cancer deaths in males and approximately 50% in females [12]. The observation that shifts in smoking habits well explain the different patterns of trends in lung cancer mortality in various Italian geographical areas does not exclude some influence by other, mainly occupational, lung carcinogens. It nevertheless provides further confirmation of the central role of cigarette smoking on lung cancer rates in various populations and geographical areas.

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Evidence for Bimodal Distribution of Breast Carcinoma ER and PgR Values Quantitated by Enzyme Immunoassay

Betty G. Mobbs, Judy-Anne W. Chapman, Donald J.A. Sutherland, Eamonn Ryan, Eugene R. Tustanoff, Teik C. Ooi and P.V. Narasimha Murthy

Breast carcinoma oestrogen receptor (ER) and progesterone receptor (PgR) values obtained by radioligand binding assays have commonly been observed to have approximate log-normal distributions. We examined the distribution of log-transformed receptor values obtained by enzyme immunoassay for 5468 primary breast carcinomas in five Ontario laboratories. In each laboratory, it was found that the frequency histograms for the log transformed receptor values were not unimodal, and generally were suggestive of bimodality. This was not affected by stratification by age or inferred menopausal status (≤ 49, ≥ 50 years), and could not be explained by kit characteristics. However, the low point in the distribution varied from 5 to 63 fmol/mg cytosol protein, depending on the receptor, patient age and laboratory. The tendency towards biomodality was more distinct for ER than for PgR. It remains to be determined whether the low points on the frequency histograms have clinical relevance for discriminating between hormone-sensitive and hormone-insensitive tumours.
Eur J Cancer, Vol. 29A, No. 9, pp. 1293–1297, 1993.

INTRODUCTION

QUANTITATION OF the oestrogen and progesterone receptor (ER and PgR) content of breast carcinomas is well recognised as an aid in the management of breast cancer patients. Until recently, measurement of steroid receptor concentration has relied on the use of radioligand binding methods (RBA), in which the radiolabelled steroid is bound to the steroid binding site of the receptor protein. It has been observed that the distribution of receptor values in large populations of tumours assayed by this method is markedly skewed. This asymmetry may be reduced with a logarithmic transformation ([1, 2] and our own unpublished data).

Recently, receptor assays using monoclonal antibodies to epitopes on the receptor protein other than the steroid binding site have been developed. These have a number of technical advantages over radioligand binding methods [3, 4]. Many

groups of investigators have observed an excellent correlation between the results of the enzyme immunoassays (EIA) and the RBA (reviewed in [5]). It might, therefore, be expected that the population of receptor values obtained by EIA would also be log-normally distributed. However, it was found in the laboratory by one of the authors that the distribution of the logs of receptor values obtained by EIA was not log-normal, but suggested bimodality. Since this laboratory is one of five in Ontario which participate in a common receptor assay programme, it was of interest to examine the distribution of receptor values in all five laboratories to see if this tendency was a general occurrence.

MATERIALS AND METHODS

The data collected were from all assays carried out on primary breast carcinomas for a complete year for each laboratory between March 1989 and October 1990. In all, a total of 5468 specimens were included in the analysis. All assays were carried out using Abbott ER-EIA and PgR-EIA monoclonal assay kits. The great majority of specimens were shipped in dry ice or in the gas phase of a liquid nitrogen tank to the service laboratories: in some cases where the hospital was very close to the service laboratory, specimens were placed in ice after removal and brought to the service laboratory within 1 h. All specimens were snap-frozen in liquid nitrogen and stored either in liquid nitrogen or at −70°C until assay. The frozen specimens were either pulverised or minced before homogenisation in chilled buffer made up according to the kit instructions. All laboratories used a Polytron homogeniser, either for one 10 s or two 5 s bursts at half maximum speed with a cooling interval between bursts. Lab. B prepared the cytosol in two steps, with an initial centrifugation at 3000 *g* for 10 min, followed by the recommended 100 000 *g* spin for 1 h. Lab. D prepared cytosol by centrifugation at 50 000 *g* for 100 min, while lab. E carried out centrifugation at 100 000 *g* for 30 min, instead of 1 h. This laboratory also used a modified Bradford method for protein estimation, instead of the recommended Lowry method used by the others. The standard and quality control preparations supplied with the kits were assayed in duplicate by all laboratories. Tumour cytosols were also assayed in duplicate by labs A, B and D, and as single aliquots by labs. C and E. Apart from these minor variations, all laboratories followed the kit instructions closely.

