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Original Article

Immunohistochemical Evaluation of P-Glycoprotein and Its Correlation to The Response to Neo-Adjuvant Chemotherapy in Stage III Breast Carcinoma Patients

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Abstract

Objective: The aim of this work was to evaluate the immunohistochemical expression of P-glycoprotein and its correlation to the response to chemotherapy with antraciclin in women affected by stage III breast carcinoma. **Methods:** In this transversal study, 88 prontuaries of patients affected by local ductal infiltrative advanced carcinoma who had received neo-adjuvant chemotherapy with antraciclin, excepting the inflammatory ones, had been analyzed from June 1996 to November 2003, in the Clinic of Clinical Oncology of CAISM/UNICAMP. Tumors was biopsed before the treatment (core or incisional biopsy or) and submitted to immunohistochemical examination, system envision peroxidase, using C494 (Signet) and C219 (Signet) anti-P-glycoprotein monoclonal antibodies. Cytoplasmic or transmembrane coloration in at least 10% was considered positive. The positive external control used was of human kidney normal tissue. Clinical response was evaluated before and surgery, and after at least two chemotherapy cycles and data were correlated with P-glycoprotein expression. Fisher accurate test or qui-square was used to evaluate the possible associations. **Results:** P-glycoprotein positivity in the sample was 23.86%. The objective clinical response to chemotherapy was similar in cases with and without P-glycoprotein expression, considering the primary tumor (57.1% versus 58.2%), the armpits (67.7% versus 78.7%) and total response (57.1% versus 65,7 %, p = 0,604). **Conclusion:** The relation between P-glycoprotein expression and the clinical response to neo-adjuvant chemotherapy was not found, suggesting that this marker is not to be considered a predictive factor of response to chemotherapy with antraciclin.

Key words: Breast cancer. Neo-adjuvant chemotherapy. P-glycoprotein.

Introduction

Locally advanced breast cancer frequency in the developing countries accounts for 30% to 50% of the diagnosed cases, including inflammatory carcinomas.[1,2] The more used initial treatment is chemotherapy, that can reduce tumoral volume in about 60% to 80% of the cases, increase lifespan, for it treats precociously systemic illness,[3,4] makes possible the accomplishment of conservative surgeries and the evaluation *in vivo* of tumoral cells chemosensibility.[5,6]

Partial or complete responses are associated toe greater lifespan[7,8] and the more effective antiblastic association schemes are those that contain antracyclines.[9,6] Paclitaxel- or docetaxel-based schemes associated to antracyclines increase the responses, mainly the complete ones, in up to 28% of cases.[10,11] Antracyclines are therefore the more used drugs, but the observed responses do not meet expectations,[12] perhaps because neoplasic cells had developed resistance to drugs.[12]

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The evaluation of resistance to antracyclines could define which tumors will not respond to treatment and would allow choosing other more effective drugs. The resistance intracellular mechanism includes alterations in the expression of the specific target of each cytotoxic agent, by means of the amplification of the agent coding gene.[13,14] MDR ("multi drug resistant") gene is associated to multiple resistance the drugs, generally those derived from natural products like vincristine and doxorubicin and would be responsible for P-glycoprotein (P-gp) production, that diminishes the drugs intracellular concentration.[15,16] Two P-gp coder genes, "MDR1 and MDR3", were isolated from human cells, and only the first has been associated to multiple-drug resistance.[17,18] P-gp is a permanent component of the cellular membrane and it has a transport and excretion function. The increase of its expression that occurs in some tumoral cells results in an increase of the drug efflux and a reduction of its intracellular concentration.[19,20] Immunoperoxidase techniques had demonstrated the presence of P-gp in normal and tumoral tissues and its superexpression as associated to resistance to chemotherapeutical drugs.[21-27] Considering that P-gp is the marker that up to now better characterizes the resistance mechanism to drugs like antracyclines, that the more used neo-adjuvant chemotherapy contains adriamicyn and that stage III breast carcinoma is very prevalent in Brazil, it is useful to evaluate P-glycoprotein immunohistochemical expression and its correlation to the response to chemotherapy with antraciclin in women affected by stage III breast carcinoma, for it could select patients whose tumors are sensible to this drug.

