8. Lawrence BV, Lipton A, Harvey HA, *et al.* Influence of estrogen receptor status on response of metastatic breast cancer to aminoglutethimide therapy. *Cancer* 1980, **45**, 786–791.
9. Vollenweider-Zerargui L, Barrelet L, Wong Y, Lemarchand-Beraud T, Gomez F. The predictive value of estrogen and progesterone receptors' concentrations on the clinical behavior of breast cancer in women. Clinical correlation on 547 patients. *Cancer* 1986, **57**, 1171–1180.
10. Walker KJ, Bouzubar N, Robertson J, *et al.* Immunocytochemical localization of estrogen receptor in human breast tissue. *Cancer Res* 1988, **48**, 6517–6522.
11. King WJ, Greene GL. Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. *Nature* 1984, **307**, 745–747.
12. Press MF, Greene GL. Localization of progesterone receptor with monoclonal antibodies to the human progestin receptor. *Endocrinology* 1988, **122**, 1165–1175.
13. Greene GL, Press MF. Immunochemical evaluation of estrogen receptor and progesterone receptor in breast cancer. In: Ceriani RL, ed. *Immunological Approaches to the Diagnosis and Therapy of Breast Cancer*. New York, Plenum Press, 1987.
14. Bacus JW, Grace LJ. Optical microscope system for standardized cell measurements and analyses. *Applied Optics* 1987, **26**, 3280–3293.
15. Bacus S, Flowers JL, Press MF, Bacus JW, McCarty KS. The evaluation of estrogen receptor in primary breast carcinoma by computer-assisted image analysis. *Am J Clin Pathol* 1988, **90**, 233–239.
16. McClelland RA, Finlay P, Walker KJ, *et al.* Automated quantitation of immunocytochemically localized estrogen receptors in human breast cancer. *Cancer Res* 1990, **50**, 3545–3550.
17. McCarty KS Jr, Miller LS, Cox EB, Konrath J, McCarty KS Sr. Estrogen receptor analyses: correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med* 1985, **109**, 716–721.
18. McClelland RA, Berger U, Miller LS, Powles TJ, Coombes RC. Immunocytochemical assay for estrogen receptor in patients with breast cancer: relationship to a biochemical assay and to outcome of therapy. *J Clin Oncol* 1986, **4**, 1171–1176.
19. Allred DC, Bustamante MA, Daniel CO, Gaskill HV, Cruz AB Jr. Immunocytochemical analysis of estrogen receptors in human breast carcinomas. *Arch Surg* 1990, **125**, 107–113.

Acknowledgements—We thank D. Brocklehurst, P. Cowen, I. Ellis, R. Hawkins, K. Healey, J. Teasdale and R.A. Walker of the British Quality Control Group for help in assessment of slides.

Eur J Cancer, Vol. 27, No. 6, pp. 715–720, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Determination of Oestrogen Receptors: Application of the Passing–Bablock Linear Regression Technique for Comparison of Enzyme Immunoassay and Radioligand Binding Assay in 1841 Breast Cancer Tumours

S. Romain, C. Dussert and P.M. Martin

To test the qualities of two assays in the same laboratory on the same tumours, a single-point dextran-coated charcoal radioligand binding assay (RLA-DCC) and the Abbott enzyme immunoassay (EIA) were used to perform oestrogen receptor determinations on cytosols from 1841 breast cancers over a 2-year period. Statistical analysis of the data was performed by the Passing–Bablock linear regression technique. The final regression curve between EIA (y) and RLA-DCC (x) yielded $y = 1.187x$ fmol/mg of protein. However, a high variability in this correlation was observed from 1986 to 1988. This variability could be explained by calibration problems in the immunoassay kits and changes in our technical team. The binding assay appears to be more sensitive to the technicians' experience than the immunoassay. Other technical points are discussed, particularly cytosol preparation and KCl presence or absence in the homogenisation buffer. Finally, the Passing–Bablock and the least squares regression procedures are compared. The conditions allowing optimal correlation and routine determination reliability are defined and the correlation variability is discussed.
Eur J Cancer, Vol. 27, No. 6, pp. 715–720, 1991