Assay reproducibility within and between laboratories was assessed by each laboratory carrying out multiple assays on different days on kit quality control preparations of the same batch number. Reproducibility within each laboratory was good, as was the concordance between laboratories (Table 1). Overall, 95% of the multiple assay results fell within the range recommended by Abbott for the quality control preparations used, and the values were commonly within 10% of the expected value. All laboratories also participate in an Ontario-wide quality control programme using lyophilised cytosols. It should be noted that assay of the Abbott quality control preparations does not involve pulverisation, homogenisation or protein assay.

Table 1. Reproducibility of EIA assay within and between laboratories

Laboratory	ER (fmol/ml) Mean ± S.D.*	PgR (fmol/ml) Mean ± S.D.*
A	66 ± 8	51 ± 2
B	60 ± 7	52 ± 5
C	78 ± 11	60 ± 8
D	69 ± 6	54 ± 7
E	63 ± 5	50 ± 6
Expected value	73	55

* Based on multiple assay runs performed on different days, using quality control preparations with the same batch number in all laboratories.

For statistical analysis, all receptor values (expressed in terms of fmol/mg cytosol protein) were transformed to $\log_{10}$ values after the addition of 0.5 to allow for the log of zero [6]. The transformed values were then plotted as frequency histograms. A few receptor values (< 10%) were too high to be read accurately on the spectrophotometer. Each of these specimens was given the maximum known value, which was always > 350 fmol/mg protein. For presentation, the receptor axis midpoint values were back transformed into fmol/mg protein. The log receptor values were also plotted after stratification by age ≤ 49, ≥ 50 years. The W-statistic was used to test for the normality of the log distributions.

RESULTS

The frequency histograms representing all ER and PgR data from each laboratory are presented in Figs 1 (a–e) and 2 (a–e), respectively. There was strong evidence against log normality in each instance (P values for W-statistic < 0.001) with local maxima at both extremes of the frequency distributions that, as a group, are suggestive of bimodality. The evidence for bimodality is stronger for ER than for PgR. Furthermore, the bimodality is more distinct in some laboratories than in others. The concept of bimodality was still supported when data from each laboratory were plotted separately for patients ≤ 49 and ≥ 50 years (data not shown). It should be noted that because approximately 10% of values which were too high to be measured accurately, the right-hand peak in each histogram should be slightly broader than that shown.

Some laboratories had well-defined low points in the frequency histograms while others had broader regions of low frequency. The observed ER low points varied from 6 to 32 fmol/mg cytosol protein and the PgR low points from 5 to 63 fmol/mg cytosol protein. In some laboratories, the low points varied for different age groups. When data from all the laboratories was pooled, the bimodality of data distribution was obscured due to the differences in low points. Table 2 contains the median untransformed receptor values for each laboratory after stratification by age (≤ 49, ≥ 50 years). As expected from previous reports (e.g. [7, 8]), the median ER values were substantially greater for the

