

Antonio Franco Montoro, Osvaldo Giannotti Filho,
Darcy Marchione Monteiro, Flávio Franco Montoro,
Maria Aparecida Pinho, Marco Túlio Assis Figueiredo

Hormonal receptors in mammary carcinoma: comparison between quantitative and qualitative methods

Fundação Oncocentro de São Paulo. Breast Clinic at Hospital Alemão Oswaldo Cruz - São Paulo, Brazil

Alternatives to the traditional hormone receptor dosages for prognostic evaluation and clinical approach to breast cancer have been proposed for immunohistochemical determinations. For correlation purposes, such procedures were compared in 37 patients presenting 5 to 15 years of survival. Considering 30 fm/mg as the positivity index, the disagreement between both methods reached 35.1% with estrogen and 48.5% with progesterone receptors. When the positiveness level was changed to 20 fm/mg, the discrepancies were reduced to 32% with ER and increased to 57% with PgR. This study leads us to not recommend the immunohistochemical method applied to paraffin sections as an alternative procedure to the dextran-charcoal dosage for prognosis and therapeutic management of mammary carcinoma.

UNITERMS: Breast carcinoma. Prognosis. Hormonal receptors assays. Correlation of methods.

INTRODUCTION

The hormonal dependence of breast cancer has been known and discussed for over 100 years. In 1889, Schinzinger¹ in Germany, was the first to call attention to this fact and Beatson² in Scotland, in 1896, presented a paper showing cases of mammary carcinoma remissions after bilateral oophorectomy. However his results did not exceed 30% of patients. After the laboratory isolation of cortisone, hormonal ablative surgery to control mammary cancer has been extended to also include adrenalectomy and hypophysectomy.

Meanwhile, endocrine additive treatments were started and successively estrogens, androgens,

progesterone, anti-hormones like tamoxifen, aromatase inhibitors, and recently LH-RH analogs, were used in order to control breast cancer.

All these attempts had in common one question: to what percentage would the patients respond to this hormone manipulation? Without a selective criterion the results were similar to those shown by Beatson, ie only one third were responsive.

By 1970 there were clinical and biochemical parameters to predict therapeutic results that would allow the drawing of conclusions regarding different grades of hormonal dependence. Among them, the disease-free interval proved to be worthwhile. When the time elapsed between the curative mastectomy and the first recurrence of disease was longer than five years, the responsiveness rate to endocrine manipulation increased to two thirds of the patients.

In the early seventies, Jensen³ identified the estrogen receptor (ER) in the mammary tumor cell as a reliable parameter to evaluate the grade of hormone dependence right at the beginning of treatment, without having to wait for five years of evolution.

Address for correspondence:

Antonio Franco Montoro
Praça Amadeu Amaral, 47
São Paulo/SP - Brasil - CEP 01327-010

Jensen believed that the mammary carcinoma hormonal dependence was positive when the biochemical dosages indicated a receptor amount above three femtomoles/milligram of neoplastic tissue. Lately, another estrogenic derived marker has been identified, the progesterone receptor (PgR). Its positivity index was initially placed at five fm/mg of tissue⁴

With further research progress it became evident that the index of 3 or 5 fm/mg was too low to characterize the degree of hormone dependence of the neoplastic tissue⁵. This index was gradually elevated to 10, 20 and 30 fm/mg. Even Jensen⁶, as his own experience increased, came to the conclusion that 50 fm/mg would be the ideal index to make a correct prognostic evaluation and prediction of therapeutic response to the endocrine treatment.

Meanwhile, in addition to quantitative methods for receptor dosages, immunohistologic qualitative techniques were developed following the pioneer research started by Pertschuk⁷. When the tumor receptor content was not established during surgery, such information could possibly be determined through paraffin sections. Thus, the hormonal dependence of breast cancer pointed out by the receptor presence, in both estrogen and progesterone conditions, being positive or negative, could represent an alternative approach to improve the mammary carcinoma management.

In this study, we present the results of quantitative biochemical dosages of hormonal receptors in frozen tumor tissue and qualitative immunohistochemical analyses in paraffin sections carried out on the same group of patients.

MATERIAL AND METHODS

Out of a series of 100 women treated for mammary carcinoma with an asymptomatic survival of 5 to 15 years, with receptor dosages for estrogen and progesterone (Dextran-Charcoal method) and with the immunohistochemical determinations of estrogen and progesterone antibodies (P29 Biogenex), we selected 37 patients presenting reliable paraffin sections.

This group varied in age from 30 to 72 years old; the clinical stages were: CS 0: 1; CS I:9; CS II: 21; CS III: 6 cases. Axillary lymphonode metastases were present in 18 and absent in 19 patients.

The hormonal receptor values ranged from zero to 762 fm/mg for ER, and from zero to 1,629 fm/mg for PgR. The positiveness value was set at 30 fm/mg. For qualitative determinations, the results were considered as positive or negative for both conditions.

The median survival rate of this series reached 105.5 months. It was analyzed for prognostic purposes and correlated to the clinical stage of disease as well with the axillary status and its receptor positiveness, according to both quantitative and qualitative procedures. In clinical stage zero with non palpable tumor the survival rate was 120 months. When the axilla was negative 113.7 months. In receptor positive above 30 fm/mg it was 112.7, and only 109.4 months for the patient presenting qualitative positive results.