INTRODUCTION

THE CLINICAL usefulness of oestradiol receptors (ER) assays in breast cancer specimens has been clearly established by correlations with patients' response to endocrine therapies and prognosis. For many years, ER determinations have been routinely performed in many breast cancer centres [1]. For optimal disease management, it seems important to accurately distinguish between ER negative and ER positive patients and to quantify the amount of ER present in the tumour tissue. Until recently, ER were quantified exclusively by radioligand binding assay. The Abbott enzyme immunoassay (EIA) using monoclonal antibodies now provides an alternative approach for ER assays in breast cancer [2–4]. Several groups have compared this enzyme immunoassay with conventional radioligand binding

Correspondence to S. Romain.
The authors are at the Laboratoire d'Oncologie Biologique-Biopathologie Tissulaire, Faculté de Médecine de Marseille-Secteur Nord, Boulevard Pierre Dramard, 13326 Marseille Cedex 15, France.
Revised 27 Feb. 1991; accepted 8 Mar. 1991.

assays [5–15]. Investigations were conducted on a small series of samples or as part of a multicentre study. Considerable variation in the correlations reported by each group has been found. In addition, no long-term study has yet been published. From 1986 to 1988, 1841 consecutive ER determinations were performed in our laboratory using both EIA and dextran-coated charcoal single point radioligand binding assay (RLA-DCC) to compare the two methods. This study made it possible for us to analyse the previously described variability in correlation.

MATERIALS AND METHODS

Tumours and cytosols

From 1986 to 1988, samples from 1841 patients with histologically recognised breast cancer were collected in our laboratory for routine assay of ER. Samples were stored in liquid nitrogen until used. In 1986 and at the beginning of 1987, tumours (protocol A, n = 1005) were homogenised in a phosphate buffer (80 mmol/l phosphate, 0.4 mol/l KCl, 12 mmol/l monothioglycerol, 1 mmol/l EDTA, 20 mmol/l sodium molybdate, 10% glycerol, pH 7 at 4°C) using a polytron. At the end of 1987 and in 1988, tumours (protocol B, n = 836) were homogenised in a Tris buffer (10 mmol/l Tris-HCl, 0.5 mmol/l dithiothreitol, 1.5 mmol/l EDTA, 10 mmol/l sodium molybdate, 10% glycerol, pH 7.4 at 4°C) either with a polytron (n = 455) or with a microdismembrator (n = 381). Cytosols were obtained by centrifugation of the homogenates at 105 000 g for 60 min at 4°C and were immediately stored in liquid nitrogen. For all samples, ER determinations were performed on the same day using both EIA and RLA-DCC. Cytosol protein concentration was determined by Lowry's method [16].

RLA-DCC assay

Radioligand ER assays were performed using the dextran-coated charcoal method and a saturating single dose assay which was first validated against the Scatchard plot assay [17–22]. Briefly, 100 µl of cytosol were incubated with ^{3}H-oestradiol (5 nmol/l) either separately or with 500 nmol/l diethylstilbestrol. Incubations were performed overnight at 4°C and were stopped by adding 120 µl of DCC suspension [1.25% charcoal (Sigma), 0.625% dextran T70 (Pharmacia), 1% gelatin (Merck)] to the reaction mixtures. After 30 min, the charcoal was pelleted by centrifugation at 800 g for 10 min and the supernatant was then counted for radioactivity. During the study, EORTC and French Receptors Study Group interlaboratory quality controls were systematically assayed.

Abbott enzyme immunoassay

Enzyme immunoassays were performed with the Abbott ER-EIA kit using the instructions and materials provided by the manufacturer.

Statistical methods

The Passing–Bablock linear regression procedure was used to statistically compare the two assay methods (y = EIA, x = RLA-DCC with values expressed as fmol/mg protein) [23, 24]. In contrast to the usual linear regression procedures, the results did not depend on assigning the methods to x and y and the procedure did not involve special assumptions regarding the distributions of the expected values or the errors terms. For the chronological analysis, the Passing–Bablock linear regression procedure was compared with the least squares linear regression.

