# Chapter 12
# Prognostic and predictive factors

The prediction of the clinical course of a primary breast tumor is very difficult. Some patients are cured by local therapy, and survive for many years. Other patients experienced early recurrence of the disease and died shortly after. It would obviously be useful to be able to identify individual patients who have a high or low risk of relapse in order to plan the appropriate management of the patient's breast cancer. Patients with a low probability of recurrence could be spared the potential toxicity associated with therapy, while patients, whose tumors are most likely to recur could be given the option of aggressive adjuvant therapy.

Collaboration between clinicians, pathologists and biologists is essential in order to select each patient's treatment, according to prognostic factors.

## Prognostic factors may be classified in three categories

- Variables, which predict distant relapse and survival, the most accurately.
- Risk factors for local recurrence, essentially following breast conservative treatment,
- Predictive factors of response to a particular adjuvant systemic therapy.

The diagnosis of breast carcinomas is now done at an earlier clinical stage than in the past. The treatment approach has changed and conducted to adapt the therapy to the particular clinical stage of each patient, taking into consideration the factors, which affect prognosis (mastectomy or conservative treatment, adjuvant chemotherapy and/or hormonal treatment,...). At the same time, in the past decades, more knowledge of natural history in the development of breast tumors has led to the description of numerous biological factors involved in tumorogenesis. Their potential interest for prognostic assessment was researched with the development of many new biological tests, which may have confused the clinician who had to choose the most useful.

Traditional (well-established) prognostic factors including clinical, pathological factors, hormonal receptor and her2 status is usually the only information routinely used in international clinical trials and recommended since 1990 by the National Institute of Health (NIH) consensus conference (1991) and integrated in some models of prediction, useful in indicating an adjuvant treatment and its choice, retained or proposed models in different consensus conferences (Nottingham, saint Gallen, saint Paul) or on line (http:, adjuvant online). Its estimation is more than ever considered as a hot subject, with the extension of therapeutic possibilities related to the development of new targeted treatment and especially due to the presence on the market of kits obtained thanks to the development of microarray techniques that guarantee a more accurate prediction. The problems are multiple, related to

the methodology, the commercial part and finally related to the modifications of tumor characteristics induced by the screening, not yet included in the prediction molecules.
Our purpose is to give a practical view of prognostic factors in breast cancers.

## Definition of a useful prognostic factor

A prognostic factor may be defined as a factor able to give information on the clinical outcome of each patient.

**Multistep research had to be carried out before being able to recognize a prognostic variable.**

1. The factor must have a clinical or biological significance, and be related to tumor progression and/or metastases potential.
2. It must be easily identified for all patients. The method of detection must be accurate, reproducible, and widely applicable.
3. It must have a significant prognostic value demonstrated on a large and representative series of patients (particularly with homogeneous treatment protocols, and with a sufficient follow-up period). It must also be shown to be independent from other well-established prognostic factors by using appropriate statistical analysis, such as multivariate analysis (Cox model). New statistical techniques are being studied in order to better define the prognosis (neural network analysis).
4. After the first description of an independent prognostic value for survival of a new factor, this relationship must be found in a second set of patients. After this, the prognostic value must be confirmed in prospective studies before being used by the clinician.
5. Some biological factors have a prognostic value for survival, but are also predictive of response to treatment. So, the prognostic value of a factor may change with the type of adjuvant treatment, which must be taken into account in the analysis.
6. This factor must be easily used by the clinician in order to be integrated in the treatment protocol and must have clinical interest.

The relation between feasibility, cost and additional prognostic information must be analyzed. The definition of an additional prognostic factor thereby requires close co-operation between clinicians, pathologists, biologists and statisticians. Numerous biological factors with prognostic implication were recently described with univariate statistical analysis, but very few have independent prognostic significance demonstrated using multivariate statistical analysis including all well-defined clinical or pathological prognostic factors. Rare prospective studies are now available. For these reasons, complementary work needs to be done to clarify the value of these biological factors.
Many reviews have noted the weak statistical quality of the prognostic studies and of the meta-analysis allocated to them, that conducted to publication of recommendations, under

the term of REMARK (JNIC 2005) concerning the methodology rules, which should be followed for the evaluation of new prognostic factors. But these rules apply only to unique factors. With the development of the microarray techniques which can simultaneously analyze thousands of different genes, new statistical methodology problems are updated, some major and not yet resolved (Koscielny 2008) and recommendations have started to be edited for them (duval 2008).

# Prognostic factors of survival

## Well-Established (Traditional) Prognostic Factors

### A- Clinical factors

The TNM staging records the size of the tumor (T), the axillary clinical involvement (N) and the presence of distant metastasis (M). Tumors with distant metastasis (M+) at diagnosis, have a worse prognosis with a 5-year survival rate lower than 20%.

For non-metastatic tumors (M0), the degree of evolution of the tumor is classified in three groups: PEV0 (stable tumor), PEV1 (rapidly growing tumor), PEV2 and 3 (inflammatory cancer). Survival is higher for PEV0 than PEV1 and lower for PEV2-3 group.

The fixation of the tumor to deepest structure is a factor of bad prognosis with a 5-year survival rate of 40% compared to 70% in its absence ($p < 0.01$). Skin fixation is also a poor clinical factor.

The size of the tumor (T) is found to be an important prognostic parameter in many studies. The 5-year survival rate is about 45% for operable cases with tumor size between 5 and 7 cm, while it is more than 80% when the size is less than 2 cm ($p < 0.01$).
The clinical axillary node status (N) allows us to distinguish cancer with metastatic fixed nodes (N2) with a disease-free survival rate less than 40%, while it is nearly 80% when nodes are not palpable (N0) and intermediate when palpable (N1). However, clinical axillary status is not correlated with histologic involvement as 40% of palpable nodes are not metastatic and 40% of metastatic nodes are not palpable. So, in multivariate analysis, the prognostic value of clinical node status was found to be less important than histologic node involvement.

