Review Article

AN OVERVIEW OF PROGNOSTIC MARKERS IN BREAST CANCER

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ABSTRACT

For many years axillary lymph nodes metastasis was the most important prognostic factor to predict the overall and disease free survival and guided the oncologists for institution of chemotherapy in breast cancer cases. However this factor alone or in combination with other parameters like, age of the patient, tumour size, histological type and tumour grade failed to predict the prognosis accurately in number of these cases. In pursuit to achieve the perfection, many new parameters which are biological or molecular in nature have been discovered. It is claimed that these factors not only have the capability to predict the prognosis but are also able to identify 'high risk' group of patients. However analysing all these parameters in a given case is not only cost prohibitive but also not essential. In order to solve this problem few important biological parameters have been reviewed, which are considered to cover all the important facets of the breast cancer. The parameters reviewed are ER, PR, S phase fraction, DNA ploidy, MIB-1 antibody, p53, C-erb-2, nm23, Cathepsin, Topoisomerase II alpha.

MJAFI 1999; 55 : 129-132

KEY WORDS:-Biological parameters, Breast cancer, ER and PR.

Introduction

Currently approximately 75,000 cases of breast cancer are estimated to occur in Indian women every year. It is estimated that 1 out of every 55 urban Indian women develops breast cancer by age of 64 years [1]. This rate is much lower in comparison to white American population which is between 80 to 104 per 100,000 population [2]. It is well known that chemotherapy forms an important component of adjuvant therapy for breast cancer, but decision regarding the administration of chemotherapy is the most important therapeutic dilemma. Until few years ago the mainstay regarding this decision used to be axillary lymph node status. However it has been observed that 25 to 30% patients amongst these node negative cases do develop early metastasis [3]. Thus there is a 'high risk' group amongst these node negative cases. Many parameters are available to predict the prognosis in breast cancer. The presumed correlates of good prognosis, as far as morphological markers are concerned, are the smaller tumour size [4]; special histological types of breast cancer in comparison to commonest type i.e. infiltrating duct carcinoma-No special type {IDC-NST} [5]; low tumour grade based on modified Scarf Bloom Richardson {SBR} system [6]; and absence of lymph node metastasis [7]. Few other parameters like vascular invasion, tumour necrosis, stromal elastosis are also available. But all these morphological parameters either singly or in

combination in form of prognostic index [7] have failed to identify the 'high risk' group and prognostic prediction is not always correct in a given case of breast cancer based on these parameters. Estrogen receptor {ER} and progesterone receptor {PR} have shown some promise to solve this problem, but in recent years many other new parameters which are not morphological, but are biological or molecular in nature, have been identified. These biological parameters appear to fulfill this gap and shall be able to identify the 'high risk' group. We have reviewed few important biological parameters, which appear promising not only from prognostic point of view but are of practical importance for the oncologists for patient management. They are as follows:-

{a} Steroid hormone receptors :	ER, PR
{b} Cell kinetics and ploidy and related factors	S phase fraction
Ploidy status
MIB-1 antibody
{c} Tumour suppressor factor :	p53 mutation
{d} Growth factor receptor :	c-erb-B2
{e} Factors predicting :
Metastasis	Cathepsin-D
nm 23
{f} Parameters indicating
drug resistance :	Topoisomerase II alpha {TPI}

Review of the parameters

{a} **Steroid hormone receptors:** -The ER and PR are two steroid hormone receptors, which have been extensively studied in breast cancer. Excellent kits for

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immunohistochemistry {IHC} procedure of various companies are available, which are applicable on routinely processed paraffin blocks. The overall hormone receptor positivity as reported in Western literature, is around 60 to 75% and Press MF has advocated that even if one of them is positive i.e. either ER or PR, the given case should be considered as hormone receptor positive [8]. Most Studies agree that expression of steroid hormone receptors, indicate favourable prognosis and patients have an overall survival advantage. It is also accepted that it usually indicates a favourable response to hormonal therapy. It has also been observed, only 34% tumours respond to chemotherapy when only ER is positive, but response rate goes to 78% if both receptors are positive [9]. Thus knowing the ER and PR status will not only be helpful from prognostic point of view but will also guide the oncologist for the essential management. Therefore it is recommended that IHC staining for both the receptors should be carried out routinely. However as the prognosis and response is not uniform in all the cases of breast cancer in relation to hormone receptor status, it is clear that the study of only these two parameters is not adequate and other parameters must be analysed for more accurate prognostication and appropriate management.