The standard algorithm computation of the linear regression procedure of Passing–Bablock appears to be rather tedious since its execution time increases very quickly with the input data size. Moreover, this algorithm needs a lot of memory space. Both of these facts prevent its use on usual desk microcomputer as far as large data groups are to be analysed. This led us to develop a new rapid and memory preserving algorithm for the Passing–Bablock test (C.D., S.R. and P.R.M.). It is based on a probabilistic study of a random set of data extracted from the original ones. This study is then used to cut the data into buckets where the standard algorithm may be used. With this procedure, both computation time and storage requirements are reduced to the square root of those needed by the standard algorithm, with exactly the same numerical results. Calculations were then easily performed on a desk microcomputer even for large sample sizes.

Statistical differences for quantitative parameters within the population were determined by the Mann–Whitney non-parametric test.

Table 1. Breast cancer cytosols: correlation between ER concentrations determined using EIA and RLA-DCC assays (Passing–Bablock)

	n	Slope	95% CI	Intercept
Cytosols A				
All	1005	1.933	1.930–1.935	0
Beginning 1986	289	1.333	1.330–1.333	0.333*
End 1986	268	1.844	1.837–1.850	0.156*
Beginning 1987	448	2.340	2.333–2.345	0.979*
Cytosols B				
All	836	1.187	1.186–1.188	0
End 1987	434	1.176	1.174–1.178	0
1988	402	1.197	1.195–1.999	0

Study by protocol (A vs. B) used in preparation of cytosols and by analysis date taking into account several lots of EIA reagents and modifications in the laboratory technical assistance. CI = confidence interval.

* Statistically significant from 0.

RESULTS

Chronological study

In our laboratory, a great variability over time was observed for the correlation between EIA and RLA-DCC (Table 1). The overall correlation was better for protocol B cytosols (Fig. 1) than for protocol A cytosols. However, a good correlation was found for protocol A cytosols at the beginning of 1986 although a dramatic drop in the correlation was observed at the end of 1986 and at the beginning of 1987.

Quality controls in our laboratory performed under the auspices of the EORTC (Fig. 2) and the French Hormonal Receptor Group for the RLA-DCC technique demonstrated the high stability of the results obtained from 1986 to 1988. At the end of 1986 and at the beginning of 1987, values obtained in our laboratory for EIA kit controls were quite different from theoretical values given by Abbott, the manufacturer (Fig. 3).

However, the average value of RLA-DCC for protocol A cytosols was lower than that for protocol B cytosols, while the average value of EIA remained virtually constant (Table 2).

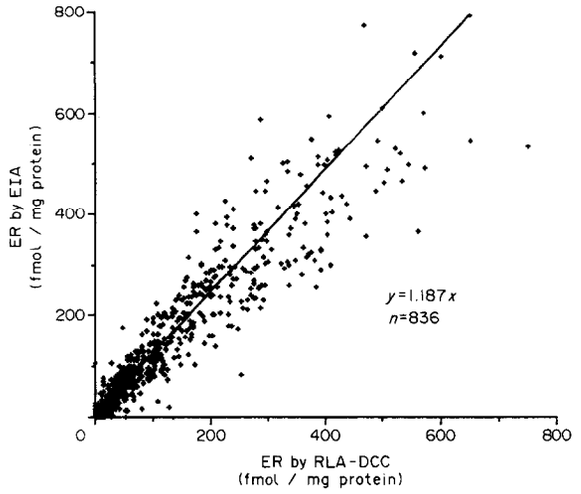


Fig. 1. Breast cancer cytosols, protocol B. Correlation between ER concentrations determined using the EIA and RLA-DCC assays. Regression lines established by Passing–Bablock method.

Comparison of Passing–Bablock and least squares linear regression procedures

Although the correlation obtained from the Passing-Bablock regression method (Table 1) and the correlation obtained from the least squares regression (Table 3) both demonstrated the temporal variability of the correlation between EIA and RLA-DCC, the regression curves obtained were quite different. The linear regression procedure was more appropriate for our analysis for the previously stated reasons and this procedure was therefore chosen for the following analyses.

Effect of cytosol preparation procedure (Table 4)

The analysis of the regression between EIA and RLA-DCC for protocol B yielded a slope of 1.115 for homogenisation using a polytron and 1.215 for pulverisation by microdismembrator recommended by the EORTC. These two slopes are significantly different ($P < 0.001$, t test).