A young age at diagnosis (< 35 years) was usually described as a factor of poor prognosis. However, it could be linked to the higher percentage of node involvement for these patients. In the other age ranges, no prognostic value was found.

No prognostic value was associated with menopausal status or localization of a tumor in the breast.

## B- Pathological factors

### 1. The anatomic size

Tumor size reflects the natural history of the cancer. When tumor size increases, the rate of metastases increases. The size can be evaluated by clinical examination, radiologic imaging and by pathologic analysis; the anatomical size has been the reference. For the radiologic imaging, the size determined by ultra sound has a better correlation to the anatomical one. The MRI, thanks to contrast taking, brings an excellent, more sensitive, evaluation, but disturbed by taking non specific contrast of benign lesions or of normal breast according to the time during the cycle.

The anatomic size is measured on the surgical specimen and represents the largest diameter of the tumor including the fine satellite spicules. The 5-year disease free survival rate is less than 60% in tumors larger than 25 mm, while it is more than 75% in smaller tumors ($p < 0.01$). Although the size is well correlated with the number of involved lymph nodes, the independent prognostic value of the size remains in multivariate analysis.

A second evaluated measure is the complete size of tumor extension, incorporating the in-situ and the infiltrating components, size without prognostic value but it gives important information to the surgeon to select the type of surgery, conservative or mastectomy.

With regards multifocal tumors; such neoplasms proved to generally represent a clonal proliferation of a single tumor. The diameter of the largest focus determines the real tumor size, but this is currently debated, some have proposed to use the summation of the sizes or the surfaces of each nodule, identifies (andea 2002, Coombs 2005). Multifocal tumors have a higher propensity for dissemination than unifocal tumors of the same dimension.

### 2. Histologic type

According to the WHO classification, ductal and lobular carcinomas represent the majority of invasive carcinomas (85%). For a long time, most undifferentiated carcinomas were described as having a poorer prognosis than differentiated forms. Among rare kinds of carcinomas, pure mucinous, tubular, and papillary carcinomas have a very favorable prognosis with a greater than 95% 5-year survival. The typical medullary carcinoma, defined by five histologic criteria, represents only 1/3 of carcinomas with inflammatory stroma and had a particularly good prognosis with a 92% 10-year survival.

On the other hand, d'autres formes sont particulièrement agressives. The metaplastic carcinoma et plus particulièrement ses variantes à cellules fusiformes ou à différentiation cartilagineuse ou osseuse, bien qu'elles s'accompagnent d'un taux relativement faible d'envahissement ganglionnaire métastatique. On peut également citer la forme micropapillaire infiltrante, récemment décrite qui comporte souvent des emboles extensifs et un envahissement ganglionnaire important et fréquent.

**3. Histologic grade**

Numerous methods of grading were described including nuclear characteristics and differentiation. The histoprognostic grading of Scarff, Bloom and Richardson (SBR) described in 1957 is most often used. It is a combination of three scores, i.e. degree of differentiation, degree of nuclear size variation (anisonucleosis) and mitotic activity. The initial description was more clearly defined by Contesso and its use extended to each form of invasive carcinoma except medullary carcinoma. More recently, Elston and Ellis have proposed ont proposé l'adoption de critères précis pour chacun de ces parameters afin d'améliorer la reproductibilité du grade. Ainsi le compte mitotique s'effectue sur 10 grands champs consécutifs et sa valeur est adaptée à la surface du champ microscopique (EE). Good histopathologic technique, especially fixation, and training pathologists in grading are required for precise evaluation and good reproducibility (65-80%) of the grading.

One recent modification was (modified SBR) to include only anisonucleosis and mitosis in a two parameters classification (Le Doussal) as mitotic score was found to be the most important factor of discrimination. *Celle ci à l'intérêt de distinguer au sein du groupe des carcinomas de grade II, ceux à potentiel plus agressif.*

*Le Grade étant un des facteurs pronostiques les plus puissants, différentes analyses génomiques ont essayé d'identifier les gènes qui lui sont associés. Une signature génomique a été publiée et aujourd'hui commercialisée, constituée pour majorité de gènes liés à la prolifération. Si l'équivalence pronostique n'est pas encore prouvée, elle s'oppose au grade SBR dont le but est de disposer d'un outil simple et universel, évaluable à partir de n'importe quelle tumeur, même de façon rétrospective et quelle qu'en soit sa taille, alors que le grade génomique requiert un fragment congelé, ce qui en limite considérablement sa portée.*

**4. Node involvement**

The number of involved axillary lymph nodes is the strongest predictor of clinical outcome in nearly all prognostic studies. Nodal disease appears physiologically to be a confirmation of the metastatic potential of the tumor.

The presence of nodal metastases is found in 40% of cases. The 10-year disease free survival rate of N+ cases was lower (45% for cases with more than 3N+ and 55% for cases with 1 to 3 N+) than N- cases (70%) ($p = 0.01$).

A precise analysis of lymph-node involvement requires a complete removal of axillary lymph nodes by the surgeon (at least 7), a methodical gross dissection and macroscopic serial slicing of each lymph node. En l'absence de métastase ganglionnaire, le nombre de ganglions prélevés est un élément pronostique, plus celui-ci est élevé, meilleur est le pronostic. En cas d'identification de métastases, le pronostic est d'autant plus mauvais que le nombre de ganglions métastatiques est élevé.

The presence of micrometastases is also linked to a higher risk of relapse, for ductal but not lobular type. Different works have proven that immunohistochemical staining could improve the detection of micrométastases, but this is cost effective and time consuming for a complete axillary dissection. Capsular invasion and rupture is related to the number of lymph nodes involved and has no prognostic value by itself, as a histiocytic reaction.

**Sentinel Node Biopsy (SLN)**

Even though it is not yet considered as a validated technique, it is today applied in first intension by most of the working teams. To guarantee its credibility, it is necessary to adopt a particular technique of histological analysis requiring multiple cut section levels and immuno-histo-chemical staining. This latter, guarantees more frequent detection of metastasis, especially the small size ones, less than 2mm.

