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EORTC Receptor Study Group Report

Steroid Receptor Distribution in 47 892 Breast Cancers. A Collaborative Study of 7 European Laboratories

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Seven laboratories of the EORTC Receptor Study Group reported the distribution of oestrogen (ER) and progesterone receptors (PR) routinely assayed in breast cancer cytosols. A low interlaboratory variability was demonstrated for the median values, and for the frequency of positive tumours as measured by enzyme immunoassay (EIA). Larger variations were found for the frequency of positive tumours, as measured by radioligand binding assay (RLA). They are probably due to differences in the cut-off levels and in the sensitivity of the assay. Analysis of the variability over time clearly demonstrated that the ER-EIA values initially increased compared with RLA. A possible source of variations could be the calibration drift in the ER-EIA kit. In conclusion, quality assessment of steroid receptors should be monitored by comparison of both common standards and distributions routinely obtained in each laboratory. In-house analysis over time is also essential for reagent survey.

Key words: breast cancer, oestrogen receptor, progesterone receptor, interlaboratory variation
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INTRODUCTION

WHEN MULTICENTRE trials are performed, analyses of steroid receptors must be comparable both in the participating laboratories and over time. The EORTC has been involved in the standardisation and quality control of steroid receptor assays in breast cancer tissues [1–7]. However, while the use of external lyophilised controls [5] evaluates both the reproducibility of assay procedures and the method of computation of data, neither the process of tumour sampling collection and storage before reaching the laboratory nor the preparation of cytosols can be evaluated by this approach. The distributions of steroid receptors routinely analysed in each laboratory thus give a more realistic view of the interlaboratory comparability of the results. Provided that the natural history of breast cancer is the same in different geographical areas, similar distributions of receptor concentrations and similar frequencies of receptor positivity would be expected in different laboratories.

In this study, seven laboratories cooperating in the EORTC Receptor Study Group reported the distribution of oestrogen (ER) and progesterone receptor (PR) levels routinely assayed in breast cancer cytosols between 1977 and 1993. Large numbers of patients were included. Because receptor concentrations are significantly associated with other patient characteristics [8–11], analyses were performed according to age of the patients (totally objective data), histological type and histological grade of the tumours. Variations over time were also analysed. Data obtained with the radioligand binding assay (RLA) and the enzyme immunoassay (EIA) were compared.

MATERIALS AND METHODS

Tissues

Seven laboratories provided receptor values from breast cancer specimens routinely analysed for ER and PR between 1977 and 1993. The samples were considered to be representative for the population of samples submitted to each laboratory. When available, age of the patients, histological grade and histological type of the tumours were also provided. Overall, 99 665 steroid receptor data were analysed (ER-RLA n = 27 226; ER-EIA n = 24 547; PR-RLA n = 25 464; PR-EIA n = 22 428). Tissues were frozen in liquid nitrogen and stored at −80° to −196°C until assayed. ER and PR measurements were performed on cytosols prepared according to the recommended EORTC procedure [1, 2].