Moreover, to study the direct effect of KCl on EIA and RLA-DCC assays, 35 B cytosols (prepared without CKl) were diluted

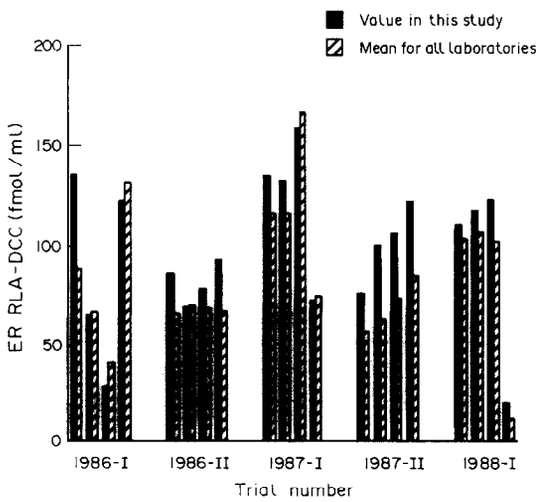
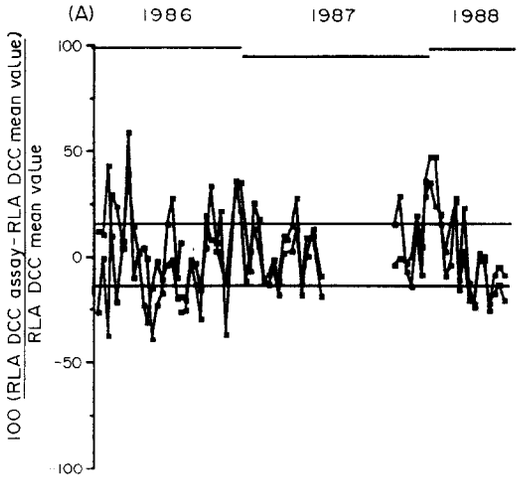


Fig. 2. EORTC interlaboratory quality controls: ER RLA-DCC assay.



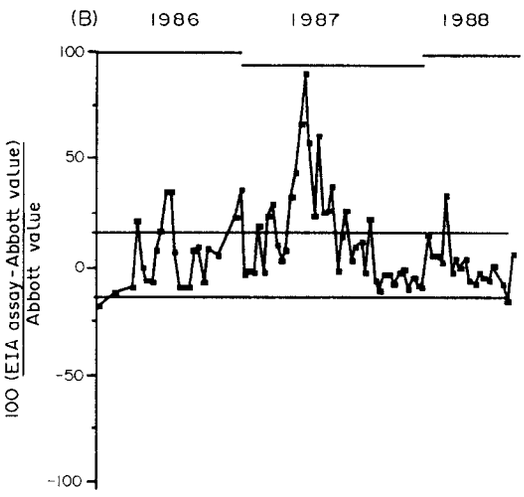


Fig. 3. Intralaboratory quality controls. (A) ER RLA-DCC assay: cytosols prepared from rabbit uterus, variation percentage compared to RLA-DCC mean values. (B) ER EIA assay: kit controls, variation percentage compared to theoretical values given by Abbott. Straight lines represent variations from +15% to −15%.

in buffer B with or without KCl (final concentration 0.4 mol/l) and were analysed by EIA and RLA-DCC. Without KCl, the correlation between EIA and RLA-DCC was high (slope = 1.086) but when KCl was added during incubation, lower ER levels were found using RLA-DCC than when EIA was used (slope = 1.876).

Regression analysis obtained with protocol B cytosols from premenopausal and postmenopausal patients

A difference in the relationship between EIA and RLA-DCC for B cytosols was found between tumours from premenopausal women and those from postmenopausal patients (Table 5).

DISCUSSION

Several groups have compared the Abbott enzyme immunoassay with conventional radioligand binding assays [5–15]. Considerable variation has been observed in the correlations reported

Table 2. Breast cancer cytosols: distribution of ER values

	n	Mean	P(0,25)	Median	P(0,75)	P*
RLA-DCC						
All						
Cytosols A	1005	71	4–5	26–27	77–78	
Cytosols B	836	100	8–9	42–43	149	< 0.001
Premenopausal						
Cytosols A	161	31	4–5	20–21	41–42	
Cytosols B	171	45	5–6	27–28	62–63	0.01
Postmenopausal						
Cytosols A	351	92	4–5	37–38	109–111	
Cytosols B	368	133	9–10	94	217	< 0.001
EIA						
All						
Cytosols A	1005	113	5–6	56–57	153–154	
Cytosols B	836	115	5–6	52–53	180–182	NS
Premenopausal						
Cytosols A	161	70	4–5	46–47	102–104	
Cytosols B	171	59	4–5	30–31	85–88	NS
Postmenopausal						
Cytosols A	351	145	6–7	67–68	200–203	
Cytosols B	368	154	7–8	95	259	NS