{b} Cell kinetics and ploidy and related factors:-

{i} S phase fraction and DNA ploidy:-Flow cytometry measurement of ploidy {DNA content} and proliferative capacity {S Phase fraction} are the most well characterized prognostic factors and secondary to hormone receptor status. These measurements have been shown to predict disease free survival and overall survival {DFS and OS} in node negative and node positive breast cancer [10]. Recently it has been reported that these measurements may also be helpful in predicting response to therapy in patients with advanced disease [11]. Using flow cytometry, a tumour with normal amount of DNA is 'DNA ploid' and tumour with nonploid amount is 'DNA aneuploid'. DNA flow cytometry also allows the estimation of percentage of cells that are in specific phase of growth cycle and S phase fraction can be obtained which indicates the proliferative capacity of the tumour. As far as prognostic significance of DNA ploidy and S phase fraction is concerned, it has been usually seen that aneuploid tumours and higher S phase fraction may be associated with a shorter DFS and OS [11].

(ii) MIB-1 antibody: -Proliferative activity of the tumour can also be studied in histological sections and antibodies to Ki-67, i.e. MIB-1 and proliferating cell nuclear antigen {PCNA} are available. MIB-1 is

an antibody against a formalin resistant epitope of Ki-67 and it appears to be a very promising marker for proliferation. Weidner et al has recently brought out very good correlation of Ki-67 antigen expression by using the novel paraffin reactive MIB-1 antibody in breast carcinoma and have also correlated it with mitotic figure index and other indicators of tumour proliferation [12]. In view of these findings it is suggested that by carrying out MIB-1 antibody IHC staining, we can get a very good idea about tumour proliferation. It has been claimed that, MIB 1 antibody IHC staining probably is even better than flow cytometry and positivity of MIB-1 indicates poor prognosis [12].

{c} P53 mutation: -The next important biological parameter for breast cancer is the determination of p53 mutation. p53 is basically a tumour suppressor gene located on chromosome 17. It acts as a 'Guardian of genome' but when it undergoes mutation, the mutant p53 acts as an oncogene [13]. The half-life of mutant p53 protein is much longer and it accumulates in the nucleus of the cell and can be detected in routinely processed paraffin blocks of the tumour tissue. The p53 was thought to have a lot of prognostic significance and few studies have shown p53 positivity to be associated with poor survival specially lymph node negative case [14]. However now it appears to have been established that, p53 alteration occurs in highly malignant breast cancers rather than advanced stage or node positivity [15]. Thus p53 mutation status has shown conflicting results in the breast cancer cases and it's value as a prognostic marker till today remains undetermined [16].

{d} Amplification of c-erb-B2: c-erb-B2 is an oncogene which was discovered in 1980, but the prognostic significance was demonstrated by Slamon et al in 1987[17]. Three lines of investigators discovered c-erb-B2. The neu oncogene was detected in neuroblastomas of rats, c-erb was a human gene discovered by its homology to retroviral gene v-erb-B and screening human genomic library for homology with v-err-B lead to isolation of HER -2[18]. When DNA sequences were determined subsequently of all these three genes, c-erb-B2, HER-2 and nue were found to represent the same gene. c-erb-B2 is located on human chromosome 17. c-erb-B2 protein is a normal component of cytoplasmic membranes. There is experimental evidence that c-erb-B2 protein is involved in pathogenesis of breast neoplasia and overproduction of otherwise normal c-erb-B2 can transform a cell line into malignant phenotype [18]. Most studies of c-erb-B2 oncogene activation do not specify histological subtypes of infiltrating breast carcinoma

and incidence of amplification varies considerably. It is as low as 7% in tubular and lobular carcinomas to as high as 82% in Paget's disease. It is around 22% in Infiltrating Duct Carcinoma-No Special Type. The assessment can be carried out by IHC technique on paraffin blocks and c-erb-B2 is located on cytoplasmic membrane and only rimming of cytoplasmic membrane is evaluated as positive staining. To be considered positive, at least 10% of tumour cells must exhibit positive labelling and most of the studies in breast cancer have indicated that overexpression (10% cells) of c-erb-B2 is associated with poor prognosis [19].

{e} Factor predicting metastasis:

{i} Cathepsin D: Cathepsin D is a lysosomal acidic protease, that is present in all normal cells and has been implicated in the process of tumour invasion and metastasis by virtue of its ability to degrade cellular matrix and latent forms of other proteolytic enzymes [20]. Apart from cytosol assay, Cathepsin D can be assessed by IHC technique on paraffin embedded blocks fairly accurately. It was originally proposed as an indicator of poor prognosis, but many studies are available which have reported inconsistent results. All these studies were reviewed by Nadji et al and it was seen that, these studies have used whole tumour extracts and carried out the analysis by cytosol assays, where one cannot distinguish between Cathepsin D content of tumour cell and that of host cells [21]. Nadji et al carried out analysis of Cathepsin D by IHC technique on paraffin blocks, and observed that the higher content of Cathepsin D in stromal cells of breast tumour and not the tumour cells correlated very well with aggressive behaviour in breast cancer cases.