Material and Methods

The prontuaries and biological material of 88 women registered in the Clinical Oncology Clinic of the Center of Integral Attention to Woman's Health of UNICAMP, affected by measurable IIIB and IIIB invasive ductal breast carcinoma, excluding the inflammatory ones, had been retrospectively evaluated from June 1996 to November 2003. From eighty-eight patients, seventy-nine had received three cycles of chemotherapy before surgery; two had received two cycles because of intolerance to schemes with adriamicyn and seven received 6 cycles due to the favorable responses these patients presented. The schemes contained adriamicyn (60mg/m2) and ciclofosfamide (600mg/m2), or fluorouracil (600mg/m2), adriamicyn (60mg/m2), ciclofosfamide (600mg/m2). P-gp glycoprotein was evaluated before chemotherapy by immunohistochemistry. Cytoplasmic or transmembrane coloration in 10% or more of the cells was considered positive. Clinical response to treatment was evaluated before surgery and defined as: complete response - disappearance of all measurable injuries; partial response - reduction of at least 30% of the value of measurable injuries' greatest diameters, without the emergence of new injuries; objective response - complete and partial values added; steady response: a reduction lesser than 30% or an increase lesser than 20% of the values of measurable injuries' greatest diameters; and progression - an increase of at least 20% of measurable injuries' greatest diameters, or the appearance of new injuries. Tumor and axillary lymphonodes initial and final clinical measures were the greatest diameters, in millimeters, specified in the prontuaries. Global clinical response was established as the addition of breast and axillary tumor.

Immunochemistry Technique, Data Collection and Statistical Analysis

4mm cuts were made on paraffin blocks that were placed in blades, unparaffined and hydrated. Endogenous peroxidase inhibition was carried through in a hydrogen peroxide 10% solution. Antigenic recovery was made in a 10mM citrate tampon (pH6) at 95°C in a vapor pressure pan. The blades were incubed at 4°C in humid chamber, with C219 (dil1/25) Signet and C494 (dil1/40) Signet primary antibodies; all monoclonals produced in mice and diluted in PBS. The marking was carried through with envision peroxidase system and the coloration with DAB (3,3 diaminobenzidin, Sigma, D5637) until the visualization of a brownish coloration in the cuts (Figures 1 and 2). The positive control was normal human kidney tissue.

Data from the patients' prontuaries had been transcribed to the Register Form elaborated by the investigators. For data processing, a codified

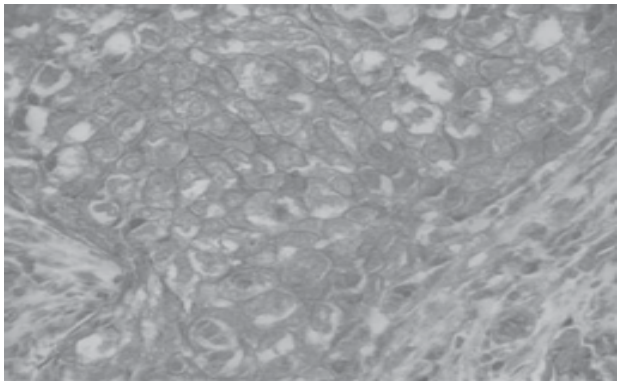

Figure 1 - 400x zoom showing P-gp expression in the cytoplasm of Invasive ductal carcinoma cells (C494)

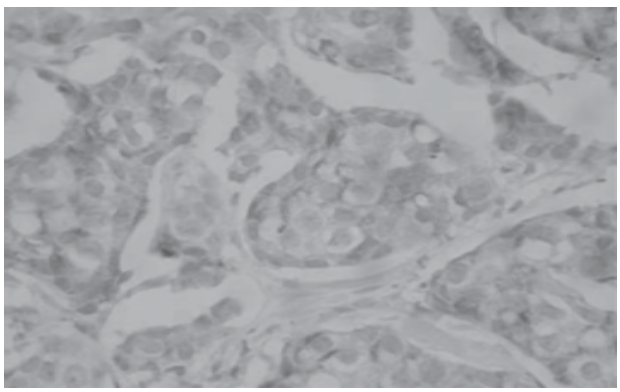

Figure 2 - 400x zoom showing P-gp expression in transmembrane of Invasive ductal carcinoma cells (C219)

form for registered data was prepared. The program Excel-Version 2000 was used to create a database for collected information. Statistic analysis was carried through Statistica program. The association test for 0 categorical variable was carried through using qui-square, with Yates correction, when appropriate, or Fisher Accurate Test.[28]

The principles enunciated in the Declaration of Helsinki (2000) and in Resolution 196/96 of the National Health Council (Brazil, 1996) were respected as regards maintaining patients' identity secrecy. The project was approved by the Ethics in Research Committee of FCM/UNICAMP.