Results expressed as fmol/mg protein.
* Mann-Whitney *U* test.
P(0,25) = 25% percentile, P(0,75) = 75% percentile.

by each group. However, comparison of the results is difficult as these investigations were performed for a small series of samples or as part of a multicentre study. In addition, several points should be discussed.

The statistical methodology must be standardised. In previous studies, the highest and lowest ER levels were often eliminated from calculations and the analysis was then performed for varying concentration ranges depending on the group. In addition, zero values which are important for the regression must be removed from the direct logarithmic transformation used to normalise distribution. A log transformation $[(x + 10)/10]$ makes it possible to avoid this bias, but it is

Table 3. Breast cancer cytosols: correlation between ER concentrations determined using EIA and RLA-DCC assays (least squares)

	n	r	Slope (S.D.)		Intercept (S.D.)	
Cytosols A						
All	1005	0.479	0.43	(0.02)	82.06	(5.22)
Beginning 1986	289	0.716	0.71	(0.04)	34.08	(6.71)
End 1986	268	0.441	0.15	(0.02)	64.54	(6.28)
Beginning 1987	448	0.790	1.58	(0.06)	40.73	(7.49)
Cytosols B						
All	836	0.942	1.07	(0.01)	7.69	(2.14)
End 1987	434	0.940	1.06	(0.02)	10.78	(2.99)
1988	402	0.945	1.09	(0.02)	5.60	(3.04)

Study by protocol (A vs. B) used in preparation of cytosols and by analysis date taking into account several lots of EIA reagents and modifications in the laboratory technical assistance.

Table 4. Breast cancer cytosols B. Correlation between ER concentrations determined using EIA and RLA-DCC assays (Passing–Bablock)

	n	Slope	95% CI	Intercept
Polytron	455	1.150	1.148–1.153	0
Microdismembrator	381	1.215	1.214–1.217	0
Without KCl	35	1.086	1.086–1.087	−2.657*
With KCl 0.4 mol/l	35	1.876	1.876–1.876	1.610*

Study by device used for homogenisation and KCl concentration added in buffer.
* Statistically significant from 0.

difficult to appreciate correspondances between EIA and RLA-DCC with this type of transformation. The standard linear regression procedures have often been used previously. However, they require both an error free independant variable and normally distributed error terms with a constant variance. Biased results may therefore be obtained (a) if the data are analysed along the whole range of concentration, because these strong hypotheses are generally not verified; (b) if the data are split into different concentration ranges before analysis, because the splitting is artificial and arbitrary; or (c) if, in addition, extreme points unduly influence calculations. In contrast, the Passing–Bablock regression procedure can be used to test the equivalency of oestradiol receptor measurements from two different analytical methods. No specific assumptions on the distributions of the expected values or the errors terms are necessary: the sampling distribution is arbitrary and the error distribution must simply be the same type for the two methods and the variances must remain proportional. Moreover, all measurement points have equal weight in the estimation of the regression line. Generating algorithms which make the Passing–Bablock linear regression procedure as user-friendly as the usual linear regression procedures should increase this method's application. In our study, direct analysis by a regression using the least squares method and analysis by a Passing–Bablock regression both demonstrated the temporal variability of the correlation between EIA and RLA-DCC. However, intercepts with values very different from 0 were found for protocol A cytosols with the least squares regression method. The regression lines obtained from the Passing–Bablock procedure appeared to better reflect the studied phenomenon. Standard linear regression tests are likely more poorly adapted to smaller sample sizes.

