PROGNOSIS OF BREAST CANCER:
A SURVIVAL ANALYSIS OF 1184 PATIENTS WITH 4–10 YEARS FOLLOW-UP, ILLUSTRATING
THE RELATIVE IMPORTANCE OF ESTROGEN RECEPTORS, AXILLARY NODES, CLINICAL STAGE
AND TUMOR NECROSIS

By

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Abstract

Prognostic indicators, measured at diagnosis, are important in breast cancer. They help clinicians select optimal treatment, provide rational bases for stratification of treatment trials and assist analysis of response to treatment. Univariate statistical survival curves have identified many such indicators. However, they do not explain why some patients, classified as favoured by one or other factor(s), experience early treatment failure, nor why a substantial number with unfavourable signs remain recurrence-free many years later. This study was undertaken to identify independent prognostic factors with the use of multivariate regression.

A Cox proportional hazards model of disease-specific survival was based on 1184 primary breast cancer patients referred to the Cancer Control Agency of B.C. between 1975 and 1981 (median follow-up 60 months). Significant univariate associations with overall survival were found for estrogen receptor concentration ([ER]), axillary nodal status (N0, N1-3, N4+), clinical stage (TNM I, II, III, IV), histologic differentiation and confluent tumor necrosis (minimal, marked). These factors were assessed at primary diagnosis. A subset of 859 patients with complete data on these variables and also histologic type, menopausal status, age, tumor size and treatment was used to fit the multivariate model. Nodal status was the most important independent factor but three others, TNM stage, [ER] and tumor necrosis, were needed to make adequate predictions. A derived Hazard Index defined risk groups with 8-fold variation in survival. Five-year predicted survival ranged from 36% (N4+, loge[ER]=0, marked necrosis) to 96% (N0, loge[ER]=6, no necrosis) with TNM I and 0% to 70% for the same categories in TNM IV. This wide variation occurred across all stages. Study of post-recurrence survival (369 patients) yielded a model with only three independent predictors: [ER], nodal status and tumor necrosis.

Survival - overall, recurrence-free and post-recurrent - is predictable by modelling a few factors measureable at diagnosis. Use of ER concentration, rather than the more common ER status (+ or -), greatly strengthens the model. Presence of ER was also shown to be increasingly important as 'protective', attenuating the effect of other factors, as risk of mortality increases.
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INTRODUCTION

BREAST CANCER, THE MAGNITUDE OF THE PROBLEM

Breast cancer has been and continues to be the most commonly diagnosed malignancy in females. The estimated number of new cases in Canada in 1987 was 11,400, or 25% of all major-site cancers. Breast cancer is also a leading cause of cancer deaths; an estimated 4,400 female (or 20% of total cancer deaths) died of the disease in 1987.

At a provincial level, breast cancer constitutes an estimated 27% of all female cancers occurring in British Columbia (1,300 new cases) in 1987. About 490 British Columbian females died of breast cancer in the same year, equivalent to 19% of all female cancer deaths.

Time trends of age-standardized incidence and mortality rates for breast cancer have been recorded in Canada from 1970 to 1982, and from 1970 to 1985, respectively (Canadian Cancer Society, 1987). Both trends show minor fluctuations over the years, but a progressive upward trend is evident at 1982 in the incidence curve and at 1985 in the mortality curve. The age-standardized incidence rate has ranged from 62 to 70 per 100,000 population. The age-standardized mortality rate has ranged from 23 to 24 per 100,000 population.

NATURAL HISTORY OF BREAST CANCER

Breast cancer is one of the most interesting human malignancies because of its extremely varied natural history. The majority of patients in western societies present with resectable disease, but most will eventually die of the cancer after an often protracted course. The manifestation of distant metastases after adequate local control is a significant marker of impending death if left untreated. Even if systemic treatment are given at this point, median survival rarely exceeds 24 months (Bonadonna & Valagussa, 1983), and once metastatic dissemination is established the probability of cure is negligible.

Breast cancer follows an unpredictable course (Keys et al., 1983). At one end of the spectrum there are patients whose cancer progresses so rapidly that available treatments will not alter the
Introduction

outlook. Patients at the other end of the spectrum may survive twenty years after initial
treatment by simple mastectomy and radiotherapy (Dixon et al., 1985). Long term survivors are
still confronted with a greater than expected frequency of deaths when follow-up beyond twenty five
years has been done (Brinkley & Haybittle, 1984). Early reports on the natural duration of
untreated breast cancer found that the average duration of survival was about 38 months, and
although no cases were spontaneously cured, the clinical course of these untreated patients was
variable (Haagensen, 1986).

While the etiology of human breast carcinoma remains speculative, there is some understanding
of processes of neoplastic transformation of normal breast epithelial cells to a state of
autonomous growth. Regardless of the evoking stimulus, a prevailing condition in the early
biologic history of the carcinoma is unregulated proliferation (Robbins et al., 1984). Although
there are no definite morphologic precursors of intraductal carcinoma and lobular carcinoma in
situ, some ductal and lobular hyperplastic lesions may predispose to in situ carcinoma development
(Gould & Morales, 1986). Page et al. (1985) found that the subsequent risk of invasive breast
cancer after atypical ductal or lobular hyperplasia is four to five times that of age-matched
female from the general population. This risk is also about half that often cited for lobular
carcinoma in situ and ductal carcinoma in situ (Rosen et al., 1978).

Even when neoplastic transformation has taken place and is evident microscopically, there is
uncertainty regarding the progress of lobular carcinoma in situ in any given patient. Haagensen
(1986) advocated the replacement term of "lobular neoplasia", since the majority of patients who
have had this condition diagnosed do not subsequently develop invasive cancer after "treatment" by
biopsy only. However, 17-25% of the patients similarly treated do subsequently develop invasive
carcinoma over a period of 25 years (Haagensen, 1986). This observation is consistent with the
fact that a high frequency of multicentricity is often found in association with lobular neoplasia
(Rosen et al., 1978). It also questions the ability of light microscopy to definitely exclude the
presence of small foci of invasion, even with extensive examination of the tissue blocks (Millis,
1983).
Introduction

Although some benign breast lesions are significant risk factors for breast cancer development (Shingleton & McCarty, 1987), the critical difference between benign lesions and malignant tumors is that the latter have the capacity and propensity for local invasion and systemic dissemination. These events may or may not coincide with exponential tumor growth, although tumor cells may enter lymphatic and vascular channels (potential routes for metastases) after about 20 net doublings (Coppin & Swenerton, 1983). However, the doubling time of individual breast tumors may vary considerably and has been reported to range from 23 to 209 days. Cases without axillary metastases also had longer average doubling time (128 days) than cases with axillary metastases (85 days) (Gershon-Cohen et al., 1963).

The malignant phenotype of breast tumor cells enables local dissemination by direct infiltration of breast parenchyma, primarily along mammary ducts, and invasion of intramammary lymphatics (Hortobagyi et al., 1987). The role of the lymphatics is important because they are avenues by which tumor emboli reach regional lymph nodes. A series of interdependent events is involved in successful establishment of metastases by hematogenous spread, such as: invasion, detachment, embolization, survival in circulation, arrest in distant capillary beds, extravasation, evasion of host defences, and progressive growth (Robbins et al., 1984).

One view of the spread of breast cancer holds that breast cancer is a systemic disease even before clinical presentation (Fisher, 1984), while the other suggests that the disease follows a sequential course whereby localized disease secondarily gives rise to regional disease, and if not controlled, will give rise to distant disease (Hellman & Harris, 1986). The difference between the two theories has important implications for any approach to treatment. According to the latter view early diagnosis and effective local and regional control ultimately influence outcome; in contrast, the former view suggests that outcome will not be altered by early diagnosis and effective local-regional therapy since such therapy would likely be ineffective when the tumor has metastasized before clinical detection, and would be unnecessary if the tumor remains localized (Hellman & Harris, 1986).
Introduction

An important concept has been developed by Nowell (1976) concerning the acquired genetic variability within developing clones of tumors. These act together with host selection pressures to evolve increasingly malignant subclones. Breast carcinoma is a biologically heterogeneous tumor, since tumor metastasis occurs at varying rates and to varying sites and organs (Haagensen, 1986). Often a metastasis is accompanied by new and diverse clonal variants which are likely to be more resistant to treatment than the cells in the original tumor, particularly when cytotoxic drugs which are also mutagenic are used (Fidler, 1984). The use of preoperative cytotoxic chemotherapy with the intention of eradicating micrometastases and/or decreasing the probability of emergence of resistant clones is thus theoretically plausible (Ragaz et al., 1985).

Tumor heterogeneity in both the primary tumor and its metastases is one reason for therapeutic failure with presently available modalities. Another reason why metastatic breast cancer is nearly impossible to cure is attributable to its selective metastatic target organs; saturation with drug that is sufficiently specific to destroy tumor cells but not normal cells is often difficult in many organs (Fidler, 1984). There are several sites and organs to which breast cancer cells metastasize at greater frequency. Pulmonary and osseous metastases are more common than hepatic and cerebral metastases, which in turn are more common than metastases to endocrine organs (Hortobagyi et al., 1987).

The stimuli for specific "homing" of tumor cells are as yet unclear. The anatomic relationship between organs is only a partial explanation for the pattern of metastases; osseous metastases frequently occur without preceding pulmonary metastases (Haagensen, 1986). The clinical manifestation of metastatic disease in the same organ may differ among individual patients. For example, pulmonary metastases may take the form of nodular disease which is more responsive to treatment than a diffuse, lymphangitic form (Hortobagyi et al., 1987).

In the absence of an established cure for breast cancer, the optimal approach to patient management will be based on greater understanding of the many determinants of indolent versus aggressive disease. Prognostic studies of breast cancer contribute to this greater understanding.
The following section is a review of prognostic factors related both to the patient and the breast tumor that have received relatively large interest.

PROGNOSTIC FACTORS

CLINICAL OR PATIENT RELATED FACTORS

AGE AT DIAGNOSIS AND MENOPAUSAL STATUS

Age-specific breast cancer incidence in North America and northern Europe increases over the entire age range, with a relatively slower increase after 45 to 54 years (Kelsey, 1983). Breast cancer in females age 30 and less accounts for only 1.5% of all breast cancers in the female population, in contrast to 67% after age 50 (Leis, 1978).

Older patients have been reported to have a more favourable prognosis than younger patients as a result of a less virulent disease, especially in the "elderly" (Schaefer et al., 1984). However, conflicting reports showed that younger women fare better than older (Rutqvist & Wallgren, 1983), possibly as a result of diminished host resistance among the old patients (Mueller et al., 1978).

There is also evidence to refute any correlation between age and prognosis (Haybittle et al., 1982; Hortobagyi et al., 1983). Specific age groups have been singled out in some analyses to have superior survival, such as the age group of 35 to 49 in contrast to poorest survival in the "≤34" and "≥75" age groups (Host & Lund, 1986), and the age group of 45 to 49 has the best relative survival (ratio of observed to expected survival) while the youngest (<30 years) and oldest (>75) groups had the worst survival (Adami et al., 1986).

Evaluation of the prognostic importance of age is complicated mainly for methodological reasons. A consistent cut-off point at which old age is defined is lacking, and the minimum age of "old" women has ranged from 60 to 80 years (Schaefer et al., 1984). Different investigators have used different age grouping in their analyses, and some have been inappropriate such as grouping all patients younger than age 50 together (Host & Lund, 1986). Aside from small sample size with short or incomplete follow-up and possible patient selection, another crucial inadequacy in some studies is the improper correction or lack of correction for causes of mortality other than breast
cancer (Adami et al., 1986). Competing causes of death and overall life expectancy are critical considerations in older patients. Furthermore, the confounding effect of presenting clinical stage has not always been considered.

Certain biologic characteristics are more commonly associated with older patients. Histologic types associated with relatively slower growth rate, such as papillary carcinoma and colloid carcinoma, may occur more frequently in older patients (Haagensen, 1986). Primary tumors of patients age 75 or above may be predominantly diploid (von Rosen et al., 1987). There is an inverse relationship between patient age and proliferative activity that reflects tumor growth rate (Meyer et al., 1978). Furthermore, patient age has a significant correlation with estrogen receptor (ER) content, independent of menopausal status (Elwood & Godolphin, 1980; McCarty et al., 1983).

The likelihood of menopausal status being an important prognostic factor is minor, particularly if account has been taken of its strong associations with age and estrogen receptor level. The role of menopausal status as a risk factor for breast cancer development is less dubious, since this risk is generally found to be significantly elevated when menopause occurs after age 55, particularly in combination with greater than 40 menstrual years (Shingleton & McCarty, 1987).

OBESITY

There are several reports of obesity, according to the Quetelet's index, having an adverse influence on prognosis (Tarttner et al., 1981; Boyd et al., 1981). Whether this relationship is independent of other factors is uncertain. There is controversy on the correlation of obesity with estrogen receptor level, since both a positive correlation between obesity and estrogen receptor positivity (postmenopausal woman only) (Kalish, 1984) and a lack of any significant correlation (Hildreth et al., 1983) have been reported. In a multivariate analysis in which the effects of hormone receptors, node involvement, tumor differentiation and age have been assessed concomitantly with obesity, Papathestas et al. (1986) observed that obesity did not contribute independent prognostic information to disease-free survival when the group was analysed as a whole. However,
in subset analysis, obesity was a significant independent factor in women 50 years or older and also in those with four or more positive nodes.

Despite the lack of consensus on the role of obesity, this condition may have some degree of influence on tumor biology in a subgroup of women. This subgroup is more likely to be postmenopausal women whose chief source of sex hormones is from the adrenal glands as androstenedione, which is catalytically converted to estradiol by aromatase in peripheral tissues such as muscle, fat, liver and the breast tumor itself (Santen et al., 1987). Hormone production and metabolism may be modulated by obesity level, and ultimately influence the growth rate of hormonally dependent tumors. Estrogen receptors may be implicated in this pathway. There is also uncertainty on the degree of obesity which constitutes serious risk to post-treatment survival.

**CLINICAL STAGE**

With the exception of a higher yield of nonpalpable carcinomas from breast screening programs (Seidman et al., 1987), the majority of the breast cancer patients present with a lump. A proportion of patients will present with manifestations of advanced disease such as edema, ulceration of the skin, or regional lymph nodes that are palpable and fixed or matted together. Yet another proportion of patients will already have distant metastases detectable by palpation, biochemical markers or imaging techniques.

A system to categorize patients early in their clinical course is necessary. Clinical staging is a multilevel scheme that combines major physical findings to produce homogeneous prognostic groups. The scheme is relatively simple and facilitates the selection of the most appropriate type of primary treatment.

Several clinical staging systems have been devised (Keys et al., 1983) but the TNM system approved by the American Joint Committee (1978) has been widely adopted. This pretreatment classification encompasses three aspects of breast carcinoma: "T" refers to the primary tumor, with a number to describe specific variations in the measured size and character of the tumor; "N"
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refers to homolateral lymph nodes, with a number to indicate their degree of involvement; "M" describes the presence or absence of distant metastasis (Robbins, 1978).

The merit of the clinical TNM staging system is in its strong relationship with survival from primary treatment. The crude 5-year survival of patients varies with clinical TNM stage: 85% for stage I, 66% for stage II, 41% for stage III, and 10% for stage IV (Henderson & Canellos, 1980). The system serves as a general indicator of the tumor burden at diagnosis, but does not necessarily reflect the rate of growth or the intrinsic aggressiveness of the disease. A considerable proportion (25 to 35%) of patients with early or limited disease as measured by clinical TNM stage I will die within 10 years of mastectomy, usually as a direct result of distant metastases (Haagensen, 1986).

A major undesirable feature of clinical TNM staging concerns the relatively inaccurate clinical assessment of regional lymph node involvement. It has been found that 39% of clinically negative nodes were positive on pathologic examination and 24% of clinically positive nodes were pathologically negative (Fisher et al., 1975). There is also an inherent misconception associated with the TNM system that there is sequential spread from breast to axilla to distant metastases, with its connotation of early or late diagnosis (Coppin & Swenerton, 1983).

HISTOPATHOLOGICAL OR TUMOR RELATED FACTORS

AXILLARY NODAL METASTASES

Rather than by reliance on the clinical judgment of nodal status by palpation, the seriousness of disease may more accurately be assessed by microscopic examination of the actual presence of tumor cells in the axillary lymph nodes. It is in fact the single most important prognostic factor in primary operable breast cancer (Fisher & Slack, 1970; Fisher et al., 1980a) known to date. The number of positive lymph nodes strongly correlates with overall prognosis and risk of disease recurrence. The 5-year treatment failure rates for patients found to have no positive lymph nodes, 1-3 positive lymph nodes and 4 or more positive lymph nodes are 13%, 39% and 69% respectively (Fisher et al., 1980a). An important biologic concept supported by experimental observation is that
metastases to lymph nodes reflect an interrelation between host and tumor that permits the
development of metastases, rather than that they are the instigators of distant disease (Fisher,
1984). Fisher (1984) also emphasized that regional lymph nodes are involved because of biologic
rather than anatomic reasons.

The presence and extent of nodal metastases reflect a definite risk of disease recurrence.
This is an impetus to administer systemic therapy, in an attempt to increase the likelihood of
tumor eradication when tumor burden is low and drug resistant clones have not appeared (Lippman,
1985). The U.S. National Cancer Institute concluded at a 1985 Consensus Development Conference
that positive lymph nodes, regardless of menopausal status, is a condition requiring either
adjuvant chemotherapy or adjuvant endocrine therapy (Lippman & Chabner, 1986).

Current techniques for assessing nodal involvement are based on light microscopic examination
of several tissue sections. Serial sectioning has improved the detection of micrometastases by
light microscopy, but no significant correlation between micrometastases and survival has been
found (Fisher et al., 1978b; Rosen et al., 1981). However, a recent study in which monoclonal
antibodies directed against epithelial cell antigens were used for immunohistochemical detection of
nodal micrometastases, reported a significant relationship between the presence of micrometastases
and both recurrence and survival, at least in the subset with infiltrating ductal carcinoma
(Trojani et al., 1987).

Since the information is dependent on axillary dissection, the questions of how many nodes and
from what levels the nodes need to be retrieved were of surgical importance. It has been found
that complete axillary clearance is not necessary, particularly when a meticulous search for lymph
nodes in the axillary contents and careful microscopic examination of one section from each
identified lymph node have been done (Kingsley et al., 1985). Careful examination may also result
in detection of extracapsular extension of nodal metastases, a condition associated more frequently
with four or more positive nodes and considered to have adverse influence on prognosis (Fisher et
al., 1976).
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Axillary sampling of three to five nodes is sufficient to determine accurately whether the nodes are positive or negative, but the quantification of nodal involvement requires removal of ten nodes from at least levels 1 and 2 (Fisher, 1984). Gaglia et al. (1987) advocate a complete axillary dissection based on the finding of a relatively high frequency (19%) of "skip" metastases. They found the level of node involvement was an independent predictor of disease-free survival from number of positive nodes and tumor size. In contrast, Veronesi et al. (1987) found only a small incidence (20 of 519 cases) of skip metastases, and reported that the predictive value of the first level of metastatic involvement is considerable. When the first level nodes were clear, the chance that metastatic nodes were present at the second and third levels was negligible. When the nodes at the first level were positive, there was a 40% chance that metastases were also present at the higher levels.

LYMPHOCYTIC INFILTRATION AND SINUS HISTIOCYTOSIS

Although the existence of immunologic surveillance in human carcinomas is not clearly established (Robbins et al., 1984), there are certain signs to suggest host immunologic response to the presence of breast cancer cells. Higher frequencies of positive local lymphoreticuloendothelial responses, such as lymphoid-plasma cell periductal reactions and positive sinus histiocytosis, are found associated with in situ carcinomas than stage II invasive cancers (Black & Zachrau, 1978). This may suggest that a loss of antigenicity of the cancer tissue or a loss of specific hypersensitivity of the host is associated with disease progression.

Earlier studies that emphasized the prognostic importance of those immunologic features (Leis, 1978) were not consistently substantiated by later studies. However, the condition of germinal center predominance was found as an important marker for poor prognosis both at 5 years and at 10 years after surgery in patients with no positive lymph nodes, independently of the prognostic influence of tumor necrosis, histologic grade and tumor size (Fisher et al., 1980a; Fisher et al., 1984). Similar prognostic importance of germinal center predominance was not evident in node positive patients.
Introduction

In an attempt to assess the impact of host reactivity on prognosis more accurately, Wernicke & Podesta (1984) combined the effect of sinus histiocytosis in the regional lymph nodes and stromal mononuclear reaction in the primary tumor, and developed a score called host defense factors. Their study found that absence of host defense factors, along with metastatic lymph nodes and the presence of a high degree of aggressiveness (assessed also by a scoring method based on histologic features), associated with significantly poorer 5-year survival, compared with patients with aggressive tumors, nodal metastases and positive host defense factors.

HISTOLOGIC TYPES

There are two major divisions in the classification system of breast carcinoma. The nomenclature adopted was originally intended to reflect the site of origin. Those carcinomas considered to arise in lobules were classified as "lobular", while those arising in ducts were "ductal". This major criterion, based on the anatomical origin of malignancy, is still cited in modern text books (Robbins et al., 1984), although it has been proposed that most carcinomas of all types arise from the terminal duct lobular unit (Wellings, 1980). Due to the considerable overlap in the site of origin, the major pathological criterion to distinguish tumor types thus depends more on histologic and cytological characteristics than on the precise anatomic location of earliest changes (Azzopardi, 1979).

The predominant tumor type is infiltrating ductal carcinoma, and further subcategorization is possible with consideration of the different macroscopic and microscopic morphologies. Approximately 70% of infiltrative ductal carcinomas do not have special features (not other specified, NOS) (Millis, 1983), but there are those that have distinctive growth patterns to segregate them into special categories.