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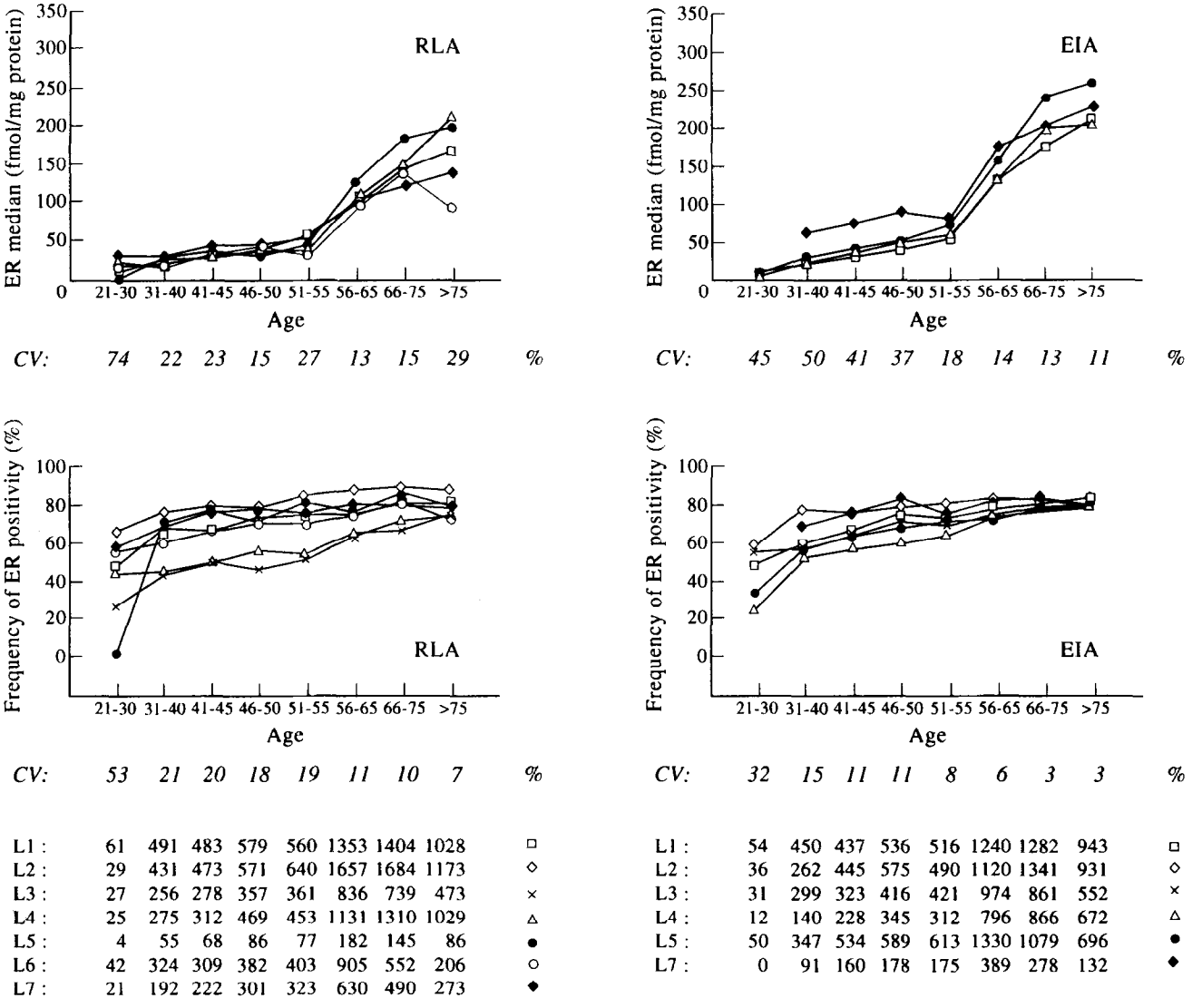


Figure 1. Distribution of ER according to the age of the patients. Numbers in italics represent coefficients of variation (CV) between laboratories. The numbers in Roman type below the figures are the number of patients of the participating laboratories. Laboratories 2 and 3 were not included in the median distribution because they expressed their data in fmol/μg DNA.