The variability may be partially due to differences in the number of premenopausal and postmenopausal patients in the groups studied. In previously published studies the results did not include a patient description and the analysis was often

Table 5. Breast cancer cytosols B. Correlation between ER concentrations determined using the EIA and RLA-DCC assays (Passing–Bablock)

	n	Slope	95% CI	Intercept
Premenopausal	171	1.361	1.358–1.362	−1.976*
Postmenopausal	368	1.163	1.161–1.165	0

Study by patient's hormonal status.
* Statistically significant from 0.

general. However, our results show that the slope of the regression curve between EIA and RLA-DCC was higher for premenopausal than for postmenopausal patients. Thus, overnight incubation with radioactive ligand seems not sufficient to achieve exchange of endogenous radioinert ligand for the labelled ligand, whereas EIA is independent of endogenous hormones in the patients' tissue. A correlation analysis should therefore take hormonal status into account.

The EORTC Receptor group has been involved in the standardisation and quality control of steroid receptor assays in breast cancer tissues. However, no alternative to the RLA-DCC method has yet been described and differences between laboratories remain. These differences, particularly in cytosol preparation, may result in correlation variations. We thus first show that homogenisation by polytron yields a slope closer to 1 than the pulverisation of samples by microdismembrator. Pulverisation by microdismembrator is the method recommended by the EORTC to avoid denaturation of the heat-labile receptors. However, the polytron is generally used for homogenisation in the USA and cytosols prepared in the same conditions as the EIA standards are likely to give a better correlation between EIA and RLA-DCC.

Next, we show that KCl, whose presence in the homogenisation buffer makes it possible to extract the majority of receptors from their nuclear site, directly leads to insufficient measurements when the RLA-DCC technique is used under standard conditions. Indeed, the number of oestradiol binding sites as measured using dextran-coated charcoal adsorption is known to decrease in proportion to the conversion of receptor from a low salt (8S) to a high salt complex (4S) resulting in a lower apparent binding and an uncontrolled variability [25, 26] whereas the ER-EIA has been demonstrated to be independent of KCl in concentrations of up to 0.8 mol/l [27].

Finally, our study examined assays performed under routine conditions by the same team over a 2-year period and revealed a high variation in correlations between EIA and RLA-DCC over time. Our study thus confirmed that to genuinely evaluate a new technique, procedures must be routinely performed over a long period of time. The high correlation observed during the initial period could be explained by the distribution of carefully selected EIA material and by the extreme diligence of the technicians. The latter variability could have three possible explanations. Firstly, variability could be explained by calibration problems in the immunoassay kits. In fact, the reliability of the EIA technique depends on calibration of the standard and the stability of the kits. Unfortunately the user is at the mercy of the manufacturer's quality controls. At the end of 1986 and the beginning of 1987, the manufacturer could not guarantee the kit's quality and had to withdraw a batch of kits. While we only kept assays from kits which had been "guaranteed", no real quality control on the standardisation was performed. Laboratories using the EIA must therefore validate this assay against RLA-DCC assays in their own setting and routinely perform quality controls.

Another possible source of variation could stem from the use of kits which are almost out of date and whose standard has lost some of its efficiency. Here, high cost of the EIA kits is a major problem to be considered before regular purchase.

Changes in our technical team may be another source of variation. Changes occurred in our team at the end of 1986. As shown by the international study carried out by Abbott on a series of prestandardised cytosol tests, a training period is necessary before using the EIA technique (one of our technicians participated in this study headed by an Abbott trainee). The RLA-DCC technique is even more sensitive to variations in technicians' methods. In fact, our chronological study showed that a less stable distribution of ER values was obtained with RLA-DCC than with EIA. Interlaboratory quality controls have shown the high stability of the results we obtained with RLA-DCC from 1986 to 1988. However, these controls are not really representative of the results routinely obtained using patient tissues. In fact, quality controls must be reconstituted with water containing glycerol and not with the laboratory homogenisation buffer and they were performed by our most highly trained technicians using multipoint titration and Scatchard calculation.

When multicentre trials are performed, receptor analyses must be comparable both in the participating laboratories and over time. Measuring oestradiol receptors is technically difficult and interlaboratory quality controls such as those suggested by the EORTC should be made in all laboratories performing these assays. These controls should be carried out under the normal assay conditions to be representative of series quality and laboratory procedures. Only the RLA technique makes it possible to directly measure binding sites and define affinity constants. The reliability of the EIA method depends on the manufacturer's quality controls and should thus be regularly validated in comparison to the RLA biochemical method. Our experience has shown that the assay of steroid receptors requires a trained staff along with both standardised homogenisation techniques and statistical analysis methods to evaluate the EIA technique compared to the radioligand binding technique.