Correspondence to B.G. Mobbs.
B.G. Mobbs is at the Department of Surgery, University of Toronto, Room 7336, Medical Sciences Building, Toronto, Ontario M5S 1A8; J.-A.W. Chapman is at the Henrietta Banting Breast Centre, Women's College Hospital, Toronto, Ontario M5S 1B2; D.J.A. Sutherland is at the Toronto-Bayview Cancer Clinic, Sunnybrook Medical Centre, 2075 Bayview Avenue, Toronto, Ontario M4N 3M5; E. Ryan is at the Department of Clinical Chemistry, McMaster Medical Centre, Hamilton, Ontario, L8N 3Z5; E.R. Tustanoff is at the Receptor Laboratory, Victoria Hospital, London, Ontario N6A 4G5; and T.C. Ooi and P.V.N. Murthy are at the Ottawa Civic Hospital, 1053 Carling Ave., Ottawa, Ontario K1Y 4E9, Canada.
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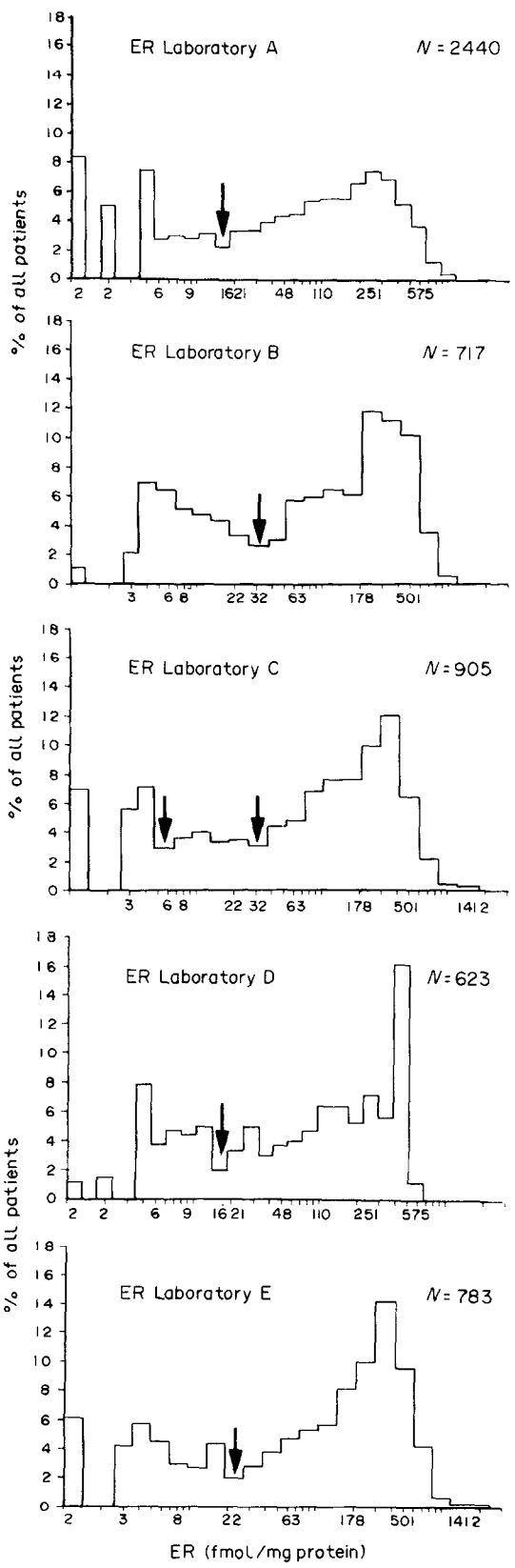

Fig. 1. Frequency histograms of ER values from each laboratory. *N* = number of assays. Arrows indicate low points of histograms.

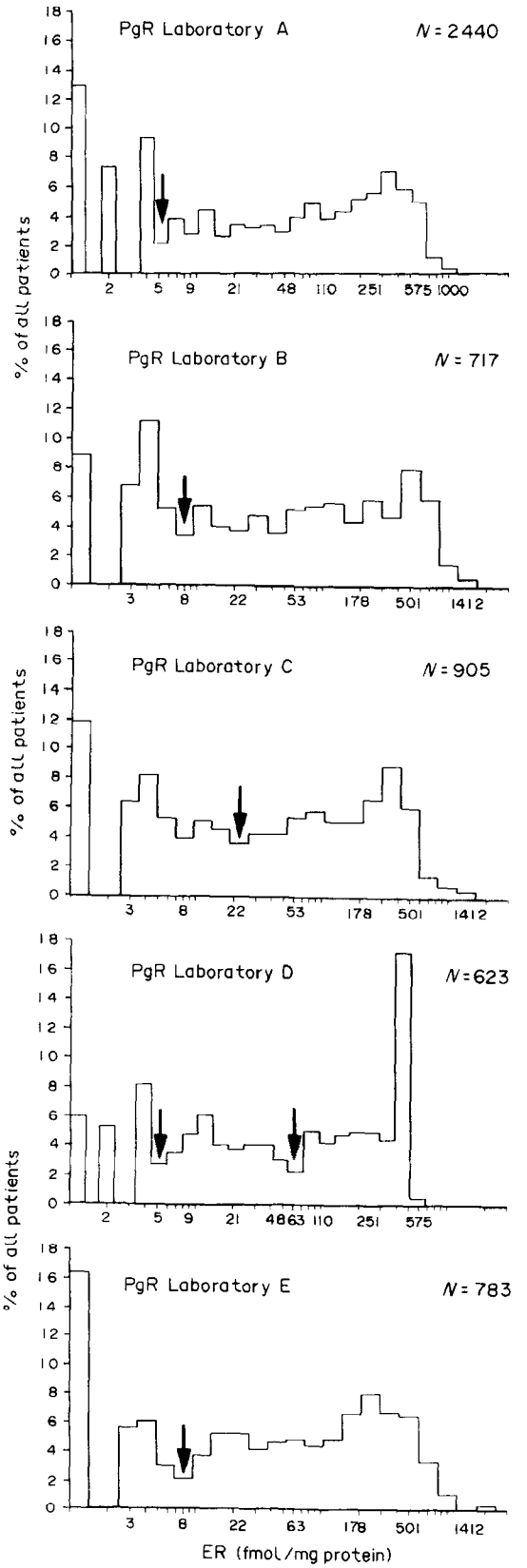

Fig. 2. Frequency histograms of PgR values from each laboratory. *N* = number of assays. Arrows indicate low points of histograms.