The risk of invasion by tumor cells of the remaining part of the axillary lymph nodes increases with the increase in number of metastatic sentinel nodes and the size of detected metastasis. However, even in case of presence of minimal lymphatic metastasis <0,02 mm (pN0i+), the risk of invasion of other lymph nodes reaches or exceeds 10%. Different trials are taking place to evaluate the therapeutic importance of complementary axillary dissection in case of sentinel node metastasis.

Many automated instruments for sentinel lymph node analysis are now acailable on the market. The principle is based on the combined detection of the genetic material after amplification of some cytokeratin and mucin genes. It allows in nearly 30 min a study of the sentinel node, which should be chewed up. Their sensitivity is however limited to metastasis having a size of more than 0.2 mm and doesn't allow the quantification of the metastatic invasion, classified as pN0 mol+.

**5. The so called molecular classification:**

Emerged from the microarray studies, this classification recognizes 5 main types that will be associated with different prognosis, bad for the "basal-like" and Her2 types, intermediate for the luminal B and "normal-like" types and good for the luminal A types.

Their prognostic value appears limited, due to the limited number of studied patients and the heterogeneity of the analyzed population regarding the treatment and follow up. There is also a strong correlation existing between this classification and the classic factors, the luminal A type was classified as grade SBR 1, RE and RP+ while the basal forms are of grades SBRIII, RE and RP negative. But the presence of different histological precursors according to the classes and also the presence of different metastatic sites, directed the way of thinking towards the possibility of the presence of different diseases, this will encourage the identification of the class type for each lesion.

**6 Other histologic features**

The presence of endolymphatic invasion was shown as a poor prognosis factor. Le problème de la faible reproductibilité de son identification, l'incidence des cas rapportés variant de 1 à plus de 40% selon les études, est aujourd'hui résolu grâce au développement d'anticorps spécifiques des cellules endothéliales lymphatiques. Ce paramètre prend une importance de plus en plus grande en raison de la diminution de la taille des tumeurs et de l'incidence des métastases ganglionnaires induit par le dépistage.

The absence of clastosis and the presence of necrosis were described as poor prognostic factors but are also difficult to analyze routinely. The lymphocyte infiltrate is without prognostic value.

**6. Hormonal receptors**

Estrogen and progesterone receptors have been used since the late 1970's to predict the outcome of breast cancer patients. But the presence of these receptors is also correlated with response to endocrine therapy. So the value of hormonal receptors as prognostic factors of survival is more difficult to determine because they are also predictive of response to treatment. Their value for prognostic assessment will be analyzed first in this chapter.

**- Estrogen receptor (ER)**

Estrogens exert their action on target cells by diffusing through the cell membrane and binding to their specific receptor. ERs are located close to the nucleus.

Oestradiol itself is thought to regulate the number of ERs in normal breast and in tumor. Thus, higher levels of ER are seen in the first half of the menstrual cycle than in the second half, and this cyclical variation is lost in breast cancer. About 70% of carcinomas are ER positive.

**- Progesterone receptor (PR)**

Estrogens regulate progesterone receptors. The presence of the PR is generally coupled to functional growth regulated by estrogens. About 50% of carcinomas are PR positive. For the 2 receptors, the distribution is: 46% ER+ PR+, 23% ER-PR-, 25% ER+ PR-, 6% ER-PR+.

Expression of ER and PR can change from primary tumor to metastasis. Loss of expression is noted in 20% of metastatic tumor cells that were positive in the initial primary tumor. Disease progression is associated with hormone profile change with less favorable outcome.

**- Measurement of hormonal receptors**

Currently a variety of methods are able to characterize ER and PR in breast cancers. In routine practice, immunohistochemistry is the gold standard. Other techniques are the biochemical assays which characterize the protein product, using ligand-binding assay or receptor antibody assay done in clinical laboratories and more recently the molecular assay, still in development, which analyses the transcription level. They are carried out on tumor homogenates and usually require freezing of the tumor specimen.

The immunohistochemical technique, initially applied to frozen tissue, could now be performed on paraffin-embedded tissue and on fine-needle aspirate. With paraffin-embedded tissue, a good fixation (in formalin, for example) and heating retrieval incubation

(microwave, ...) are needed. For imprints or aspirates, cells are fixed and slides are conserved at – 20 °C.

The comparison between the biochemical, molecular and immunohistochemical assays shows an excellent correlation between these techniques. Each has relative merits. The biochemical assays were used first and have been the accepted standard for ER and PR quantification. The molecular assay can assess other genes of prognostic value. Biochemical and molecular techniques are quantitative and accurate techniques but are carried out on a tissue sample homogenate with no information on the nature of the sample and need a relatively large amount of tumor for radio legend assay.

The immunohistochemical technique provides semi quantitative evaluation and is expressed in different ways but gives information about the cell types expressing receptor (malignant vs. benign or normal breast) and can be done on small biopsy specimens, even on individual tumoral cells and is able to evaluate tumoral heterogeneity of the expression. The quality and reproducibility of the analysis has greatly improved with the development of the antigen retrieval technique and more sensitive revelation's system, the commercialization of entirely automatized devices which control every steps of the process and the extent of European quality assurance program and guidelines for the immunochemistry.

Biological and immunohistochemical techniques provide complementary information regarding receptor content. The second one is simpler, less expensive, could also be applied on paraffin-embedded tissue or cytological samples, and provides concordant information with biochemical assay. Some of the molecular assays seem very correlated with the immunohistochemistry and might be pronostic for relapse (bavre 2008). However, their interest seems more in looking for resistance or sensitivity marker s for treatment more than for prognostic analysis, these techniques being restricted by the quantity of evaluated cells.

**- Hormonal receptor and prognosis**

The value of hormonal receptors as prognostic indicators remains controversial. Many of the initial studies included women treated with various therapies. It is, therefore, difficult to separate the prognostic value from the predicting response to therapy.

In the studies restricted to women without adjuvant therapy, the presence of ER gives an advantage (but does not reach significance) in some studies or does not show any prognostic value at all.