The association between histologic types and prognosis has been studied. While patients with infiltrating ductal carcinoma NOS may exhibit a range of prognoses, there are several histologic subtypes that generally correlate with a favourable prognosis. Tubular carcinomas represent about 5% of invasive breast cancer (Azzopardi, 1979). When considered in their pure form without
Introduction

Admixture of undifferentiated elements, tubular carcinomas rarely metastasize to axillary lymph nodes and have a very favourable prognosis (Carstens et al., 1985). Pure colloid carcinomas, about 2% of all invasive disease, also have a low incidence of nodal metastases and favourable long term prognosis (Haagensen, 1986). When the tumor contains large areas of mucin but also has areas with infiltrating carcinoma devoid of extracellular mucin, Rasmussen et al. (1987) observed that the lymph node status and recurrence-free survival of patients with these tumor characteristics closely resemble those with infiltrative ductal NOS.

Medullary carcinomas are characterized by a cytologically aggressive microscopic appearance, a moderate to marked lymphocytic infiltrate and are frequently accompanied by extensive necrosis. However, Ridolfi et al. (1977) found that application of a strict diagnostic criteria for this histologic subtype showed a lower incidence of axillary nodal metastases and higher 10-year survival rates as compared to invasive carcinoma NOS. The survival was found to be favourable even when nodal metastases were present. It has been stated that if medullary carcinomas are lethal, they cause death with relative rapidity (Azzopardi, 1979). It is evident that rigid diagnostic definitions of histologic subtypes are clinically important.

Classic infiltrating lobular carcinomas with a distinctive "Indian file" or targetoid pattern (Robbins et al., 1984) occur with an incidence of about 10%. However, identification of variants of this tumor type is not always clear (Dixon et al., 1982), and the prognostic effect has not been clearly defined. Dixon et al. (1982) observed that when variant forms of infiltrating lobular carcinomas were compared with classical forms, prognosis was the best in the classical form, followed by alveolar, mixed, and solid variant types. The prognostic difference between these forms were different even after adjusting for clinical stage. An incidence of 29% treatment failure has been associated with infiltrating lobular carcinoma, as compared to a 36% treatment failure rate of infiltrating ductal carcinoma NOS (Fisher, 1984).

There is consensus on the extremely poor prognosis of inflammatory carcinoma, which represents less than 1% of all invasive cancers and is distinctively characterized by massive involvement of dermal lymphatics and rapid growth (Millis, 1983). This type is also associated with early
metastases to axillary nodes and distant metastases, and survival is usually very short regardless of therapy (Haagensen, 1986).

OTHER HISTOLOGIC FEATURES

Other aspects of tumor morphology also associate with prognosis. After the architecture of the tumor has been described by the histologic type, the general degree of differentiation of the tumor may also be expressed as an indicator of malignancy. Bloom and Richardson (1957) considered the degree of gland formation or tubule formation and the degree of nuclear atypicality marked by nuclear pleomorphism, hyperchromatism and mitoses. It is unknown why these features were used or if the features should be given the same weight in assessing grade (Stenkvist et al., 1983). Rank et al. (1987) regarded as inappropriate the summing of different parameters to yield a total malignancy grade, since the tripartite gradation was found not to be equidistant. Furthermore, they observed that only cell pleomorphism and neither tubule formation nor mitoses carried independent prognostic information.

A tumor grading system generally distinguishes patients with poorly differentiated tumors who have poorer survival from those with well differentiated tumors who have better survival (Fisher et al., 1984). A prognostic significance of tumor grade was found in a series of node-positive patients who had received adjuvant therapies, independent of the prognostic effects of other factors (Davis et al., 1986). Tumor grade was also found to add important prognostic information to lymph node stage and tumor size in another series (Haybittle et al., 1982).

The major limitation of assessment of tumor differentiation, like other histopathological features, is the inherent observer subjectivity. Interobserver reproducibility has been a major concern (Gilchrist et al., 1985) and careful standardization of grade assessment will increase interobserver consistency and clinical confidence in this prognostic factor (Davis et al., 1986). This system is also limited by the predominance of tumor grade II or moderately differentiated tumors.
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Two types of tumor necrosis have been observed, the most common one associates with the intraductal components and the less frequent one is the infarct-like necrosis in areas of invasive cancer (Fisher et al., 1980a). Fisher et al. (1978a) found that the latter type of necrosis alone or in combination with the former correlated with higher treatment failure and was independent of tumor size. Extensive tumor necrosis is also correlated with high risk of recurrence within two years of diagnosis in stage I patients with estrogen receptor negative breast carcinoma (Bauer et al., 1983).

The precise biologic mechanism for the adverse effect of tumor necrosis is unknown. However, some degree of biologic aggressiveness may be ascribed to a tumor with a high growth rate. It has been observed, at least in the rat mammary carcinoma induced by dimethylbenz(a)anthracene, that high cellular proliferation associates with an increased tissue pressure which impairs capillary perfusion. The resultant necrosis and degradation of macromolecules further increase the oncotic interstitial fluid pressure (Tveit et al., 1984).

The prognostic role of peritumoral lymphatic and blood vessel invasion has been regarded by some to be important (Bettelheim et al., 1984; Dixon et al., 1985). But others such as Sears et al. (1982) did not find any significant correlation with risk of recurrence. Fisher et al. (1984) also found that the peritumoral vascular invasion was of much less prognostic importance than the extent of axillary nodal metastases. This may be consistent with the notion that tumor cells found within vascular channels in a microscopic specimen, although ominous, cannot be equated with metastases (Robbins et al., 1984).

Several methodological factors may account for the lack of consensus between studies, such as differences in patient population characteristics, adequacy of tissue sampling, the histologic criteria used for lymphatic and blood vessel invasion, and interobserver variations, while the use of immunohistochemical techniques may be more reliable than conventional staining methods (Lee et al., 1986).

Tumor elastosis is a common feature of infiltrating ductal and infiltrating lobular carcinomas and assumes two main forms. Focal elastosis has a mainly periductal location and appears to
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predominantly affect pre-existing structures with elastic tissues such as blood vessels. Diffuse elastosis occurs when elastic fibre is produced at sites where previously little or none had been present (Azzopardi, 1979). Dixon et al. (1985) found patients who had survived up to 20 years with breast cancer to have significantly more elastosis than patients who had survived less than 10 years after diagnosis. This correlation with survival could not be verified by Rasmussen et al. (1985), who found that elastosis lacks an important correlation with recurrence-free survival despite its association with estrogen receptor content and grade of anaplasia.

TUMOR BIOLOGY

A measure of tumor aggressiveness is provided by the study of tumor cell kinetics. The thymidine labelling index (TLI) has been employed as an indicator of the fraction of tumor cells that synthesize DNA, the S-phase fraction, upon in vitro exposure to tritiated thymidine (Meyer & Lee, 1980). Tubiana et al. (1984) demonstrated prolonged recurrence-free and total survival with a low TLI. The importance of TLI was independent of tumor size, histologic grade, and the number of involved lymph nodes.

Meyer (1986) confirmed the role of TLI, and found it was a prognostic factor for recurrence-free survival in node negative patients, independent of stage and ER content. Labelling index was also important to both the recurrence-free survival and overall survival of node negative patients, independent of tumor size and ER status (Silvestrini et al., 1986). Meyer (1986) believed that the significant positive correlation between TLI and tumor size is evidence for a biologic association between high S-phase fraction and rapid growth, although he acknowledged that growth rate is also modified by the rate of loss of cells (Meyer & Lee, 1980; Robbins et al., 1984).

Breast cancer cell kinetics may also be derived from flow cytometry, which quantitatively measures the proportion of cells in the synthetic S-phase of the cell cycle and also measures the degree of DNA abnormality (aneuploidy) in the cells (McGuire & Dressler, 1985). Early studies have
found an important correlation between DNA ploidy and relapse (Hedley et al., 1984) and survival (Coulson et al., 1984).

The independent prognostic role of DNA ploidy has not been firmly established. Dowle et al. (1987) assessed tumor DNA content in patients who have not had adjuvant therapy and found that patients with diploid cancers had significantly better short-term survival but this effect disappeared after a median follow-up of 84 months. Tumor DNA content was also found not to be independent of tumor size, histologic grade and lymph node stage in predicting survival.

In another series Kallioniemi et al. (1987) found that DNA ploidy was a significant prognostic factor even for overall 8-year survival in both node-negative and node-positive patients, but mainly in the ER+ subset. A multivariate analysis showed that DNA ploidy, primary tumor size, nodal status and progesterone receptor (PgR) content represented the only significant independent prognostic factors for this series of patients. The authors reflected that the inconsistent prognostic value of DNA ploidy obtained in different studies may be due to differences in treatment type given and to methodological variation in detecting tumor aneuploidy (Kallioniemi et al., 1987).

**ROLE OF STEROID HORMONE RECEPTORS**

Analyses of ER in human breast cancer has various clinical uses, most notably patient selection for endocrine therapy and prediction of the clinical course of the disease. Jensen and his colleagues (1967) showed that breast cancer tissue possessed the same specific receptor mechanism for estrogen as did other estrogen target tissues. Their work presented a working model of steroid action at the molecular level, applicable to female reproductive tissue including the breast. It was an extension of Folca's observation that breast cancer patients with a favourable response to adrenalectomy incorporated more radioactive hexestrol into their tumors in vivo than those who did not respond (Folca et al., 1961). Such research contributed a mechanistic explanation for Beatson's observation (1896) that oophorectomy caused tumor regression in two premenopausal patients with advanced breast cancer.
MODELS OF STEROID HORMONE ACTION

Jensen's model (Jensen, 1974) states that response of target tissues to steroid hormones depends on the presence of specific, high affinity protein receptors in the cytoplasm, to which activating hormone binds to exert an inductive effect on protein synthesis including the estrogen receptors themselves. They found that most but not all women whose breast tumors contained these receptors responded to endocrine ablation or hormone administration, whereas neither manipulation effected significant remission in patients with negligible amounts of receptors. Others soon confirmed the results (Maass et al., 1972; Leung et al., 1973; Savlov et al., 1974). The ER status, then, met the need for a method of a priori prediction of those patients with a reasonable chance of hormonal treatment success (Jensen & DeSombre, 1977).

The first formal hypothesis on the intracellular fate of the steroid hormone was proposed independently by Jensen and Gorski in the late 1960's, and is often referred to as the "two-step translocation" model. The original observation was that radiolabelled estradiol was retained in uterine tissue by binding to cytoplasmic proteins with high specificity and high affinity (a molar dissociation constant of $10^{-10}$ to $10^{-13}$). The ligand-receptor complex then underwent activation and temperature dependent translocation into the cell nucleus with subsequent gene regulation.

Studies done during the subsequent decade have produced apparently convincing biochemical evidence to support the hypothesis, the main premise of which was that only hormone-bound receptors are found in the nucleus, while cytoplasmic ER can exist in hormone-free or hormone-bound states.

Sheridan et al. (1979) questioned the extranuclear existence of ER. Based on autoradiographic and hormone binding experiments, they proposed an "equilibrium" model: in the absence of hormone, unbound soluble receptors exist in both the nucleus and cytoplasm. A portion of these receptors may be loosely bound to nuclear components. When the hormone is present, the proportion of nuclear receptor is greater since binding of hormone to the soluble nuclear receptor causes activation and a tighter association with nuclear components. The decrease in soluble nuclear receptors causes a
movement of soluble cytoplasmic receptor into the nucleus so that a new equilibrium can be established. Therefore, the quantity of receptor in any one state is determined by the free water content and physical parameters of the compartment.

The current model holds that both unoccupied and occupied ER are bound to nuclear elements (possibly nuclear matrix), and the degree of association varies only upon binding with steroid (Gorski, 1986). The loose association of unbound ER results in extraction with the cytosol during tissue homogenization under low salt conditions. There is no evidence for strong association of the hormone with any particular cytoplasmic component as it traverses the cytoplasm to the nucleus. The extranuclear binding previously observed is attributable to interaction with much lower affinity, so called Type II binding sites (Clark et al., 1978).

There are two lines of supporting evidence. Firstly, Welshons et al. (1984) used a cell enucleation technique to produce a nucleoplast (nucleus plus a rim of cytoplasm), which had been pulled away from the cytoplast (cytoplasm surrounded by an intact cell membrane). The cytoplasts (identified by the presence of the cytoplasmic enzyme lactic dehydrogenase) were found to contain little ER. However, unoccupied ER was present in much greater quantity in the nucleoplasts. The amount of cytoplasm remaining in the nucleoplast did not correlate with receptor concentration, ruling out the possibility of cytoplasmic contamination.

Secondly, King & Greene (1984) have demonstrated strong immunocytochemical evidence of exclusive nuclear staining with ER-specific monoclonal antibodies in frozen, fixed sections of different estrogen target tissues, including human breast tumors. The lack of cytoplasmic staining could not be attributed to destruction of receptors during fixation and embedding since the experimental procedures were tested under a variety of conditions without materially affecting the results.

METHODS FOR QUANTITATION OF ESTROGEN RECEPTOR

Sucrose density gradient (SDG) was the first ER assay done on rat uterus (Toft & Gorski, 1966) and then human breast cancer tissues (Jensen et al., 1971). The cytosol is incubated with
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$^3$H-estradiol and layered onto 5-20% or 10-30% sucrose gradients. Any nonspecific binding is determined by parallel gradient layered with cytosol that has been incubated with excess unlabelled competitor and $^3$H-estradiol. The gradients are ultracentrifuged for a length of time dependent upon the g force, usually at least 15 hours. Hormone-receptor complex sediments in the 8s region under low salt conditions. Albumin, sex hormone binding globulin and other proteins which bind estrogen sediment at 4s. However, gradient preparation and fractionation are time consuming (Mirecki & Jordan, 1985) and the long centrifugation time may promote receptor degradation. Furthermore, it is nearly impossible with SDG to do other than a one point assay (Chamness & McGuire, 1979).

The dextran-coated charcoal (DCC) assay is a radioligand binding assay that involves incubation of $^3$H-estradiol (or other radioactive hormone) with a homogenized cytosol. Nonspecific binding is determined by parallel incubation with excess unlabelled competitor. Unbound or loosely bound hormone is removed by dextran-coated charcoal prior to liquid scintillation counting. A multipoint assay is usually done with various concentrations of the labelled ligand. The data is quantitatively analysed by Scatchard or Woolf plots (Keightly & Cressie, 1980) that give a dissociation constant and the number of binding sites at saturation.

The establishment of international quality control programs generally ensures good reliability and reproducibility, and the DCC assay correlates well with the SDG analysis. There has been a strong emphasis on standardization of the DCC protocol and identification of potential sources of variation.

A potential pitfall concerns an early event in the assay: tissue handling. Steroid receptors are thermolabile and the tissue specimen is best placed into liquid nitrogen for transport and a $-70^\circ$C freezer for permanent storage. Maintenance of low temperature (0-4$^\circ$C) is essential during the assay manipulation to prevent receptor degradation.

Histological verification of malignancy in tissue that is being analysed is important. The type of instrument used to homogenize the tissue may also affect results. Higher ER values are found in tissues prepared with a Micro-Dismembrator as compared to the use of a Polytron or an
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Ultraturrax (Thorpe, 1987a). The latter two types of homogenization device may produce excessive shearing forces that disrupt more cell organelles and thus yield higher cytosolic protein concentrations; the additional proteins solubilized may also increase proteolytic activity (Thorpe, 1987a).

Since receptor values are commonly expressed in relation to the amount of protein present, standardization of the protein assay is necessary. The interlaboratory coefficient of variation of protein analysis could be reduced below 15% by the standardized use of one type of protein assay and a standard protein solution (Koenders & Thorpe, 1986). Borjesson et al. (1987) compared quantitative results between five laboratories and reported that protein measurement is only a minor source of variation between laboratories.

Tumors have varied degrees of vascularization, which results in the presence of serum protein components in cytosolic preparations from pulverized tissue. Failure to account for such serum protein "contamination" would cause underestimation of the number of receptor sites. The E.O.R.T.C. Breast Cancer Cooperative Group (1973, 1980) endorses a mathematical correction for serum protein contamination. The albumin content is estimated and a factor representing the usual ratio of albumin to total protein, both in plasma and the interstitial fluid, is used to express final results as ER per milligram cytosol protein.

The enzyme immunoassay for ER (ER-EIA) is one of two new techniques that evolved from the development of a library of monoclonal antibodies that recognize at least twelve unique epitopes distributed throughout the length of the estrogen receptor protein (Press & Greene, 1987). The ER-EIA is a "sandwich" technique with two antibodies that recognize distinct antigenic sites, one near the DNA binding domain of the ER and the other near the steroid binding domain. Both occupied and unoccupied receptors are theoretically detectable.

The ER-EIA correlates well with DCC results and is a potentially more useful assay on the basis of the following features: it is highly specific, radioactive ligands are not used, it requires a minimal amount of cytosol, the assay procedures are simple and total standardization is possible (Leclercq, 1987).
Like the DCC assay, the ER-EIA is unable to provide information on tumor heterogeneity, i.e., the proportions of ER-rich and ER-poor cells. The ER immunocytochemical assay (ER-ICA), uses antibodies for visualizing the location of the receptors and is a viable alternative to receptor measurement. Briefly, the method involves incubating the tissue sections with a primary antibody, a bridging antibody and a peroxidase-antiperoxidase complex followed by a chromogen (Ozello et al., 1986). Two parameters are derived: proportion of stained cells and staining intensity.

A generally good correlation has been found between DCC and ER-ICA (King & Greene, 1984; McCarty et al., 1985; Di Fronzo et al., 1986). The ER-ICA can not only detect tumor heterogeneity but can distinguish between invasive and noninvasive components (DeSombre et al., 1986). Another advantage to this method is its adaptability to very small tumor specimens, even fine needle aspirates (Reiner et al., 1986).

There are several disadvantages to the ER-ICA which offset its potential utility. The ER determination is semiquantitative; at best a scoring index (staining intensity x percentage of stained cells) has been used (McCarty et al., 1985). The number of stained nuclei may indicate whether ER is present and the predominant ER status of the cell population examined, but variation in staining intensity is only a crude indicator of the ER quantity and is subject to observer interpretation. Finally, the ER-ICA has mainly been used on frozen sections; its utility is not certain with neutral formalin-fixed paraffin-embedded specimens, apparently a result of loss of antigenic ER expression (King et al., 1985).

FACTORS THAT INFLUENCE RECEPTOR MEASUREMENT

Heterogeneity within a breast tumor influences not only potential response to hormonal treatment but also receptor quantitation. Individual epithelial cells may or may not retain the functional characteristic of receptor production during malignant transformation (McGuire & Chamness, 1973). A particular tumor specimen may have enough cells containing receptors to yield a positive assay, but cells whose growth is not under hormonal control (i.e., ER- cells) may dominate and influence the clinical course while the ER+ cells are selectively eliminated by hormonal
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therapy (Leake, 1984). A microsample technique was recently used to examine intratumoral regional variability in ER levels, and showed that the average coefficient of variation for intratumoral ER level was a striking 86% and that 35% of overall ER+ tumors lacked receptors in some regions of the sample (van Netten et al., 1985). Measuring this heterogeneity is beyond the capacity of the conventional biochemical ER assay.

Temporal ER stability in vivo also relates to tumor heterogeneity. An 85% concordance has generally been found in sequential determinations, independent of the length of elapsed time between assays. This indicates that ER status of the primary tumor can predict hormonal response of a recurrent lesion which may be inaccessible to biopsy (Hahnel & Twaddle, 1985). In the absence of adjuvant therapy the receptor phenotype rather than receptor quantity remains generally the same as before a recurrence, even though primary breast tumors are comprised of both ER+ and ER- cells. A study by Jakesz et al. (1985) examined the effect of intervening therapy on receptor levels in subsequent biopsies. They reported reductions in both ER and PgR levels sufficient to change from positive to negative in patients who received endocrine intervention. This resulted in a discordance rate of 45%. The effect of intervening cytotoxic chemotherapy is less clear (Toma et al., 1985), since ER status changed at a rate of 32% but PgR status of the second biopsy did not. This and other studies reporting the trend of ER+ conversion to ER- (Lee, 1982; Hull et al., 1983; Raemaekers et al., 1984) support the hypothesis that selective pressure is exerted against ER+ cells by hormonal therapy, resulting in receptor loss as the disease progresses and more autonomy (Leake, 1984). It must be emphasized that only nodal and soft tissue metastases were studied due to their accessibility for biopsy. Those ER+ tumors that tend to first metastasize to osseous sites (Campbell et al., 1981) may not display similar receptor status conversion.

Prospective studies are needed to clarify the clinical significance of receptor changes over time. The reliability of the receptor status of the primary tumor for therapeutic and prognostic purposes could then be ascertained. In addition to adequate sample size, assay methodology for sequential biopsies must be nearly identical for proper interpretation. An assessment of whether or not the receptor change is accompanied by increasing histologic differentiation would be
informative. It is unclear whether prognosis is different in patients whose tumors have undergone either a spontaneous or treatment induced change from ER+ to ER- status, as compared to patients with ER- tumors which remain ER- at the second biopsy.

Several other factors influence the level of steroid hormone receptors. Both the incidence and quantity of ER in breast tumors are higher in postmenopausal than in premenopausal women (Lippman, 1976; Wittliff, 1984). This relationship is confounded by age (Clark et al., 1984). In fact, age but not menopausal status was significantly associated with ER concentration in a multiple regression analysis. Elwood & Godolphin (1980) demonstrated that ER level becomes significantly higher with increasing age within both pre- and postmenopausal categories; patients of similar age (45-54 years old) had similar ER levels, regardless of menopausal status. After age was controlled for, ER status was found not to correlate with variables such as use of exogenous hormones, parity, age at first birth, ethnic origin, body weight and family history of breast cancer. At variance with these results was a study by Lesser et al. (1981), who reported a statistically significant correlation between age and receptor level only in the postmenopausal group. However, no adjustment was made for age as a confounding factor in the study. Well known associations between ER status and contraceptive hormone usage and postmenopausal estrogen usage illustrate this point. Oral contraceptives are used by premenopausal and hence younger women, thus the age factor rather than the use of such hormones per se may be the primary contribution to the lower ER concentration among women who had ever used them or were using them at diagnosis. Similarly, lower ER levels seen in postmenopausal women who were menopausal estrogen users at diagnosis relative to those who had used estrogens but not at time of diagnosis may be due to relative age differences, with the latter being the older group. The significant association between race and ER status may also be an artifact of age.