Table 1. *Cut-off values employed in each participating laboratory*

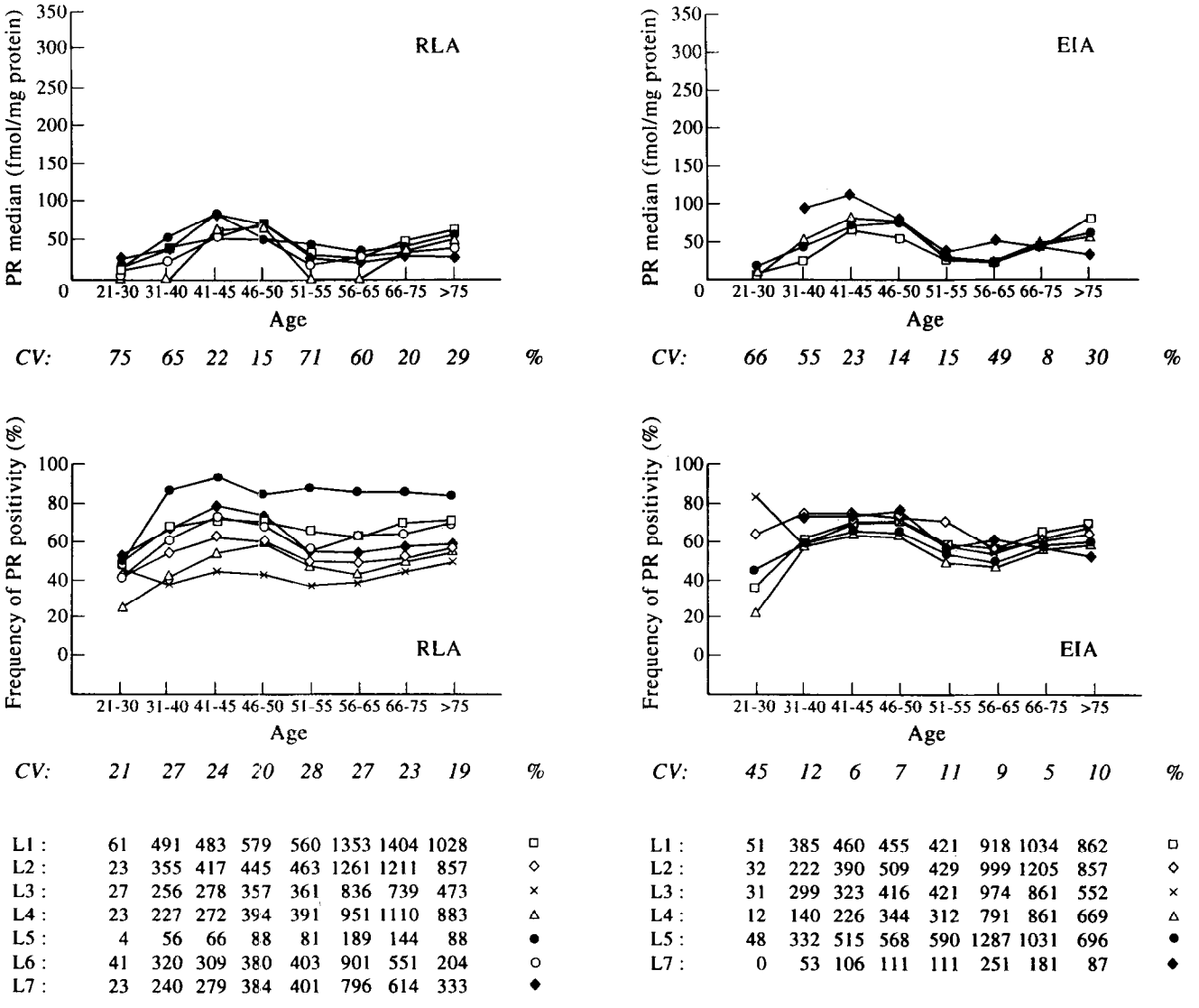
Laboratory	Period	Cut-off values ER-RLA	ER-EIA	PR-RLA	PR-EIA
1	78–93	10	10	10	10
2	77–92	0*	0*	0*	0*
3	82–92	0.1*	0.1*	0.1*	0.1*
4	80–92	10	25	10	25
5	87–93	10	20	10	20
6	82–92	10		10	
7	81–93	15	20	15	20

* Expressed in fmol/μg DNA.

ER and PR assays

Radioligand binding assay was performed in most instances according to the recommended EORTC dextran-coated charcoal procedure (DCC) with Scatchard plot analysis [1–7]. In some cases, radioligand assay was conducted via a single saturating dose assay. Data obtained by isoelectrofocusing (IEF) were provided by laboratories 2, 3 and 4 for ER, and laboratories 2 and 3 for PR [12, 13].

ER and PR enzyme immunoassays were performed with Abbott kits (Abbott Laboratories, Chicago, U.S.A.) using the instructions and materials provided by the manufacturer.



CV:	*75*	*65*	*22*	*15*	*71*	*60*	*20*	*29*	%
L1 :	61	491	483	579	560	1353	1404	1028	□
L2 :	23	355	417	445	463	1261	1211	857	◇
L3 :	27	256	278	357	361	836	739	473	×
L4 :	23	227	272	394	391	951	1110	883	△
L5 :	4	56	66	88	81	189	144	88	●
L6 :	41	320	309	380	403	901	551	204	○
L7 :	23	240	279	384	401	796	614	333	◆

CV:	*66*	*55*	*23*	*14*	*15*	*49*	*8*	*30*	%
L1 :	51	385	460	455	421	918	1034	862	□
L2 :	32	222	390	509	429	999	1205	857	◇
L3 :	31	299	323	416	421	974	861	552	×
L4 :	12	140	226	344	312	791	861	669	△
L5 :	48	332	515	568	590	1287	1031	696	●
L7 :	0	53	106	111	111	251	181	87	◆

CV:	*21*	*27*	*24*	*20*	*28*	*27*	*23*	*19*	%
L1 :	61	491	483	579	560	1353	1404	1028	□
L2 :	23	355	417	445	463	1261	1211	857	◇
L3 :	27	256	278	357	361	836	739	473	×
L4 :	23	227	272	394	391	951	1110	883	△
L5 :	4	56	66	88	81	189	144	88	●
L6 :	41	320	309	380	403	901	551	204	○
L7 :	23	240	279	384	401	796	614	333	◆