Table 2. Median receptor values by age

	N	ER (fmol/mg protein)		PgR (fmol/mg protein)	
Age		≤ 49	≥ 50	≤ 49	≥ 50
Laboratory					
A	2440	22	99	40	32
B	717	25	152	27	37
C	905	18	126	32	33
D	623	32	126	47	37
E	783	28	180	23	40

older age group, and median PgR values were generally similar for both age groups across the five laboratories.

DISCUSSION

Receptor binding assay results have been shown to be approximately log normally distributed ([1, 2] and our unpublished data). We have here presented evidence ($P \ll 0.001$) against both ER and PgR EIA values having log normal distributions. The data distributions from all five laboratories were suggestive of bimodality with the interlaboratory variation in the low frequency region possibly being due to minor variations in cytosol preparation.

We have ruled out two factors which might contribute to the type of distribution observed. Firstly, the age (≤ 49, ≥ 50) and inferred menopausal status of the patients did not affect the distribution pattern. Secondly, there was no evidence that kit variation was responsible, as bimodality of values was observed within individual kit batches. It is also unlikely that a shift with time in the immunoreactivity of the ER-EIA antibodies such as has been observed by one group of investigators [9] would occur within 1 year's data.

The EIA usually gives higher values than the RBA [5] possibly because the commonly used RBA methods detect only unoccupied receptors, while the EIA detects both unoccupied and occupied receptors. The values in the first EIA peak may be those in which no binding was detected by RBA due to occupation of receptor by endogenous oestrogen.

In addition, while there is usually a high correlation ($r > 0.9$) across the whole range of RBA and EIA values, some investigators have found a much lower correlation in the lower part of the range ([4, 5] and our own data) suggesting a different relationship between immunoactivity and steroid binding capacity in tumours in the low range of receptor content. A number of investigators have reported variant ER, or variant ER mRNA [10–13]. It is conceivable that oestradiol-binding mutant ERs may occur which have altered binding properties for the antibodies used in the EIA. A reduction or dysfunction of one or more of the EIA binding epitopes for a subpopulation of tumours could lead to the situation illustrated in Fig. 3(a). If this reduction/dysfunction did not affect the hormone binding site a log normal RBA distribution might be observed—Fig. 3(b).

The clinical relevance of our observations remains to be investigated. In particular, the significance of the low point in the distribution of EIA values is of interest: it is possible that this may represent a more useful 'cut-off' point to discriminate between hormone-sensitive and -insensitive tumours than the values in common current use (10 fmol/mg protein). Given the somewhat variable low point between laboratories, this could indicate that the 'cut-off' point should not be the same for all laboratories until methodology is further standardised, particularly with regard to cytosol preparation.

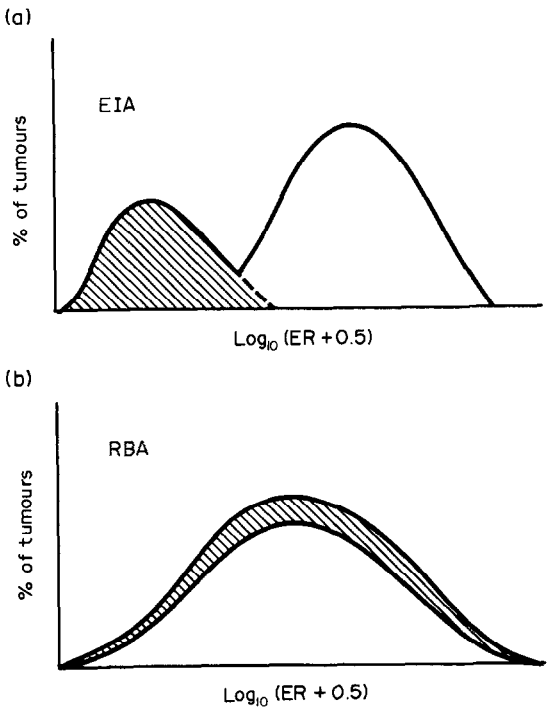

Fig. 3. Hypothetical distribution of receptor values assayed by EIA (upper panel) and RBA (lower panel). The shaded areas represent tumours which may be able to bind hormone normally, but have reduced or dysfunctional epitopes for the H222 and/or D547 monoclonal antibodies.