The mechanism that underlies the age-receptor relationships is not yet fully elucidated, although it has often been said that masking of receptor sites by high endogenous estrogen level in the premenopausal state is partially responsible for the phenomenon (Wittliff, 1984). This is not supported by Edery et al. (1981), who concluded that estrogen regulates its own receptor synthesis.
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since estradiol concentration is higher in ER+ than ER- tumors. Receptor "masking" is also not supported by Saez et al. (1978), who suggested that lower ER in premenopausal women may be due to counteracting effects of cyclic progesterone on the stimulatory action of estrogens. Menstrual cycle variation of ER and PgR have been observed in normal uterus and normal breast cells. This variation corresponds with the inductive effect of estrogen on its own receptors and PgR, while progesterone not only down-regulates its own receptors but is also antagonistic to the estrogenic effects. Progesterone acts by negative feedback to the pituitary to suppress ovarian release of estrogen, by stimulating enzymatic conversion of estradiol to a less active estrone, and possibly by direct inhibition on breast cell proliferation (Mauvais-Jarvis et al., 1986).

Whether this phenomenon of endogenous progesterone limiting ER synthesis is the physiological basis for the inverse correlation between receptor level and patient age is not certain. High concentrations of plasma estradiol (Saez & Chouvet, 1984) or cytosolic estradiol (Drafa et al., 1983) are found not to significantly reduce measurable cytosolic ER levels. Steroid-bound receptors have a tighter association with the nuclear matrix and remain in the nuclear fraction upon homogenization. In contrast, older women can be postulated to have higher ER levels because the majority of their receptors are loosely bound within the nuclear compartment and are readily extractable in low salt buffers. This may partially explain why patient age and menopausal status are not independent prognostic factors (Caldarola et al., 1986; Sutherland & Mather, 1986).

There is no definite advantage to analysis of both the cytosol and nuclear fractions to measure total ER content. No clear additional information accrues by knowing the nuclear ER level and studies have generally found a positive correlation between cytosolic and nuclear ER content (Thorsen, 1979; Vandewalle et al., 1983).

It is possible that in the postmenopausal state, where cyclical variations of progesterone and estrogen are absent, syntheses of both ER and PgR may be prompted by extraglandular origins of estrogens. In support of this concept it was found that ER+, PgR+ postmenopausal women demonstrated a positive correlation between ER levels and circulating estrogens (Mason et al.,
1985). All these results appear to conflict with the observation that patients with higher plasma estradiol have fewer numbers of binding sites (Nagai et al., 1979).

Since one of the biological consequences of steroid binding to ER is promotion of PgR synthesis, measurement of PgR level may more accurately reflect the integrity of the receptor system and hormone dependency of the tumor (Horwitz & McGuire, 1978). However, PgR is less stable than ER and assay reproducibility is poorer (Ryan et al., 1985). Monoclonal antibody assays for PgR may overcome these problems (Bilous et al., 1987).

Tumor receptor levels are also influenced by systemic treatment. For example, tamoxifen causes a reversible G₁ blockade of the cell cycle; the hypothesized mechanism is that tamoxifen establishes an equilibrium between antiestrogen binding sites and nuclear ER, increases the ER concentration and stimulates some PgR synthesis (Jordan, 1986). Given in an adjuvant setting, tamoxifen may enhance any existing heterogeneity in the tumor, i.e. ER+ cells will be more positive but ER- cells are not affected. Cytotoxic chemotherapy may have the reverse effect, that is, ER- cells which generally exhibit more rapid proliferation in vitro (Silvestrini et al., 1979) may be selectively eliminated over ER+ cells. In both cases ER-ICA may provide useful information on subsequent tumor specimens since tumor heterogeneity is demonstrable with this method.

A critical factor that inherently affects ER determination is the malignant epithelial cell content of the specimen. This epithelial cellularity is important since ER is found in tumor cells as opposed to stromal elements or nontumorous tissue (Underwood, 1985). The conventional biochemical assay homogenizes all cells in the tumor. Results are commonly expressed on a per milligram cytosol protein basis which at best approximates the amount of ER measured minus any nontumor cell protein that has varying affinity for the radiolabeled estradiol, without an accurate assessment of cellularity of the specimen. The consequence may be clinically significant if a false negative assay is generated by a tumor specimen containing sparse numbers of ER+ carcinoma cells (van Netten et al., 1985). This problem has been studied by a field-by-field graticule cell counting method (Mumford et al., 1983), and by semiquantitative estimation of the proportion of tumor cells in order to correct for measured ER (Howat et al., 1983; Black et al., 1983). These
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studies do not entirely reinforce each other. Howat et al. found cellularity to be unrelated to the mere presence or absence of ER but the highest ER values were found in the most cellular tumors, statistically significant in the postmenopausal and overall patient groups. Mumford et al. (1983) reported a correlation between cellularity and ER concentration in postmenopausal, but not premenopausal and overall groups.

CORRELATION OF ER WITH PATHOLOGY

From a pathologist's point of view, histological characteristics of a tumor are a measure of the cancer's anatomical extent and its biological aggressiveness. A biochemist's view is that presence of ER or PgR is evidence the breast cancer cell, like its normal counterpart, has retained its dependence on estrogenic stimulation for growth, in contrast to one that might not produce receptor because it has escaped hormone dependency (Jensen, 1981). The common belief is that the greater the mimicry of normal breast epithelium, the less the anticipated biological aggressiveness. A causal relationship cannot yet be ascribed to the presence of certain histological features and ER in the primary tumor, but the question of whether there are correlations between the pathologist's findings and the biochemist's findings was posed nearly as soon as the predictive importance of ER was realized. The earliest correlations tested were between ER and the two features: histological type and histological grade. There is no strong association between major histologic types and ER status, perhaps with the exception of variants such as tubular and papillary carcinomas that have a tendency to be ER+ (Fisher et al., 1980b; Howat et al., 1983), and medullary carcinoma that associates with low or negative ER (Silfversward et al., 1980). Significant correlations between ER status and cellular differentiation are usually found (Fisher et al., 1980b; Millis, 1980) even when this differentiation is expressed as an ultrastructural index that is different from histological grading (Le Doussal et al., 1984).

Three other morphological factors, elastosis, necrosis and fibrosis have received attention. A positive correlation between the presence of elastosis and clinically significant amounts of ER is usually found (Glaubitz et al., 1984). A recent study found a direct quantitative relationship
between the two but refuted any prognostic significance of elastosis (Rasmussen et al., 1985).

Tumors having marked necrosis tend to be ER-; apparently only viable cells harbor the receptors. Extensive necrosis may be indicative of aggressive growth and is one manifestation of vascular invasion. A marked degree of tumor necrosis associating with infiltrative ductal carcinoma (NOS) is directly correlated with higher rates of locoregional recurrence, metastases or death (Fisher et al., 1978a). Carter et al. (1978) found that tumors with both necrosis and an infiltrating border associate with a higher 10-year mortality rate than circumscribed tumors without necrosis. The influence of tumor fibrosis on prognosis is uncertain, but a positive correlation between degree of fibrosis and ER content has been found (Howat et al., 1983). In contrast to these histological parameters, the anatomic extent of the primary disease as indicated by clinical staging, primary tumor size and nodal status is not correlated with ER.

Correlation studies between tumor ER and the kinetics of cell proliferation, such as measured by thymidine labeling index, have consistently shown an inverse relationship (Silvestrini et al., 1979; Straus et al., 1982). Thymidine labeling index also positively correlates with tumor size, nuclear anaplasia, tumor necrosis, and inflammatory cellular infiltrate (Meyer, 1986). Theoretically, inability to retain ER and high rates of cellular replication are the results of tumor cell dedifferentiation (Meyer et al., 1977). The mean proportion of cells engaged in DNA synthesis (S-phase fraction) increases in ductal carcinoma as the tumor becomes more poorly differentiated, and this fraction is still higher in the predominantly ER- medullary carcinoma (Moran et al., 1984). The prognostic role of cell kinetics was also confirmed, but it appears that while recurrence-free survival is better in all patients with low labeling index than those with high labeling index, low labeling index confers overall survival advantage in only premenopausal and perimenopausal patients (Silvestrini et al., 1985). However, the number of postmenopausal patients examined was relatively small in this subset analysis (node-negative patients only).
CLINICAL APPLICATIONS OF RECEPTORS

In 1974 a gathering of twelve independent research groups produced a consensus that ER is a more reliable predictor of hormonal response than more exclusionary clinical factors, such as age less than 35 years or perimenopausal status, short duration of relapse free survival, visceral, lymphangitic or cerebral metastases, or failure of previous hormonal therapy (Lippman, 1976). Even with guidance by these factors, rarely would earlier forms of ablative and additive endocrine manipulation yield an enduring response in more than one-third of patients. Despite different assay methods and different thresholds for a "significant" receptor level, patients classified ER+ responded to endocrine therapy at a rate of 52-58%, whereas ER- tumors responded at about 5% (McGuire et al., 1975). The independence of ER from other prognostic factors like nodal status and clinical stage (DeSombre et al., 1979), was impressive.

A 1979 NIH Consensus Development Conference confirmed ER to be a significant predictor of response to endocrine therapy as well as an important prognostic factor (DeSombre et al., 1979). Recent evidence also points to a correlation between ER level and efficacy of adjuvant tamoxifen, independent of menopausal status and stage (Breast Cancer Trials Committee, 1987).

The combined knowledge of PgR with ER improves response prediction. However, the correlation is not perfect since approximately 25% of ER+/PgR+ patients did not show an objective response to endocrine manipulation (Osborne et al., 1980). Several possibilities may account for this observation. PgR may simply serve as a marker for high tumor ER content (Mirecki & Jordan, 1985). Existence of other growth control mechanisms, such as endogenous growth factor production or enhanced exogenous growth factor sensitivity, may not be under hormonal regulation (McGuire et al., 1987). There are also large interlaboratory differences in cut-off points defining ER status (Godolphin, 1982) and differences in judgment of objective responses (Lippman, 1976).

Early studies correlating ER and treatment response were predominantly based on conventional radioligand binding assays. Results from the more recent ER-EIA and ER-ICA methods are still being investigated, and similar correlations are being compared to the standard DCC assay in order to establish the clinical utility of the newer methods. McCarty et al. (1985) reported a strong...
correlation between ER-ICA results and response to endocrine therapy given for metastatic disease, but the number of patients studied was only 23 and the correlation was made retrospectively.

There is no agreement on the role of ER in prediction of the response to cytotoxic chemotherapy. A response rate to chemotherapy was reported higher in ER- than ER+ patients (Lippman et al., 1978) and vice versa (Kiang et al., 1978). However, it is agreed that premenopausal patients respond to adjuvant chemotherapy through effects of ovarian suppression (Padmanabhan et al., 1986). This may be consistent with the study results of Thorpe (1987b) in which younger women (<50 years) had a greater fraction of nuclear ER (as determined by ER-EIA with high salt extraction) than women age 50 or older.

The prognostic value of ER was established in the mid-1970's. Identification by ER status allows for discrimination of those patients at higher risk for disease recurrence, independent of certain clinical characteristics (menopausal status, size of primary, tumor nodal status). A very high risk group of ER- patients with positive axillary nodes who might benefit from adjuvant therapy was defined (Knight et al., 1977; Maynard et al., 1978). Subsequent studies also found ER+ patients to have significantly longer recurrence-free survival regardless of lymph node involvement (Allegra et al., 1979; Cooke et al., 1979; Forrest et al., 1980).

More dissension arose among studies which examined subset behavior. The observation that a difference in the recurrence-free survival between ER+ and ER- subgroups was seen only in the premenopausal group regardless of nodal status (Samaan et al., 1981), was contradicted by Crowe et al. (1982) who found that recurrence-free survival was significantly longer only in the postmenopausal, ER+, node negative group. Howat & Barnes (1981) found no difference between recurrence rates of ER+ and ER- patients with negative nodes and consequently stratified the node positive group into "1-3 nodes" and "≥4 nodes". They subsequently found that ER+ patients with 1-3 nodes had a recurrence rate similar to patients with no nodes; that ER- patients with 1-3 nodes recurred at a higher rate, but the rate between ER+ and ER- subgroups was no longer different when patients with ≥4 nodes were examined.
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The other extreme of the debate suggests it is futile to use ER to predict risk of relapse. No difference was found in the recurrence-free survival between ER+ and ER- patients when analysed by menopausal status (Butler et al., 1985) or by stage (Skinner et al., 1982). To further confuse the issue differences which existed during early months of follow-up did not persist when prognostic data were scrutinized much later (Howat et al., 1983; Saez et al., 1983; Adami et al., 1985).

There is much greater agreement that ER positivity confers advantage to overall survival after initial surgery. ER+ patients are found to have considerably longer survival than ER- patients (Stewart et al., 1983; Howell et al., 1984; Vollenweider-Zerargui et al., 1986). Some studies implied that ER+ patients experienced longer survival because they responded favourably to hormonal therapy given at disease recurrence (Alanko et al., 1985; Howat et al., 1985). Those ER+ patients who did not demonstrate objective response had post-recurrent survival comparable to ER- responders or ER- nonresponders (Howell et al., 1984; Williams et al., 1986). It is however arguable whether response is a direct and sole explanation for prolonged survival after recurrence, or whether patients defined to be ER+ at primary diagnosis are prognostically better than ER- patients even when hormonal therapy was not given. One explanation for ER+ nonresponders may be that the loss of response is related to receptor quantity changes over the course of disease in some breast tumors. Another possibility is that an objective response to treatment is difficult to ascertain accurately in patients with bone metastases (Stoll, 1985) that are more often disseminated from ER+ primary tumors (Campbell et al., 1981). Therefore response to treatment may not necessarily or reliably identify the varied prognosis of patients who have recurred.

In spite of the greater prognostic value of quantitative ER, correlation with prognosis is often based on ER status. The study conducted by Godolphin et al. (1981) was one of the few to recognize the importance of considering the concentration of ER. A relatively large subgroup of 358 patients who had received no adjuvant therapy after mastectomy was analysed and a highly significant longer recurrence-free survival with higher ER concentration was seen.

A multivariate analyses by Clark et al. (1983a) of stage II patients showed positive nodes and PgR concentration to be the only two important predictors for recurrence-free survival, even though
Introduction

ER status and ER concentration were also significant when univariately analysed. An additional set of patients \((n=229)\) with a different demographic profile who were not treated in a randomized clinical trial was subsequently analysed with the previous patient group \((n=189)\). Not only did ER status attain statistical significance as a predictor for recurrence-free survival but it was actually more important than PgR in predicting overall survival (Clark et al., 1983b). The significance of PgR as a prognostic factor has not always been confirmed (Howell et al., 1984; Howat et al., 1985) perhaps because cut-off points for defining PgR positivity have ranged from 5 to 15 fmol/mg cytosol protein or greater. Studies based on patients who did not receive systemic adjuvant treatment could not establish a significant prognostic role for PgR, while others found the contrary in patients given systemic adjuvant treatment (Raemaekers et al., 1987).

Inconsistencies in the literature concerning the true value of hormone receptors as prognostic factors are primarily due to methodological differences between studies. Comparisons are made difficult by different criteria. It is seldom unequivocally clear that study samples subjected to prognostic analyses were free from a variety of biases. The routes through which patients entered study cohorts are rarely described and criteria for exclusion from the final sample are not always specified. Occasionally survival experience of patients enrolled in clinical trials (Leake et al., 1981; Clark et al., 1983a) forms the basis for correlational studies of ER and prognosis but it is questionable if such studies are sufficiently representative of the general breast cancer patient population. However, even when a cohort is fairly well defined, it is critical to have a sample size large enough to detect important differences in outcomes, especially when subsets are to be analysed.

Studies that reported a more favourable recurrence-free survival for ER+ than ER- patients have been criticized for inadequately short follow-up. A common measure is the median length of follow-up, which has been as short as 14 months (Kinne et al., 1981) and 18 months (Knight et al., 1977). The obvious shortcoming is that sufficient time has not elapsed for those patients whose delayed recurrence may be influenced by possession of ER in the primary tumor. On the other hand, long term follow-up, such as a duration of 10 years reported by Aamdal et al. (1984), is not
Introduction

necessarily good and may even be misleading if the end point evaluated is not death due to metastatic breast carcinoma. In their study, nearly 25% of patients were over 70 years of age and 64% were postmenopausal. Since such a high proportion of the study sample was elderly patients who had been followed for up to 10 years it was critical but apparently unrecognized that only disease-specific deaths be used to account for long term prognosis of breast cancer patients.

Although most studies describe objective criteria for identifying recurrences and death, the controversy regarding influence of ER on recurrence-free survival may have stemmed in part from imprecision in the date of first recurrence, a time point over a protracted clinical course which is liable to observer variation (Editorial, 1984). In contrast, the date of death is usually confirmable from death certificates or autopsy reports. A study cohort should be identified at a sufficiently early and uniform point in the natural history of the disease. A concern relates to the use of the date of primary surgery as opposed to the date of diagnosis to assemble such a cohort. Most studies are retrospectively analysed so the time from mastectomy is often chosen, for it can be conveniently traced. The trend in Western Canada during the last decade is toward longer delay time between diagnosis and primary surgical treatment. While this may not be internationally true, one must be aware that waiting time from diagnosis to mastectomy may vary widely such that interstudy comparisons of recurrence-free survival cannot be done reliably. Further complications arise if ER results from primary tumors and metastases are combined such as in a study by Alanko et al. (1985) who correlated recurrence-free survival with ER results from 84% of patients who had the assay on a primary tumor and 16% on metastases. Criteria for defining ER positivity also vary greatly, from greater than 3.1 and 4.1 fmol/mg cytosol protein for premenopausal and postmenopausal patients respectively (Walt et al., 1976), to greater than 18 (Brooks et al., 1983) and 20 (Vollenweider-Zerargui et al., 1986).

OBJECTIVES OF THIS PROJECT

Prognostic factors in breast cancer are worthy of study; they predict the course and outcome of
Introduction
disease, enable allocation of adjuvant treatment to patients at high risk of recurrence and are important in stratification of clinical trials.

An earlier study (Godolphin et al., 1981) involving a portion of the present series of patients clearly showed a significant quantitative relationship between ER and recurrence-free survival, post-recurrence survival and overall survival. Of importance was the observation that further stratification of patients in the conventional ER negative category (≤ 1 and 2-9 fmol/mg protein) refined the 3-year survival estimate: 35% for patients with ≤ 1 fmol/mg protein versus 68% for patients with 2-9 fmol/mg protein. A proportional hazards model was also derived which iterated the predictive significance of both ER concentration and clinical TNM staging information for survival. Due to limited data on histopathology (e.g. degree of differentiation of the tumor), its prognostic value relative to that of ER was not elucidated.

This project was based on a larger set of data and longer follow-up time. The first objective was to establish the degree of prognostic impact of a set of patient-related and tumor-related variables measured at the time of initial diagnosis. This was accomplished by examining the tumor receptor content and clinicopathologic factors under univariate conditions according to the product-limit method. However, this only serves to elucidate how the prognostic factors act on survival but fails to clarify how they interact to influence survival. The second objective was therefore to assess the relative importance of these prognostic factors in predicting the risks of disease recurrence and death. This analysis involved the development of Cox proportional hazards models, based on the principles of multiple regression allowing each variable to be tested independently while effects of all other variables were taken into account. Predictors for survival after the first disease recurrence were evaluated in the same manner.
MATERIALS AND METHODS

PATIENTS

The study consists of 1,184 female patients with primary breast carcinoma, who were referred to the Cancer Control Agency of British Columbia (CCABC) in Vancouver between 1975 and 1981 by physicians in Vancouver and other regions of British Columbia. These 1,184 patients (75% of the total in the database) were selected on a consecutive basis by satisfying the inclusion criteria of satisfactory ER determination on the primary tumor, known dates of diagnosis and no previous, concomitant, or later malignancy regardless of site (including bilateral breast cancer) except nonmelanoma squamous cell and basal cell carcinomas of the skin.

Postoperative clinical follow-up was complete on all but 21 patients, and conformed to a definite schedule. Patients who were surgically treated, with or without postoperative radiation, received complete physical examination one month after radiation therapy (at CCABC), every three months until the second year (alternating between CCABC clinic and referring physician), every six months from third to fifth year (by referring physician) and once a year thereafter. Chest X-ray and mammogram of the opposite breast were repeated annually. Patients who received adjuvant chemotherapy underwent detailed physical examination at the start of each course of chemotherapy. While patients were on an adjuvant regimen blood count and liver function tests were repeated at each visit; chest X-ray and carcinoembryonic antigen were assessed every three months. Bone scan and mammogram of the opposite breast were performed annually or sooner if indicated. Results of follow-up by referring physicians were reported on a standardized form which was returned to the CCABC. If recurrent disease was suspected or evident patients were referred back to the CCABC for evaluation and therapy planning. Histopathologic diagnoses of malignancy consistent with the primary tumor or evidence of metastatic disease on radiologic scans were used to confirm recurrent disease.
Materials & Methods

TREATMENT RECOMMENDATIONS

Patients of this series have received treatment based on a published guideline of current treatment recommendations for various cancer types (CCABC, 1982).

