Pergamon

European Journal of Cancer Vol. 30A, No. 6, pp. 730–732, 1994

0959–8049/94 $7.00+0.00

0959-8049(94)E0071-B

Interlaboratory Comparison of Oestrogen Receptor Data

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BREAST CANCER biopsies display a broad spectrum of oestrogen receptor (ER) concentrations (Figure 1). Because ER is both heat-labile and susceptible to proteolytic degradation, it is difficult to reproducibly quantitate ER in tumour tissues. Consequently, it is also difficult to compare results from different laboratories. It is at once unfortunate and alarming that this difficulty persists when international standard protocols have been defined, but often ignored, for more than a decade.

The need to standardise assay methodology for ER was recognised more than 20 years ago when the first workshop for this express purpose was held under the auspices of the EORTC [1]. Eventually, a *bone fide* Receptor Study Group evolved and is presently recognised as a subcommittee of the EORTC Breast Cancer Group. One of the early accomplishments of the study group was to define optimal conditions for cytosol preparation and assay incubation [2] (Table 1, first entry). These conditions became a standard, often referred to in the literature. The group still conducts trials testing various aspects of assay methodology. Additionally, it sponsors biannual quality control trials that are open to all. Despite these efforts, great variation persists in assay results and Romain and her colleagues' paper in this issue of the *European Journal of Cancer* (pp. 740–746) describes and displays these shortcomings.

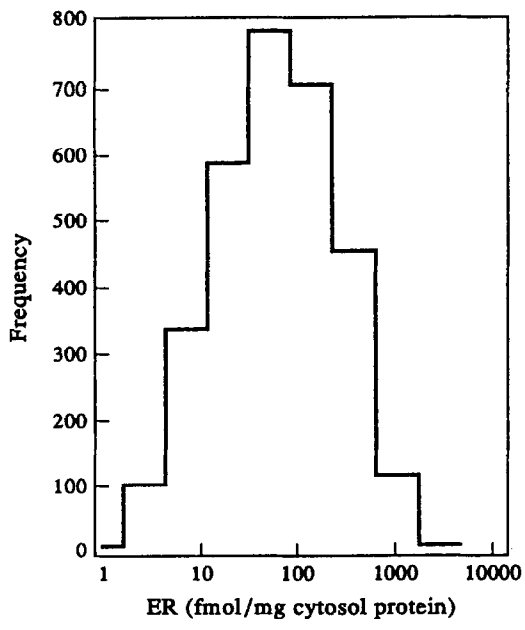

Figure 1. Distribution of ER concentrations determined in a single laboratory for 3728 patients with primary breast cancer who are enrolled in the Danish Breast Cooperative Group (DBCG) protocols 77 and 82. Only patients < 70 years of age are enrolled in the DBCG-82 protocols: therefore, only patients < 70 years are included in this figure. The overall frequency of ER positivity among the patients is 74%. The median value of ER is 42 fmol/mg cytosol protein (lowest and highest quartiles: 9 and 142; range: 0–2542).

Currently, there are two major methods to detect and quantitate ER. These two methods differ in principle: the classical ligand binding assay (LBA), in which the amount of radioactive oestradiol bound to ER is measured, and the commerically available enzyme immunoassay (EIA) method that relies on recognition of specific epitopes in the ER by monoclonal antibodies. The recommended procedures for preparation of tissue and assay conditions for the two methods also differ (Table 1, first and second entries).

When Romain and colleagues observed an approximately two-fold difference in median ER concentrations in populations of consecutive breast cancer biopsies determined using ER-EIA in Marseille and Nice, they looked for potential sources of this variation. In so doing, they considered various aspects of the assay methodology: (a) composition of homogenisation buffer; (b) method of homogenisation (microdismembrator versus polytron); (c) cytosol protein concentration during assay incubation; and (d) composition of diluent. Having eliminated the first two as possible explanations for the difference, we are, however, left wondering as to the source of the differences observed between Marseille and Nice. We must assume it is associated with circumstances of dilution of cytosol prior to assay incubation and/or other aspects of tissue handling not dealt with here.