Results

Patients included in the study had a median age of 49 years, and 52 of them were in pre-menopause. The initial tumoral size was bigger in the breast that in the armpit. As regards clinical stage, about 2/3 of the patients had stage IIIB tumors, corresponding to 75% of T4 a, b injuries. Only 30.68% of the patients did not have clinical axillary metastasis and more than half presented axillary lymphonodal conglomerates (Table 1). P-gp expression was positive in 23,86% of the patients. Neo-adjuvant chemotherapy schemes used were AC, 66 patients and FAC, 22 patients. Managed chemotherapy cycles median was 3; with a minimum of 2 and a maximum of 6. Tumoral size median, after primary chemotherapy, were 50mm on the breast and 0mm on the armpit. Total diameter was 50mm. Clinical response evaluated before surgery presented objective responses in 51 patients regarding breast tumors (57,1%), in 48 patients in axillary tumors (76,2%) and 56 patients in the global tumor (63,6%). Complete response was presented by 7 patients as regards the primary tumor, 24 patients regarding the armpit tumor and 6 patients regarding both (breast + armpit). Partial breast armpit and global clinical response were observed in 44, 24 and 50 patients, respectively. Breast, armpit and global steady illness were presented by 37, 12 and 32 patients, respectively. Progression was observed only in the armpit. Initially, axillary tumor was measurable in 61 patients. After chemotherapy, progression occurred in only three cases; two patients were initially N0 and one N2. The evaluation of the association between P-gp expression and clinical breast, armpit and global responses was not significant in this study (Tables 2, 3, 4).

Table 1 - Distribution of patients according to illness characteristics

Characteristics	n (88)	%
Tumoral size median (mm)		
Breast (extremes)	72,5 (20-150)	
Armpit	20 (0-70)	
Stage		
IIIA	22	25
IIIB	66	75
Primary Tumor		
T2	10	11,36
T3	17	19,32
T4a	2	2,27
T4b	59	67,05
Axillary Lymphonodes		
N0	27	30,68
N1	16	18,18
N2	45	51,14

Table 2 - Evaluation of the association between breast clinical response to neo-adjuvant chemotherapy and P-gp expression

Response	P-gp			
	Positive		Negative	
	n	(%)	n	(%)
Objective response	12	57,10	39	58,20
No response	9	42,90	28	41,80

Table 3 - Evaluation of the association between armpit clinical response to neo-adjuvant chemotherapy and P-gp expression

Response	P-gp			
	Positive		Negative	
	n	(%)	n	(%)
Objective response	11	67,75	37	78,70
No response	5	31,25	10	21,30

p 0,418

Table 4 - Evaluation of the association between global clinical response to neo-adjuvant chemotherapy and P-gp expression

Response	P-gp			
	Positive		Negative	
	n	(%)	n	(%)
Objective response	12	57,10	44	65,70
No response	9	42,90	23	34,30

Discussion

The study showed that P-gp frequency in local advanced breast carcinoma patients was 23,86%, and P-gp positivity varies from 10% to 50% of breast tumor cases and is related with the worse prognostic for the illness.[24,29,30]

Complete response rates depend on primary tumor size: 21% for T1 T2 tumors patients and 7% for those with T3 and T4.[12,31,32] In the present study, primary tumor median was 7cm, and complete clinical response reached 7.95%. The presence of compromised axillary lymphonodes is not a predictive factor for local-regional response in local advanced or unresectable carcinomas.[33] In the present study axillary lymphonodal compromising also was not a predictive factor for clinical response to neo-adjuvant chemotherapy. The identification of factors associated to the response to chemotherapy could assist in the election of patients with a greater probability of objective response and in the definition of better therapeutic strategies. Complete response was identified as a favorable prognostic factor in relation to the clinical evolution of locally advanced breast carcinoma patients.[34] Chemotherapy schemes containing antracyclines, mainly doxorubicin, promote a rate of complete clinical response from 10% to 51% and a rate of pathological responses from 3% to 30%. These responses are similar in all involved places as regards potentially operable primary tumor, that is, breast and axillary ones.[5,35,36] In the present study, breast, armpit and global clinical objective responses were 57,95%, 76.20%, 63.64% and 7.95%, and global clinical complete responses were 38.1% and 6.82%, respectively, results inferior to the described results in studies previously cited, perhaps because of a greater tumoral volume.