PRIMARY SURGERY

The type of surgery was selected primarily by the TNM classification of the breast tumor. Simple mastectomy with lower axillary nodal dissection was recommended for "T1s, N0" patients, while "T0, T1a, T2a, N0, N1" patients should have had a modified radical mastectomy. Tumor operability was defined for tumors smaller than 5 cm, which means "T1b, T2b" tumors that were tethered or fixed to underlying pectoral fascia would undergo total mastectomy. Tumors greater than 5 cm ("T3a, T3b") were considered inoperable and treated mainly by radiotherapy. Inflammatory carcinoma was also an inoperable disease.

ADJUVANT RADIOThERAPY

Patients treated with simple mastectomy or local surgical excision (except in situ carcinoma) received adjuvant radiotherapy to the chest wall, internal mammary chain, axilla and supraclavicular regions.

Patients treated with modified radical mastectomy or radical mastectomy received adjuvant radiotherapy if they had central or inner half lesions and/or positive axillary lymph nodes, or if considered to have a relatively high probability of local recurrence (e.g. large tumor size, T1b or T2b tumors).

Patients over 70 years of age and considered surgical risks may have received radiation or systemic therapy as primary treatment.

ADJUVANT CHEMOTHERAPY AND HORMONE THERAPY

Axillary nodal status (pathological involvement) largely dictated the adjuvant treatment plan.
Materials & Methods

Menopausal status and estrogen receptor status of the patient and to some extent, presence of high risk histologic features, modified the treatment plan.

Menopausal status was defined as follows: premenopausal (a menstrual period had occurred within the previous year); perimenopausal (early postmenopausal, the last period had occurred within one to five years); late postmenopausal (last period was more than five years ago). Serum follicular stimulating hormone was determined when the menopausal status was uncertain.

For node negative patients, regardless of menopausal status, without evidence of lymphatic, vascular or perineural invasion, no systemic treatment was required. When there was evidence of lymphatic, vascular or perineural invasion, premenopausal patients received adjuvant chemotherapy, while postmenopausal ER+ patients received adjuvant tamoxifen and postmenopausal ER- patients received adjuvant radiotherapy. Adjuvant radiotherapy was recommended if the tumor was located in the central area or the inner half of the breast.

Systemic therapy was given for node positive patients, regardless of menopausal status or any lymphatic, vascular or perineural invasion. Premenopausal patients received adjuvant chemotherapy, and postmenopausal ER+ or ER unknown patients received tamoxifen.

LOCALLY ADVANCED DISEASE (TNM III, TNM IV)

Adjuvant radiotherapy was usually given for local control. Premenopausal ER+ patients underwent ovarian ablation while postmenopausal ER+ patients received tamoxifen. Under this classification, ER- patients regardless of menopausal status received adjuvant chemotherapy.

RECURRENT DISEASE AFTER PRIMARY TREATMENT

Locoregional recurrent disease in the absence of distant metastasis was treated with excision if possible, along with radiation therapy. Hormonal therapy as treatment for first disease recurrence was considered suitable if the patient was postmenopausal or at advanced age, whose primary or recurrent tumor contained high ER level, a disease-free interval of at least 2 years, and whose
Materials & Methods

recurrence occurred at sites which were not life threatening such as chest wall, pleura, lymph nodes, and bone.

For patients who had liver or pulmonary lymphangitic recurrent disease, cytotoxic chemotherapy was considered most appropriate. This modality was also indicated for patients who had a disease-free interval of less than 2 years, pre- or perimenopausal with low or negative tumor ER, but ovarian ablation may have been added if the tumor was ER+.

MEASUREMENT OF PROGNOSTIC VARIABLES

RECEPTOR ANALYSIS

Tumors were frozen and transported in liquid nitrogen, then stored at -75°C. Prior to analysis they were trimmed to remove fatty, fibrotic and necrotic material. All procedures including trimming were maintained at <4°C. Tumors were pulverized with a Braun Mikro-dismembrator, homogenized in buffer and centrifuged at 39,000 g for 15 minutes to isolate cell cytosol which was incubated with ³H-estradiol with or without competitor (diethylstilbestrol). Unbound and loosely bound hormone was removed with dextran-coated charcoal and remaining bound ³H-estradiol measured by liquid scintillation counting. The Scatchard plot was used in earlier years to calculate receptor concentration, but in later years the Woolf plot was used (Keightly & Cressie, 1980). Both plots yielded results that were quantitatively the same, expressed as femtomoles of bound estradiol per milligram of protein.

Receptor concentration was corrected for variable serum protein in the cytosol preparation. This is in accordance with the recommendation of the E.O.R.T.C. Breast Cancer Cooperative Group (1973, 1980). A correction factor of 1.67, based on the average ratio of total protein and albumin in normal sera, was applied to the albumin concentration determined by radial immunodiffusion. The tissue cytosol protein was calculated as the difference between measured total protein and the estimated serum protein (Jacobson, 1981).

All ER analyses were performed in the same laboratory, where the technique has remained essentially unchanged throughout the study period. In-house quality control measures (Godolphin &
Materials & Methods

Jacobson, 1980) and participation in a national quality control program (Ryan et al., 1985) have indicated a reliable and reproducible assay over the years of data collection for this study. The laboratory operates at a coefficient of variation of < 13% at an ER concentration of 20 to 40 fmol/mg protein.

On the basis of the resultant ER data, patients were stratified into subgroups according to the ER ranges of: ≤1, 2-9, 10-159, and ≥160 fmol/mg protein. These were the same cut-off points used previously (Godolphin et al., 1981). The absolute ER concentration was also examined for prognostic significance.

CLINICAL AND PATHOLOGICAL STAGING

Clinical TNM stage was assigned according to Union Internationale contre le Cancer (UICC) criteria (American Joint Committee, 1978), based on presurgical clinical assessment of the patient by a clinician. The information obtained from this procedure was recorded on a questionnaire (Appendix A), which included a clinical measurement of the tumor size; with or without tethering or fixation to underlying pectoralis fascia or chest wall; signs of edema, ulceration and/or satellite nodules; palpability and extent of physical involvement of regional lymph nodes (mobile or fixed homolateral axillary nodes, palpable supraclavicular or infraclavicular nodes); presence or absence of distant spread as verified by metastatic work-up. Patients who did not have complete information on the above were not clinically staged.

Pathological staging was based on histologic assessment of the number of axillary nodal metastases. This information was taken from the original pathology report when an axillary dissection had been done, and categorized as none (N0), 1-3 positive nodes (N1-3), and 4 or more positive nodes (N4+). Information was considered indeterminate when an axillary dissection had not been performed or when the pathologist could not ascertain nodal involvement in the examined specimens.
Materials & Methods

HISTOLOGIC FEATURES

At the time of ER analysis, tumors were trimmed of fatty and necrotic tissue before a representative piece of the tumor was dissected for biochemical analysis. An adjacent section of the specimen was then cut and processed for haematoxylin-eosin staining.

A pathologist semiquantitatively evaluated histologic features including: (1) histologic type of the dominant growth pattern; (2) degree of differentiation classified into “well”, “moderate”, and “poor” categories, assessed by extent of tubule formation, nuclear pleomorphism and mitotic activity; (3) degree of confluent necrosis seen under low power magnification and categorized as “absent”, “minimal”, and “marked”; (4) degree of fibrosis assessed in a similar manner and listed as “minimal”, “moderate”, and “marked”. Assessment was independent of and “blind” to the results obtained from the ER analysis.

Tumor size as recorded in the pathology report was the largest diameter of the tumor and was categorized into three levels: < 2 cm, 2 to 5 cm, > 5 cm.

OTHER CLINICAL INFORMATION

Collection of epidemiological data and data related to present illness (breast cancer) had been initiated in 1980 (Godolphin et al., 1981). Information was abstracted from the medical records of the patients and the format used is displayed in Appendix B. The information was coded in a standardized manner and was subsequently stored as a computerized data base. The follow-up information in this data base was updated between 1985 and 1986, when observation of patient survival was prolonged and patient status could be ascertained for subsequent analysis.

OUTCOME MEASUREMENT

All patients were followed till death or last reported date of having been seen at the CCABC or by their private physicians. Survival parameters examined in this project were defined as follows:
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(1) overall survival (OS) refers to the time from diagnosis to breast cancer-specific death (as primary cause of death from death certificate or autopsy report), or last follow-up for patients who were alive;

(2) recurrence-free survival (RFS) was the time from diagnosis to first confirmed locoregional relapse (chest wall, ipsilateral regional nodes, ipsilateral breast of patients not having had a mastectomy) or distant dissemination (bone, visceral organs, brain); this interval was also determined for patients who had not recurred at all during the study period, but excluded patients who presented with metastatic disease at diagnosis;

(3) post-recurrence survival (PRS) was the time from a defined recurrence till breast cancer-specific death or last follow-up.

 Patients alive with evidence of disease, lost to follow-up (less than 2% of the total group), and dead from other causes were treated as censored data. Patients presenting with clinical TNM IV and persistent disease were excluded from RFS and PRS analyses.

DESCRIPTIVE STATISTICS

The present patient series is comprised of 1,184 women, about half of which have been studied after follow-up to 1980 (Godolphin et al., 1981). This "old" group had a maximum follow-up of ten years. A subsequent "new" group of patients which had a minimum follow-up of four years was added to the first group. The median follow-up of the combined group was 60 months. The overall survival rate was slightly higher (P=0.04) for the additional series (Figure 1), possibly related to earlier detection or more effective treatment.

Survival trends according to stage and ER concentration were examined separately for the old group and the new group, before combining the two groups into one series. Table I shows that the correlations of survival with ER level, nodal status, and TNM stage were highly significant and followed similar trends in both sets of data. The merging into one consecutive series for all subsequent analyses was thus justified.
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Characteristics of these 1,184 patients and their frequency distribution are shown in Table II. Four hundred and fifty seven patients (38%) had recurrences and their specific characteristics are displayed in Table III. The number of patients per univariate analysis of OS, RFS, and PRS differed according to the completion of data on the individual variables.

Patients from the total group of 1,184 were excluded from the multivariate analysis if any of the following information was not available: clinical TNM stage, axillary nodal status, ER quantity, age, menopausal status, and histopathologic variables such as histologic type, degree of differentiation, degree of confluent tumor necrosis, and tumor size. The exclusion resulted in 859 patients to be studied. There was a significantly larger proportion of patients with advanced TNM stage in the excluded group Table IV. These patients were most often excluded because an axillary dissection had not been done and hence pathologic nodal status was unknown.
Figure 1 Overall survival by the year of diagnosis. The number at risk in each group were: (old group) 1975-1978 = 543; (new group) 1979-1981 = 641. Curves discontinued when fewer than 10 patients under follow-up; P=0.04.
Table I Overall survival by ER, nodes and clinical stage in old and new groups. Five-year percent rates (number of patients at risk in parentheses)

<table>
<thead>
<tr>
<th>ER concentration (fmol/mg protein)</th>
<th>Old group</th>
<th>New group</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1</td>
<td>33 (13)</td>
<td>57 (22)</td>
</tr>
<tr>
<td>2-9</td>
<td>50 (54)</td>
<td>63 (51)</td>
</tr>
<tr>
<td>10-159</td>
<td>75 (170)</td>
<td>77 (149)</td>
</tr>
<tr>
<td>≥160</td>
<td>79 (67)</td>
<td>84 (53)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Nodal status</th>
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<th>New group</th>
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<td>89 (118)</td>
</tr>
<tr>
<td>N1-3</td>
<td>73 (91)</td>
<td>75 (92)</td>
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<tr>
<td>N4+</td>
<td>57 (56)</td>
<td>48 (41)</td>
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<th>Clinical TNM stage</th>
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<th>New group</th>
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<tr>
<td>I</td>
<td>84 (134)</td>
<td>88 (63)</td>
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<tr>
<td>II</td>
<td>72 (146)</td>
<td>76 (168)</td>
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<tr>
<td>III</td>
<td>29 (10)</td>
<td>48 (36)</td>
</tr>
<tr>
<td>IV</td>
<td>22 (8)</td>
<td>38 (5)</td>
</tr>
</tbody>
</table>

* P < 0.0001
### Table II  Patient characteristics, overall survival study. The number of patients in each category is given. Median follow-up of 1,184 patients = 60 months.

<table>
<thead>
<tr>
<th>Age at diagnosis (years)</th>
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<th>45-54</th>
<th>55-64</th>
<th>≥65</th>
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<td>291</td>
<td>318</td>
<td>351</td>
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<td>341</td>
<td>782</td>
<td>61</td>
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<th>ER concentration (fmol/mg protein)</th>
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<th>2-9</th>
<th>10-159</th>
<th>≥160</th>
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<td></td>
<td>103</td>
<td>278</td>
<td>573</td>
<td>208</td>
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<thead>
<tr>
<th>ER status</th>
<th>ER+</th>
<th>ER-</th>
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<tbody>
<tr>
<td></td>
<td>771</td>
<td>391</td>
<td>22</td>
</tr>
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<table>
<thead>
<tr>
<th>Clinical TNM stage</th>
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<th>II</th>
<th>III</th>
<th>IV</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>322</td>
<td>589</td>
<td>158</td>
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<thead>
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<th>Pathologic nodal status</th>
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<th>N1-3</th>
<th>N4+</th>
<th>Unknown</th>
</tr>
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<tr>
<td></td>
<td>381</td>
<td>343</td>
<td>250</td>
<td>210</td>
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</table>

<table>
<thead>
<tr>
<th>Primary treatment</th>
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<th>Surgery + radiation</th>
<th>Biopsy only</th>
<th>Biopsy + radiation</th>
<th>Unknown</th>
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<tbody>
<tr>
<td></td>
<td>428</td>
<td>598</td>
<td>62</td>
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<th>Chemotherapy</th>
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<td></td>
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<td>157</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Alive</th>
<th>Dead of breast cancer</th>
<th>Dead of other causes</th>
<th>Lost to follow-up</th>
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<tbody>
<tr>
<td></td>
<td>677</td>
<td>390</td>
<td>96</td>
<td>21</td>
</tr>
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</table>

-44-
Table III  Patient characteristics, post-recurrence survival study. The number of patients in each category is given. Median follow-up after recurrence = 18 months.

<table>
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<tr>
<td>Age at diagnosis (years)</td>
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<td>128</td>
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<tr>
<td>Menopausal status</td>
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</tr>
<tr>
<td>Pre-menopausal</td>
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<tr>
<td>Post-menopausal</td>
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<td>Unknown</td>
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<td></td>
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<td>ER conc. in primary (fmol/mg protein)</td>
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<td>2-9</td>
<td>10-159</td>
<td>≥160</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>110</td>
<td>206</td>
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<td>ER status</td>
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<tr>
<td>ER+</td>
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<tr>
<td>ER-</td>
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<td></td>
</tr>
<tr>
<td>Unknown</td>
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</tr>
<tr>
<td>Clinical TNM stage</td>
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<td>III</td>
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</tr>
<tr>
<td>I</td>
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</tr>
<tr>
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<tr>
<td>III</td>
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</tr>
<tr>
<td>Pathological nodal status</td>
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</tr>
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</tr>
<tr>
<td>N1-3</td>
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</tr>
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<td>253</td>
<td>11</td>
<td>33</td>
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<tr>
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<td>Endocrine</td>
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<td>167</td>
<td>84</td>
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<td>Outcome</td>
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<td>Dead of other causes</td>
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<td>Lost to follow-up</td>
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</table>

* Both endocrine and cytotoxic chemotherapies given, sequentially.
Materials & Methods

Table IV Distribution of patients by characteristic at primary diagnosis.
The excluded group were missing one or more data considered for the stepwise regression analysis. The distribution of ER status is given in this table, however, all statistical analyses were performed with the actual concentration.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Studied group</th>
<th>Excluded group</th>
<th>P*</th>
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<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
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<td>ER status:</td>
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<tr>
<td>ER+ (≥10 fmol/mg)</td>
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<td>(68)</td>
<td>199</td>
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<tr>
<td>ER- (&lt;10 fmol/mg)</td>
<td>277</td>
<td>(32)</td>
<td>104</td>
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</tr>
<tr>
<td>I</td>
<td>266</td>
<td>(31)</td>
<td>56</td>
</tr>
<tr>
<td>II</td>
<td>474</td>
<td>(55)</td>
<td>115</td>
</tr>
<tr>
<td>III</td>
<td>95</td>
<td>(11)</td>
<td>63</td>
</tr>
<tr>
<td>IV</td>
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<td>(3 )</td>
<td>51</td>
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<tr>
<td>N0</td>
<td>365</td>
<td>(42)</td>
<td>76</td>
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<tr>
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<td>288</td>
<td>(34)</td>
<td>55</td>
</tr>
<tr>
<td>N4+</td>
<td>206</td>
<td>(24)</td>
<td>44</td>
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<td>Premenopausal</td>
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<td>Postmenopausal</td>
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<td>(70)</td>
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<td>(10)</td>
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<tr>
<td>40-49</td>
<td>187</td>
<td>(22)</td>
<td>58</td>
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<td>50-59</td>
<td>238</td>
<td>(28)</td>
<td>71</td>
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<tr>
<td>60-69</td>
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<td>≥70</td>
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<td>(12)</td>
<td>77</td>
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### Materials & Methods

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<th>Feature</th>
<th>Value 1</th>
<th>Value 2</th>
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<td>Tumor differentiation:</td>
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<td></td>
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<td>well or moderate</td>
<td>610 (71)</td>
<td>183 (66)</td>
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<td>poor</td>
<td>249 (29)</td>
<td>94 (34)</td>
</tr>
<tr>
<td>Tumor necrosis:</td>
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<td></td>
</tr>
<tr>
<td>absent or minimal</td>
<td>750 (87)</td>
<td>245 (88)</td>
</tr>
<tr>
<td>marked</td>
<td>109 (13)</td>
<td>32 (12)</td>
</tr>
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<td>infiltrating ductal</td>
<td>760 (88)</td>
<td>243 (85)</td>
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<tr>
<td>infiltrating lobular</td>
<td>85 (10)</td>
<td>25 (9)</td>
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<tr>
<td>others</td>
<td>14 (2)</td>
<td>18 (6)</td>
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<td>Tumor size (cm):</td>
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<td>&lt;2</td>
<td>180 (21)</td>
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<td>2-5</td>
<td>627 (73)</td>
<td>138 (60)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>52 (6)</td>
<td>36 (16)</td>
</tr>
</tbody>
</table>

* $\chi^2$ for proportions.
STATISTICAL METHODS

UNIVARIATE SURVIVAL ANALYSIS

The product-limit method was used to calculate survival curves. The information conveyed by survival curves is the probability of not dying of breast cancer, or not having recurrent disease, at different times in the absence of other causes of death. The product-limit method is based on the concept of cumulative probability of survival, that is:

Probability of surviving 365 days = (P1)(P2)(P3)...(P365), where:

\[ P_i = \frac{\text{number at risk on day } i - \text{number of deaths on day } i}{\text{number of patients at risk on day } i} \]

and "at risk" refers to the state of being still alive and under observation. This approach yields the best estimate of the probability of survival and is subject to less random variation than any alternative formula (Peto, 1984).

The second important principle of the product-limit estimate is that patients with censored observations are included to be at risk of death only up to their time of censoring (Coldman & Elwood, 1979). Censoring accounts for those patients whose dates of death (or recurrence) are unknown. The dates may be unknown because they are still alive at the time of analysis, or have been lost to follow-up, or have died of other causes, thus precluding the end point under study. Censored observations are assumed to occur randomly (Coldman & Elwood, 1979). All that can be said about the lifetime of these censored patients is that it is greater than the observed value. This allows all patients to contribute individually different survival times to the calculation and minimizes the standard error. Thus a survival curve which maximizes the likelihood of the observed data is produced (Peto, 1984).

The standard error (S) of the product-limit curve is an estimate of the precision of the survival curve. The range of error of the curve at a given time is calculated with Greenwood's formula which accommodates losses to follow-up:

\[ S = \left\{\frac{P^2 \sum (D_i / N_i(N_i - D_i))}{2}\right\}^{1/2} \]
Materials & Methods

where \( D_i \) = number of deaths on the \( i \)th day; \( N_i \) = number at risk on the \( i \)th day; \( \Sigma_i \) = summation up to and including the time at which curve \( P \) is estimated (Peto, 1984).

A formal statistical test is needed to confirm if the observed differences in the survival curves are significant or merely chance variations. The presence of censored observations in the data preclude the use of procedures such as \( t \)-tests, analyses of variance and least-squares regression, all of which involve averages of the observations (Breslow, 1984).

Comparison of survival curves that is based on a fixed point in time is also not efficient, since valuable information about survival differences which exist earlier or later is not considered and the arbitrary choice of the time point (Breslow, 1984) allows serious bias when a point with maximal observed difference between curves is selected.

The use of nonparametric methods which are free of assumptions about the nature of the underlying survival distribution is efficient; they test for equality based on whole curves. Two such tests were used: the Mantel-Cox test (Mantel, 1966; Cox, 1972) and the generalized Wilcoxon test (Breslow, 1970). The two tests differ in the way the observations are weighted but are both valid in large samples whether the censoring patterns are equal or unequal (Benedetti et al., 1983). Only the Mantel-Cox statistics which weigh later observations more heavily are reported unless they were much discrepant from generalized Wilcoxon statistics.

An extension of the logrank test to a test for trend (Mantel, 1963) has also been used when the test groups represented an ordered sequence such as age groups. Since the test for trend includes a correction for continuity its associated chi-square value is generally smaller than the global chi-square that tests the overall comparison of the different groups (Kalbfleisch & Prentice, 1983). The purpose is to achieve a more powerful test based on partitioning from the global chi-square to obtain a single degree of freedom statistic (Breslow, 1984).

MULTIVARIATE SURVIVAL ANALYSIS

The Cox proportional hazards model was used as a tool for studying the effect of one prognostic factor on survival while adjusting for and evaluating the effects of the other prognostic factors at
the same time. Unlike linear and nonlinear regressions or logistic regression this is a specialized regression model proposed by Cox (1972) which accommodates censored survival data.