A correlation was demonstrated between ER and PR positivity and low tumor differentiation, low histoprognostic grade and low proliferative index. The presence of PR also has favorable predictive value in patients with node-negative breast cancers treated without adjuvant therapy, but does not achieve statistical significance.

The prognostic utility of both ER and PR has been reported to be strongest in pre-menopausal women. However, the relative importance of ER and PR is still controversial. The PR level is more sensitive than ER levels for predicting recurrence stage II tumors. The difference of prognosis linked to PR became insignificant with time.

Overall, all this data supports the fact that PR and ER positivity correlated with better survival. However, this advantage may be less than 10% over a long period of observation and disappears in multivariate analysis including tumor differentiation, grade, and proliferative rate.

**7. c-erbB-2**

c-erbB-2 (or HER2 neu) gene located on chromosome 17, codes for a transmembrane glycoprotein, which has homology with the epidermal growth factor receptor (EGF-R). In normal cells, only one copy of the gene is expressed. In breast carcinomas, a c-erbB-2 gene amplification with an elevated number of copies of the gene has been seen in 10% to 25% of the cases. High levels of c-erbB-2 protein or mRNA are linked to gene amplification but could be observed without it following transcriptional disorders. Different methods, immunohistochemistry on fixed and embedded tumors, is situ hibridation (FISH, CISH, SISH), Rt-PCR, CGH array, can detect an amplification. In routine practice, immunohistochemistry is generally realized in first intention, using a score from 0 to 3+ which combine intensity of the cytoplasm membrane staining and percentage of invasive stained cells. Intermediate score 2+ corresponds to tumors with an uncertain her2 status which requires additional study. Similarly, FISH with a number of her2 copies between 4 to 6 or with a ratio between number of copies of her2 and chromosome 17 centromere from 1.8 to 2.2 require additional study.

In 1987, Slamon demonstrated that amplification of the c-erbB-2 gene is associated with shorter survival. There is a strong association between c-erbB-2 amplification/over-expression and other established poor prognostic factors: ER-, PR-, involved axillary lymph nodes (N+), poor histoprognostic grade, inflammatory carcinomas, high mitotic activity, DNA aneuploidy. c-erbB-2 amplification/overexpression is a poor prognostic factor usually found in women with axillary lymph-node involvement. For node-negative patients, the prognostic utility of c-erbB-2 was not demonstrated and contradictory results were published.

c-erbB-2 evaluation is essential to the selection of treatment, her2 amplified tumor could benefit from antiher2 treatment. Positive Her2 tumors seem to be more sensitive to chemotherapy and specialy anthracyclin based regime.

**MULTIVARIATE ANALYSIS OF THE TRADITIONAL PROGNOSTIC FACTORS**

Many of the previous factors described are interrelated at different degrees. Only a multivariate analysis (like Cox model) allows us to determine the relative importance of each factor.

**I. Non Operable Carcinomas** (Table 14.1.)

**1. Inflammatory carcinomas**

In a series of 103 cases, treated at the Institut Gustave-Roussy with primary irradiation, followed in certain cases by mastectomy and/or by radiation castration, the histologic grade evaluated on drill biopsies is the only important predictor for disease-free survival. The kind of inflammation (localized or diffuse) is at the limit of significance. Age, clinical tumor size, tumor fixation, clinical node status, and histologic type were not significant.

**2. Non inflammatory carcinomas**

In a series of 289 cases, comprising 141 with rapid clinical growth and 148 either with tumor size >7 cm or skin fixation, treated at the Institut Gustave-Roussy by primary irradiation,

followed in some cases by mastectomy and/or by radiation castration, the clinical node status (N) and the histoprognostic grade were the only statistically independent predictive factors for disease-free survival. Clinical size is at the limit of significance, but age, rapid clinical growth (PEV1), histologic type, and skin fixation were not significant.

**II. Operable carcinomas (T1-T3, <7 cm, N0 - 1)** (Table 14.2.)

In the experience of the Institut Gustave-Roussy, 612 patients were treated between 1967 and 1974, by mastectomy, radiotherapy and radiation castration for pre- and peri-menopausal node positive patients but without chemotherapy. Among the criteria evaluated: age, clinical size, clinical node status, histologic type, histoprognostic grade and node involvement, the multivariate analysis showed two independent prognostic factors for poor 10-year disease-free survival: the presence of histologically involved lymph nodes (N+) ($p = 0.00001$) and a high histoprognostic grade ($p = 0.0001$). Clinical tumor size showed a strong trend, but does not reach significance ($p = 0.08$) (Table 14.2.). When axillary invasion and grade are used together, the relative risk of relapse is significantly different between patients with grade I - N+ 3 or grade II - N- tumors with 72% 10-year disease free survival and patients with grade II - N+ > 10 or grade III - N+ > 4 tumors with 27% survival ($p = 10\text{-}3$) (Table 14.3).

In studies including hormonal receptor status, the only independent factors for disease-free and overall survival are histologic grade, lymph node status and tumor size. A more detailed analysis showed that the most significant prognostic factors are the number of lymph nodes involved and the histologic grade. The presence of estrogen receptor is without prognostic value while the progesterone receptor is found a prognostic factor for metastasis-free survival at 2 and 5-year but lost its significance at 10 years. The prognostic value of hormonal receptor seems more important in series of patients, which did not include histologic grade. This could be partly due to the correlation between ER and PR negativity and high histologic grade. In addition, the prognostic value of hormonal receptor varies with time.

**Node-negative carcinomas** (Table 14.4)

A series of 1322 patients with node-negative tumors treated by surgery without adjuvant chemotherapy had a metastasis-free survival rate of 72% at 10 years. After multivariate analysis including hormonal receptors, the most important factors of good prognosis were a low SBR grade ($p = 10\text{-}6$) and small tumor size, whether considered from a clinical ($p = 10\text{-}6$) or a pathological ($p= 10\text{-}3$) point of view (Table 14.4)

Other studies including a high number of cases show that the tumor size, the nuclear grade and to a lesser extent, the histologic type are important prognostic factors. The 5-year disease-free survival rate is lower in ER- tumors but only by 8-9%. This leads us to conclude that hormonal status alone was not a sufficiently important prognostic factor to select patients who need adjuvant chemotherapy.