*{ii} nm 23:-*nm 23 gene in contrast to Cathepsin D, is associated with metastasis suppressive ability. Steeg and coworkers identified it by screening DNA libraries from murine melanoma cell lines of varying metastatic potential. Very few studies are available about nm 23, but transfection experiments with breast carcinoma cell lines have shown that by restoring the expression of nm 23, the metastatic potential can be reduced by 50 to 90% [22]. In breast cancer, reduced nm 23 expression correlated with higher metastatic potential. A case is considered negative if any lesional epithelium is unstained for nm 23 protein and nm 23 negativity was found to be correlated significantly with increasing grade of lymph node metastasis in one study [23].

{f} Topoisomerase II alpha (TPI) : Topoisomerase II enzymes are nuclear enzymes which play an

important role in DNA multiplication, formation of chromosome scaffolds, chromatin organization and may be involved in DNA transcriptase repair [24]. Topoisomerase II has alpha and beta isomers and alpha II, is not detectable in G 0 phase of cell cycle but its activity increases dramatically during S phase, peaks in G-2M phase and then declines. Increased expression of TPI has been observed to be associated with most aggressively and highly proliferative neoplasms [25]. Jarvinen et al have recently shown that positivity for TPI IIa in breast cancer is associated with aneuploidy [26] and Hellamen et al have shown higher rate of TPI IIa activity in high tumour grade [27]. The most important factor amongst all these studies of TPI, is also related to cancer chemotherapy and positivity is associated with resistance to anti-cancer drugs, which acts as TPI IIa inhibitors [28].

After reviewing these multiple parameters, one may feel that the list is very long, but many more parameters are arising on the scene. These are pS2; plasminogen activators and inhibitors; factor VIII immunostaining for angiogenesis; cell cycle regulators like Cyclin D1; adhesion molecules like E-cadherin; Heat shock proteins; Laminin receptors and so on. However, either these parameters have not shown very consistent results with prognosis or detect the same facet of the breast cancer which has been covered by above mentioned parameters and hence were not reviewed.

Recommendations

Based on the above review of the various biological parameters, it is clear that, it is not possible to predict the prognosis of a given case of breast cancer based on analysis of a single parameter and a battery of parameters must be used in a given case. But it is not possible to analyze all the above mentioned parameters. Hence while recommending the parameters for routine use, factors which have been considered are; the cost effectiveness of a marker, its almost unequivocal utility as a marker to detect a particular facet of breast cancer and at the same time the markers should be able to be carried out on routinely processed paraffin blocks, by any histopathology laboratory involved in breast cancer diagnosis.

Hence the prognostic factors recommended for routine use are, ER, PR (hormone receptors), MIB 1 antibody (proliferation status), c-erb-B2 (indicating oncogene activation), cathepsin D (promoting tumour metastasis) and nm 23 (indicating tumour suppressor potential). The markers thus indicating 'high risk' group or poor prognosis in breast cancer are, negative immunostaining of ER, PR, nm23 and; positive stain-

ing of c-erb-B2, cathepsin D and MIB-1 antibody. The diametrically apposite pattern of staining of these parameters on the other hand indicates better prognosis.