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Risk Factors for Epithelial Ovarian Cancer in Women Under Age 45

Alessandra Tavani, Eva Negri, Silvia Franceschi, Fabio Parazzini and Carlo La Vecchia

Risk factors for ovarian cancer in young women were investigated using data from a case–control study conducted between 1983 and 1992 in Milan, northern Italy, on 194 women below age 45 with histologically confirmed incident cancers of the ovary and 710 controls admitted to the same network of hospitals for acute non-gynecological, non-hormonal and non-neoplastic diseases. An elevated relative risk (RR) of ovarian cancer was found among women reporting 12 or more years of education [RR 1.6, 95% confidence intervals (CI) 1.0–2.03] and belonging to the highest social class (RR 1.8, 95% CI 1.1–3.0). Women whose mothers had had ovarian cancer had a multivariate RR of 2.7 (95% CI 0.7–10.5) compared to those with no family history. Menarche above age 13 and irregular menstrual cycles were significantly protective against ovarian cancer (RR 0.6 for both risk factors). There was a significant inverse relationship with abortions, the RR being 0.6 both for spontaneous and for induced abortions, while protection of parity was not significant. Higher risks of ovarian cancer were observed in women having first or last birth when older than 30 years (RR 2.0 and 2.4, respectively, compared to those delivering under age 25). A significant trend toward an increased risk of ovarian cancer was also observed with decreasing time since last birth. Compared with women whose last birth occurred 10 or more years before diagnosis, the RR was 2.1 (95% CI 1.1–3.9) for those reporting a birth during the last 5 years. The RR for oral contraceptive users was 0.7 (95% CI 0.5–1.0) and the protection increased with duration, with RR of 0.3 (95% CI 0.1–0.7) for 5 or more years of use. This study indicates that, although the incidence of ovarian cancer is higher in older women, recognised risk and protective factors are similar below age 45. An excess risk in the few years after a term delivery is also suggested. *Eur J Cancer*, Vol. 29A, No. 9, pp. 1297–1301, 1993.

INTRODUCTION

IN DEVELOPED areas of the world ovarian cancer is common, being among the five leading sites of incidence and mortality in most countries [1–3]. Several studies have assessed the role of menstrual, reproductive and hormonal factors on ovarian cancer and a few aspects of its epidemiology are now well understood [3]. Ovarian cancer has been inversely associated with parity [4] and oral contraceptive (OC) use [5, 6]. Early age at first pregnancy and abortions are also potential weak protective factors [4], while family history of ovarian cancer increases the risk [7–9]. A positive association of ovarian cancer with late age at menopause has been shown in several although not all studies [3], while evidence for a detrimental effect of early age at menarche is less consistent. [10, 11].

Information on the determinants of ovarian cancer in women below age 45 is scanty; trends over the past 20 years in developed countries show substantial declines in ovarian cancer mortality in young women, possibly because of the protection afforded by OC use [12, 13]. Only one study [14], to our knowledge, was restricted to ovarian cancer under age 50, and suggested that the number of live births, number of incomplete pregnancies and OC use were protective factors. However, only the combination of those factors in a total measure of "anovulatory" or "protected time" showed a significant inverse association. Thus, in the present study we investigated and quantified risk factors for ovarian cancer in young women, focusing on the role of family history of ovarian cancer, menstrual and reproductive factors and OC use, using data from a case–control study conducted in Milan, northern Italy.

Correspondence to A. Tavani.
A. Tavani, E. Negri, F. Parazzini and C. La Vecchia are at the Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milano; S. Franceschi is at the Centro di Riferimento Oncologico, Via Pedemontana Occ., Aviano (PN); F. Parazzini is also at the II Clinica Ostetrica e Ginecologica, Università di Milano, Italy; and C. La Vecchia is also at the Institute of Social and Preventive Medicine, University of Lausanne, 1005 Lausanne, Switzerland; and at the Institute of Medical Statistics, University of Milan, 2013 Milan, Italy.
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