Introduction

A variety of patient (age and menopausal status) and tumor characteristics (lymph node status, histological size, type and grade) can provide prognostic information useful in the management of patients with breast cancer (1). In addition to the above «classical» prognostic factors, it has been generally accepted today that determinations of receptors for estrogen (ER) and progesterone (PR) in breast cancer tissue are an important task in the management of breast cancer patients. Simultaneous knowledge of all these biomarkers may help in medical decision making. However, the amount of tumor material available from breast carcinoma can make impossible determination of estrogen-regulated biomarkers together with estrogen and progesterone receptors. To assess whether we could replace our current five-point ligand binding assay for measurement of estrogen and progesterone receptors with a single-point ligand binding assay, we compared simultaneous measurements in same samples of breast carcinomas by both methods. A linear regression analysis shows that single-point assay can be confidently used instead of five-point assay. In addition, there were no variations over time in estrogen and progesterone receptors phenotypes, as well as in estrogen and progesterone receptors contents determined by single-point assay. Accordingly, the results clearly demonstrate the validity of intralaboratory quality control and give a possibility for the establishment of interlaboratory quality control of single-point ligand binding assay.

Key words: estrogen and progesterone receptors, single-point ligand binding assay, breast cancer.
PR determination by a single-point instead of five-point biochemical method and, therefore, to enable determination of the other molecular biomarkers from the same breast carcinoma cytosolic fraction.

**Material and methods**

**Tumors**

Excised tumors were obtained from breast cancer patients treated by ablative breast surgery at the Institute of Oncology and Radiology of Serbia. None of the patients have received preoperative radiotherapy, chemotherapy or endocrine therapy. At the time of surgery, tumor specimens were divided into two representative parts by the pathologists. One part of the specimen was proceeded for histology and the other was immediately placed into liquid nitrogen for biochemical receptor assays. ER and PR contents were obtained by the single-point and five-point biochemical method using 49 and 36 breast carcinoma samples, respectively. Quality control of the single-point biochemical assay was performed using 500 consecutive breast carcinoma samples.

**Standard biochemical assay: five-point assay**

Storage of tumors specimens, homogenization of the tissue, cytosol preparation, incubation of cytosol with radiolabeled steroid hormone and separation of receptor-bound steroid hormone from excess of free steroid hormone, were performed exactly according to procedures recommended by the EORTC (6-12). The saturation kinetics of ER and PR breast carcinoma was analyzed using five concentrations of each 3H-estradiol (2, 4, 8, 16 and 32 nmol/L) and 3H-ORG 2058 (4, 8, 16, 32 and 64 nmol/L).

Binding observed in the presence of excess of unlabeled steroid hormone is related to nonreceptor or nonspecific (low affinity, high capacity) binding of the ligand. Specific binding was estimated as the difference between total and nonspecific binding (Figure 1A). The saturation kinetics data from Figure 1A were used (13) for determination of the dissociation constant (Kd) and the number of specific binding sites (Bmax) of the ER and PR (Figure 1B). Usually, the number of specific binding sites is expressed in fmol of labeled steroid hormone bound per mg cytosol protein. Cytosol protein concentration was determined by the method of Lowry at al. (14).

**Single-point assay**

To verify precise quantification of the number of specific binding sites of the ER and PR, it is necessary to use the highest, saturable hormone concentration from five-point assay. The number of specific binding sites of ER and PR is estimated as the difference between total and nonspecific binding. Standardization of single-point assay was performed in accordance with recommendations of EORTC (15).

**Statistical methods**

A linear regression analysis was used to compare five-point and single-point assays for ER and PR content determination.

Non-parametric statistic methods were used to analyze the obtained results within single-point biochemical assay. Chi-square test was performed to examine distribution of phenotypes of steroid receptor status among the whole group and each subgroup, or
among subgroups. Mann-Whitney U-test was performed to examine the distribution of characteristic quantitative receptor contents or the distribution of all receptor individual contents.

Results

Comparison of five-point and single-point assays

Results of comparison of five-point and single-point assays for estrogen receptor (A) and progesterone receptor (B) contents are shown in Figure 2.

Intralaboratory standardization of single-point assay

Intralaboratory standardization was performed in accordance with recommendation of EORTC (9).

Qualitative manner of standardization related to examination of distributions of ER and PR phenotypes within the whole group and each subgroup, as well as within subgroups is shown in Figure 3. Homogeneity was striking in distribution of each phenotype among the whole group and each subgroup, or among subgroups.

Quantitative manner of standardization related to examination of the distribution of the 5th, 25th, 50th, 75th and 95th percentile values of estrogen receptor or progesterone receptor contents within the whole group and each subgroup, as well as within subgroups, is shown in Figure 4. Homogeneity was striking in distribution of represented ER and PR contents among the whole group and each subgroup, or among subgroups. These findings were confirmed by the analyses of all individual quantitative values of ER and PR (data not shown).
Discussion

The aim of this study was to investigate potential usefulness of single-point biochemical method for ER and PR determination in breast carcinomas. Our reported method-comparison study generally shows that single-point assay is equivalent to five-point assay in relation to the content of both ER and PR. Consequently, we could use single-point assay instead of five-point assay for ER and PR determination, thus making possible determination of the other molecular biomarkers from the same breast carcinoma cytosol fraction.

Our further analysis in this study was aimed at examining of intralaboratory quality control of single-point biochemical assay. We analyzed distributions over time of ER and PR status in qualitative and quantitative manner. No variations have been observed over time in ER and PR phenotypes, as well as in ER and PR quantitative values.

It is important to point out that consideration of interlaboratory variations of ER and PR phenotypes may not be suitable due to two reasons, so crucial for the biological nature of breast carcinoma, which are often ignored (16). Firstly, the ER and PR quantitative contents have a wide range of values. Secondly, the threshold quantitative value used to define receptor positivity, which varies from 3 to 20 fmol/mg, belongs to the level with the highest frequencies in distribution of the ER and PR levels. Therefore, the distribution of ER and PR quantitative values gives a more realistic view of the interlaboratory comparability.

Studies of estrogen-regulated proteins may offer an insight into the molecular pathogenesis of estrogen-dependent carcinomas and they may help in medical decision making, as well. There is a sufficient number of small pilot studies for most of the estrogen regulated biomarkers of “new generation” (17–19) and there is a wealth of information on the more established “classical” biomarkers. It is time for extensive prospective studies in which the majority of the old and new biomarkers would be assessed together. Such studies are necessary to fully evaluate and validate significance of each biomarker and to determine the combinations of biomarkers which can identify accurately subgroups of patients with different courses of disease. In this way, both under and overtreatment can be avoided.

Acknowledgments

This work was supported by the Ministry for Science and Technology of Serbia, contract No 13M13. The authors truly thank Ms Andelka Lukač, Milica Matas and Jelica Perović for excellent technical assistance.
ODREĐIVANJE RECEPTORA ZA ESTROGEN I PROGESTERON BIOHEMIJSKOM METODOM JEDNA TAČKA VS. PET TAČAKA

Dragica Nikolić-Vukosavljević, Milan Markićević, Sladana Todorović
Institut za onkologiju i radiologiju Srbije, Beograd, Jugoslovija


Ključne reči: receptori za estrogen i progesteron, biohemijski metod sa jednom tačkom, kancer dojke.

References


Received: October 8, 2001
Accepted: December 4, 2001