificate information was available, but this must surely influence the results.

In the excellent, meticulous study of Mueller *et al.* [8] on 3558 breast cancer cases, stage and old age were significant determinants on the length of survival and cause of death. They mentioned that 12% of all causes of death was unrelated to breast cancer, being only 4% in younger patients but 23% in the oldest age group.

We may therefore conclude that age seems to be a real prognostic factor. A poor prognosis in the youngest and eldest patients may be considered, but we have no explanation of the results.

HISTOPATHOLOGICAL MARKERS AND PROGNOSTIC FACTORS

The prognostic factors are all well known, most of them appearing in Table 3. Histopathological factors as markers are, on the other hand, mostly overlooked. Certainly, *in situ* carcinomas, both of ductal and lobular types, are the most convincing known markers today. Thus, as regards lobular carcinoma *in situ*, we know from the literature that about one-third of all only biopsy-treated cases later develop invasive carcinoma [9]. Concerning microfocal intraductal carcinoma, the literature [10, 11] seems to indicate the same frequency of later development of carcinoma.

Regarding histopathological prognostic factors, the most essential factors are node involvement or not, size of tumour, and nuclear and histological grading. Most marker investigations compare their possible significance to these factors, but not always with success. Therefore a short critical evaluation of the parameters seems desirable.

Our people often forget or do not know that the histopathological statement 'node-negative' is rather uncertain in daily routine investigations. Saphir and Amromin [12] in 1948, and later E. Fisher *et al.* [13], by step sectioning the rest of the lymph nodes in so-called node-negative cases, showed micrometastases in about 30% of all cases. The latter found no significant influence on recurrence and stated that detection of micrometastases by extending histopathological methods may be more academic than practically or therapeutically significant. This statement was founded on only 5 yr of follow-up, and from our knowledge about doubling time 10 or even 15 yr may be necessary for such a strong statement. In 1981 Rosen *et al.* [14], in T_1 tumours, confirmed Fisher's results after 6 yr of follow-up, but after 12 yr a significantly worse prognosis was found in cases with micrometastases compared to node-negative patients. Furthermore, the recurrence

was now similar to real node-positive patients, i.e. cases with macrometastases.

Therefore lymph node status is not as discriminating a prognostic factor in daily routine work as it probably could be. Knowledge about the pathologic procedure is really essential in evaluating the predictive value of the statements.

Concerning size of tumour, all agree that small tumours have a better prognosis than large tumours. Other factors, first and foremost node status, are necessarily incorporated for real evaluation of the predictive value of tumour size. In 1969 B. Fisher *et al.* [15] very clearly showed that node-negative patients with tumours less than 3 cm in diameter had a recurrence rate within 5 yr of 15%, compared to 26 for all tumours more than 3 cm in diameter. In node-positive patients the percentage difference in recurrences was higher, about 25%. This higher difference is probably totally explained by the larger ratio of cases with four or more lymph nodes involved in large tumours compared to small tumours. We also found these rather small differences in recurrences in the Danish trial (DBCG) [16]. In 522 premenopausal and 1070 postmenopausal node-negative cases the differences in recurrence within 3 yr were only 4 and 9% respectively. Among node-positive patients we found, just like Fisher *et al.* [15], a larger difference. We know that some investigations cannot even confirm tumour diameter as a prognostic factor, possibly because of small samplings or perhaps differences in the way of estimating tumour size. It is common knowledge that the tumour diameter decreases by fixation and increases by measuring on histological slides by 10–30%. Furthermore, differences in clinical compared to pathological evaluation of tumour size may be more evident than Fisher *et al.* [15] found. In the Danish trial we made a comparison of the clinical and pathological evaluation of tumour size (Table 4). In 19% the tumour sizes were unequally assessed, meaning that the difference in assessment was more than 1 cm. Naturally, such differences, and in some investigations possibly differences in methods, may influence the results.

The third essential prognostic factor, the histologic and nuclear grading, has in many retrospective (e.g. [17, 18]) and a few prospective investigations [19, 20] found to be significantly related to recurrences and survival, and to a lot of other prognostic factors. This is certainly true in investigations where a single pathologist, or a small group of pathologists, does the job. Even in multicentric investigations a significant relation can be found [19].