The effects of cytosol dilution on assay results are difficult to interpret. They appear to depend on the type of sample analysed (MCF-7 versus patient biopsies) as well as composition of the diluent (homogenisation buffer versus kit speciment diluent versus kit specimen diluent + 4-monohydroxytamoxifen). The compositions of the specimen diluent and homogenisation buffer used for dilution differ significantly (Table 1). Implementation of such different solutions for dilution can seem artifactual and manipulative. Nevertheless, the point that exposure of epitopes for binding to the H222 and D547 monoclonal antibodies depends on the milieu in the sample is beautifully illustrated using this tactic. As pointed out by the authors, we are left with more questions: are the differences clinically relevant? Is the ER-EIA assay more sensitive to slight procedural changes than the ER-LBA? How can consistency in results be assured? How can it be monitored? Perhaps most importantly, what can we

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Received 7 Jan. 1994; accepted 8 Feb. 1994.

Table 1. Conditions of preparation of tissue for ER analysis

Source	Method of homogenation	Ratio tissue: buffer	Homogenisation buffer	Buffer for dilution and/or incubation
EORTC[2]	Microdismembrator	< 1 : 8	10 mM $K_2HPO_4KH_2PO_4$ 1.5 mM K_2EDTA 3 mM NaN_3 10 mM MTG 10 mM Na_2MoO_4* 10% glycerol pH 7.5	Same as homogenisation buffer
ER-EIA[6]	Pulverisation with mortar; polytron	1 : 10	10 mM Tris 1.5 mM EDTA 1 mM MTG 5 mM Na_2MoO_4 pH 7.4–7.5	20 mM Na_2HPO_4† 0.8 M KCl 40 mM Na_2MoO_4 5 mg BSA/ml 70 mg bacitracine/ml
Marseille[3]	Microdismembrator	1 : 10	10 mM Tris-HCl 1.5 mM Na_2EDTA 0.5 mM DTT 10 mM Na_2MoO_4 10% glycerol pH 7.4	Homogenisation buffer or specimen diluent from ER-EIA kit
Nice[3]	Polytron	1 : 10	Same as Marseille	Same as homogenisation buffer

*Optional inclusion of sodium molybdate added to EORTC recommendations in 1986 [6]; influence on results reported in 1987 [3].
†Denoted as 'Specimen Diluent' in ER-EIA kit; used to dilute sample in homogenisation buffer by factor of two prior to assay incubation.

do to make interlaboratory assay results comparable? These questions are still central to the work of the EORTC Receptor Study Group.

Despite the critical importance of establishing assay standards which are generally observed, most labs perform an individualised, modified version of the published standard ER assay methodology. In the best of cases, these modifications are described in the literature and are thus accessible to others for evaluation. However, often they are not, and too little attention is paid to the consequences these modifications have on results. Hopefully, steroid receptor assays routinely performed for clinical use are run in laboratories which conscientiously maintain consistency in in-house assay methodology. However, while this consistency yields the required reproducibility of in-house assays, it is not in itself sufficient when data from different laboratories are compared.

Consider ways in which intra and interlaboratory consistency in assay results can be monitored. Three routine ways of doing this are the following: (a) in-house interassay controls give a good overview of daily reproducibility; (b) in-house comparison of the distribution of receptor concentrations achieved over different periods of time give an idea of long-term assay reproducibility (with the caveats sketched below); (c) external quality control by analysis of a common standard, as is possible through the EORTC Receptor Study Group programme, shows interlaboratory comparability. All three methods should be routinely practiced. But while many laboratories practice (a) and (c), option (b) is still only rarely implemented.

A note of caution must be added with regard to the approach outlined in point (b): because receptor concentrations are significantly associated with other patient characteristics (e.g. age, menopausal status and grade of anaplasia), any change in the demography of the patient population will inevitably be reflected in the overall distribution of receptor concentrations (and, thus, frequency of receptor positivity). Such a change could be envisioned if, for example, mammography succeeds in bringing a larger number of women with small, well-differentiated tumours to the operating theatre. Nevertheless, the usefulness of comparing distributions of ER concentrations over different periods of time has recently been emphasised by the discovery that reference values in the ER-EIA kit had drifted significantly over time. This discovery only occurred when results reported in the Romain paper as well as those from other European centres—that higher and higher frequencies of receptor positivity were being detected—provided the impetus for scrutinising and re-evaluating the ER-EIA kit.

In spite of efforts to optimise and standardise assays, interlaboratory results will inevitably differ. We must recognise a degree of individuality in the way each laboratory performs the assay. Perhaps there should be a readily available archive of assay protocols. The EORTC Receptor Study Group offers one possible forum for establishing such an archive. How, then, should we deal with individuality? The earliest solution to this problem was to report results from receptor assays in a binary fashion: a biopsy was either receptor-positive or receptor-negative. This method does not work well, however. The frequency of ER positivity in large populations of primary breast cancer patients in the literature varies by at least 20% (highest values = ~80%)[3]. Provided that interlaboratory differences in assay methodology do not grossly affect quantitation of receptors, minor differences in assay results can be minimised by normalisation of data *using a reference standard*. The feasibility of this approach has already been demonstrated in a study conducted by the EORTC Receptor Study Group [4]. However, if preparation of cytosols or the assays themselves are performed under suboptimal conditions, normalisation ceases to be an option: zero values cannot be normalised!