CV:	*45*	*12*	*6*	*7*	*11*	*9*	*5*	*10*	%
L1 :	51	385	460	455	421	918	1034	862	□
L2 :	32	222	390	509	429	999	1205	857	◇
L3 :	31	299	323	416	421	974	861	552	×
L4 :	12	140	226	344	312	791	861	669	△
L5 :	48	332	515	568	590	1287	1031	696	●
L7 :	0	53	106	111	111	251	181	87	◆

Figure 2. Distribution of PR according to the age of the patients. Numbers in italics represent coefficients of variation (CV) between laboratories. The numbers in Roman type below the figure are the number of patients of the participating laboratories. Laboratories 2 and 3 were not included in the median distribution because they expressed their data in fmol/μg DNA.

Quality control was ensured by testing with internal controls and EORTC standards.

Statistics

Both the steroid receptor median values and the frequency of positivity were analysed. The cut-off levels routinely employed in each institution to classify tumours as positive or negative are shown in Table 1.

Conversion of ER concentrations into normal distributions was performed by the Kaleidagraph programme. The values at which the normal distributions intercept were employed to define limits between subgroups.

RESULTS

Distribution of ER and PR according to patient age

The distributions of ER and PR levels measured in each participating laboratory with regard to age of the patients are presented in Figures 1 and 2.

Similar distributions of ER and PR in relation to age of the patients were observed in all laboratories. Moreover, ER and PR distribution patterns were similar for RLA and EIA. Overall, positive tumours increased with age, with a sharp concentration rise after 50 years. Changes as a function of age were different for PR, with higher PR median values and more positive tumours between 40 and 50 years.

In almost all age groups, a relatively low interlaboratory variability was demonstrated for ER and PR median values. Clearly, with both RLA and EIA, interlaboratory coefficients of variation (CV) below 25% are not unusual. The results obtained with the EIA show a similar variation to those of the RLA.

The interlaboratory CV for the frequency of ER and PR positive tumours as measured by EIA did not exceed 15%, except for the group 21–30 years with, however, the lowest receptor concentrations and only a few patients.

Larger interlaboratory variations were found for the frequency of ER positive tumours as measured by RLA (CV mean value 20%). Laboratories 3 and 4, using the IEF procedure, reported significantly lower percentages of ER positive tumours than did the other laboratories, although the ER median values obtained by laboratory 4 were in agreement with the others. Excluding laboratories 3 and 4, the CV mean value for the frequency of ER-RLA positivity decreased to 13%.