The Cox proportional hazards model is formulated in terms of the effects of the prognostic factors (herein called covariates) upon death (hazard) rates rather than times to death (Hopkins, 1983). It is expressed in the form of:

$$H(t,x) = H_0(t) \exp ( \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_j x_j)$$

to model the effects of the $\beta$'s upon the death (hazard) rate ($H$); where $t$ is the time from diagnosis, $x$ is a vector of prognostic variables (covariates) $x_1 \ldots x_j$, and $H_0(t)$ is an unknown baseline hazard function when all covariates are equal to zero. This model is semiparametric since $H_0(t)$ represents an arbitrary base-line hazard that does not need to be specified in order to estimate the regression coefficients. The parametric aspect of this model is the proportional hazards assumption: the full hazard is the product of the baseline hazard and a function of the covariates (Byar, 1982). It is assumed that the covariates taken together have a log-linear effect on the hazard, and the same multiplicative effect with $H_0(t)$ on the hazard applies at all points in time. The additive covariate effects in the form of an exponential function was used as it guarantees that $H(t) \geq 0$ for all values of $\beta$ (Byar, 1982).

Cox (1972) suggested a maximum likelihood procedure to estimate the regression coefficients. A partial likelihood was first expressed by observing $t_1 < t_2 < t_3 \ldots t_k$ as k distinct failure times. For the particular failure at time $t_i$, conditionally on the risk set $R(t_i)$ all patients whose survival times were at least $t_i$, the conditional probability of failure on the observed patient according to her hazard is:

$$l_i = \frac{\exp \left( \sum_{j=1}^{p} \beta_j x_{ji} \right)}{\sum_{j\in R_i} \exp \left( \sum_{j=1}^{p} \beta_j x_{ji} \right)}$$

The denominator is the sum of the hazards for herself and those at risk up to time $t_i$. When there are no tied death times, each distinct death time contributes to the partial likelihood. Censored observations contribute to this likelihood estimation because censored data enter the denominator for as long as the patients with censored times are still at risk.
Materials & Methods

These conditional probabilities were multiplied together for each of the k death times to yield a partial likelihood function (Cox, 1975). The partial likelihood function was maximized to give estimators of β through an iterative Newton-Raphson method (Hopkins, 1983). First, a global chi-square test of fit was based on the null hypothesis that all regression coefficients equal zero (i.e. no effect on survival). The algorithm then began by undergoing halvings and progressively approached the true value of β. The algorithm terminated when the relative improvement in the partial likelihood function was less than the value of the convergence criterion or when the maximum number of iterations had been reached.

A forward stepwise procedure was chosen for this project to determine the most significant set of covariates and the relative importance of each in the model. The method used the likelihood ratio as the criterion for adding significant covariates to the model (Krall et al., 1975). The maximum likelihood value, LL(β), was used as a measure of importance of covariates not yet in the regression equation. The first covariate to enter the regression was selected on the basis of having the largest LL(β). A likelihood ratio test was performed at the next and subsequent steps to determine if the last covariate entered added significantly (i.e. gave the next largest LL(β)) to those already selected. The procedures continued to fit one additional covariate at a time until the regression was satisfactory (Lee, 1980).

Since a difference in two ER concentrations in the negative range may not have the same prognostic effect as an equivalent difference in two ER concentrations in the positive range, ER data was used as a continuous variable after logarithmic transformation. Categorical variables (k categories) were dummy coded into k-1 strata (Lee, 1980). For example, with TNM I as a referent stratum, S1 was a dummy variable that assumed the value 1 for TNM II, 0 otherwise; S2 was another dummy variable that had a value of 1 for TNM III, 0 otherwise; S3 was the final dummy variable with a value of 1 for TNM IV and 0 otherwise. Both differentiation (0 = well or moderate, 1 = poor) and necrosis (0 = absent or minimal, 1 = marked) were coded as binary variables since preliminary analysis indicated no significant difference in survival between the combined subgroups.
Materials & Methods

The Cox proportional hazards models were generated with EPILOG (Epilog version 3, 1986). The predicted survival functions based on different covariate patterns were obtained with the program RISK (Thomas, 1980). Program P:2L of BMDP (Dixon, 1983) was used to check the proportionality assumption of the model with stratification of covariates. This was done with a graphical method, which was based on a plot of ln(-ln \( \hat{s}(t;\hat{x}) \)) against survival time t, where \( \hat{s}(t;\hat{x}) \) is the Kaplan-Meier estimate (Kaplan & Meier, 1958) of \( s(t;\hat{x}) \) and \( \hat{x} \) is the mean vector of the covariates of a certain stratum, when a covariate is stratified (Kay, 1977). If the proportionality assumption holds, the plots should display approximately constant differences between strata.
SURVIVAL WITH BREAST CANCER: THE IMPORTANCE OF ER QUANTITY
CORRELATION BETWEEN ER STATUS AND CLINICAL VARIABLES

Associations between ER status and other clinical variables are presented in Table V. Menopausal status and patient age at diagnosis were significantly associated with ER status. The frequency of ER positivity is higher in postmenopausal women (P<0.005) and in older women (P<0.0001). There was no relationship between ER status and the number of positive lymph nodes, or between ER status and clinical TNM stage.

OVERALL SURVIVAL

Overall survival for the total group is shown in Figure 2. A median survival of 110 months was observed. At the time of analysis, 390 patients had died of breast cancer, 677 patients remained alive, 96 patients died of other causes while 21 patients were lost to follow-up. Overall patient survival was 70% at 5 years.

The importance of ER quantity as a prognostic indicator was studied in detail. Many laboratories use 10 fmol/mg cytosol protein as the usual cutoff to dichotomize receptor levels into ER+ and ER-. The data are shown this way in Figure 3. There was no convergence even at 7 years post-diagnosis and the difference between the two groups was highly significant. However, there was a much greater difference in survival with stratification by ER concentration (Figure 4). The difference between the curves for ER≤1 and ER=2-9 was significant (P=0.03). The curves for ER=2-9 and ER=10-159 were also different (P<0.0001). A borderline significance was reached (P=0.07) when the curves for ER=10-159 and ER≥160 were compared.

The importance of ER level was examined with stratification by clinical TNM stage, pathological lymph node category and menopausal status, to assess whether the influence of receptors on survival was independent of these factors. The quantitative pattern of better survival with higher ER was
## Results

### Table V  Relationship of ER status with other variables

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>ER+ (%)</th>
<th>ER- (%)</th>
<th>$\chi^2$</th>
<th>P-value</th>
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<td><strong>Nodal status</strong></td>
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<td></td>
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<td>N0</td>
<td>381</td>
<td>260 (68)</td>
<td>121 (32)</td>
<td>0.86</td>
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<td>N1-3</td>
<td>343</td>
<td>235 (69)</td>
<td>108 (31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4+</td>
<td>250</td>
<td>163 (65)</td>
<td>87 (35)</td>
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<td><strong>Clinical TNM stage</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>I</td>
<td>322</td>
<td>232 (72)</td>
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<td>6.53</td>
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<td>II</td>
<td>589</td>
<td>398 (68)</td>
<td>191 (32)</td>
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</tr>
<tr>
<td>III</td>
<td>158</td>
<td>96 (61)</td>
<td>62 (39)</td>
<td></td>
<td></td>
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<td>IV</td>
<td>75</td>
<td>53 (71)</td>
<td>22 (29)</td>
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<td><strong>Menopausal status</strong></td>
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<td>208 (61)</td>
<td>133 (39)</td>
<td>8.24</td>
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<td>&lt;45</td>
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<td>108 (50)</td>
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<td>111 (39)</td>
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<td>55-64</td>
<td>312</td>
<td>220 (71)</td>
<td>92 (29)</td>
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<td></td>
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<tr>
<td>$\geq$65</td>
<td>347</td>
<td>267 (77)</td>
<td>80 (23)</td>
<td></td>
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</tr>
</tbody>
</table>
Figure 2 Overall survival of 1,184 patients. Curve discontinued when fewer than 10 patients under follow-up.
Figure 3 Overall survival by ER status. The number at risk in each group were: ER+ = 771; ER- = 391. Curves discontinued when fewer than 10 patients under follow-up; P<0.0001.
Figure 4 Overall survival by ER concentration. The number at risk in each group were: ER>160 = 208; ER=10-159 = 573; ER=2-9 = 278; ER≤1 = 103; (ER concentration in fmol/mg protein). Curves discontinued when fewer than 10 patients under follow-up; P<0.0001.
Results

maintained (Tables VI-VIII) within each subcategory. The differences in survival between the ER levels were significant in each case, although the relationship in clinical TNM stage IV was based on relatively small numbers.

Patient survival was influenced by the number of positive lymph nodes and clinical TNM stage (Figures 5 & 6). A 5-year survival rate of 89% was observed in patients who had negative lymph nodes, compared to 73% and 53% for the nodal categories of 1-3 and ≥4, respectively. Patients with clinical TNM stage I disease had a favourable 5-year survival rate of 86%, compared to 74%, 42% and 26% for stages II, III and IV, respectively. Menopausal status was not a significant prognostic factor for overall survival (Table IX), nor for recurrence-free survival and post-recurrence survival. Patient age at diagnosis also had no significant influence on overall survival ($\chi^2$ for trend = 1.97; P=0.16) (Table IX), nor for recurrence-free survival and post-recurrence survival.

Tumor size was a prognostic discriminant for overall survival (Figure 7). Although the survival curve defined by tumor size less than 2 cm was slightly different from that for tumor size 2 to 5 cm, the difference between these curves and the survival curve for tumor size greater than 5 cm was significantly different (P<0.0001).

Since it was previously reported that a concentration difference exists in ER among women of different ages (Elwood & Godolphin, 1980), the influence of age on survival rates was examined within the same four ER concentration strata (Table X). None of the four age groups had better survival when their ER level was the same, albeit a borderline significance occurred when ER=2-9. In contrast, when their age group was the same, patients with higher ER levels had better overall survival.

RECURRENT-FREE SURVIVAL

ER positivity (ER>10) associated with a significant prolongation of RFS, but convergence with the ER- group appeared about 6 years post-diagnosis (Figure 8). Stratification in the ER+ range yielded little more prognostic information (Figure 9) and the difference between the ER≤1 group and
Results

Table VI  Overall survival by ER level and clinical TNM stage.

<table>
<thead>
<tr>
<th>ER (fmol/mg protein)</th>
<th>No. Dead</th>
<th>Censored</th>
<th>%Survival (number at risk)</th>
<th>3 years</th>
<th>5 years</th>
<th>7 years</th>
<th>9 years</th>
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<tr>
<td>≤1</td>
<td>27</td>
<td>33</td>
<td>67</td>
<td>81 (22)</td>
<td>66 (13)</td>
<td>66 (2)</td>
<td>-</td>
</tr>
<tr>
<td>2-9</td>
<td>63</td>
<td>27</td>
<td>73</td>
<td>79 (45)</td>
<td>74 (32)</td>
<td>68 (6)</td>
<td>68 (1)</td>
</tr>
<tr>
<td>10-159</td>
<td>160</td>
<td>17</td>
<td>83</td>
<td>94 (143)</td>
<td>90 (105)</td>
<td>81 (29)</td>
<td>71 (3)</td>
</tr>
<tr>
<td>≥160</td>
<td>61</td>
<td>3</td>
<td>97</td>
<td>98 (57)</td>
<td>98 (43)</td>
<td>98 (10)</td>
<td>-</td>
</tr>
<tr>
<td>Clinical TNM stage II*</td>
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<td></td>
</tr>
<tr>
<td>≤1</td>
<td>46</td>
<td>48</td>
<td>52</td>
<td>65 (27)</td>
<td>57 (19)</td>
<td>29 (2)</td>
<td>-</td>
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<tr>
<td>2-9</td>
<td>145</td>
<td>37</td>
<td>63</td>
<td>77 (105)</td>
<td>62 (63)</td>
<td>58 (11)</td>
<td>50 (2)</td>
</tr>
<tr>
<td>10-159</td>
<td>290</td>
<td>25</td>
<td>75</td>
<td>90 (250)</td>
<td>80 (169)</td>
<td>68 (28)</td>
<td>62 (3)</td>
</tr>
<tr>
<td>≥160</td>
<td>101</td>
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<td>79</td>
<td>96 (88)</td>
<td>84 (61)</td>
<td>74 (13)</td>
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<td>Clinical TNM stage III*</td>
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<td>40</td>
<td>46 (16)</td>
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<td>-</td>
</tr>
<tr>
<td>10-159</td>
<td>67</td>
<td>48</td>
<td>52</td>
<td>68 (42)</td>
<td>52 (28)</td>
<td>49 (2)</td>
<td>49 (2)</td>
</tr>
<tr>
<td>≥160</td>
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<td>45</td>
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<td>70 (18)</td>
<td>53 (11)</td>
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<td>Clinical TNM stage IV</td>
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<td>0</td>
<td>25 (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-9</td>
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<td>89</td>
<td>11</td>
<td>14 (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10-159</td>
<td>35</td>
<td>74</td>
<td>26</td>
<td>49 (16)</td>
<td>33 (8)</td>
<td>8 (1)</td>
<td>-</td>
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<tr>
<td>≥160</td>
<td>18</td>
<td>61</td>
<td>39</td>
<td>51 (8)</td>
<td>44 (5)</td>
<td>23 (2)</td>
<td>-</td>
</tr>
</tbody>
</table>

* P<0.0001
### Table VII: Overall survival by ER level and pathologic nodal status.

<table>
<thead>
<tr>
<th>ER (fmol/mg protein)</th>
<th>No. of patients</th>
<th>Dead</th>
<th>Censored</th>
<th>% Survival 3 years</th>
<th>% Survival 5 years</th>
<th>% Survival 7 years</th>
<th>% Survival 9 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative nodes (NO)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>33</td>
<td>36</td>
<td>64</td>
<td>76 (25)</td>
<td>66 (15)</td>
<td>50 (2)</td>
<td>-</td>
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<tr>
<td>2-9</td>
<td>88</td>
<td>16</td>
<td>84</td>
<td>92 (77)</td>
<td>86 (51)</td>
<td>82 (7)</td>
<td>-</td>
</tr>
<tr>
<td>10-159</td>
<td>174</td>
<td>11</td>
<td>89</td>
<td>98 (165)</td>
<td>93 (118)</td>
<td>84 (18)</td>
<td>79 (1)</td>
</tr>
<tr>
<td>≥160</td>
<td>370</td>
<td>4</td>
<td>96</td>
<td>99 (68)</td>
<td>96 (48)</td>
<td>96 (9)</td>
<td>96 (1)</td>
</tr>
<tr>
<td>1-3 positive nodes (N1-3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>35</td>
<td>57</td>
<td>43</td>
<td>57 (18)</td>
<td>40 (9)</td>
<td>35 (3)</td>
<td>-</td>
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<tr>
<td>2-9</td>
<td>73</td>
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<td>75 (53)</td>
<td>59 (31)</td>
<td>54 (7)</td>
<td>54 (2)</td>
</tr>
<tr>
<td>10-159</td>
<td>171</td>
<td>21</td>
<td>79</td>
<td>92 (149)</td>
<td>83 (103)</td>
<td>75 (23)</td>
<td>60 (4)</td>
</tr>
<tr>
<td>≥160</td>
<td>60</td>
<td>25</td>
<td>75</td>
<td>90 (51)</td>
<td>83 (38)</td>
<td>70 (8)</td>
<td>58 (1)</td>
</tr>
<tr>
<td>≥4 positive nodes (N4+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>18</td>
<td>78</td>
<td>22</td>
<td>44 (8)</td>
<td>39 (7)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-9</td>
<td>69</td>
<td>58</td>
<td>42</td>
<td>52 (30)</td>
<td>40 (17)</td>
<td>35 (4)</td>
<td>35 (1)</td>
</tr>
<tr>
<td>10-159</td>
<td>125</td>
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<td>51</td>
<td>73 (88)</td>
<td>59 (56)</td>
<td>43 (11)</td>
<td>43 (2)</td>
</tr>
<tr>
<td>≥160</td>
<td>32</td>
<td>31</td>
<td>69</td>
<td>90 (27)</td>
<td>68 (17)</td>
<td>68 (4)</td>
<td>-</td>
</tr>
</tbody>
</table>

* P < 0.0001
### Results

**Table VIII** Overall survival by ER level and menopausal status.

<table>
<thead>
<tr>
<th>ER (fmol/mg protein)</th>
<th>No. of patients</th>
<th>Dead %</th>
<th>Censored %</th>
<th>3 years %</th>
<th>5 years %</th>
<th>7 years %</th>
<th>9 years %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Premenopause</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>40</td>
<td>60</td>
<td>40</td>
<td>57 (22)</td>
<td>44 (13)</td>
<td>26 (1)</td>
<td>-</td>
</tr>
<tr>
<td>2-9</td>
<td>93</td>
<td>35</td>
<td>65</td>
<td>71 (62)</td>
<td>63 (39)</td>
<td>61 (7)</td>
<td>61 (1)</td>
</tr>
<tr>
<td>10-159</td>
<td>191</td>
<td>30</td>
<td>70</td>
<td>87 (161)</td>
<td>78 (110)</td>
<td>66 (26)</td>
<td>54 (3)</td>
</tr>
<tr>
<td>≥160</td>
<td>6</td>
<td>0</td>
<td>100</td>
<td>100 (5)</td>
<td>100 (4)</td>
<td>100 (1)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Postmenopause</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>33</td>
<td>53</td>
<td>47</td>
<td>58 (33)</td>
<td>48 (21)</td>
<td>33 (3)</td>
<td>-</td>
</tr>
<tr>
<td>2-9</td>
<td>108</td>
<td>45</td>
<td>55</td>
<td>69 (108)</td>
<td>53 (61)</td>
<td>48 (12)</td>
<td>44 (2)</td>
</tr>
<tr>
<td>10-159</td>
<td>288</td>
<td>28</td>
<td>72</td>
<td>86 (288)</td>
<td>76 (203)</td>
<td>65 (37)</td>
<td>63 (5)</td>
</tr>
<tr>
<td>≥160</td>
<td>155</td>
<td>21</td>
<td>79</td>
<td>94 (155)</td>
<td>84 (107)</td>
<td>77 (26)</td>
<td>67 (2)</td>
</tr>
</tbody>
</table>

* P<0.0001
Results

Figure 5  Overall survival by pathologic nodal status. The number at risk in each group were: NO = 381; N1-3 = 343; N4+ = 250. Curves discontinued when fewer than 10 patients under follow-up; P<0.0001.
Results

Figure 6 Overall survival by clinical TNM stage. The number at risk in each group were: TNM I = 322; TNM II = 589; TNM III = 158; TNM IV = 75. Curves discontinued when fewer than 10 patients under follow-up; P < 0.0001.
### Table IX  Overall survival by menopausal status and age.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients</th>
<th>Dead %</th>
<th>Censored %</th>
<th>%Survival (number at risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 years</td>
<td>5 years</td>
<td>7 years</td>
</tr>
<tr>
<td><strong>Menopausal status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopause</td>
<td>341</td>
<td>35</td>
<td>65</td>
<td>79 (258)</td>
</tr>
<tr>
<td>Postmenopause</td>
<td>782</td>
<td>32</td>
<td>68</td>
<td>82 (593)</td>
</tr>
<tr>
<td><strong>Age at diagnosis (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>124</td>
<td>40</td>
<td>60</td>
<td>77 (92)</td>
</tr>
<tr>
<td>40-49</td>
<td>245</td>
<td>34</td>
<td>66</td>
<td>80 (189)</td>
</tr>
<tr>
<td>50-59</td>
<td>309</td>
<td>34</td>
<td>66</td>
<td>77 (220)</td>
</tr>
<tr>
<td>60-69</td>
<td>329</td>
<td>31</td>
<td>69</td>
<td>83 (255)</td>
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<tr>
<td>≥70</td>
<td>177</td>
<td>28</td>
<td>72</td>
<td>83 (128)</td>
</tr>
</tbody>
</table>
Figure 7  Overall survival by tumor size. The number at risk in each group were: < 2 cm = 234; 2-5 cm = 765; >5 cm = 88. Survival curves discontinued when fewer than 10 patients under follow-up; P<0.0001.
Table X  Overall survival by ER level and age at diagnosis.  Five-year percent rates (number of patients in parentheses)

<table>
<thead>
<tr>
<th>Age</th>
<th>ER concentration (fmol/mg protein)</th>
<th>X²&lt;sup&gt;*&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤1</td>
<td>2-9</td>
<td>10-159</td>
</tr>
<tr>
<td>&lt;45</td>
<td>45 (31)</td>
<td>63 (75)</td>
<td>73 (106)</td>
</tr>
<tr>
<td>45-54</td>
<td>37 (31)</td>
<td>65 (77)</td>
<td>78 (163)</td>
</tr>
<tr>
<td>55-64</td>
<td>63 (20)</td>
<td>43 (69)</td>
<td>76 (151)</td>
</tr>
<tr>
<td>≥65</td>
<td>45 (21)</td>
<td>50 (57)</td>
<td>76 (153)</td>
</tr>
</tbody>
</table>

X²<sup>**</sup> 5.3  7.9  1.9  3.5
P-value 0.18  0.05  0.42  0.33

Overall X² by Mantel-Cox statistics:

*  ER controlled for age.
** age controlled for ER.
Results

Figure 8 Recurrence-free survival by ER status. The number at risk in each group were: ER+ = 710; ER- = 342. Curves discontinued when fewer than 10 patients under follow-up; P=0.0001.
Figure 9  Recurrence-free survival by ER concentration. The number at risk in each group were: ER $\geq$ 160 = 208; ER = 10-159 = 531; ER = 2-9 = 246; ER $\leq$ 1 = 88; (ER concentration in fmol/mg protein). Curves discontinued when fewer than 10 patients under follow-up; $P=0.0001$. 
Results

ER = 2-9 group was apparent only after two years from diagnosis. This difference was not statistically significant (P = 0.12).