In this group of patients, 30% will relapse in the 10 first years and traditional prognostic factors were not sufficient to predict those with a higher risk. For these reasons, the research for new prognostic factors was essentially focused on this population of patients.

These previous prognostic factors are well-established and remain widely used to select patient protocol. However, the need for other predictive factors and progress in breast cancer

biology has led to the description of new prognostic factors, which have to be compared with the traditional ones.

**New Prognostic Factors**

Numerous biological factors allow a better understanding of tumor growth, cancer invasion, and tumorogenesis. They could be classified into markers of proliferation, oncogenes or markers of metastatic potential. Some of them are not confirmed as prognostic factors of survival; others are closely related to traditional prognostic factors. For these reasons, only the most important biological factors which could become additional prognostic factors and which would be useful for the clinician will be described below.

**Circulating tumor cells**

The technological breaking through of the molecular biology and the techniques of reduction, have allowed the creation of instrument detecting the tumor cells in the general circulation. The detection of these cells is done either by size, the epithelial cells being larger than the elements present in the blood or by antigenic affinity by using some cytokeratins and / or mucines which are only expressed on cells of epithelial origin. The number of detected elements is few; they are essentially detected during the metastatic phase. The presence of epithelial elements in the circulation of control group formed by healthy persons supports the use of a threshold value (cut off value) in case of techniques which doesn't perform morphologic analysis. Their presence or absence before starting the treatment and their modification during the treatment will represent independent prognostic elements (Cristofanilli).

**Micro array**

The automated instruments, simultaneously analyzing thousands of genes, have been used to study breast cancers, first, classifying them by homology of expression (unsupervised analysis) which has allowed the identification of the so called molecular classes then for prognostic reasons, classifying them by the differential of expression from different groups of patients, one that have recurrences and the other who doesn't have (supervised analysis). Different genomic fingerprints have thus been described, based on radically different concepts. So, some are only correlated to prognosis (Amsterdam), others to the genomic grade, but also to the repair (wound signature) to the tumor stem cells, to the stroma reaction … etc. They are all different; these different fingerprints have only one gene in common between them. Many methodological difficulties was raised, from one hand, the gene selection, the constituents (koscielny), from the other hand, taking in consideration the tumor diversity, their analysis being done on a fragment which is not always representative of the lesions diversity. Finally, most of the genes incorporated in each of these signatures are related to the proliferation.

But are the results obtained by these DNA chips (or microprocessors?) superior to the simple evaluation of the proliferation where the low cost and the simplicity of their performance cannot compared to these sophisticated genomic tools? From the other hand, is the mARN analysis more important than that of the protein, being the final step of the procedure?

Finally, can we really determine a group of genes having a valid prognostic value for all patients and during all the phases of carcinogenesis?

**Proliferation**

The cellular division being one of the most controlled events of the cellular machinery, the observed proliferation in the tumors requires escaping from the whole group of these controls which also requires multiple genetic anomalies.

Mitotic activity was the first factor of poor prognosis described. This criterion is included in histoprognostic grade or nuclear grade.

Other techniques were used to estimate proliferation. Firstly, the labeling index is determined after in vitro incorporation of tritiated thymidine in proliferative cells. This auto radiographic method is difficult to perform, because it is time consuming and can only be applied on fresh tissue. For these reasons, the labeling index (LI) is rarely used routinely, although several studies confirm the independent poor prognostic value of a high LI in non operable as well as in operable node negative tumors.

The evaluation of DNA content per cell and the percentage of cells in the S phase of the cell cycle using a mathematical model by flow cytometry have been widely studied. However there value was inconstant according to the studies.

Immunohistochemistry on fixed and embedded tissue is the favored method of proliferation evaluation. The antibody Ki 67 recognizes a nuclear antigen, expressed in cells into GI, S, G2 and M phases of the cell cycle, and not into GO. The percentage of proliferating cells is calculated as the ratio of cells with nuclear staining by the total number of cells. Different thresholds are used, according to the purpose, low (about 10%) for prognostic value, higher (25%) for predictive response to chemotherapy.

A high proportion of Ki 67 positivity correlates with poor tumor grade and absence of ER. It indicates poor prognosis, but its independent prognostic value is debatable.

Ki 67 staining is correlated with the labeling index and to S phase fraction. Ki 67 may provide an alternative approach for measuring proliferation.

Problem related to screening

**II. Other Markers**

Proliferating cell nuclear antigen (PCNA) is a nuclear protein. Its level was shown to be correlated with DNA synthesis. PCNA staining could be measured by immuno-histochemistry. The results concerning its prognostic value are very “divergent”.

DNA polymerase-alpha appears earlier in G1 than Ki 67 and is a marker of the kinetics of the tumor. Its increase might be a poor prognostic factor.

The incorporation of bromodeoxyuridine (BrdU) allows analysis not only of the S phase fraction but also the potential doubling time of the tumor. The value of the potential doubling time has yet to be confirmed.

**III. Oncogenes and anti-oncogenes**

Human cancers result from the accumulation of somatic DNA cell alterations. Genetic changes such as amplification, mutation, translocation, and deletion lead to phenotypic

changes produced by protein overexpression, loss of activity, or altered activity and give characteristics of cancer.

**2. p53 tumor suppressor gene**

p53's function is to regulate the passage through the cell cycle, DNA repair and to program cell death. Normal or wild-type p53 is expressed in low levels in all normal cells helping to co-ordinate a complex system of response to any DNA damage and protect cells from DNA alterations that could lead to neoplasm.