Responses were better in lymphonodes that in the breast, perhaps because tumoral cells present in lymphonodes are more undifferentiated clones.[12,37] P-gp can be a clinically important marker in the evaluation of tumors treatment, prognostic and evolution. The easiness of its evaluation by the immunoperoxidase technique, even after inclusion in paraffin and the possibility of using normal tissue as control allow its use in the diagnosis of multiple-drug intrinsically resistant tumors. [22,38] The prevalence of P-gp expression in breast cancer is arguable, varying from non detectable by Western Blot method, with C219[39] monoclonal antibody, to 85% of positivity using C494 antibody.[40] Generally this protein positivity varies from 10,6% to 19.1%, depending on the used antibody.[41]

In the present study, the prevalence of P-gp was 10,2%, when C 219 antibody was used, 19.3% with C494 and 23,86% considering the positivity with at least one of the employed antibodies. These data are similar to Wang's (1997),[41] that found a positivity of 10,6% with JSB1 monoclonal antibody, 12.8% with C494 and with at least one of them 25%.

In breast cancer, P-gp expression is more common in local advanced tumors[42,43] and its positivity could be a predictive factor of response to chemotherapy; however, this correlation has not been observed and perhaps P-gp is more a marker of tumor aggressiveness than of response to treatment.[42] But the study showed a significant

relation between the response to chemotherapy with antraciclin and P-gp expression.[24]

In the present work, no relation was found between P-gp and the response to neo-adjuvant treatment. Responses observed when it was positive were similar to those in which the protein was negative. Thus, one cannot consider P-gp expression correlated to response to chemotherapy with antraciclin, perhaps because multiple-drug resistance is multifactorial, involving several genes with both with isolated or associated action,[21] but inquiries are justified that seek to identify genes responsible for drugs transport, evaluating their proteins' amplification and superexpression, that could predict tumor sensitivity to chemotherapeutic schemes.[44,45]