1. Desombre ER, Carbone PP, Jensen EV, *et al.* Special report: steroid receptors in breast cancer. *N Engl J Med* 1979, **18**, 1011–1012.
2. Greene GL, Jensen EV. Monoclonal antibodies as probes for estrogen receptor detection and characterisation. *J Steroid Biochem* 1982, **16**, 353–359.
3. Greene GL, Nolan C, Engler JP, *et al.* Monoclonal antibodies to human estrogen receptor. *Proc Natl Acad Sci USA* 1980, **77**, 5115–5119.
4. King WJ, Greene GL. Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. *Nature* 1984, **307**, 745–747.
5. Craig Jordan V, Jacobson HI, Keenan EJ. Determination of estrogen receptor in breast cancer using monoclonal antibody technology: results of a multicenter study in the United States. *Cancer Res* 1986, **46**, 4237s–4240s.
6. Delarue JC, Mouriesse H, Contesso G, *et al.* Récepteurs estrogéniques dans les cancers du sein. Comparaison de deux méthodes. *Ann Biol Clin* 1986, **44**, 629–633.
7. Di Fronzo G, Miodini P, Brivio M, *et al.* Comparison of immunochemical and radioligand binding assays for estrogen receptors in human breast tumors. *Cancer Res* 1986, **46**, 4278s–4281s.
8. Goussard J, Lechevrel C, Martin PM, *et al.* Comparison of monoclonal antibodies and tritiated ligands for estrogen receptor assays in 241 breast cancer cytosols. *Cancer Res* 1986, **46**, 4282s–4287s.
9. Goussard J, Lechevrel C, Roussel G, *et al.* Dosage et formes moléculaires des récepteurs d'oestrogènes analysés par ligands tritiés et anticorps monoclonaux. *J Biophys Biomec* 1986, **10**, 133–134.
10. Heubner A, Beck T, Grill HJ, *et al.* Comparison of immunochemical estrogen receptor assay, estrogen receptor enzyme immunoassay, and radioligand-labeled estrogen assay in human breast cancer and uterine tissue. *Cancer Res* 1986, **46**, 4291s–4295s.
11. Leclercq G, Bojar H, Goussard J, *et al.* Abbott monoclonal enzyme immunoassay measurement of estrogen receptors in human breast cancer: an european multicenter study. *Cancer Res* 1986, **46**, 4233s–4236s.
12. Nicholson RI, Colin P, Barrie Francis A, *et al.* Evaluation of enzyme immunoassay for estrogen receptors in human breast cancer. *Cancer Res* 1986, **46**, 4299s–4302s.
13. Pousette A, Gustafsson SA, Thörblad M, *et al.* Quantitation of estrogen receptor in seventy-five specimens of breast cancer: comparison between an immunoassay (Abbott ER-EIA monoclonal)