It would better serve the goal of making receptor data from

different laboratories readily comparable if these data were reported in a more meaningful framework. Rather than stating receptor concentration and/or receptor status (the latter of which is in itself often rather arbitrary), the fractile of distribution of receptor concentration could be stated: knowledge that a patient belongs to the lowest or highest 15% of the distribution of all receptor concentrations is probably more meaningful clinically than knowledge that there were 9 or 700 fmol ER/mg cytosol protein in the tumour tissue; it is certainly more informative than mere classification of the patient as being either ER negative or ER positive. Additionally, this approach lends itself to establishment of clinically relevant cutoff limits. Statements such as "Patients with the lowest *x*th per cent of ER concentrations experienced shortest recurrence-free survival," can be directly compared among centres. Another attractive aspect of this latter approach is that problems with interpretation of the numerous clinical studies in the literature that arise due to the broad spectrum of receptor positivities are diminished.

We need to solve these problems. The clinician and the patient are those in the greatest immediate dilemma. The clinician relies on laboratory results in order to decide treatment. Clinicians must participate in the debate if we are to reach a viable solution. Meanwhile, the burden of standardising and normalising assay results to make receptor data reliable and comparable rests upon us, the laboratory scientists. We also bear the burden of continuing to identify and describe points in assay methodology leading to interlaboratory variation so others can be alerted to potential pitfalls.

Romain's study will hopefully revive interest in standardisation of basic elements of assay methodology. Precise description of tissue preparation and assay conditions used in each investigation is a basic premise of good science. Unfortunately, this premise is not always present. Accurate and complete reporting should be a requirement respected by investigators submitting papers for publication, and enforced by reviewers and journal editors. We can hope eventually to accumulate a data base in the literature that will enable us to interpret data in a broader framework.

While the mechanism of steroid hormone action has never appeared to be simple, recent research indicates higher levels of complexity than envisioned barely 10 years ago. Interactions among at least several classes of molecules (e.g. ligands, transcription activation factors, heat shock proteins) take place in the modulation of steroid hormone receptor action. With this complexity in mind, it is easy to understand that even slight changes in assay methodology influence results significantly.

The point of all biochemical assay methodologies is to quantitate receptor content. The number of receptors determined depends on the condition used during homogenisation and fractionation as well as during assay incubation. Spatial organisation of the molecules is also influenced by the buffer composition: epitopes will be more or less exposed depending on the buffer used. A clearer picture of the mechanism of hormone action as well as the clinical usefulness of receptor determinations will emerge most rapidly if we use well-documented methodologies that lend themselves to interlaboratory comparisons. Failure to do this so many years after guidelines were painstakingly hammered out is scientifically short-sighted. It impedes information exchange and thereby, denies optimal treatment to the 1 in 8–10 women who will in their lifetimes be diagnosed with breast cancer.

1. EORTC Breast Cancer Cooperative Group. Standards for the assessment of estrogen receptors in human breast cancer. *Eur J Cancer Clin Oncol* 1973, **9**, 379–381.
2. EORTC Breast Cooperative Group. Revision of the standards for the assessment of hormone receptors in human breast cancer: report of the second EORTC workshop, held on 16–17 March 1979, in the Netherlands Cancer Institute. *Eur J Cancer Clin Oncol* 1980, **16**, 1513–1515.
3. Thorpe SM. Steroid receptors in breast cancer: sources of interlaboratory variation in dextran-charcoal assays. *Breast Cancer Res Treat* 1987, **9**, 175–189.
4. Blankenstein MA, Benraad TJ. Multilaboratory assessment of tissue prognostic factors in breast cancer: the EORTC Receptor Study Group experience. *Can J Oncol* 1992, **3**(Suppl. 2), 58–64.
5. Koenders A, Thorpe SM, on behalf of the EORTC Receptor Group. Standardization of steroid receptor assays in human breast cancer. IV. Long-term within- and between-laboratory variation of estrogen and progesterone receptor assays. *Eur J Cancer Clin Oncol* 1986, **22**, 945–952.
6. Abbott Laboratories. Abbott ER-EIA Monoclonal kit instructions. 1989.