Mutation in the p53 gene results in the synthesis of a mutated p53 protein, which has a prolonged half-time. The accumulation of p53 protein could be visualized in the nucleus by immunohistochemistry and is an indirect indication of the mutation, although there are exceptions, such as gene mutation with no protein synthesis or accumulation of the normal protein.

p53 accumulation is detectable in 36% to 46% of breast cancers. It is linked to negative hormonal receptor status, to high grade tumors, to high S phase fraction, and to c-erbB-2 positivity. p53 positivity is an independent factor of poor prognosis for overall and disease-free survival. For node-negative tumors, overexpression is a poor prognostic factor, which is not always found to be statistically independent when histologic grade is included in the analysis. Its role has yet to be confirmed.

**IV. Tumor invasion markers**

**1. Angiogenesis**

Tumor growth requires the formation of new vessels. Angiogenesis permits a tumor to spread by giving a channel to tumor cells. The number of vessels, stained by immunohistochemistry is correlated to node involvement. Some authors evaluate VEGF or VEGR receptors by immunohistochemistry and biochemical techniques in the tumor or in the serum. Several studies confirm the poor prognostic value of a high number of microvessels for disease-free and overall survival particularly for node-negative patients. However, there is no consensus on the method of counting microvessels and more work is necessary to determine the place of this promising factor.

**PREDICTIVE FACTOR OF LOCAL RECURRENCE**

Recent progress in diagnosis has increased the incidence of small tumors, which may be treated by tumorectomy followed by radiotherapy. However, recurrent tumor in the remaining breast was found in 10-15% of the patients. Essentially, pathological findings influenced recurrence: the quality of the surgical excision and the presence of an extensive in situ component.

One factor is the presence of an extensive ductal in situ component (EIC). A tumor is defined as EIC+ when intraductal carcinoma is present both in the invasive tumor (comprising at least 25% of the tumor area) and in the surrounding normal breast tissue. The likelihood of finding residual foci of carcinoma is 70% in EIC+ cases and 28% in EIC- cases when a mastectomy was carried out following tumorectomy. In most studies, the extensive in situ component (EIC+) is the most important factor of relapse in multivariate analysis. Five-year breast relapse rate is significantly higher for patients treated by tumorectomy and radiotherapy with EIC+ carcinomas (24%) than for those with EIC- tumors (6%) ($p =$

0.0001). However, when surgical margins is associated, EIC+ tumors with a complete resection have a similar rate of relapse than EIC- tumors.

The adequacy of the surgical margins appears to be the main other important pathological factor of relapse. The 10-year local relapse rate was 9% when the resection was complete and 24% when it was incomplete. The adequacy of the surgical margins could be estimated by gross examination or by histologic examination but the quality of the analysis is better at microscopic level by inking the borders of the tumorectomy. Smitt proposed a classification of the margins as positive (invasive or in situ carcinoma on the inked specimen margins), close (2 mm), negative (>2 mm free margins). In this study, the re-excision of the margins for patients with close or positive margins increased the 10-year local control (97% vs. 84% without re-excision). The adequacy of the resection was also a factor of better survival.

The young age of the patient, lymphatic invasion and high histoprognostic grade seem to a lesser extent predictive of local failures.

**PREDICTIVE FACTORS OF RESPONSE TO TREATMENT**

Prognostic factors of survival may help to select patients eligible for adjuvant chemotherapy. Some of them are also useful indicators for selection of patients who are likely to respond to a particular form of therapy. For these reasons, the relative importance of prognostic factors of survival described in former series of patients treated exclusively with surgery and radiotherapy may change with addition of adjuvant chemotherapy. Predictive Factors help to determine the probability of response to a particular drug class. There are numerous proteins and genes involved in breast cancer growth, proliferation and metastasis. Exploration of their role in predictive response to various therapies may help draw the tumor profile and plan appropriate treatment for them.

**I. Response to Hormonal Treatment**

Standard methods

Oestradiol is one of the most important tumor growth factors. Numerous studies on patients with metastatic disease demonstrated a tumor rate of response dependent on the steroid receptor status of the tumor (10% ER-, 50% ER+, 75% ER+ PR+ tumors). The same correlation was also demonstrated when steroid receptor status was determined by immunohistochemistry (Table 14.6).

pS2 protein is induced by estrogen. pS2 must be shown to be more of a predictive factor of response to hormonal treatment than a prognostic factor for survival. pS2 protein may indicate a functional estrogen regulatory system. In ER+ PR+ tumors, pS2 may identify patients, who have an intact ER pathway. pS2 protein identifies an ER+ PR+ subgroup of patients who were more likely to respond to hormonal treatment.

New techniques

Micro arrays (oncotype)

**II. Response to Chemotherapy**

Histologic type lobular vs others

Grade and mitotic count

P53

Molecular classification

Micro arrays

Her2 patients

Several biological factors were studied in metastatic, locally advanced tumors or large breast tumor treated by pre-operative CT.

In a series of 89 patients with large breast tumors (>3 cm) treated at the Institut Gustave-Roussy with neoadjuvant chemotherapy in order to reduce the tumor size and to begin systemic treatment earlier, the predictive factors of clinical tumor regression were analysed (Table 14.6). The high mitosis rate is linked to better clinical response. A high S phase fraction was correlated with a clinical response 75%. The initial overexpression of c-erbB-2, or the accumulation of p53 determined by immunohistochemistry was not predictive of response to treatment. This data does not confirm the hypothesis of a role of c-erbB-2 in chemoresistance determined in series treated by adjuvant chemotherapy. Preclinical and some clinical data suggest that tumors with p53 mutation may be particularly sensitive to taxanes and relatively resistant to anthracyclines.

In literature, proliferative activity evaluated by flow cytometry (S-phase fraction) or by labeling index was always associated with a higher rate of objective clinical response (>50%). DNA aneuploidy and/or high histologic grade were not found to be predictive of higher clinical response.

The expression of multidrug resistance (MDR) phenotype is associated with chemoresistance. The initial expression of P-glycoprotein, which is encoded by MDR gene, is related to a worse response to preoperative chemotherapy but the series of patients is small.

Topoisomerase II alpha (topo 2$\alpha$) gene detection is associated with resistance to doxorubicin treatment. It is also usually associated with HER-2/Neu gene amplification. Simultaneous detection of topo 2$\alpha$ and HER-2/Neu can be done by PCR.