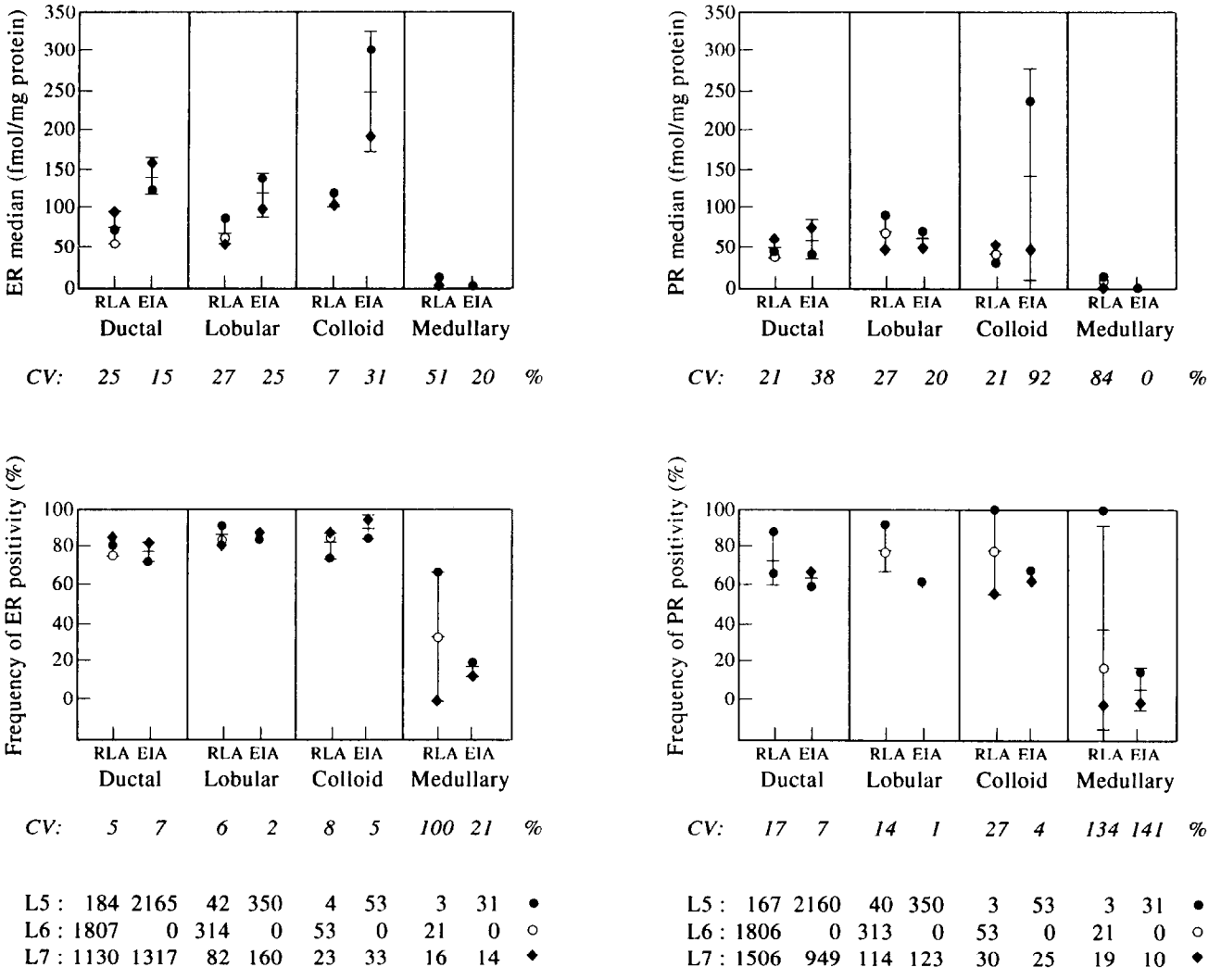


Figure 3. Distribution of ER and PR according to the histological type of the tumours. Bars shown in the figures are means and the standard deviation. Numbers in italics represent coefficients of variation (CV) between laboratories. The numbers in Roman type below the figure are the number of patients of the participating laboratories.

Large interlaboratory variations were also found for the frequency of PR positive tumours, as measured by RLA (CV mean value: 24%). The lowest percentages of PR-RLA positive tumours were observed in laboratories 3 and 4, as for ER. In contrast, high percentages of PR positive tumours were reported by laboratory 5 with RLA. The PR median values reported by laboratories 4 and 5 with RLA were in agreement with the other laboratories.

Comparison of EIA and RLA (excluding the IEF procedure) was performed for ER and PR median values. In each class of age, higher ER median levels were found by EIA as compared with RLA. In contrast, a very good agreement between EIA and RLA could be observed for PR median values. For ER, the ratio between EIA and RLA was higher than 1.39, except for the 21–30 year group. For PR, the ratio between EIA and RLA ranged from 0.97 to 1.35.

Distribution of ER and PR according to the histological type of the tumours

Figure 3 shows the distributions of ER and PR levels measured by laboratories 5, 6 and 7 with regard to the histological type of tumour.

Overall, no major difference could be observed between ductal and lobular carcinomas. Higher ER median values were found in colloid carcinomas. Medullary tumours rarely contained significant levels of receptors.

The distributions of ER and PR in ductal and lobular carcinomas were highly comparable in all laboratories, and with both RLA and EIA. Large interlaboratory variations were observed in colloid and medullary tumours. This could well be due to the small number of patients available for analysis.

Distribution of ER and PR according to the histoprognostic grade of the tumours

In laboratories 5, 6, and 7, the distributions of ER and PR were analysed with regard to the histological grade of the tumours, according to Bloom and Richardson [14] (Figure 4). In all laboratories, a clear relationship was found between ER and the grade of the tumour. Lower median concentrations of ER and lower frequencies of ER positivity were found in grade III tumours. This was observed with both RLA and EIA. Overall, a significant negative correlation was also observed between PR and the grade of the tumours. However, in laboratory 5, only small variations in the frequency of PR positive tumours (94 versus 93 versus 90%) were obtained using the RLA assay.