Table 4. *Comparison of clinical and pathological tumour size assessment in* 1624 *patients*

	Tumour size (cm)			
	0–2.5	2.5–5	>5	Total
Equally assessed	321	416	81	818
Unequally assessed (all)	359	344	103	806
Unequally assessed (>1 cm)	46	203	63	312 (19%)

The grading method includes two very serious problems: (1) heterogeneity of a tumour; and (2) inter- and intraobserver variability.

Regarding heterogeneity, Bloom and Richardson [17] mentioned the problem but denied its practical relevance. Haagensen [21] and Schiødt [22] found a significant difference in various tumour areas in 11 and 10% respectively. Poulsen *et al.* [23] even demonstrated a difference of 33%. Therefore in this connection tumour heterogeneity may also have a much larger influence on grading results than previously thought.

As regards observer variabilities, older and more recent studies indicate them to be about 30% for interobserver variation and between 10 and 30% for intraobserver variation [24–26]. When six pathologists, educated at the same institute, later evaluate 200 ductal carcinomas, they only agreed on 14.4% of the cases [27].

A preliminary result from the Danish trial (Table 5) shows a comparison between one pathologist and more than 100 participating pathologists from the whole of Denmark. This comparison is disappointing as regards the diagnostic value of common multicentric investigations, with a kappa value of about 0.4.

Table 5. *Histological grading: a comparison between one pathologist and many*

		Mr Andersen		
		I	II	III
25 participating departments	I	41	23	2
of pathology, i.e. more than	II	28	87	20
100 pathologists	III	0	22	15

Kappa = 0.391.

All together, therefore, grading in large materials certainly has a significant relation to recurrences and survival, but it cannot be a decisive factor for stratification of patients to adjuvant therapy.

In the workshop the accepted posters primarily reflected this essential subjectivity problem. Thus many studies included DNA measurements, morphometric factors and mitotic index. Baak *et al.* [28] found an adjunctive prognostic value of these histomorphometric parameters to lymph node status and tumour size. Kuenen-Boumeester *et al.* [29] applied morphometric measurements

on FNA smears and found that variation in nuclear area together with axillary metastasis was the most important prognostic factor. In preliminary studies including quantitative cytometric techniques (nuclear size, chromatin pattern, and DNA) together with flow-cytometry, van de Velde *et al.* [30] showed that node-negative breast cancer patients can be divided on the basis of these objective and reproducible measured tumour characteristics. Therefore these and other studies may in future give us more objective methods that may be predictive for the single patient, but it is as yet too early to be definite.

It is evident that lymph node involvement or not is without any doubt the most essential single prognostic factor, all the other prognostic factors being only of minor adjunctive value.

BIOCHEMICAL MARKERS AND PROGNOSTIC FACTORS

For a surgical pathologist and a surgeon it is certainly the most difficult topic, incorporating an overwhelming amount of different research methods. In Table 6 the biochemical markers are traditionally divided into two main groups, *viz.* the tumour-derived markers and the tumour-associated markers. Among the normal breast tissue products are, for instance, casein and lactalbumin, and among oncofoetal proteins, CEA. With hormones we are first and foremost thinking of calcitonin and HCG.

What characterizes the ideal marker? It must be sensitive enough to detect a very small tumour cell burden. It must be specific for breast cancer. Measurement may be correlated to tumour burden. Furthermore, it must be easy and economical.

Besides knowledge about the ideal marker, it is necessary to realize the successive stages in testing a potential marker. These stages are: firstly, successful testing in metastatic disease; secondly, evaluation in localized disease both in the pre- and postoperative situation; thirdly, evaluation in high-risk groups; and lastly, evaluation in the general female population. Most studies seem to include only the first and, mostly, the second stage.