Taxol (Taxane) has an anti angiogenic effect. It causes tumor necrosis, microtubulin-associated protein level may predict sensitivity to taxanes.

Mib-1 proliferation marker is decreased after neoadjuvant chemotherapy. It could be estimated to evaluate the degree of neoadjuvant effectiveness. P27 tumor suppressor gene involved in control of G1-S transition may predict response to chemotherapy.

HER-2 overexpression may denote sensitivity to anthracyclines and anthracycline dose intensity and less responsiveness to CMF. However, only strongly positive HER-2 overexpression (+++) is considered, while lack of HER-2 overexpression have similar results with low and standard doses. Breast International Group Herceptin Adjuvant (HERA) still questions whether HER-2 3+ should receive 1 or 2 years of trastuzumab or not, irrespective of adjuvant chemotherapy regimen given.

Thymidylate synthase and dihydropyridine dehydrogenase levels may predict response to fluoropyrimidnes. So far, they have been tested in colorectal cancer.

**CONCLUSION**

The ultimate goal of the research of prognostic factors is to better tailor the treatment of the patient to the clinical, pathological and biological characteristics of the tumor.

For the clinician, the main questions are how useful are these prognostic factors for daily routine practice and how to interpret them. The interrelation between the biological factors in breast cancers is important and complicates the determination of the relative value of each

factor. For these reasons, multivariate statistical analysis only allows one to determine independent prognostic factors. The relative value of the new biological parameters had to be compared to the well defined prognostic factors.

The prognostic value of factors determined in a series of patients receiving no adjuvant therapy may be modified when hormonal treatment or chemotherapy are given, if this factor is predictive of response to treatment.

The cost of measuring a factor as well as the reproduction of the measure must be taken into account. The value of well-established prognostic factors determined after good histopathologic technique has been confirmed by a large number of studies and remains the most important.

For operable breast tumors, the axillary involvement, the histoprognostic grade and the size of the tumor remain the most important prognostic factors. The value of S-phase fraction, angiogenesis and p53 overexpression has to be confirmed by prospective studies (Table 14.7).

For patients who have conservative treatment, the main factors of local breast relapse are : the presence of an extensive intraductal component and the involvement of the margins of the tumorectomy (Table 14.7).

However, these prognostic factors may be modified with the use of new therapy. The response to hormonal treatment is well correlated with the presence of hormonal receptors. The response to chemotherapy is associated with high proliferation, but other predictive factors remain to be studied (c-erbB-2, MDR, ..) (Table 14.7).

Research will not tend towards a multiplicity of factors of prognostic assessment but to a precise determination of the most useful and reproducible factors using precise statistical methodology and to better treatment decisions individualized for each patient. The relative value of well-established prognostic factors may be modified in the future with the use of biological parameters as predictors of response to treatment and even as targets for new treatments (immunotoxins, angiogenesis inhibitors....).

**Table 14.1. Disease-free survival of 398 inoperable carcinomas treated at the Institut Gustave-Roussy**

| Criteria | Inflammatory (n= 109) p value | Non inflammatory (n= 289) p value |
|---|---|---|
| Age | NS | NS |
| PEVO/1 | - | NS |
| PEV2/3 | 0.09 | - |
| Tumor fixation | NS | NS |
| Clinical size | NS | 0.07 |
| Clinical nodes | NS | $10^{-4}$ |
| Histologic type | NS | NS |
| SBR grade | 0.001 | 0.03 |

IGR (Contesso 1987)

**Table 14.2. Disease-free survival at 10 years of 612 operable carcinomas Treated at the Institut Gustave-Roussy**

| Criteria | P value |
|---|---|
| N+ | $10^{-5}$ |
| SBR grade | $10^{-4}$ |
| Clinical size | 0.08 |
| Age | NS |
| Clinical nodes | NS |
| Anatomic size | NS |
| Histologic type | NS |

IGR (Contesso 1987)

**Table 14.3. Disease-free survival rate at 10 years for operable breast carcinomas**

| Histologic Grade | Node involvement N- | N+ 1-3 | N+ 4-10 | N+ >10 |
|---|---|---|---|---|
| I | 72% | 72% | 63% | 51% |
| II | 72% | 63% | 51% | 27% |
| III | 63% | 51% | 27% | 27% |

IGR

**Table 14.4. Metastasis-free survival rate at 10 years for 1322 N- carcinomas**

| Clinical size | SBR grade I | II | III |
|---|---|---|---|
| < 1 cm | 97% (78)* | 80% (93) | 78% (32) |
| 1- 3 cm | 89% (228) | 70% (432) | 57% (183) |
| > 3 cm | 80% (50) | 57% (130) | 53% (96) |

IGR

**Table 14.5. Multivariate Analysis of the Prognostic Value of S Phase Fraction**

| Study year | N | Follow-up (years) | N histo | Multivariate Analysis DFS | OS |
|---|---|---|---|---|---|
| Kallionemi 1988* | 297 | 6 | N+/- | - | 0.0001 |
| Stal 1989* | 472 | 7 | N+/- | 0.05 | 0.03 |
| Toikkanen 1989 | 351 | 25 | N+/- | - | 0.02 |
| Gnant 1992 | 241 | 10 | N+/- | NS | 0.04 |
| O'Reilly 1992 | 169 | 8 | N+/- | NS | NS |
| Fisher 1991 | 398 | 10 | N+/- | 0.04 | 0.08 |
| Muss 1989 | 101 | 4 | N- | NS | 0.04 |
| O'Reilly 1990 | 169 | 5 | N- | 0.0005 | - |
| Sigurdsson 1990 | 367 | 4 | N- | 0.03 | 0.0001 |
| Bosari 1992 | 147 | 4 | N- | 0.0001 | - |