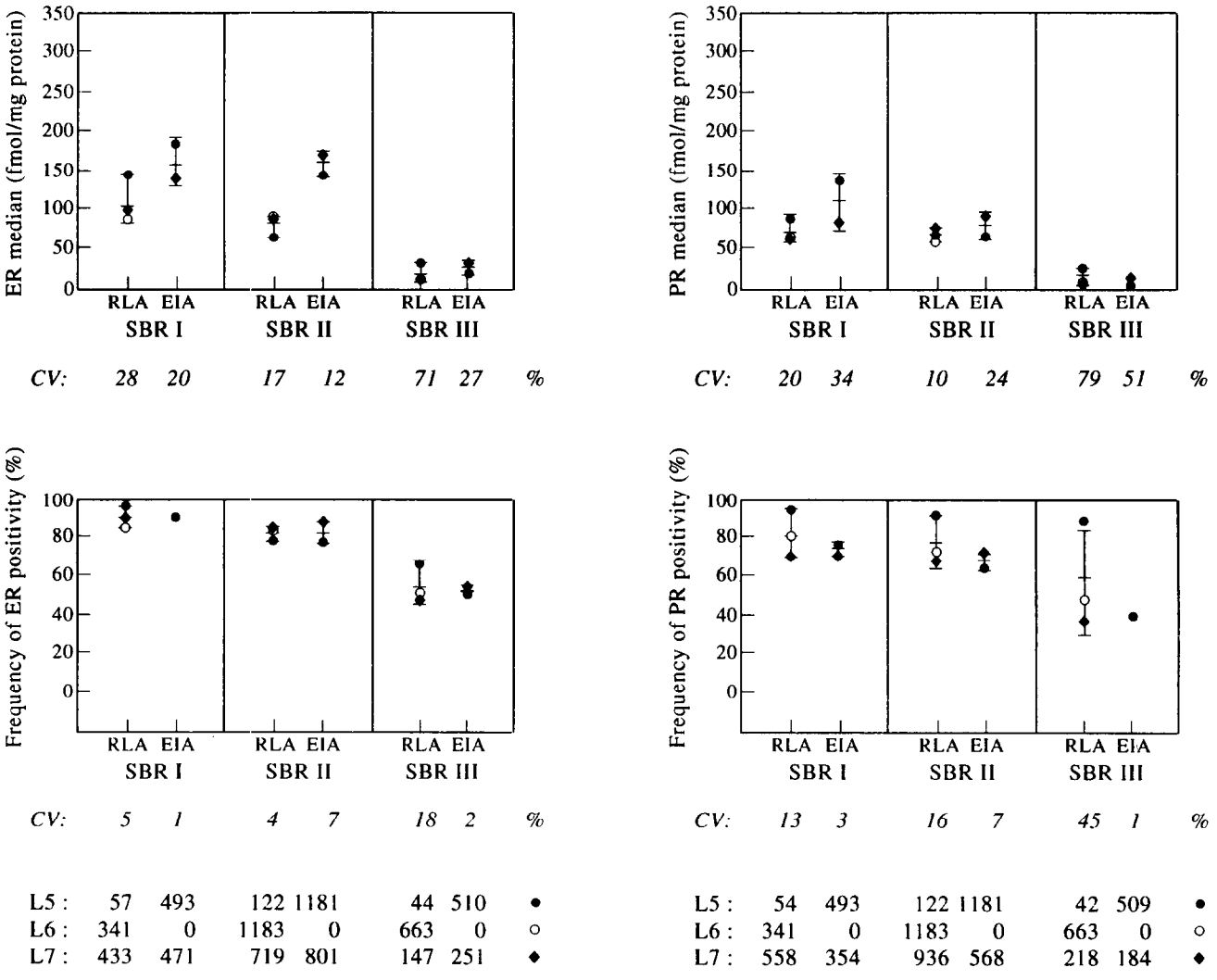


Figure 4. Distribution of ER and PR according to the SBR grade of the tumours. Bars shown in the figure are means and the standard deviation. Numbers in italics represent coefficients of variation (CV) between laboratories. The numbers in Roman type below the figures are the number of patients of the participating laboratories.

Distribution of ER and PR according to year of assay

In laboratories 1, 4, 5, 6 and 7, the distribution of ER and PR was analysed with regard to the year of assay (Figure 5).

For RLA, an initial increase was observed in laboratories 1 and 7 for both ER and PR levels. It was restricted to PR in laboratory 6. For EIA, ER values initially increased in all laboratories compared with RLA. Then, they decreased back to the level of RLA, except in laboratory 4. In contrast and during the same time, stability could be observed in laboratory 6 using the RLA. In laboratories 1, 5 and 7, the ER-EIA values of 1993 were much lower than in 1992.