The comparison between the two methods is shown in Tables 1 and 2. The concordance rate for ER was 64.8% and for PgR 51%. Considering that numerous authors accept 20 fm/mg as positive for both hormone receptors, we also used this index. As a result, the estrogen receptor showed a reduction of discrepancy from 35.1 to 32%. For the progesterone receptor there was a discrepancy increase to 57%.

DISCUSSION

Jensen³ noticed that the longer survival and better response rates to hormonal manipulation were directly proportional to quantitative receptor levels. The higher the index the greater the benefit. Similarly, adrenalectomy and

Table 1
Correlation with ER results. The qualitative determinations disagreed by 35.1% with the quantitative dosages

Method	#	%	Method	#	%
DC+ IH+	14/20	70.0	DC+ IH-	6/20	30.0
DC- IH-	10/17	58.8	DC- IH+	7/17	41.1
Concordance	24/37	64.8	Discrepancy	13/37	35.1

DC: Dextran charcoal dosage
IH: Immunohistochemical assay

Table 2
Correlation with PgR results.
For this condition the disagreement reached 48.5%

Method	#	%	Method#	%
DC+ IH+	3/18	16.6	DC+ IC -	15/18 83.3
DC- IH -	15/17	88.2	DC - IH +	2/17 11.7
Concordance	18/35	51.0	Discrepancy	17/35 48.5

DC: Dextran charcoal dosage
IH: Immunohistochemical assay

castration procedures exhibit increased responses when compared to endocrine additive therapies.

After the introduction of the PgR condition as another marker for breast cancer hormonal dependence evaluation, such observations were confirmed^{4,8}, especially by Osborne⁹, who demonstrated that the progesterone receptor was more sensitive than ER as a prognostic marker, because its value is independent of the pre or post menopausal status, while the ER condition shows up only after the menopause.

The literature on the comparison between the quantitative and qualitative methods has shown discordant results¹⁰⁻¹². Janssens et al¹³ concluded that histochemistry determinations and the biochemical dosages were not concordant when the latter were considered positive at 10 fm/mg of tissue. On the other hand, Pertschuk et al¹⁴ utilizing the same index of 10 fm/mg found a 80% concordance between both methods. The authors used only estrogen receptors and the immunohistochemistry test was performed on frozen biopsy tissue. Leal et al¹⁵ using identical methods found 92% in agreement. Panko et al¹⁶ however, found a concordance of 63% and came to the conclusion that the selection of patients for endocrine therapy should not be made on the basis of immunohistochemistry methods.

The diversity of opinions and conclusions about comparative studies was explained by Osborne¹⁷. This author assigned these conflicting results, especially in relation to the estrogen receptors, to the tumor heterogeneity, where hormone responsive and non-responsive cells were present in variable proportions. For Osborne, these varying fractions were responsible for the 15% to 34% non-concordance of his results. He added that the clinical responses related to immunohistochemistry were non-satisfactory in 40% of his patients. Borjesson et al¹⁸ came to the same conclusion. Thorpe et al¹⁹ found that the uniformization of the techniques of citosol preparation

contributed to the reduction of the differences seen in the dosages performed by several laboratories, especially concerning the progesterone receptors.

Ozello et al²⁰ confronted the quantitative method with immunohistochemistry executed in frozen tissues, imprints and paraffin block sections. Except for the latter, the results between the two methods were concordant.

It must be said that one reason for disagreement between quantitative and qualitative methods using frozen sections is the low index criterion for positiveness. Parl and Posey²¹ verified 89% in concordance when the index for receptors was taken at 3 femtomoles. Kinsel et al²² using 10 fm/mg as parameter found the agreement just below 70%.

Finally, Hasson et al²³ stressed that the material to be submitted either to quantitative or qualitative methods should be taken early from the biopsy fragment and not from the surgical specimen. Besides, a further measure to reduce the discrepancies is that the tissue should be taken from different areas of the tumor. This precaution applies to either the quantitative or the qualitative determinations.

CONCLUSIONS

Our study of correlation between the quantitative method with dextran charcoal technique for ER and PgR and the qualitative immunohistochemical procedure using antibodies antireceptors for estrogen and progesterone on paraffin sections, reached the conclusion that the concordance between the two methods is not satisfactory. Thus, we do not recommend the immunohistochemical determination applied to paraffin sections as an alternative to the quantitative procedure for the prognostic evaluation of mammary carcinoma.

RESUMO

À tradicional dosagem dos receptores hormonais para avaliação prognóstica e abordagem clínica no câncer de mama, métodos alternativos para determinação imunohistoquímica vêm sendo propostos. Com finalidade comparativa, tais procedimentos foram correlacionados em 37 pacientes exibindo 5 a 15 anos de sobrevida. Tomando como índice de positividade 30 fm/mg a discordância entre os métodos alcançou 35,1% com os estrógenos e 48,5% com os receptores de progesterona. Quando o nível de positividade foi alterado para 20 fm/mg o desencontro de resultados reduziu-se para 32% com o ER, elevando-se para 57% com o PgR. Este estudo nos permitiu concluir pela não recomendação do método imunohistoquímico com laminais fixadas em parafina, como um procedimento alternativo à dosagem com carvão-dextrã, para prognóstico e abordagem terapêutica no carcinoma mamário.

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