Nodal status (number of positive nodes) and clinical TNM stage are prognostic discriminators for risk of disease recurrence. The 5-year RFS rates for the three nodal categories were N0 = 75%, N1-3 = 60%, N4+ = 32% (P < 0.0001). A similar pattern was seen for clinical TNM stage where I = 73%, II = 56% and III = 30% (P < 0.0001) (Figures 10 & 11).

There was a significant trend (Mantel & Byar, 1974) of longer RFS with increasing ER, even after controlling for important covariates like nodal status, clinical TNM stage and menopausal status (Table XI). However, it is notable that the recurrence free survival increment was greatest when the groups with ER ≤ 1, ER = 2-9 and ER = 10-159 were compared. The group with ER ≥ 160 had similar recurrence rates to that with ER = 10-159.

To determine if the effect of ER on RFS is independent from treatment effects, relative recurrence ratios were calculated with adjustment for the type of systemic adjuvant therapy. A significant association was found in patients who had not received systemic adjuvant therapy and in those given adjuvant chemotherapy. However, there was no significant ER effect on RFS in patients given adjuvant endocrine therapy (Table XI).

POST-RECURRENCE SURVIVAL

Four hundred and fifty-seven patients had an objectively determined recurrence. The survival after disease recurrence for these patients is described in Figure 12. Their median survival was 22 months. The relationship between ER concentration in the primary tumor and prolongation of PRS was highly significant (Figure 13). The median survival of 46 months in the ER ≥ 160 group was much more favourable than in the other three (ER = 10-159: 27 months; ER = 2-9: 16 months; ER ≤ 1: 12 months).

The influence of other prognostic factors on this relationship was evaluated by the Cox proportional hazards model. ER concentration, therapy, number of involved axillary nodes and clinical TNM stage were significant univariate predictors of PRS (Table XII). The PRS curves as a function of pathological nodal status ascertained at the time of primary diagnosis are shown in
Figure 10 Recurrence-free survival by pathologic nodal status. The number at risk in each group were: NO = 374; N1-3 = 331; N4+ = 238. Curves discontinued when fewer than 10 patients under follow-up; P<0.0001.
Figure 11 Recurrence-free survival by clinical TNM stage. The number at risk in each group were:
TNM I = 321; TNM II = 573; TNM III = 138. Curves discontinued when fewer than 10 patients under follow-up; P<0.0001.
## Results

**Table XI** Relative recurrence ratio. Ratio of observed to expected by ER concentration, adjusted for stage, menopausal status and adjuvant systemic treatment

<table>
<thead>
<tr>
<th>ER concentration (fmol/mg protein)</th>
<th>X²*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1</td>
<td>2-9</td>
<td>10-159</td>
</tr>
<tr>
<td>All patients</td>
<td>1.52</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Nodal status

| Nodal status | 1.70 | 1.15 | 0.82 | 1.08 | 8.12 | <0.005 |

Clinical TNM stage

| Clinical TNM stage | 1.52 | 1.20 | 0.85 | 1.00 | 8.95 | <0.005 |

Menopausal status

| Menopausal status | 1.55 | 1.20 | 0.88 | 0.90 | 13.39 | <0.005 |

Adjuvant therapy:

- **Endocrine**
  - 3.40 | 0.51 | 0.90 | 1.06 | 1.03 | 0.31

- **Chemotherapy**
  - 2.28 | 1.00 | 0.81 | 1.44 | 4.05 | 0.04

- **No adjuvant**
  - 1.24 | 1.29 | 0.86 | 0.97 | 4.75 | 0.03

* X² test for trend (Mantel & Byar, 1974)

** Relative recurrence ratios of all patients unadjusted for other factors
Figure 12 Post-recurrence survival of 457 patients. Curves discontinued when fewer than 10 patients under follow-up.
Results

Figure 13  Post-recurrence survival by ER concentration. The number at risk in each group were:
ER≥160 = 84; ER=10-159 = 206; ER=2-9 = 110; ER<1 = 49; (ER concentration in fmol/mg protein).
Curves discontinued when fewer than 10 patients under follow-up; P<0.0001.
Table XII  Stepwise regression analysis of post-recurrence survival. Cox proportional hazards model of 390 patients with complete data on ER, pathological nodal status, clinical TNM stage and therapy

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable</th>
<th>Likelihood ratio $\chi^2$</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ER concentration</td>
<td>32.3</td>
<td>3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Pathological nodal status (Nodes)</td>
<td>20.0</td>
<td>2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Clinical TNM stage (TNM)</td>
<td>6.7</td>
<td>2</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>First-line therapy (Therapy)</td>
<td>10.9</td>
<td>3</td>
<td>0.012</td>
</tr>
<tr>
<td>2</td>
<td>ER + Nodes</td>
<td>21.2</td>
<td>2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>ER + TNM</td>
<td>7.1</td>
<td>2</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>ER + Therapy</td>
<td>6.3</td>
<td>3</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>ER + Nodes + TNM</td>
<td>4.3</td>
<td>2</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>ER + Nodes + Therapy</td>
<td>5.2</td>
<td>3</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>ER + Nodes + Therapy + (ER x Therapy)</td>
<td>11.8</td>
<td>9</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>ER + Nodes + (ER x Nodes)</td>
<td>5.4</td>
<td>6</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>ER + Nodes + TNM + (ER x TNM)</td>
<td>9.6</td>
<td>6</td>
<td>0.14</td>
</tr>
</tbody>
</table>
Results

**Figure 14.** The difference between the post-recurrent survival of the three nodal groups was highly significant (P=0.0001). A significant difference in PRS occurred between clinical TNM stage groups (Figure 15).

The importance of a single factor in PRS prediction was assessed in relation to the other factors by stepwise regression analysis. This analysis was limited to the examination of interactions between ER, nodal status, clinical TNM stage and type of treatment. The first variable selected to be an independent prognostic factor was ER, followed by nodal status (Table XII). An insignificant interaction term was found in the Cox proportional hazards model (P>0.2) for ER concentration and type of therapy, suggesting that quantitative ER was predictive of PRS regardless of therapy. This is supported by a significant relationship between ER and PRS in the group of 141 patients who were not given any endocrine therapy (P=0.001). Quantitative ER retained its significance in association with PRS after stratification by clinical TNM stage and nodal status (Tables XIII & XIV). However, results from interaction tests of ER with nodal status and ER with clinical TNM stage showed that the effect of ER on PRS was not dependent on the other two factors (Table XII).

**HISTOLOGIC FEATURES OF BREAST CANCER: CORRELATION WITH ER AND PROGNOSIS**

**ER STATUS AND HISTOLOGIC CHARACTERISTICS**

Infiltrating lobular carcinoma associated with a greater proportion of ER+ tumors (P=0.01) than infiltrating ductal carcinoma or other histologic types. Therefore associations between ER status and histologic features were tested after controlling for histologic type. Table XV displays odds ratios for ER positivity to associate with histologic variables. These associations were significant within each histologic type. ER+ tumors are more likely to be well or moderately well differentiated. ER positivity was also related to greater degrees of fibrotic reaction and absent or minimal necrosis. Necrosis was also associated with tumor size. Smaller tumors (<2 cm) tended to have little or no necrosis and larger (2-5 cm and >5 cm) tumors were more often necrotic (Figure 16). Necrosis, however, was not related to the number of positive axillary nodes, menopausal
Figure 14  Post-recurrence survival by pathologic nodal status. The number at risk in each group were: $N_0 = 100$; $N_{1-3} = 133$; $N_{4+} = 154$. Four patients with known nodal status and ER concentration had PRS $< 1$ month. Curves discontinued when fewer than 10 patients under follow-up; $P = 0.0001$. 

Results
Figure 15 Post-recurrence survival by clinical TNM stage. The number at risk in each group were: 
TNM I = 98; TNM II = 250; TNM III = 86. Four patients with known TNM stage and ER concentration 
had PRS < 1 month. Curves discontinued when fewer than 10 patients under follow-up; P=0.01.
### Results

**Table XIII** Survival after first recurrence by ER level and nodal status.

<table>
<thead>
<tr>
<th>ER (fmol/mg protein)</th>
<th>No. of patients</th>
<th>Dead %</th>
<th>Censored %</th>
<th>2 years</th>
<th>3 years</th>
<th>4 years</th>
<th>5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative nodes (NO) *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>12</td>
<td>75</td>
<td>25</td>
<td>29 (3)</td>
<td>14 (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-9</td>
<td>22</td>
<td>55</td>
<td>45</td>
<td>50 (11)</td>
<td>46 (8)</td>
<td>46 (3)</td>
<td>46 (2)</td>
</tr>
<tr>
<td>10-159</td>
<td>39</td>
<td>44</td>
<td>56</td>
<td>71 (23)</td>
<td>62 (17)</td>
<td>47 (11)</td>
<td>47 (9)</td>
</tr>
<tr>
<td>≥160</td>
<td>24</td>
<td>12</td>
<td>88</td>
<td>88 (12)</td>
<td>77 (6)</td>
<td>77 (4)</td>
<td>77 (1)</td>
</tr>
<tr>
<td>1-3 positive nodes (N1-3) *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>21</td>
<td>81</td>
<td>19</td>
<td>25 (4)</td>
<td>12 (2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-9</td>
<td>31</td>
<td>81</td>
<td>19</td>
<td>33 (7)</td>
<td>23 (4)</td>
<td>6 (1)</td>
<td>-</td>
</tr>
<tr>
<td>10-159</td>
<td>53</td>
<td>58</td>
<td>42</td>
<td>57 (24)</td>
<td>44 (17)</td>
<td>38 (9)</td>
<td>33 (6)</td>
</tr>
<tr>
<td>≥160</td>
<td>28</td>
<td>54</td>
<td>46</td>
<td>66 (13)</td>
<td>56 (10)</td>
<td>51 (9)</td>
<td>37 (4)</td>
</tr>
<tr>
<td>≥4 positive nodes (N4+) *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>12</td>
<td>73</td>
<td>17</td>
<td>42 (5)</td>
<td>31 (2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-9</td>
<td>41</td>
<td>80</td>
<td>20</td>
<td>28 (9)</td>
<td>8 (2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10-159</td>
<td>77</td>
<td>70</td>
<td>30</td>
<td>43 (27)</td>
<td>29 (15)</td>
<td>21 (8)</td>
<td>12 (3)</td>
</tr>
<tr>
<td>≥160</td>
<td>19</td>
<td>53</td>
<td>47</td>
<td>57 (7)</td>
<td>57 (4)</td>
<td>42 (3)</td>
<td>42 (1)</td>
</tr>
</tbody>
</table>

* P<0.005
Results

Table XIV Survival after first recurrence by ER level and clinical TNM stage.

<table>
<thead>
<tr>
<th>ER (fmol/mg protein)</th>
<th>No. of patients</th>
<th>Dead %</th>
<th>Censored %</th>
<th>% Survival (number at risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 years</td>
<td>3 years</td>
<td>4 years</td>
</tr>
<tr>
<td>Clinical TNM stage I*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>9</td>
<td>89</td>
<td>11</td>
<td>26 (2)</td>
</tr>
<tr>
<td>2-9</td>
<td>23</td>
<td>70</td>
<td>30</td>
<td>40 (7)</td>
</tr>
<tr>
<td>10-159</td>
<td>46</td>
<td>59</td>
<td>41</td>
<td>58 (22)</td>
</tr>
<tr>
<td>≥160</td>
<td>17</td>
<td>12</td>
<td>88</td>
<td>93 (8)</td>
</tr>
<tr>
<td>Clinical TNM stage II*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>27</td>
<td>67</td>
<td>33</td>
<td>39 (8)</td>
</tr>
<tr>
<td>2-9</td>
<td>62</td>
<td>76</td>
<td>24</td>
<td>32 (18)</td>
</tr>
<tr>
<td>10-159</td>
<td>114</td>
<td>54</td>
<td>46</td>
<td>55 (49)</td>
</tr>
<tr>
<td>≥160</td>
<td>44</td>
<td>48</td>
<td>52</td>
<td>66 (20)</td>
</tr>
<tr>
<td>Clinical TNM stage III*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>9</td>
<td>100</td>
<td>0</td>
<td>11 (1)</td>
</tr>
<tr>
<td>2-9</td>
<td>23</td>
<td>78</td>
<td>22</td>
<td>32 (5)</td>
</tr>
<tr>
<td>10-159</td>
<td>34</td>
<td>71</td>
<td>29</td>
<td>50 (15)</td>
</tr>
<tr>
<td>≥160</td>
<td>18</td>
<td>56</td>
<td>44</td>
<td>53 (8)</td>
</tr>
</tbody>
</table>

* P<0.005
## Results

### Table XV  Prevalence of ER positivity by histologic variables.

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>ER+ %</th>
<th>Odds Ratio * (95% confidence)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histologic differentiation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>well to moderate</td>
<td>793</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>poor</td>
<td>343</td>
<td>64</td>
<td>0.56 (0.41-0.75)</td>
<td>0.0002 **</td>
</tr>
<tr>
<td>unknown</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tumor fibrosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>minimum</td>
<td>478</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>moderate</td>
<td>499</td>
<td>76</td>
<td>2.33 (1.77-3.06)</td>
<td></td>
</tr>
<tr>
<td>marked</td>
<td>159</td>
<td>79</td>
<td>2.62 (1.60-4.27)</td>
<td>&lt;0.0001 **</td>
</tr>
<tr>
<td>unknown</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tumor necrosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>absent</td>
<td>721</td>
<td>76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>moderate</td>
<td>273</td>
<td>62</td>
<td>0.54 (0.40-0.73)</td>
<td></td>
</tr>
<tr>
<td>marked</td>
<td>141</td>
<td>38</td>
<td>0.20 (0.14-0.29)</td>
<td>&lt;0.0001 **</td>
</tr>
<tr>
<td>unknown</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Odds ratio refers to the odds for ER+ associated with that characteristic, relative to the first category listed, with adjustment for histologic type.

** Overall $\chi^2$ statistic, for proportions and trend.
Figure 16 Relationship between of necrosis and tumor size. P<0.01.
status, clinical stage, or histologic differentiation (Table XVI).

PROGNOSIS AND HISTOLOGIC CHARACTERISTICS

Due to small numbers, patients with well differentiated tumors were combined with moderately well differentiated tumors; the resultant OS curve significantly differed from that of the poorly differentiated group (Figure 17). The 5-year survival rate of moderately well differentiated tumors was 73% compared to 63% for the poorly differentiated. There was also a difference between curves defined by extent of necrosis, although most of this difference is attributable to much poorer survival seen in the group with marked necrosis (Figure 18). Neither histologic type (P > 0.5) nor tumor fibrosis (P > 0.5) was a significant univariate predictor of survival (Table XVII), nor for recurrence-free survival.

RFS also varies with differentiation (Figure 19). The 5-year RFS rate is more favourable for better differentiated tumors (60%) than the poorly differentiated (51%). Necrosis also predicts for RFS; tumors with marked necrosis showed a greater recurrence rate than those with little or none (Figure 20).

Of the four histologic variables examined only tumor necrosis retained its prognostic ability for survival after first relapse. Patients with marked primary tumor necrosis have a worse PRS with a 3-year PRS rate of 19% compared to 39% in patients with moderate degree of necrosis and 56% for those with none (Figure 21).

A MULTIVARIATE MODEL FOR BREAST CANCER SURVIVAL

From univariate analyses, it is evident that overall survival is associated with ER quantity, number of positive lymph nodes, clinical stage, tumor differentiation, tumor necrosis and tumor size. The independence of these prognostic factors (covariates) were tested in the Cox proportional hazards model. Other prognostic factors such as histologic type, patient age and menopausal status were also evaluated. Although they were found not to have prognostic importance in the present data, these factors were tested in the multivariate analysis because they have been
Table XVI  Relationship between tumor necrosis and other variables.

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>Tumor necrosis</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absent (%)</td>
<td>Minimal (%)</td>
<td>Marked (%)</td>
</tr>
<tr>
<td>Clinical TNM stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>303</td>
<td>200 (29)</td>
<td>66 (25)</td>
<td>37 (27)</td>
</tr>
<tr>
<td>II</td>
<td>568</td>
<td>349 (50)</td>
<td>145 (55)</td>
<td>74 (54)</td>
</tr>
<tr>
<td>III &amp; IV</td>
<td>225</td>
<td>148 (21)</td>
<td>52 (20)</td>
<td>25 (18)</td>
</tr>
<tr>
<td>Nodal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>360</td>
<td>219 (37)</td>
<td>99 (43)</td>
<td>42 (35)</td>
</tr>
<tr>
<td>N1-3</td>
<td>332</td>
<td>207 (35)</td>
<td>74 (32)</td>
<td>51 (43)</td>
</tr>
<tr>
<td>N4+</td>
<td>247</td>
<td>163 (28)</td>
<td>57 (25)</td>
<td>27 (23)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopause</td>
<td>322</td>
<td>196 (29)</td>
<td>82 (32)</td>
<td>44 (33)</td>
</tr>
<tr>
<td>Postmenopause</td>
<td>752</td>
<td>489 (71)</td>
<td>174 (68)</td>
<td>89 (67)</td>
</tr>
<tr>
<td>Tumor differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>well/moderate</td>
<td>791</td>
<td>494 (69)</td>
<td>201 (74)</td>
<td>96 (69)</td>
</tr>
<tr>
<td>poor</td>
<td>342</td>
<td>226 (31)</td>
<td>72 (26)</td>
<td>44 (31)</td>
</tr>
</tbody>
</table>
Figure 17 Overall survival by histologic differentiation. The number at risk in each group were: moderate & well = 793; poor = 343. Curves discontinued when fewer than 10 patients under follow-up; P=0.0006.
Figure 18 Overall survival by extent of primary tumor necrosis. The number at risk in each group were: absent = 721; moderate = 273; marked = 141. Curves discontinued when fewer than 10 patients under follow-up; P=0.0002.
### Results

**Table XVII**  Overall survival by histologic type and tumor fibrosis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients</th>
<th>Dead %</th>
<th>Censored %</th>
<th>3 years</th>
<th>5 years</th>
<th>7 years</th>
<th>9 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 years</td>
<td>5 years</td>
<td>7 years</td>
<td>9 years</td>
</tr>
<tr>
<td><strong>Histologic type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td>1003</td>
<td>32</td>
<td>68</td>
<td>80 (754)</td>
<td>71 (503)</td>
<td>62 (96)</td>
<td>55 (11)</td>
</tr>
<tr>
<td>Lobular</td>
<td>110</td>
<td>30</td>
<td>70</td>
<td>87 (87)</td>
<td>72 (57)</td>
<td>57 (9)</td>
<td>57 (1)</td>
</tr>
<tr>
<td><strong>Tumor fibrosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>478</td>
<td>31</td>
<td>69</td>
<td>78 (357)</td>
<td>70 (237)</td>
<td>64 (35)</td>
<td>64 (5)</td>
</tr>
<tr>
<td>Moderate</td>
<td>499</td>
<td>36</td>
<td>64</td>
<td>80 (375)</td>
<td>68 (250)</td>
<td>59 (60)</td>
<td>50 (4)</td>
</tr>
<tr>
<td>Marked</td>
<td>159</td>
<td>31</td>
<td>69</td>
<td>84 (126)</td>
<td>73 (86)</td>
<td>61 (19)</td>
<td>58 (4)</td>
</tr>
</tbody>
</table>
Results

Figure 19 Recurrence-free survival by histologic differentiation. The number at risk in each group were: moderate & well = 742; poor = 299. Curves discontinued when fewer than 10 patients under follow-up; P=0.008.
Figure 20  Recurrence-free survival by extent of tumor necrosis. The number at risk in each group were: absent = 652; moderate = 246; marked = 129. Curves discontinued when fewer than 10 patients under follow-up; P=0.04.
Figure 21 Post-recurrence survival by extent of tumor necrosis. The number at risk in each group were: absent = 276; moderate = 102; marked = 63. Curves discontinued when fewer than 10 patients under follow-up; $P = 0.0002$. 

PRS: Tumor necrosis
Results

Four covariates were shown to be predictive of the overall risk of mortality and each conveyed prognostic information unique from the others (Table XVIII). Nodal status associated with the greatest likelihood value and hence was selected into the model first. The more positive estimated $\beta$ of N4+ relates to the greater hazard when this particular characteristic was present. The significance of that covariate level is indicated by a standardized regression coefficient ($\beta$ divided by its standard error); a value greater than 2.0 corresponds roughly to $P \leq 0.05$ for a standard normal deviate (Byar, 1982). Clinical TNM stage entered the regression equation next; the incremental coefficients reflect the detrimental effects of increased stage at presentation. The negative coefficient of $\log_e[ER]$ indicates the reduced risk associated with increasing ER concentration. The final variable selected was tumor necrosis. Marked confluent necrosis was associated with a greater risk of mortality than minimal or no necrosis.

Figure 22 depicts the fitted statistical model which predicts the probability of 5-year survival of an individual breast cancer patient with a particular combination of the above four parameters. The model permits prediction for any discrete time after diagnosis. The 5-year mark was chosen for illustration because the 95% confidence intervals of estimates at this time were relatively small, 5-year survival is of clinical interest and it allows comparison with other reports.

A counterbalance of the effects of these covariates on survival can be seen. For example, a particular patient with TNM I, N4+, ER = 400 fmol/mg and no tumor necrosis is predicted to have similar survival to another with TNM I who has N0, but ER = 1 fmol/mg and marked tumor necrosis. Thus "high-risk" according to nodal status or clinical TNM stage can be attenuated by the presence of high ER concentration. The proportional benefit of high ER generally increases with increasing risk due to other factors (Figure 23).