Distribution of ER according to patient menopausal status

In laboratory 5, the distribution of ER-EIA levels was analysed according to the menopausal status of the patients. In postmenopausal patients, three normal distributions fit into the frequency distribution curve of the logarithmically transformed ER values ($r = 0.97$) (Figure 6). The low, intermediate and high ER groups comprised, respectively, 22, 26 and 52% of the patients. As recommended for EIA to classify tumours as ER negative and positive, the lower limit was 16 fmol/mg protein. The upper limit defining ER intermediate and high levels in the ER positive population, was 145 fmol/mg protein. In contrast, the logarithmically transformed value of ER among premenopausal patients was approximately normally distributed.

DISCUSSION

The EORTC Receptor Study Group has been involved in the standardisation and quality control of steroid receptor assays. Special attention has been given to the development of a standardised protocol [1, 2], reproducibility [3], problems associated with analysis of low levels [4], selection of reference material [5] and long-term variability [6]. The present paper is the first in the European collaborative study in which the distributions of receptor levels of breast cancer tissues, routinely assayed in different laboratories, are compared. Analyses were performed according to the age of the patients and the histopathological characteristics of the tumours, in order to exclude the major potential differences in assay results due to patient populations or subjective uncontrolled data.

All investigators reported the same relationships between receptors, age and histopathological characteristics of the tumours. There was also no clear difference between RLA and EIA. The correlations of steroid receptors with age and histoprognostic grade are in agreement with most studies [8–11].