References

1. Jardines L, Haffty BG, Royce M, Jaiyesimi I. Cancernetwork.com. Breast Cancer. Advanced Disease. Stages III and IV breast cancer, cap. 11, 2000. Disponível em: <http://www.cancernetwork.com/contents/breast.htm# Disease>. Acesso em: 06.01.2006
2. Hortobagyi GN, Singletary E, Mcneese M. Treatment of locally advanced and inflammatory breast cancer. In: Harris JR, Lippman ME, Morrow M, Hellman S. (eds). Disease of the breast. Philadelphia: Lippincott-Raven Publishers. 1996. p.585-99.
3. van der Hage JA, van de Velde CJ, Julien JP, Tubiana-Hulin M, Vandervelden C, Duchateau L. Preoperative chemotherapy in primary operable breast cancer: Results from the European Organization for Research and Treatment Cancer trial 10902. J Clin Oncol 2001;19:4224-37.
4. Perloff M, Lesnick GJ, Korzun A, Chu F, Holland JF, Thirlwell MP et al. Combination chemotherapy with mastectomy or radiotherapy for stage III breast carcinoma: a cancer and leukemia B study. J Clin Oncol 1988; 6:261-9.
5. Dickson RB, Lippman ME. Cancer of the breast. In: De Vita Jr VT, Hellman S, Rosenberg SA. Cancer - Principles e practices of oncology. 6ª ed., Philadelphia: Lippincott Williams & Wilkins; 2001. p.1633-717.
6. Bonadonna G, Valagussa P, Brambilla C, Ferrari L, Moliterni A, Terenziani M *et al*. Primary chemoterapy in operable breast cancer: eight-year experience at the Milan Cancer Institute. J Clin Oncol 1998;16:93-100.
7. Pierga JY, Mouret E, Dieras V, Laurence V, Beuzeboc P, Dorval T *et al*. Prognostic value of persistent node involvement after neoadjuvante chemotherapy in patients with operable breast cancer. Eur J Cancer 2000;83:1480-7.
8. Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER *et al*. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. J Clin Oncol 1998; 16:2672-85.
9. Hortobagyi GN, Singletary SE, Strom EA. Treatment of locally advanced and inflammatory breast cancer, In: Harris JR, Lippman ME, Morrow M, Osborne CK. Diseases of the breast. 2ª ed., Philadelphia: Lippincott Williams & Wilkins; 2000. p.645-60.
10. Evans T, Gloud A, Foster E *et al.* Phase III randomized trial of adriamycin (A) and docetaxel (D) versus A and cyclophosphamide (C) as primary medical therapy (PMT) in woman with breast cancer. An ACCOG study. Proc Am Soc Clin Oncol 2002; 21:351, (abstr. 136).
11. Semiglazov VF, Bojok AA, Arsumanov AS, Ivanova OA *et al*. Neoadjuvant chemotherapy paclitaxel and doxorubicin (PD) versus fluorouracil and doxorubicin and cyclophosphamide (FAC) in locally advanced breast cancer clinical and pathological response. Eur J Cancer 2001;37(Suppl 6):166-7.
12. Kuerer HM, Newman LA, Smith TL, Ames FC, Hunt KK, Dhingra K et al. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin- based neoadjuvant chemotherapy. J Clin Oncol 1999;17:460-9.
13. Ferguson PJ, Cheng Y. Critical issues relating to clinical drug resistance. Cancer Bull1989; 41:7-13.
14. Teeter LD. Drug resistance and chemotherapy a perspective. Cancer Bull 1989;41:14-20.
15. Bech-Hanson NT, Till JE, Ling V. Pleiotropic phenotype of colchicine – resistant CHO cells: cross resistance and collateral sensitivity. J Cell Physiol 1976; 88:23-2.
16. Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 1976; 455:152-62.
17. Ueda K, Clark DP, Chen CJ, Roninson IB, Gottesman MM, Pastan I. The human multidrug resistance (mdrl) gene. J Biocommun 1987; 262:505-8.
18. Van der Bliek AM, Baas F, Ten Houte de Lange T, Kooiman PM, Van der Velde-Koerts T, Borst P. The human mdr3 gene a novel P-glicoprotein homologue and gives rise to alternatively spliced mRNAs in liver. EMBO 1987; 6:3325-31.
19. Pusztai L, Wagner P, Ibrahim N, Rivera E, Theriault R, Booser D, Symmans FW, et al. Phase II Study of Tariquidar, a Selective P-Glycoprotein Inhibitor, in Patients Chemotherapy-Resistant, Advanced Breast Carcinoma. Cancer 2005;104:682-91.
20. Trock BJ, Leonessa F, Clarke R. Multridrug resitnce in breast câncer: a meta-analysis of MDR1/ gp170 expression and its possible functional significance. J Natl Cancer Inst. 1997;89:917-31.
21. Cordon-Cardo C, O'brien J. The multidrug resistance phenotype in human cancer. In: De Vita V, Hellamn S, Rosenberg AS (eds). Important Advances in Oncology. Philadelpyia: JB Lippincott Company; 1991. pp.19-38.
22. Goldstein LJ, Galski H, Fojo A, Willingham M, Lai SL, Gazdar A et al. Expression of a multidrug resistance gene in human cancers. J Natl Cancer Inst 1989;81:116-24.
23. Broxterman HJ, Pinedo HM, Kuiper CM, van der Hoeven JJ, de Lange P, Quak JJ, et al. Immunohistochemical detection of p-glycoprotein in human tumor cells with a low degree of drug resistance. Int J Cancer 1989;43:340-3.
24. Chintamani, Singh JP, Mittal MK, Saxena S, Bansal A, Bhatia A, Kulshreshtha P. Role of p-glycoprotein expression in predicting response to neoadjuvant chemotherapy in breast cancer-a prospective clinical study. World J Surg Oncol 2005;3:61.
25. De Vita VT. Principles of chemotherapy. In: De Vita VT, Hellman S, Rosemberg SA. (eds). Cancer: Principles and practice of oncology, 3a. ed., Philadelphia: JB Lippincott; 1989. p.276-300.
26. Bodey B, Bodey Jr B, Groger AM, Luck JV, Siegel SE, Taylor CR, Kaiser HE. Immunocytochemical detection of the p170 multidrug resistance (MDR) and the p53 tumor suppressor gene proteins in human breast cancer cell: clinical and therapeutical significance. Anticancer Res 1997;17:1311-8.
27. Liux Song S, Shi C. Clinical significance of expression of multidrug resistance gene in breast cancer tissue – Chung Hvan I Sueh ISA 1997;7:488-90.
28. Fisher LD, Van Belle GB. Biostatistics: a methodology for the healthy sciences. New York: Jonh Wiley Sons; 1993. 91p.
29. Chung HC, Rha SY, Kim JH, Roh JK, Minj S, Kim BS, Lee KB. - p-Glycoprotein: the immediate end point of drug resistence to induction chemotherapy in locally advanced breast cancer. Breast Cancer Res Treat 1997; 42:65-72.
30. Castagna M, Viacava P, Cianfriglia M *et al*. Immunohistochemical localization of the P- glycoprotein by monoclonal antibody MAB 57 in human breast cancer. J Cancer Res Clin Oncol 1991;117(suppl 3):S92.
31. Dieras V, Fumoleau P, Romieu G, Tubiana-Hulin M, Namer M, Mauriac L et al. Randomized parallel study of doxorubicin plus