*No histologic grade included in the multivariate analysis

**Table 14.6. Predictive factors of response to CT for 89 large carcinomas (>3 cm)**

| Parameters | N | Clinical regression | P value |
|---|---|---|---|
| **TNM** | | | |
| T2 | 45 | 59% | |
| T3 | 36 | 58% | NS |
| T4 | 8 | 41% | |
| **Rapid growth**** | | | |
| PEV0 | 61 | 53% | |
| PEV1 | 28 | 67% | P=0.02 |
| **Menopausal status** | | | |
| Premenopausal | 42 | 64% | |
| Postmenopausal | 47 | 51% | P=0.03 |
| **Histologic type** | | | |
| ductal carcinoma | 83 | 62% | |
| lobular carcinoma | 5 | 39% | NS |
| in situ ductal carcinoma | 1 | | |
| **Histoprognostic grade** | | | |
| I | 4 | 58% | |
| II | 44 | 52% | NS |
| III | 38 | 63% | |
| **Mitosis (SBR)*** | | | |
| 1 | 26 | 49% | |
| 2 | 14 | 47% | 0.02 |
| 3 | 46 | 65% | |
| Not evaluated | 3 | | |
| **Fibrosis** | | | |
| 0 | 4 | 86% | |
| 1 | 52 | 61% | < 0.03 |
| 2 | 30 | 50% | |
| **Necrosis** | | | |
| 0 | 60 | 52% | |
| 1 | 10 | 72% | < 0.03 |
| 2 | 15 | 67% | |

*1 + 2 vs. 3 p= 0.005

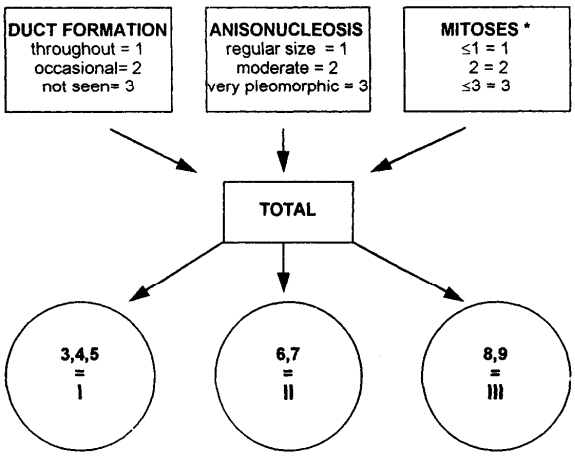
**PEV1= rapid clinical evolution (subjective clinical growth in 6 months) Denoix 1970

**Table 14.7. Relative Importance of Prognostic Factors In Breast Carcinomas**

| Factors | | Poor survival | Increase of local recurrence | Good response to treatment | |
|---|---|---|---|---|---|
| | | | | Hormonal | Chemo-therapy |
| Clinical | Size (High) | ++ | + | - | - |
| Pathological | Lymph-node involvement | +++ | - | - | - |
| | Histoprognostic grade (high) | +++ | + | + | (+) |
| | Anatomic size (high) | + | + | - | - |
| | Extensive intraductal component (EIC+)* | | +++ ++ | | |
| | Involvement of margins* | | | | |
| Biological | Hormonal receptor | + | | - | - |
| | DNA aneuploidy | +/- | | (+) | + |
| | S-phase fraction (high) | ++ | | (-) | +++ |
| | c-erbB-2 | ++/- | | | (-) |
| | p53 | ++ | | | (-) |
| | Angiogenesis (high) | ++ | | | |

( ) data have to be confirmed;
* conservative treatment

**Fig. 14.1. Scarff and Bloom grading, modified by Contesso (1987)**




* maximum number of mitoses per one field at high power field (x400) (area=0.159mm$^2$)

**An update of prognostic factors in Breast cancer**

**Immunologic and Genetic Markers**

- **Mib-1** proliferation marker is useful in subtyping of diploid tumors. It is superior to SPF.
- **CD10**: a cell surface neutral endopeptidase is negative in normal stromal cells. But in some breast cancer cases, CD10 is expressed, facilitating cancer cell invasion. This is associated with aggressive behavior.
- **p63** is a member of p53 gene family. It is involved in mammary gland development (myoepithelial cells).
- **Syndecan (CD138)** is a cell surface heparin sulphate proteoglycan. It is involved in cell proliferation, migration, and cell matrix interaction. It is expressed frequently in breast cancer and is associated with tumor aggressiveness and poor prognosis.
- **Kit protein**: Kit proto-oncogene encodes transmembrane tyrosine kinase growth factor receptors and is essential for cell differentiation. Its protein expression is lost in aberrant formation of tumor.
- **Apoptotic markers**: **Bax** is a pro-apoptotic tumor suppression gene. p53 is a direct transcriptional activator of Bax, aberration in the normal programmed cell death mechanisms is critical in the proliferation of breast cancer. Bcl-2, Bcl-x and Bax are significantly expressed in low grade ER/PR +ve tumors. This apoptotic pathway is in equilibrium in good prognostic group.

- **Metallothionein isoform3 (MT-3**) is a matrix component. Its regulation is aberrant in breast cancer and its overexpression is associated with poor progression.

**Chromosomal Changes**

- Numeric aberration of chromosome 1 and 7 indicate abnormal DNA and aggressive tumor.
- LOH 3p14 correlates with aggressive biology.
- Mismatched repair gene: inactivation of DNA repair in breast tissue could lead to tumor pathogenesis.

**Methodologies**

- **Tissue microarray DNA technology**: is a new method, which allows detection of mutated, deleted or amplified status of up to 1000 genes simultaneously. This forms a library of individual tumor profiles associated with sensitivity and resistance to specific cytotoxic treatment. Individual tumor samples can be tested for optimal regimen on the basis of their DNA fingerprint. It could be performed on tumor sample from paraffin blocks. One gene in question could be correlated with the status of numerous other genes that could affect its function.
- **Comparative Genomic Hybridization:** the genetic profile of two tissue samples could allow the study of tumor development progression or regression.
- **Automated Cellular Imaging System** can assist the quantitation of immunohistochemistry; it is a sensitive and rapid system with objective findings.