Another way of understanding the biochemical markers and the stage problem is to clarify for oneself marker priorities. What is it that the clinician really wants of a marker? First and foremost, the clinicians want a marker for early detection, i.e. before dissemination; next, a prognostic marker which predicts recurrences with a very long lead time; then a prognostic factor showing minimal tumour cell burden recurrences; and finally, a marker for monitoring of therapy.

Table 6. Biochemical markers and prognostic factors

Tumour-derived	normal breast tissue products
	oncofoetal proteins (antigens)
	hormones
	enzymes
	receptors
	cell turnover products
	viral proteins
Tumour-associated	plasma proteins
	hydroxyproline
	miscellaneous

Research papers on this topic are difficult for most of us and we are, therefore, more or less dependent on review articles: e.g. the brilliant reviews by Coombes [31] in 1978, and by Neville [32], Edgington and Nakamura [33] and Imam [34] in 1982. From these reviews it is possible to conclude that the ideal marker has not yet appeared, but in the latest reviews some optimism exists about new monoclonal and monospecific antibodies against macromolecules derived from cell surfaces—cytoplasmic or secretory components.

Our six posters about biochemical markers mostly confirmed earlier investigations. Thus Deshpande *et al.* [35], now after 3–7 yr of follow-up, continuously found a significant relation between recurrences and some changes in enzymes related to carbohydrate metabolism. In a new prospective study including more than 400 patients it seems possible to separate patients into high- and low-risk groups on the basis of the activity of these enzymes in the carcinoma.

Frühling *et al.* [36] confirmed previous evidence about recurrences and serum CEA levels. They combined the CEA results with scintigraphic investigation of regional lymph nodes and seemed to get more significant results, but the amount of material was small.

Miller *et al.* [37] investigated the relationship between tumour cyclic AMP-binding proteins and prognostic factors and prognosis. They found no relation to common prognostic factors including oestrogen receptor values, but they established a significantly higher value of cyclic AMP-binding proteins in patients developing recurrences. This study and most other biochemical studies forget information about possible heterogeneity in a carcinoma. Together with some uncertainty about selection of material and small samplings, it makes any conclusion only suggestive.

Oglobine *et al.* [38] investigated serum α-1-alkaline phosphatase and found a clear relation between negative enzyme values and absence of liver metastases. With high enzyme values, on the other hand, hepatic metastases were found in about 50% of the cases. This study indicates the special predictive significance of the negative value.

Zangerle *et al.* [39, 40] again concluded that, unfortunately, lactalbumin is not a marker for breast cancer, but their studies give us a lot of information about cell differentiation.

All together we were impressed by the very beautiful biochemical studies. We realized that they have the same selection and sampling problems as, for instance, the pathologists have. They have not yet found the real marker or prognostic factor, but they have collected essential information about tumour biology.

STEROID RECEPTORS

In his review 'Breast cancer: hormone receptors, prognosis, and therapy' in 1982, DeSombre [41] concludes: "Patients with receptor positive lesions appear to have longer disease-free intervals and prolonged survival when compared with patients whose cancers lack estrogen receptors", and he continues: "This conclusion is still somewhat controversial". In two out of the six papers quoted in this part of the review, no significantly longer survival was established among oestrogen-receptor-positive compared to oestrogen-receptor-negative patients. In a third paper only a marginally significant difference in survival related to tumour oestrogen receptor status was found within all patients.

Lack of time necessitated that in our workshop we directed our attention to hormone receptors as supposed prognostic factors, leaving the therapeutical aspect to others. Of six papers four primarily evaluated this essential problem.

In a prospective, controlled clinical trial on adjuvant therapy of stage II tumours, Rydén *et al.* [42] showed after 2 yr of follow-up (mean) a significant difference in recurrences between receptor-negative patients and patients with high values of oestrogen receptors. The same tendency was established with progesterone receptors, although not as pronounced. Now, after 3 yr of follow-up, these significant differences seem to have disappeared. In this study two-thirds of the patients are on tamoxifen treatment, but, regrettably, information concerning the effect of this adjuvant treatment is not available at the time of going to press.