A hazard index was calculated for each patient by log-linear substitution of the obtained coefficients and covariate values. For example a patient with N1-3, TNM II, $\log_e[ER] = 3$ and no necrosis would have a hazard index equal to:

$$\exp (0.47 + 0.41 + (-0.21)(3) + 0) = 1.28$$
Results

Table XVIII  Cox proportional hazards model for survival. Final steps of stepwise regression analysis on all studied variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\beta^*$</th>
<th>$\beta$/SE</th>
<th>$p^{**}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall survival model (n = 859)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodal status:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1-3</td>
<td>0.47</td>
<td>2.65</td>
<td>0.01</td>
</tr>
<tr>
<td>N4 +</td>
<td>1.17</td>
<td>6.74</td>
<td>0.0001</td>
</tr>
<tr>
<td>Clinical TNM stage:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0.41</td>
<td>2.31</td>
<td>0.02</td>
</tr>
<tr>
<td>III</td>
<td>1.14</td>
<td>5.12</td>
<td>0.0001</td>
</tr>
<tr>
<td>IV</td>
<td>2.06</td>
<td>6.89</td>
<td>0.0001</td>
</tr>
<tr>
<td>Log$_e$ [ER]:</td>
<td>-0.21</td>
<td>5.56</td>
<td>0.0001</td>
</tr>
<tr>
<td>Necrosis:</td>
<td>+</td>
<td>0.64</td>
<td>3.57</td>
</tr>
<tr>
<td>Recurrence-free survival model (n = 828)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodal status:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1-3</td>
<td>0.31</td>
<td>2.27</td>
<td>0.02</td>
</tr>
<tr>
<td>N4 +</td>
<td>1.01</td>
<td>7.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clinical TNM stage:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0.34</td>
<td>2.56</td>
<td>0.01</td>
</tr>
<tr>
<td>III</td>
<td>0.77</td>
<td>4.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Necrosis:</td>
<td>+</td>
<td>0.44</td>
<td>2.84</td>
</tr>
<tr>
<td>Log$_e$ [ER]:</td>
<td>-0.07</td>
<td>2.32</td>
<td>0.02</td>
</tr>
<tr>
<td>Post-recurrence survival model (n = 369)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log$_e$ [ER]:</td>
<td>-0.23</td>
<td>6.08</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nodal status:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1-3</td>
<td>0.52</td>
<td>2.83</td>
<td>0.005</td>
</tr>
<tr>
<td>N4 +</td>
<td>0.86</td>
<td>4.80</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Necrosis:</td>
<td>+</td>
<td>0.43</td>
<td>2.37</td>
</tr>
</tbody>
</table>

* Estimated regression coefficient for each level of the covariate.

** Global $\chi^2$ statistic that tests that all $\beta$'s = 0.
Figure 22  Predicted 5-year overall survival. By simultaneous effects of pathological nodal status, clinical TNM stage, ER concentration and tumor necrosis. The pairs of lines A, B and C designate patients with axillary nodal status N0, N1-3 and N4+, respectively. The upper of each pair designates absent or minimal tumor necrosis and the lower, marked tumor necrosis. Four ER concentrations (1, 7, 150 and 400 fmol/mg), the break points on each line from lowest to highest, were arbitrarily chosen to represent a wide range.
Results

Figure 23. Relative gain in predicted 5-year survival. As a consequence of high ER concentration (400 fmol/mg) compared to negative ER (1 fmol/mg); in cases with no necrosis.
Partition levels were set for even cut-points after preliminary examination of the data and four risk groups were formed. Survival curves representing the four risk groups are shown (Figure 24); the difference between them was highly significant (P<0.0001). The following 5-year survival was observed in each subgroup: (a) hazard index <1, (n = 366): 91%; (b) hazard index = 1.00-1.99, (n = 256): 79%; (c) hazard index = 2.00-2.99, (n = 97): 65%; (d) hazard index >3, (n = 140): 43%.

The goodness-of-fit of the model was tested graphically by comparison of the observed survival curves defined by hazard indices with predicted survival curves from the model (Figure 24). Each of the predicted curves represents the mean hazard value for the category. For example, the upper curve represents a hazard index of 0.62 which is the mean for the "<1" subgroup.

The basic proportionality assumption of the Cox model states that the relationship between the underlying hazard function and the covariates is multiplicative. This was supported by nearly constant differences of the log (-log survival) [more specifically, ln(-ln \hat s(t;\hat x)); see Materials & Methods] curves between strata of a covariate, specified at the mean of the other covariates. Figures 25-28 show each of the four covariates that were stratified. The covariate ER was treated as a binary component of ER positive and ER negative to facilitate plotting. That the plots display approximately constant differences between strata supports the proportionality assumption (Kalbfleisch & Prentice, 1980). Furthermore, interaction terms composed of the cross-products between all terms in the model were tested when the main factors (nodal status, clinical TNM stage, ER and necrosis) were in the model. The likelihood ratio \chi^2 associated with the addition of the interaction terms to the model containing the main factors was insignificant (P=0.13), indicating the effect of one covariate does not depend on the value of another.

The results of multivariate analysis of RFS are shown in Table XVIII. The recurrence-free survival model is based on 828 patients and consists of number of positive lymph nodes, clinical stage, tumor necrosis and ER concentration. It is evident that the number of positive nodes predicts for the highest risk of recurrence. Clinical stage contributes additional information on this risk. Tumor necrosis is found to be of greater prognostic importance than ER concentration for RFS. This is supported by the relatively small magnitude of the \beta associated with ER.
Figure 24 General model fit: observed survival versus predicted survival. As a function of hazard index level. Each of the predicted curves represents the mean hazard value for the category. The curves were discontinued when fewer than 10 patients remained at risk in the observed survival group.
Results

Figure 25  Log minus log survival by ER status.
Results

Figure 26 Log minus log survival by pathologic nodal status.
Figure 27 Log minus log survival by tumor necrosis.
Results

Figure 28  Log minus log survival by clinical TNM stage.
Results

concentration ($\beta = -0.07$). ER concentration also showed a less definitive role as seen previously in the univariate analysis of RFS. However, the information conveyed by ER concentration is independent of the other three covariates and still significantly improves the predictability of the model.

A relatively large number of patients have had a recurrence, thus a model specifically relates to survival after first recurrence has been developed. A model based on 369 patients consisting of only three covariates was found to best explain variation in survival after first recurrence (Table XVIII). The most influential factor of PRS was ER concentration in the primary tumor, while pathological nodal status at the time of primary diagnosis and tumor necrosis have further prognostic effects which improved the prediction. These three predictors could be shown to collaborate in modifying PRS (Figure 29). The model suggests that any nodal categories at lower ER concentrations have very poor prognosis with an additional influence by the extent of necrosis in the primary tumor.
Figure 29  Predicted 5-year post recurrence survival. By simultaneous effects of ER concentration, nodal status and tumor necrosis. The upper of each pair designates absent or minimal tumor necrosis and the lower, marked tumor necrosis. Four ER concentrations (1, 7, 150 and 400 fmol/mg), the break points on each line from lowest to highest, were arbitrarily chosen to represent a wide range.
A better understanding of the natural history of breast cancer comes by identification of factors that discriminate patient prognosis. Prognostic factors enhance our knowledge of the biological differences in tumor behavior. Hence, they are useful for the selection of optimal treatment after primary surgery and for proper stratification of patients in treatment trials.

Univariate survival analysis was one method employed in this study of breast cancer prognosis. A statistically significant relationship between overall survival and ER content in the primary tumor was found. This finding confirms an earlier study (Godolphin et al., 1981) that had shown greater prognostic information accrued from quantitative ER data. Higher ER concentration associated with longer survival throughout the entire observation period of ten years. This relationship remained strong after separate adjustments for pathologic nodal status, clinical TNM stage, menopausal status and patient age at diagnosis.

In this study the group with ER<1 had a particularly poor prognosis. This distinction from the ER=2-9 group would be concealed by the conventional classification of ER<10 as ER-, or if an insensitive assay was employed. The few studies of quantitative ER and overall survival overlooked this fine distinction in the ER- range; others have used categories such as: 0 vs 5-30 vs 30-60 (Howat et al., 1985); <5 vs 5-19 vs >20 (Vollenweider-Zerargui et al., 1986); and <3 vs 3-49 vs >50 (Clark et al., 1983b) fmol/mg. The choice of cutoff value to define ER status is also very important. Forrest et al. (1980) reported a significantly better prognosis for ER+ patients when ER positivity was defined as ≥0.1 fmol/mg wet weight, but a cutoff of 0.5 fmol/mg wet weight eliminated this difference between ER+ and ER- patients. The use of ER categories as defined in this study (≤1, 2-9, 10-159, ≥160 fmol/mg protein) was justified, in view of the significant correlation observed between ER and survival and in the previous study (Godolphin et al., 1981).

Most studies of RFS compared ER+ and ER- groups. Initial differences between ER+ and ER- RFS curves were not observed after follow-up times of 2 years (Adami et al., 1985), 3 years (Howat et al., 1983), 4 years (Saez et al., 1983), 5 years (Hahnel et al., 1979), or 7 years (von Maillot et
Discussion

al., 1982). The present data by ER status (ER- and ER+) revealed prognostic difference between ER+ and ER- patients which was most prominent for the first six years after diagnosis.

When the data was analysed by ER concentration a higher ER predicted longer RFS, especially when the groups ER=2-9 and ER=10-159 were compared. However, having ER≥160 did not prolong RFS compared to ER=10-159, which cannot be readily explained on a biological basis. It is possible that this a priori cutoff point of ER≥160 could not adequately detect a more favorable survival that may associate with tumors very rich in ER, (e.g. ER≥400). Such a high level of ER was found in only about 3% of the patients studied and therefore was not appropriate for statistical testing. Nevertheless, it is believed that the essence of analysing survival with ER concentration is to detect the highly significant trend of better survival with higher ER. Whether pair-wise differences between curves are statistically significant is relatively less important, since cutoff points for ER strata might be redefined in different data sets in order to obtain “significant” statistical difference.

ER does not act exclusively through adjuvant therapy to prolong RFS. The biological effect of ER on tumor behavior may be more enduring than the therapeutic effects of systemic adjuvant treatments. However, it was not intended to evaluate therapeutic effects of different treatments in this retrospective study. Nevertheless, better RFS with higher ER was observed among patients who did not receive any systemic adjuvant treatment. This significant trend was also seen in patients who received adjuvant chemotherapy but was not evident in those who received adjuvant endocrine therapy.

Several possibilities may account for the lack of prognostic effect by ER in the subset given adjuvant endocrine therapy. Firstly, the advantage of longer RFS might not be restricted to ER+ patients. Patients classified as ER- but whose tumor contained quantifiable amounts of ER could have derived sufficient benefit from this type of therapy to virtually eliminate RFS differences that otherwise exist when no adjuvant endocrine therapy was given. The PgR status in the tumors of these patients would have been a valuable adjunct to explain the phenomenon. Secondly, it is possible that patients in this subset represented those treated in earlier years when the efficacy
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of ovarian ablation in premenopausal patients and that of tamoxifen in postmenopausal patients was not clearly defined. It is also possible that statistical significance was not reached, with an unstable trend in the relative recurrence ratios, as a result of the small number of patients in this subset.

Patients who were ER+ recurred later and less frequently than ER- patients, at least over the five year post-surgery period during which the risk of recurrence is greatest (Giuliano, 1983). Nevertheless, there was a tendency for the higher ER curves to converge with the lower after six years of observation. This is an important reflection of different biological influences which promote disease recurrence of ER+ and ER- tumors. It is consistent with the hypothesis that ER influences the timing of a recurrence if it is to occur, rather than the likelihood of it occurring (McGuire et al., 1986). Unless primary surgical treatment is curative or it leaves such a low tumor burden that any residual disease is potentially curable by adjuvant therapy (Zelen & Gelman, 1986), the metastatic potential of all tumors exists but may be manifested at various times and dictated by tumor cell kinetics, which are generally correlated with ER status (Silvestrini et al., 1986). Thus, it is not surprising that the curves converged when the observations were extended over time. Most ER- patients have an early recurrence. Therefore, there are fewer ER- patients still at risk after prolonged observation. The survival curves for these patients show less frequent changes in the cumulative probability of survival and tend to plateau at the tail of the curves. Recurrence is delayed in ER+ patients so there are more patients at risk of disease recurrence in the latter part of the observation. This results in a gradual and continual decline of the ER+ survival curves.

It is unlikely that the overall survival curves will converge with long term follow-up. The relevant end point is disease-specific death and other causes of death are included as censored data. After prolonged observation, e.g. past 15 years from diagnosis, the survival estimates of both ER+ and ER- groups would be similarly affected by greater censoring due to competing causes of death. The pattern of the curves would be expected to remain the same because disease-specific death would continue to be a function of ER.
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Although ER is strongly correlated with patient age, there is no survival advantage in older patients. After controlling for ER level, the overall survival rates of the four age groups (<45, 45-54, 55-64, ≥65) did not differ. However, the overall survival of patients with higher ER was consistently better across the four age strata. Hence ER does not depend on age to express its influence on survival and any effect of age on prognosis may be explained by variation in ER level. This may account for the prognoses of young women (Rosen et al., 1984) and elderly women (Schaefer et al., 1984) being similar to that of other age groups. The present results disagree with a recent large study reported by Adami et al. which did not control for ER level but found the age group 45-49 to be most favoured (Adami et al., 1986). Menopausal status was also an insignificant discriminant of patient prognosis.

These findings emphasize the value of quantitative ER to breast cancer survival and verify its prognostic importance independent of nodal status and clinical TNM stage. The ER concentration ranges used in the present analysis served to demonstrate this generalized relationship of ER quantity and prognosis; they are not rigid cutoff points that define prognosis for a group of patients. The presence of tumor in axillary nodes is a very strong indication for some form of systemic adjuvant therapy, regardless of menopausal status (Lippman & Chabner, 1986). However, axillary nodal status is not an absolute prognostic factor, since approximately 13% of node-negative patients have a recurrence within five years (Fisher et al., 1980a). Measurement of the ER level may help to identify a subset of high risk patients with apparently uninvolved axillary nodes, or node-positive patients whose prognosis is not improved by usual systemic adjuvant therapy. More intensive adjuvant regimens may then be considered for these patients.

The present study also demonstrates the importance of ER to post-recurrence survival. Survival after the first relapse is associated with higher ER in the primary tumor. Others have found a significant correlation between ER status (ER+ and ER-) and time from recurrence to death (Paterson et al., 1982; Lionetto et al., 1986). The present study shows that the prognostic role of ER is further enhanced by stratification into concentration ranges. The subset with primary ER≥160 consistently had superior PRS when the 457 patients were analysed as a group and after
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stratification by nodal status and clinical TNM stage. The significant association between ER concentration and survival after recurrence was also independent of the effects of therapy given for recurrent disease. Improved response to endocrine therapy associated with higher ER is not the only reason for prolonged PRS, since survival after disease recurrence is significantly prolonged with higher ER even in patients not given endocrine therapy. Thus, the effect of primary ER on post-recurrent survival appears not to depend on the mode of systemic therapy.

That higher ER in the primary tumor associates with more favourable PRS may relate to the intrinsic biologic behaviour of the cancer. Indirect measurements of tumor growth rate, in terms of thymidine labelling index (Silvestrini et al., 1979) or mean proportion of cells engaged in DNA synthesis (McDivitt et al., 1986), are inversely correlated with ER. Thus, not only is there a tendency for ER+ tumors to recur later than ER- tumors but the biological influences signalled by the presence of ER act in the same direction even after disease recurrence.

The prognostic effect of high tumor ER on PRS has a biologically plausible basis. The degree of tumor differentiation is a strong correlate of tumor ER (Fisher et al., 1981; Alanko et al., 1984) and may be an attribute of the generally less aggressive nature of ER+ tumors as compared to ER- tumors. ER content has also been found to relate to the site of initial metastases (Campbell et al., 1981; Williams et al., 1987). It is likely that well-differentiated ER+ tumors will maintain a low growth rate even after the establishment of a metastasis, which is more often in bone and tend to have a less aggressive course than metastases to visceral organs (Coleman & Rubens, 1987). Response by ER+ tumors to endocrine therapy may vary according to site; recalcification of bone may be less frequent than soft tissue tumor regression and appears to depend on the availability of specific matrix requirements (Stoll, 1985).

In contrast, ER- tumors that tend to be poorly differentiated may have a faster growth rate. This would increase the pace of acquisition of genetic variability and instability, leading to emergence of subclones with greater growth autonomy or malignancy (Nowell, 1976). This behaviour may be maintained even after recurrence but be arrested by cytotoxic chemotherapy. Response to endocrine therapy by the hormone resistant cells is unlikely. Therefore, the advantage in survival
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after recurrence seen among patients with higher ER relies more on the biologic interplay between host and tumor than on response to specific endocrine therapy.

Pathological nodal status shows the approximate extent of tumor involvement in the axillary lymph nodes. It was another significant predictor of OS, RFS and PRS. Patients who had no involved axillary nodes showed significantly prolonged OS and RFS compared to patients who had greater numbers of involved axillary nodes. The finding that nodal status predicts PRS contradicts several reports (Paterson et al., 1982; Howat et al., 1985; Williams et al., 1986). However, Lionetto et al. (1986) reported a significantly shorter survival after relapse in patients with axillary node metastases at presentation. A multivariate analysis by Clark et al. (1985) of predictors of PRS also showed the importance of axillary lymph node status at primary diagnosis. Perhaps others, e.g., Howat et al. (1985), who categorized nodal involvement in the manner done here, did not detect a difference in PRS according to nodal status because of small sample size and staging based on anatomic involvement rather than extent (number of positive nodes) of involvement (Williams et al., 1986).

The metastatic involvement of axillary nodes at the time of primary treatment is likely another reflection of tumor aggressiveness. The presence of positive nodes signals the successful evasion of host immune and nonimmune defences in the regional lymph nodes (Fisher, 1984; Fidler, 1984). A conducive environment may be furnished by the host which facilitates the expression of specific tumor cell properties that permit dislodgment from the primary site, such as changes in cell surface chemistry associated with loss of cell adhesiveness (Kim, 1985) and enhanced tumor invasiveness by increased tumor cell motility and production of proteolytic enzymes such as collagenases specifically active against basement membrane (Liotta, 1984).

The clinical TNM stage at presentation is also a significant prognostic factor. Earlier presenting stage was associated with both better OS and RFS. This relationship was diminished when survival after recurrence was examined, although PRS was still significantly better in patients of clinical TNM stages I and II as compared to those of clinical TNM stage III.
Breast cancer prognosis is also influenced by several variables which were evaluated by microscopic examination. Histologic differentiation was a significant prognostic factor. Overall survival and time to recurrence were significantly shorter for patients with poorly differentiated tumors as compared to those with well to moderately differentiated tumors. Stenkvist et al. (1983) also observed that poor differentiation by World Health Organization classification associated with high 5-year recurrence rates. Others have also found that histologic grade predicts overall survival and early disease recurrence (Ketterhagen et al., 1984; Davis et al., 1986). Fentiman et al. (1984) found that tumor grade had little prognostic value past 5 years after diagnosis. They saw a similar prevalence of grade 3 (poorly differentiated) tumors in survivors of over 20 years and those who died within 20 years after diagnosis. Differentiation had no prognostic importance after the first relapse in the present data. Patients with better differentiated tumors had the same risk of mortality from breast cancer after it has recurred as those with poorly differentiated tumors.

Tumor necrosis was found to be an important discriminator in OS, RFS as well as PRS. Despite the subjectivity of measurement and the fact that the extent of necrosis also depends on how representative is the plane of sectioning, it was evident that patients with marked necrosis consistently fare worse than those with little or none. The demonstration of a significant prognostic distinction in tumors that exhibited marked necrosis from the other two conditions is suggestive of an intrinsically aggressive behavior of these tumors. Invasion of the vasculature by tumor may be one factor that brings about tumor necrosis; the latter is a more convenient and reliable marker since it is more readily detected microscopically. Identification of blood vessel and lymphatic invasion is hampered by various factors including shrinkage artifact as a result of tissue fixation (Lee et al., 1986).

The prognostic importance of tumor necrosis has only been examined in a few reports. Marked tumor necrosis associating with infiltrating ductal carcinoma (not otherwise specified, NOS) is directly correlated with higher rates of locoregional recurrence, metastases or death (Fisher et al., 1978a). Carter et al. (1978) found that tumors with both necrosis and an infiltrating border
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associate with a higher 10-year mortality rate than circumscribed tumors without necrosis. Fisher et al. (1980a) identified tumor necrosis, poor differentiation and tumor size greater than 4 cm to be independently important in predicting 5-year treatment failure in node negative patients and those with four or more positive nodes.

There were no differences detected between survival rates for infiltrating ductal and infiltrating lobular carcinomas. Perhaps differences between these two major types were not detected due to the preponderance of the former (87%); or perhaps only certain morphologic variants of these two types associate with better survival. In fact, Dawson et al. (1986) identified three cytologic variants among the broad descriptive term "infiltrative ductal carcinoma (NOS)", and found a variant of small cells with small nuclei, indistinct cell boundaries and amphiphilic cytoplasm associated with 25-year survival while a combination variant was less often observed in long term survivors. Dixon et al. (1985) identified certain invasive types (cribiform, tubular and medullary) as more prevalent in patients who survived for 16 to 20 years than in those who survived less than 10 years after diagnosis.

Prognostic association with other histologic types is problematic; mucin-producing colloid carcinomas, generally considered to have favourable long term prognosis, can be confused with mucin-producing lobular and papillary carcinomas or infiltrating carcinomas with a minor colloid component (Editorial, 1985). It is likely that differences in prognosis are attributable mainly to variation in stage and grade rather than histologic type, since even common infiltrating ductal carcinoma of no special type may encompass a range of morphology (Gallager, 1984).