and a [3H] estradiol binding assay based on isoelectric focusing in polyacrylamide gel. *Cancer Res* 1986, **46**, 4308s–4309s.
14. Spona J, Gitsch E, Kubista E, *et al.* Enzyme immunoassay and scatchard plot estimation of estrogen receptor in gynecological tumors. *Cancer Res* 1986, **46**, 4310s–4312s.
15. Thorpe SM. Monoclonal antibody technique for detection of estrogen receptors in human breast cancer: greater sensitivity and more accurate classification of receptor status than the dextran-coated charcoal method. *Cancer Res* 1987, **47**, 6572–6575.
16. Lowry OH, Rosebrough NJ, Farr AL, *et al.* Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
17. Breast Cancer Cooperative Group. Standards for the assessment of hormone receptors in human breast cancer. *Eur J Cancer Clin Oncol* 1973, **9**, 379–381.
18. Breast Cancer Cooperative Group. Revision of the standards for the assessment of hormone receptors in human breast cancer. *Eur J Cancer Clin Oncol* 1980, **16**, 1513–1515.
19. Koenders A, Thorpe SM on behalf of the EORTC Receptor Group. Standardisation of steroid receptor assays in human breast cancer. I. Reproductibility of oestradiol and progesterone receptor assays. *Eur J Cancer Clin Oncol* 1983, **19**, 1221–1229.
20. Thorpe SM on behalf of the EORTC Receptor Group. Standardisation of steroid receptor assays in human breast cancer. II. Samples with low receptor content. *Eur J Cancer Clin Oncol* 1983, **19**, 1467–1472.
21. Koenders A, Thorpe SM on behalf of the EORTC Receptor Group. Standardisation of steroid receptor assay in human breast cancer. III. Selection of reference material for intra and inter laboratory quality control. *Eur J Cancer Clin Oncol* 1986, **22**, 939–944.
22. Koenders A, Thorpe SM on behalf of the EORTC Receptor Group. Standardisation 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.
23. Passing H, Bablock W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, part I. *J Clin Chem Clin Biochem* 1983, **21**, 709–720.
24. Passing H, Bablock W. Comparison of several regression procedures for method comparison studies and determination of sample sizes. Application of linear regression procedures for method comparison studies in clinical chemistry, part II. *J Clin Chem Clin Biochem* 1984, **22**, 431–445.
25. Peck EJ, Clark JH. Effect of ionic strength on charcoal adsorption assays of receptor-estradiol complexes. *Endocrinology* 1977, **101**, 1034–1043.
26. Katzenellenbogen BS, Lan NC, Rutledge SK. Estrogen receptors of human endometrium: characterization of nuclear and cytoplasmic forms and comparisons with rat uterine receptors. *J Steroid Biochem* 1980, **13**, 113–122.
27. Thorpe SM, Lykkesfeldt AE, Vinterby A, Londsdorfer M. Quantitative immunological detection of estrogen receptors in nuclear pellets from human breast cancer biopsies. *Cancer Res* 1986, **46**, 4251s–4255s.

Acknowledgements—The authors thank A. Durand, M.O. Sigonnez and M. Soullière for technical assistance.

Eur J Cancer, Vol. 27, No. 6, pp. 720–723, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Malignant Ascites: Sonographic Signs of Peritoneal Carcinomatosis

Christian Goerg and Wolf-B. Schwerk

This study was performed in 65 patients with cytologically proved malignant ascites to describe and classify direct and indirect sonographic signs of peritoneal carcinomatosis. Abdominal sonography revealed tumour-associated abnormalities which account for malignant ascites in 60 cases (92%). This includes visualisation of peritoneal metastases (n = 16, 25%); matting together of bowel loops (17, 26%); distribution of fluid (19, 29%); echoes within the fluid space (3, 5%); omental matting (8, 12%); associated masses (21, 32%); lymphoadenopathy (31, 48%); and hepatic metastases (26, 40%). Sonography enables the physician to demonstrate direct and indirect signs of peritoneal carcinomatosis in almost all tumour patients with ascites and is therefore useful in determining whether the cause of ascites is malignant or benign disease.
Eur J Cancer, Vol. 27, No. 6, pp. 720–723, 1991

INTRODUCTION

PHYSICIANS WHO deal with cancer patients are frequently required to determine the aetiology of an abnormal intraperitoneal fluid. Clinical signs and symptoms often are too non-specific to be of definitive value. The conventional way to make an accurate diagnosis of ascites associated with malignancy is cytological examination of needle-aspirated ascites fluid. But other less invasive diagnostic procedures need to be developed to determine whether the course of intra-abdominal fluid collection is a malignant or benign disease.

The evaluation of ascites was one of the earliest uses of abdominal ultrasound [1], and several unspecific sonographic signs such as thickening of the gall bladder wall [2], matted bowel loops [3], loculated fluid [4] or echoes within the fluid [5] have shown that sonography is useful in determining whether the cause of ascites is malignant or benign disease. Furthermore, high resolution ultrasound enables to identify near-field structures of the abdominal wall and visualisation of peritoneal masses [6, 7].

We conducted this retrospective study to describe and classify

Correspondence to C. Goerg.
C. Goerg is at the Division of Hematology/Oncology, and W.-B. Schwerk is at the Division of Gastroenterology, Department of Internal Medicine, Philipps-University, Balderingerstrasse, D-3550 Marburg, Germany.
Revised 26 Feb. 1991; accepted 6 Mar. 1991.