In the study by Harland *et al.* [43] of 655 patients, now with a maximum period of follow-up of about 6.8 yr (mean 2.1 yr), no significant differences were found in disease-free survival whatever the ER or PR content of the primary tumour and whether or not lymph nodes were involved. As regards overall survival, a significant relation was found but only confined to node positive patients. The authors claim that such differences in survival could arise solely as a result of treatment of the relapses.

Furthermore, Blamey *et al.* [44], in 550 cases, found no difference in incidence of distant metastases between receptor-positive and -negative tumours, but size of initial metastases was significantly different with bone metastases in receptor-positive cases and visceral metastases in oestrogen-receptor-negative cases.

With an average follow-up of 55 months, Caldarola *et al.* [45] found in 179 women significantly lower recurrences only in node-positive cases. Like these authors, we think that these differences may be explained as an effect of the hormonal adjuvant therapy.

Our posters thus seem to indicate no differences in recurrence after 3–5 yr of follow-up (Table 7) whatever the ER or PR content of the carcinoma. The primary effect on recurrences may be explained by the selective effect of adjuvant hormonal therapy and earlier recurrences in ER-negative tumours. Therefore the prognostic significance of receptors is still not quite clear. The European materials seem to point to no

Table 7. Comparison of recurrences in ER-positive and -negative tumour cases

Reference	*n*	Mean follow-up (months)	Significant differences
[42]	225 premenopausal 337 postmenopausal	36	No Yes
[43]	655	25	No
[44]	468	—	No
[45]	179	55	No (node-negative) Yes (node-positive)

Table 8. Comparison of recurrences in PR-positive and -negative tumour cases

References	*n*	Mean follow-up (months)	Significant differences
[42]	170 premenopausal 254 postmenopausal	36	No
[43]	625	25	No

significance of receptor status or only a very short prolongation of the disease-free interval and survival in receptor-positive cases.

DNA AS A PROGNOSTIC FACTOR

The DNA problem was evaluated in four posters, all generally focusing on the flow-cytometry technique. In the study by Cornelisse *et al.* [46] more than 150 breast cancer cases were investigated. More than 70% of these cases were aneuploid. The diploid tumours tended to be more frequently oestrogen-receptor-positive and of histological grade I than aneuploid tumours, but not significantly so. No correlation with age or node involvement was established.

Killander *et al.* [47] evaluated the value of oestrogen receptors and DNA assay together in a study of more than 400 patients from a prospectively randomized clinical trial. The recurrence rate in postmenopausal patients was only 8% in diploid and oestrogen-rich tumours, compared to 47% in aneuploid and oestrogen-poor tumours. They found an adjunctive value of DNA assay in stage II tumours, especially in postmenopausal and T_2NO tumours.

From the four posters [46–49] and the latest DNA literature [50–54] we may conclude: the DNA values have no diagnostic relevance: about two-thirds of all breast carcinomas are non-diploid. No significant relation can be found with mean age, tumour size or node status. A trend or significant relation is found with steroid receptor content and histologic grading. Furthermore, a supposed significance of diploid tumours against non-diploid tumours as regards fewer recurrences seems to be characteristic.

TUMOUR INHOMOGENEITY

Our workshop was not able to include papers on growth rate problems or thymidine labelling in relation to prognosis, but a study by Lambert [55] discussed the very serious problem of tumour inhomogeneity. Of 22 carcinomas 19 showed significantly different labelling index values from tissue slide to tissue slide within a single tumour. The differences in labelling index were up to 4-fold. He concluded that the labelling index or any other cell kinetic index based upon evaluation of a single small sample is subject to considerable sampling error and is unlikely, therefore, to be of significant prognostic value. We may probably include histological grading, steroid receptors and other biochemical tumour markers in this sampling error problem.

It may be a little off-putting to continue studies of supposed markers and prognostic factors knowing that the last two or three decades have not given us a real marker of practical importance. But we must not forget that all these studies provide us with a lot of information about tumour history and biology and, therefore, we should necessarily continue these studies. Naturally, we must concentrate more on avoiding selection errors, sampling errors, etc. Thus in future it will be necessary that materials be picked up in prospective, randomized studies and that inhomogeneity problems of carcinomas are primarily evaluated.

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