Significant associations were found between tumor ER status and histologic characteristics. Infiltrating lobular tumors were found to be more often ER+ than infiltrating ductal tumors in the present data; this was also the observation of Rosen et al. (1975) and Muresan et al. (1986). Shousha et al. (1986) also found that an alveolar variant of invasive lobular carcinoma consistently contained high ER concentration. Better differentiated infiltrating ductal tumors were more often ER+, confirming previous reports (Fisher et al., 1981; Elwood & Godolphin, 1980; Alankö et al., 1984). In contrast to my observations Howat et al. (1983) found no association.
between stromal fibrosis and receptor status although their postmenopausal patients with marked fibrosis had low ER concentration. Stromal fibrosis, positively associated with ER+, would be considered a constructive host response which may be related to better tumor differentiation. However, I found tumor fibrosis had no significant influence on all three survival classes.

The negative association between ER positivity and confluent tumor necrosis may be modulated through the growth pattern. If necrosis occurs, it will probably affect large aggregates of cells and be less likely in single cell carcinomas such as infiltrating lobular and colloid carcinomas which are more often ER+. Even so, the association between ER+ and necrosis remained significant after controlling for histologic type.

Despite strong evidence that confirms the prognostic roles of ER concentration, clinical TNM stage, pathological nodal status, tumor differentiation, tumor necrosis and tumor size, it is difficult to summarize the long term outlook or short term risks of a patient on the basis of the above univariate associations with prognosis. The challenge is to elucidate which among a set of factors provide relevant and important prognostic information when their contributions are assessed simultaneously. An answer might also improve understanding of biological modifiers of the clinical course after primary treatment. It would also help to explain the prognostic heterogeneity of similarly treated patients.

A multivariate approach was taken in this study to identify relevant and independent factors, measurable at diagnosis, that could be used to model risk of breast cancer mortality. Four such factors emerged as the best combination; in descending order of importance they were: number of involved axillary nodes, clinical TNM stage, ER concentration and extent of tumor necrosis. The incorporation of these factors in the final model obviated the prognostic information provided by other covariates that were studied, since the effect of other factors had already been explained by one or more of the chosen covariates.

The Cox proportional hazards model has been used by others to correlate predictive factors and survival with breast cancer. The prognostic usefulness of ER status in addition to tumor stage, axillary node metastasis and histopathologic grade has been reported (Parl et al., 1984). Russo et
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al. (1987) showed improved survival predictions when nodal status, tumor size and grade and ER status were combined. Haybittle et al. (1982) found that the inclusion of tumor grade or ER status in a similar model was mutually exclusive, such that omission of either factor in the analysis permitted the inclusion of the other. My prediction model was based on a large number of patients with long follow-up and the quantitative effects of ER together with its strong correlates such as tumor differentiation and tumor necrosis were studied. The effect of tumor differentiation was overwhelmed when examined in this context and did not remain in the final equation. This was not due to a lack of prognostic importance but to a prognostic influence similar to other variable(s).

There is firm concordance amongst studies pointing to axillary nodal involvement as the single most important predictor (Fisher, 1984). In many treatment protocols metastases in axillary nodes warrant the institution of systemic adjuvant therapy. However, considerable prognostic heterogeneity within nodal categories may result in overtreatment when the sole criterion for adjuvant therapy is the presence of lymph node metastases (van der Linden et al., 1986). Adjuvant therapy is clearly indicated in patients who are considered "high-risk", but there is a need to agree upon the definition of high-risk (Cascinelli et al., 1987). It is evident from this study that "high-risk" is a composite of characteristics. Despite the inherent unreliability of clinical assessment of regional lymph node involvement (Coppin & Swenerton, 1983) clinical TNM stage was found to be independently important in conveying the severity of this risk. There is general consensus on the clinical importance of ER status of the primary tumor and it is confirmed by the present investigation that there is a quantitative relationship with prognosis.

Survival predictions based on this model suggest that the more ER present in the primary tumor, the greater the prognostic improvement in patients with dire features of advanced stage disease. Therefore a high-risk patient, by the criterion of advanced clinical TNM stage or node positivity, may be considered to have reduced her risk by virtue of high ER concentration in the tumor.

The positive influence of high ER concentration was also expressed as a reduced risk of death after disease recurrence. This favourable effect was not dependent on the mode of systemic therapy (Table XI), but appears to be an independent manifestation of the generally better differentiation.
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(Fisher et al., 1981) and slower growth rate of these tumors (McDivitt et al., 1986). Tumor necrosis, which was the only histopathologic feature of the primary tumor with enduring biological influence beyond recurrence, added further prognostic information to ER concentration.

A hazard index is a convenient quantitative measure of the summed risk estimate for a patient with a particular set of predictors. The concept of one covariate compensating for the hazardous effect of the others may be illustrated with this index. Each of the four hazard categories in Figure 24 is defined by a range of hazard index values. Patients who have different patterns of the four covariates may have the same hazard index and hence be considered to have the same degree of risk.

One important caveat to model derivation based on multivariate regression analysis in general is that this may be a subjective process. Simon (1983) noted that use of different stepwise procedures may give different sets of selected models. Greenberg et al. (1974) have used a step-down regression method to derive a prediction model. This method is supported by Mantel (1970) for its better discriminatory ability when there are highly correlated covariates. The idea is that covariates with the least predictive ability are eliminated first and eventually one ends with the several best fitting covariates in the model. The numerical coding of covariates may also be arbitrary. The criterion for covariate selection may vary at different significant levels.

Model fit evaluation has been done by others with the data splitting technique (Greenberg et al., 1974). Other methods such as graphical analysis of residuals (Simon, 1983) from fitting survival models are being presently investigated in the field of applied statistics but software for this procedure is not currently available. Simon (1984) noted that good agreement between observed and fitted results on a single set of data does not in itself demonstrate the reliability of the predictions. The accuracy of these prediction models needs to be prospectively tested on an independent set of data.

It must be emphasized that the present multivariate analyses were done with a priori definitions of the variables to be tested and with a predetermined significance level of 0.05 for variable entry into the equation and there was confirmation of the joint linear effects of the
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covariate on the hazard function. The prognostic effects of all four covariates that constitute
the model also make biological sense. However, the possibility of time-dependent covariate effects
was not investigated.

Other inherent limitations of the present retrospective study must also be noted. Although the
source of clinical data may be considered relatively homogeneous as it was derived from a major
referral center, the precise nature of the referral may yield a potential underrepresentation of
earlier stage disease or rapidly fatal disease. The application of inclusion criteria that
restricted patients who had certain predetermined characteristics to enter the study, produced
further homogeneity among patients at the expense of generalizability (Fletcher et al., 1982). The
increased yield of mammographically detected breast tumors in recent years may also affect the
natural history of the disease by an unknown magnitude. The generalizability of the present
results to future breast cancer patients is not known.

Another potential bias in this type of study relates to measurement of various parameters, such
as the prognostic variables and patient outcome. This is especially important with variables that
have been measured by subjective methods. The assessment of histopathologic features may be
influenced by the pathologist's clinical experience and personal interpretation of results. There
may be intraobserver variation in this interpretation over time but this is an aspect not readily
amenable to quantitative testing. Furthermore, the histologic assessment of features present in
the adjacent section to that used for ER analysis gives a crude summary of histologic features that
presumably represent the entire tumor.

The categorization of clinical TNM stage was also based on clinical judgment by different
clinicians who performed the presurgical physical examination. The various components of the TNM
staging system associate with certain margins of error. However, more objective means of measuring
tumor size and the extent of axillary nodal metastases are also not immune to measurement
inaccuracy. Quality control in terms of preparation of the tumor and axillary nodal specimens for
subsequent evaluation is difficult to ensure and to assess in a retrospective manner.
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The treatment variable was another potential measurement bias in this study. Treatment approaches changed from the mid-seventies to the early eighties and the absolute effect of treatment on patient outcome cannot be objectively determined outside of a prospective randomized clinical trial. Treatment given after disease recurrence may also have been heterogeneous under circumstances where individualization took precedence over standard protocols. The relationship between the effect of treatment types and other prognostic factors was thus examined in a general context in the present study.

A bias might have existed in the measurement of patient outcome, particularly when disease recurrence was considered. The date of recurrence is a less precise and less reliable parameter as compared to the date of death. The reliability is also a function of the intensity and frequency with which the patients were followed. Certain subgroups of patients deemed to have high risk disease by various definitions might have been preferentially followed more closely than others.

There is a finite probability that the individual or combined consequences of the above biases adversely affected the present results. However, the use of multivariate adjustments for the various parameters with large sample size allow clinically important statements be made on the present findings. The interpretations of these findings were also made in a biological context with acknowledgement of potential weaknesses of the study.

The four factors identified in this study are believed to represent different aspects of the complex interactions between host and tumor and reflect biologically significant characteristics of breast cancer although the underlying precise mechanisms remain speculative. The presenting clinical TNM stage indicates the anatomical progression of the disease and also signals a permissive environment that allows full expression of tumor invasive properties. The host may also provide facilitative conditions for tumor expression of metastatic properties, such as changes in cell surface chemistry associated with loss of cell adhesiveness (Kim, 1985), increased tumor cell motility and production of active proteolytic enzymes that render basement membranes defective (Liotta, 1984). These are some features of biologically aggressive tumors that predispose to
involvement of axillary nodes. They do not appear to be a function of time (Bonadonna & Valagussa, 1983).

Greater malignancy of tumors with low levels of ER correlates with a lack of morphologic and functional differentiation. It is consistent with the observation of Lippman et al. (1986) that hormone-independent breast cancer cells in culture showed greater secretion of polypeptide growth factors. It is reasonable to expect that these growth factors interact with their specific cell surface receptors to mediate cellular proliferation. Furthermore, in human breast breast cancer tissues high levels of epidermal growth factor receptors have been found to associate with low content of estrogen receptors and progesterone receptors (Fitzpatrick et al., 1984; Pekonen et al., 1988).

Tumor necrosis may result from high tumor cellular proliferation, which leads to the outgrowth of its vascular supply and/or impairment of capillary perfusion due to the increased tissue pressure (Tveit et al., 1984). This hypothesis is consistent with the significant associations found between tumor necrosis and ploidy or the mean fraction of cells engaged in DNA synthesis (McDivitt et al., 1986). Tumor necrosis may be considered a good indicator which reflects the growth potential of the tumor.

The ascertainment of pathological nodal involvement, clinical TNM stage, ER concentration and tumor necrosis is feasible at primary diagnosis. Assessment of just these four parameters appears to enable fairly accurate prediction of the long term outlook for individual patients. This in turn allows the clinician to recognize the heterogeneous prognoses of patients at any presentation stage of disease and may help in the selection of specific therapies, design of overall treatment strategies, more uniform stratification for randomized trials and proper statistical adjustments in the analyses of nonrandomized trials.
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Appendix A

CANCER CONTROL AGENCY OF BRITISH COLUMBIA

BREAST TUMOR QUESTIONNAIRE

To Doctor: ___________________________ Date: ____________

Name of Patient: _______________________ CCABC#: ____________

Address of Patient: _______________________________________

1. Location of tumor in breast:
   (Tumor not palpable __ or tumor palpable __)
   Right breast ____  Left breast ____

   Indicate Point of Origin:
   If Tumor Extensive Indicate Areas Involved:

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   (i.e. 3 cm radius from edge of nipple)

   Is Paget's Disease present? yes ____ no ____

2. Size of breast tumor: _____ x _____ cms
   Measured by (a) Caliper ____ (b) Mammogram ____ (c) other ____

3. Was the mass fixed to underlying pectoral fascia and/or muscle? Yes ____ No ____
   Was the mass fixed to the chest wall? Yes ____ No ____

4. Was the skin fixed to the tumor? Yes ____ No ____
   Was there erythema of the skin over the tumor? Yes ____ No ____
   Was there oedema of the skin over the tumor? Yes ____ No ____
   Was there any ulceration of the skin over the tumor? Yes ____ No ____
   Was the above skin involvement wide of the underlying tumor? Yes ____ No ____
Appendix A

5. Were any nodes palpable in the axilla on same side as lesion? Yes ____ No ____
   Were the nodes considered to contain growth? Yes ____ No ____
   Were the node mobile? Yes ____ No ____
   Were any of the nodes over 2.5 cm in diameter? Yes ____ No ____
   Were nodes fixed to one another or to other structures? Yes ____ No ____

6. Were there any nodes present?
   In the supraclavicular area on same side? Yes ____ No ____
   Were the nodes considered to contain growth? Yes ____ No ____
   In the infraclavicular area on same side? Yes ____ No ____
   Were the nodes considered to contain growth? Yes ____ No ____
   Was there oedema of the arm? Yes ____ No ____

7. Was there any evidence of spread to skin wide of the breast or to bone, liver, lung, or to the opposite breast etc? Yes ____ No ____
   If so, where ____________________________________________

8. If a pathological diagnosis was made prior to mastectomy, what was the interval of time in days between it and the mastectomy? 
   How was the diagnosis obtained? _____________________________

9. What type of surgery was carried out?
   Radical ____ Modified radical _____
   Simple ____ Segmental resection ____ Other ____
   Was axillary sampling done? Yes ____ No ____

10. Was a specimen sent for estrogen receptors? Yes ____ No ____

   Signature: ____________________________
   Date: ________________________________
Appendix B

Card number: [ ] 1

Patient ID number: [ ] 11111111 2-5

CCABC number: [ ] 111111111 6-11

Age at diagnosis: [ ] 12-13

Age at menarche:
99 = unknown [ ] 14-15

Age at menopause:
99 = unknown
00 = still menstruating
99 = menopausal, age unknown [ ] 16-17

Type of menopause:
1 = natural [ ] 18
2 = hysterectomy/oophorectomy
3 = hysterectomy alone
4 = other artificial
5 = unknown

Number of deliveries:
8 = 8+ [ ] 19
9 = unknown

Age at first birth: [ ] 20-21

Sex of first 4 children and of last child:
1 = M 4 = MF [ ] 22-26
2 = F 5 = FF
3 = MM 9 = unknown

Any multiple births?
1 = yes [ ] 27

Ethnic origin:
1 = Caucasian [ ] 28
2 = Oriental
3 = N.A. Indian
4 = Other

Socioeconomic status:
(BY pt’s occupation or husband’s occupation) [ ] 29
1 = high professional or managerial
2 = skilled manual, farming etc.
3 = unskilled, regular employment
4 = unemployed or irregular employment
5 = clerical, sales
6 = technical (nursing, teaching)
7 = housewife (husband’s occupation unknown)
Appendix B

Marital status: 
1 = married 
2 = not married 
3 = widowed 
4 = separated/divorced

Weight when first seen: (kgs)  

Height: (cms) 

Family history of breast cancer in 
mother, sister or daughter = 1

Previous hormone use: 
Oral contraceptives: 1 = yes 
Total length of use: 99 = unknown 

Time from last use to diagnosis: 
99 = unknown

Estrogen drugs, e.g. Premarin: 1 =yes 
Total length of use: 99 = unknown 

Time from last use to diagnosis: 
99 = unknown

Other hormonal drugs: 1 = yes 
Specify: __________

First symptoms: 
0 = none 
1 = painless lump in breast 
2 = pain or pain and lump in breast 
3 = nipple inversion 
8 = other 
9 = unknown

Date of first symptom: 

Date of first contact with patient: 

Date of first confirmed diagnosis: 

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Appendix B

Primary treatment:

Date of mastectomy: [ ] [ ] [ ] [ ] 70-73 mos. yrs.

Type of mastectomy: [ ] [ ] [ ] 74-75
1 = simple
2 = modified radical
3 = radical or extended radical
4 = other or unknown
5 = simple + RT
6 = modified radical + RT
7 = radical or extended radical + RT
8 = other or unknown + RT
10 = excisional biopsy only
11 = RT only (no surgery)
12 = excisional biopsy + RT

Residual tumor: 1 = yes [ ] 76

E.R. determination:
E.R. determination is done on primary tumor at time of diagnosis, seen at CCABC for primary treatment = 1
Seen at CCABC for primary treatment, E.R. done on later recurrence or persistent disease = 2
E.R. determination at time of diagnosis, seen at CCABC later = 3
Seen at CCABC later, E.R. done later = 4
E.R. determination is done on primary tumor at time of diagnosis, not seen at CCABC = 5

Card no.: [ ] 1

Patient ID. number: [ ][ ][ ][ ][ ][ ] 2-5

Physical staging:
Size of tumor - tumor stage: [ ] [ ] [ ] 6-7

Nodal status - nodal stage - clinical: [ ] [ ] [ ] 8-9
- nodal stage - pathological: [ ] [ ] [ ] 10-11

Pathological staging:
Pathology - in situ: [ ] 12
- infiltrating: [ ] 13
Pathology - grade 1-4, 9 = unknown [ ] 14
Appendix B

Metastases present:
1 = pulmonary
2 = bone
3 = liver
4 = brain
5 = marrow
6 = skin

Patients excluded from analysis:
Reason: 1 = bilateral breast tumor
2 = other cancer prior to breast tumor
3 = other cancer subsequent to breast tumor

If bilateral, state date of
diagnosis of 2nd primary:

If other cancer, date of diagnosis:

Adjuvant treatment:

Adjuvant hormonal therapy (or combination hormonal/other trt.)
Type:

Date of commencement:

Adjuvant chemotherapy:
Type:

Date of commencement:

Secondary treatment

Treatment target:
1 = persistent disease
2 = first recurrence, local, homolateral, or axillary nodal
3 = first recurrence, other or both
4 = other (e.g. 2nd recurrence), local or axillary
5 = other

Date of first recurrence:
9999 = date unknown
blank = no recurrence
8888 = persistent disease

Site of recurrence:
1 = pulmonary
2 = bone
3 = liver
4 = brain
5 = marrow
6 = skin
7 = local
8 = regional nodes
9 = others
Appendix B

First hormonal treatment: (and hormonal/chemo., RT. treatment)
Sequence: [ ][ ][ ] 43-44
Type: [ ][ ][ ] 45-46
Date commenced: [ ][ ][ ][ ] mo. yr. 47-50
Response: [ ][ ][ ] 51
Date of assessment of response: [ ][ ][ ][ ] mo. yr. 52-55
Date of treatment changed: [ ][ ][ ][ ][ ] mo. yr. 56-59
Reason for change: [ ][ ][ ] 60

First chemotherapy treatment: (and chemo/RT. treatment)
Sequence: [ ][ ][ ] 61-62
Type: [ ][ ][ ][ ] 63-64
Date commenced: [ ][ ][ ][ ] mo. yr. 65-68
Response: [ ][ ][ ] 69
Date of assessment of response: [ ][ ][ ][ ][ ] mo. yr. 71-73
Date treatment changed: [ ][ ][ ][ ][ ][ ] mo. yr. 74-77
Reason for change: [ ][ ][ ][ ] 78

Card no.: [ ][ ] 1
Patient ID. no.: [ ][ ][ ][ ][ ][ ][ ][ ] 2-5

First radiotherapy treatment:
Sequence: [ ][ ][ ] 6-7
Target: [ ][ ] 8
Date commenced: [ ][ ][ ][ ][ ] mo. yr. 9-12
Response: [ ][ ][ ] 13
Date of assessment of response: [ ][ ][ ][ ][ ][ ][ ] mo. yr. 14-17

Date of death or last follow-up: [ ][ ][ ][ ][ ][ ][ ][ ][ ] mo. yr. 18-21
Appendix B

Outcome:
1 = dead, from disease
2 = dead, other causes, disease present
3 = dead, unknown if disease present
4 = dead, other malignancy
5 = dead, other causes, no disease present
6 = alive, no disease
7 = alive with disease
8 = lost to follow-up

Primary E.R. determination:

Patient ID. no. [ ] 22

Date of assay: [[ ]] [ ] 28-31

Tissue of origin:
1 = breast
2 = lymph node
3 = skin
4 = other

Estrogen receptor status:
1 = positive
2 = negative
3 = indeterminate
4 = tissue unsuitable

Total protein: (fmol/mg) [[ ]] 34-36

Cytosol protein: (fmol/mg) [[ ]] 37-39

Binding index: [[ ]] 40-41

Secondary estrogen receptor determination:

Date of assay: [[ ]] [ ] 42-45

Tissue of origin:
1 = breast
2 = lymph node
3 = skin
4 = other

Estrogen receptor status:
1 = positive
2 = negative
3 = indeterminate
4 = tissue unsuitable
### Appendix B

<table>
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<td>Total protein: (fmol/mg)</td>
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<tr>
<td>Cytosol protein: (fmol/mg)</td>
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<td>Binding index:</td>
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<tr>
<td>Report sent to physician: 1 = sent, 2 = not sent, 3 = unknown</td>
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<td>Year chart abstract performed:</td>
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**Pathology of initial lesion:**

<table>
<thead>
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<tr>
<td>Non-invasive component:</td>
<td>59</td>
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<tr>
<td>Invasive component:</td>
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<td>Mass configuration:</td>
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<td>Inflammatory reaction:</td>
<td>62</td>
</tr>
<tr>
<td>Infiltrate type:</td>
<td>63</td>
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<tr>
<td>Maximum diameter of tumors: (cms)</td>
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<tr>
<td>Pathological involvement of underlying pectoral fascia and/or muscle:</td>
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</tr>
<tr>
<td>Direct extension to chest wall or skin:</td>
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<tr>
<td>Pathological grade:</td>
<td>68</td>
</tr>
<tr>
<td>Card number:</td>
<td>1</td>
</tr>
<tr>
<td>Patient ID no.:</td>
<td>2-5</td>
</tr>
<tr>
<td>CCABC no.:</td>
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</tbody>
</table>

**Outcome:**

- Source of reporting when last reported: [ ] 12
- Date of first recurrence: [ ] 13-16
- Site of first recurrence: [ ] 17
- Status of patient when last reported: [ ] 18
- Disease status when last seen: [ ] 19
- Date of death or last follow-up: [ ] 20-23
- Date of abstract: [ ] 24-27
- Abstracted by: [ ] 28
### Appendix B

<table>
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<th>Description</th>
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