REFERENCES

1. Epidemiology of Breast Cancer. The Indian scenario, In: 'Guidelines for breast cancer pathology reporting', Ed Chinnoy RF. Tata Memorial Hospital Publication No 9; 1997:1-4.
2. Parkin DM, Muir CS, Whelan SL. Cancer incidence in five continents. Vol VI, IARC Scientific publications No. 120, Lyo IARC, 1992.
3. Fisher B, Costantino J, Wickerman R, Redmond N, Wolmark R, Margolese D. Adjuvant therapy for node negative breast cancer: an update of NSABP findings. *Proc Am Soc Clin Oncol* 1993; 12:69-80.
4. Carter PL, Allen C, Henjon D. Relationship of tumour size, lymph node status and survival in 24,370-breast cancer cases. *Cancer* 1989; 63:181-7.
5. Ellis OA, Galea M, Broughton N, Krockner A, Blamey RW, Elpson CW. Pathological prognostic factor in breast cancer histologic type. Relationship with survival in a large study with long term follow-up. *Histopathology* 1992; 20:479-89.
6. Elston GK, Ellis IO. Pathological prognostic factors in breast cancer. The value of histologic grades in breast cancer. Experience from a large study with long-term follow up. *Histopathology* 1991;19:403-10.
7. Galen MH, Blamey RW, Elston CW, Ellis IO. Nottingham prognostic index in primary breast cancer. *Breast Research Diag and Treat* 1992; 220:207-19.
8. Press MF. Estrogen receptors. In: *Advances in Pathology and Lab Med Vol 6 St Lousy Mosby* 1993:117-43.
9. Benner SE, Clark GM, McGuire WL. Review: Steroid receptors, cellular kinetics lymph node status as a prognostic factors in primary breast cancer. *Am J Med Sci* 1988; 296:59-66.
10. Hedley DW, Clark GM, Cornelisse CJ, Killander D, Kute T. Consensus review of the clinical utility of DNA cytometry in carcinoma breast. *Cytometry* 1993; 14:482-485.
11. Semour L, Bezdowna WR, Meyer K. Response to second line hormone treatment for advanced breast cancer, predictive value of ploidy determination. *Cancer* 1990; 65:2270-4.
12. Weidner N, Moore DH, Vartanian R. Correlation of Ki-67 antigen expression with mitotic figure index and tumour grade in breast carcinomas using novel 'paraffin reactive MIB-11' antibody. *Human Pathol* 1994;25:337-42.
13. Lane DP, Benchimal S. p53 oncogene or antioncogene? *Gene Dev* 1990;4:1-8.
14. Isola J, Visa Korpi T, Bolli K, Kallioneimy OP. Association of overexpression of tumour suppresser p53 with rapid cell proliferation and poor prognosis in node negative breast cancer patients. *J Natl Cancer Inst* 1992; 84:1108-14.
15. Sasa M, Kandok K, Kowaki K, Movimoto T, Morden Y. p53 alteration correlation with negative ER, negative PgR and high histologic grade in breast cancer. *J Surg Oncol* 1994; 56:46-50.
16. Haersley T, Jacobson GK. An immunohistochemical study of p53 with correlation to histopathological parameters, c-erb-B2, proliferating cell nuclear antigen and prognosis. *Hum Pathol* 1995; 26:295-301.
17. Slamon DJ, Clark GM, Wong SG. Human breast cancer. Correlation of relapse and survival with amplification of HER 2/nue oncogene. *Science* 1987; 235:177-80.
18. Singleton TP, Strickler JG. Clinical and pathologic significance of the c-erb-B2 oncogene. In *Pathology Annual*, Rosen PP, Fechner RE editors, Appleton and Lange Norwalk, California 1992; Part 1:165-90.
19. Connor GE, Udey JA. Expression and refolding of recombinant human fibroblast procatepsin D DNA. *Cell Biol* 1990;19:403-10.
21. Nadji M, Fresno M, Massini M, Conner G, Herrero A, Morales AR. Cathepsin D in host stromal cells but not in tumour cells is associated with aggressive behaviour in node negative breast cancer. *Human Pathol* 1996; 27: 890-895-.
22. Steeg PS. Metastasis. *Proc Ann Meet Am Asso Cancer Res* 1995; 36:A 650.
23. Royds JA, Stephenson TJ, Rees RC. nm 23 protein expression in ductal in situ and invasive human breast carcinoma. *J Natl Cancer Inst* 1993;85:727-31.
24. Pommier Y, Bertrand R. The mechanism of formation of chromosomal aberrations: Role of eukaryocytic and DNA topoisomerase. In Krish IR editors. *The causes and consequences of chromosomal aberrations*. BOAC Rattan Florida. CR. press 1993;277-309.
25. Cummins J, Smith JF. DNA topoisomerase I and II as target for rational design of new anticancer drugs. *AA Oncol* 1993; 4:533-43.
26. Jarvinen TA, Konnen J, Delto Huikkom M, Isola J. Expression of topoisomerase II alpha in associated with rapid cell proliferation, aneuploidy and C-erb-B2 overexpression in breast. *Am J Pathol* 1996; 148 : 2073-82.
27. Helleman P, van Dam PA, Geysken M, Dosterom AJV, Buytaert March EV. Immunohistochemical of topoisomerase II alpha expression in primary ductal carcinoma breast. *J Clin Pathol* 1995;48:147-50.
28. O, Reilly S, Kennedy MJ, Rowinsky EK, Donehowen RC. Vinorelbin and topoisomerase inhibitors: Potential role of breast cancer chemotherapy. *Cancer Res Treat* 1995;33:1-17.