paclitaxel and doxorubicin plus cyclophosphamide as neoadjuvant treatment of patients with breast cancer. J Clin Oncol 2004;22:4958-65.
32. Thomas E, Holmes FA, Smith TL, Buzdar AU, Frye DK, Fraschini G et al. The use of alternative, non-cross resistant adjuvant chemotherapy on the basis of pathologic response to a neoadjuvant doxorubicin-based regimen in women with operable breast cancer; Long-term results from a prospective randomized trial. J Clin Oncol 2004;22:2294-302.
33. McCready DR, Hortobagyi GN, Kau SW, Smith TL, Buzdar AU, Balch CM. The prognosis significance of lymph node metastases after preoperative chemotherapy for locally advanced breast cancer. Arch Surg 1989;124:21-5.
34. Hortobagyi GN, Buzdar AU, Strom EA, Ames FC, Singletary SE. Primary chemotherapy for early and advanced breast cancer. Cancer Lett 1995;90: 103-9.
35. Hortobagyi GN, Singletary SE, Strom EA. Locally advanced a breast cancer, In: Harris JR, Lippman ME, Morrow M, Osborne CK. Diseases of the breast. 3ª ed., Philadelphia: Lippincott Williams & Wilkins; 2004. p.952-69.
36. Smith IE, A Hem RP, Howell A, Hiskish T, O'brien M, Mansi J. et al. Preoperative continuons infusional E Cis F (epirubicin, cisplatin and infusional 5FU) vs conventional AC chemotherapy for early cancer. A phase III multicentric randomized trial by TOPIC Trail Group. Proc Am Soc Clin Oncol 2000;19:84A, (abstr. 320).
37. Kuukasjarvi T, Karhu R, Tanner M, Kahkonen M, Schaffer A, Nupponen N et al. Genetic heterogeneity and clonal evolution underlying development of asynchronous metastasis in human breast cancer. Cancer Res 1997;7:1597-604.
38. Spoelstra EC, Dekker H, Schuurhuis GJ, Broxterman HJ, Lankelma J. P-glicoprotein drug effux pump involved in the mechanisms of intrinsic drug resistance in various colon cancer cell lines. Evidence for a saturation of active daunorubicin transport. Biochem Pharmacol 1991;1:349-59.
39. Merkel DE, Fuqua SAW, Tandon AK, Hill SM, Buzdar AU, McGuire WL. Electrophoretic analysis of 248 clinical breast cancer specimens for P-glycoprotein overexpression or gene amplification. J Clin Oncol 1989;7:1129-36.
40. Verrelle P, Meissonnier F, Fonck Y, Feillel V, Dionet C, Kwiatkowski F et al. Clinical relevance of immunohistoquimical detection of multdrug resistance P-glycoprotein in breast cancer. J Natl Cancer Inst 1991;83:111-6.
41. Wang CS, La Rue HL, Fontini A, Gariepy G, Tetu B. MDR, mRNA expression by RT-PCR in patients with primary breast cancer submitted to neoadjuvant therapy. Breast Cancer Res Treat 1997;45:63-74.
42. Pinedo HM, Giaccon G. P-glicoprotein-a marker of cancer-cell behavior. N Engl J Med 1995;333:1417-9.
43. Giaccon G, Linn SC, Pinedo HM. Multidrug resistance in breast cancer: mechanisms, strategics. Eur J Cancer 1995;31(suppl 7):S15-S17.
44. Beck WT, Grogan TM, Willman CL, Cordon-Cardo C, Parham DM, Kuttesch JF et al. Methods to detect P-glicoprotein-associated multidrug resistance in patients tumors: Consensus recommendations. Cancer Res 1996;56:3010-20.
45. Lehnert M. Clinical multidrug resistance in cancer: A multifactorial problem. Eur J Cancer 1996;32:912-30.