A relatively low interlaboratory variability was demonstrated

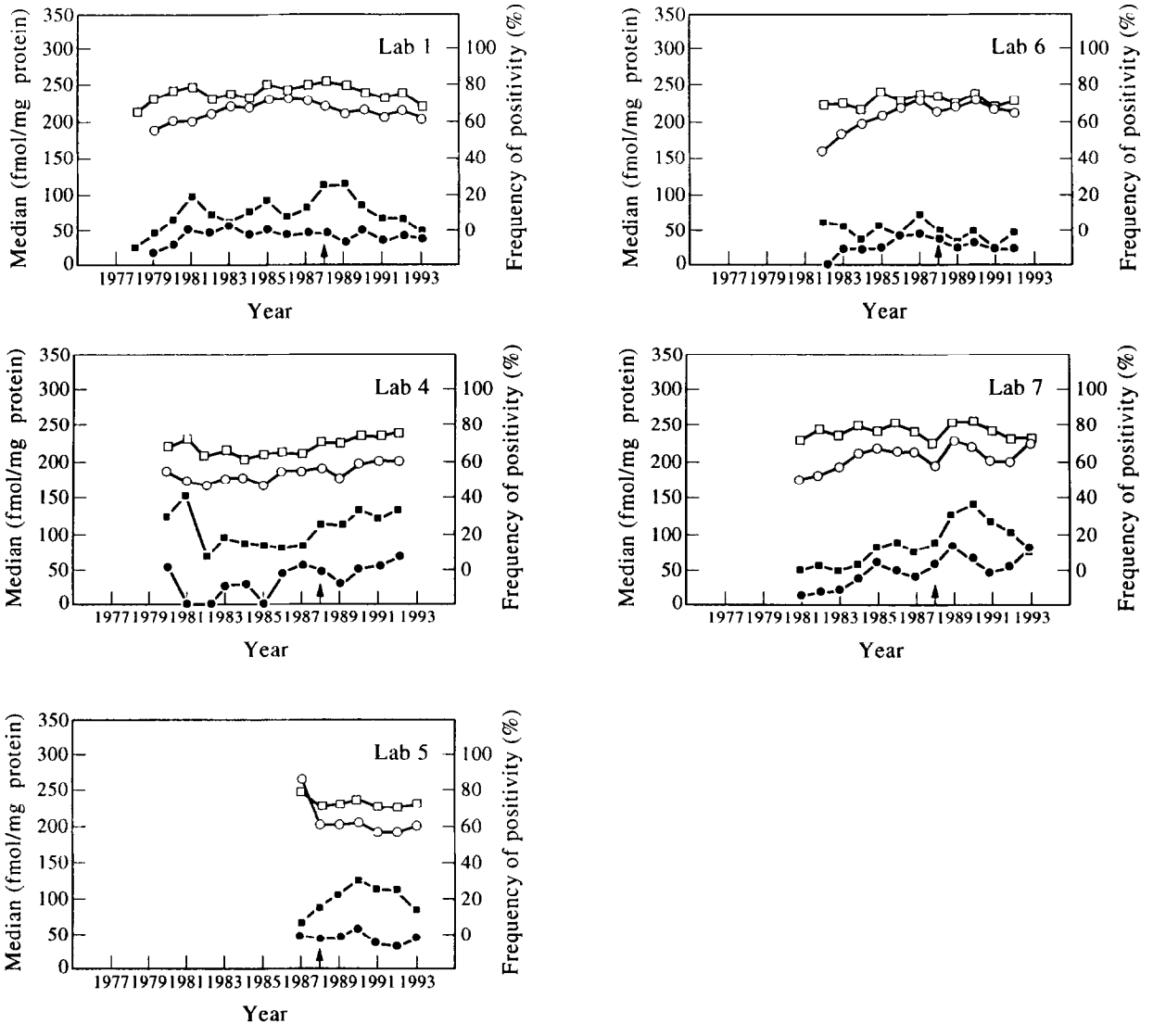


Figure 5. Distribution of ER and PR according to the year of assay. Laboratory 6 used RLA from 1982 to 1992. Laboratories 1, 4, 5 and 7 changed from RLA to EIA in 1988. The arrow indicates change from RLA to EIA. ER median value, ■; PR median value, ●; ER frequency of positivity, □; PR frequency of positivity, ○.

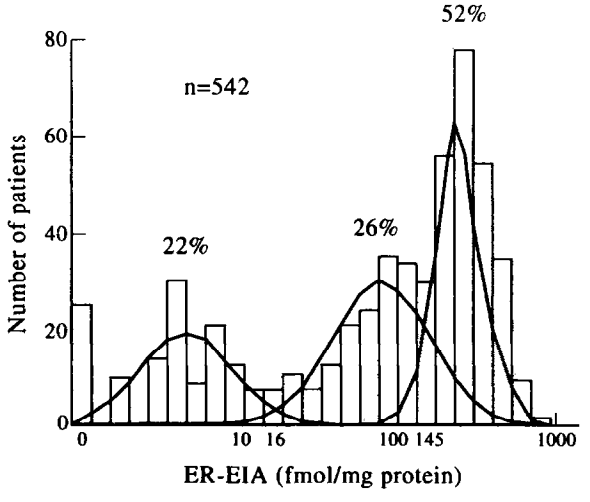


Figure 6. Distribution of ER-EIA concentrations in postmenopausal patients (laboratory 5).

for ER and PR median values with both RLA and EIA. Differences in the study populations, apart from age and histopathological characteristics, cannot be ruled out as a possible cause for the observed variability. Differences in tissue handling in the operating theatre, in processing by pathologists, in sample storage and in cytosol preparation may also have contributed to the observed differences. The CV demonstrated in EORTC interlaboratory quality control for the 22 core laboratories of the Receptor Study Group have, however, been fluctuating around 20% for the past several years. Thus, the differences in the accuracy of the receptor assays themselves are probably one of the major causes for the variability of median values observed in this study.

A very low interlaboratory variability was demonstrated for the frequency of ER and PR positive tumours, as measured by EIA. In contrast, systematic variations appeared in few laboratories with RLA. The observation that these variations were restricted to the receptor status strongly suggests that they may be due to differences in the cut-off level used to define positive tumours. Concerning laboratory 4, it is also possible that the sensitivity of RLA is lower for IEF, compared with the DCC procedure.

The variability over time in receptor measurements was analysed in five laboratories. The data clearly demonstrated that surveying routine analysis variations and reagents is essential. For RLA, an initial increase was generally observed for both ER and PR levels. This could reflect the adhesion to the EORTC standard procedure. It could also be due to the high sensitivity of the assay to the technician's experience. A lower frequency of non-malignant biopsies was also probably achieved through improved communication with the departments of histopathology. For EIA, the ER values initially increased in all laboratories compared with RLA. In contrast, during the same period, stability was observed in laboratory 6 using RLA. Moreover, although equivalent results between EIA and RLA have been previously reported in European centres [15, 16], in every subgroup analysed in this study, higher ER median values were found by EIA, compared with RLA. These data strongly support the standardisation drift in the ER-EIA kit [17]. The ER-EIA assay was subsequently recalibrated by the manufacturer in 1993 [18]. In laboratories 1, 5 and 7, the 1993 values were lower than those of 1992, in agreement with the change in standards. Surprisingly, however, the ER-EIA values already seemed to decline before 1993. A similar trend could not be observed in laboratory 4. We can therefore not exclude the possibility that the introduction of mammographic screening during the same time period may influence receptor distributions.

Interlaboratory consistency in steroid receptor results is usually monitored by analysis of common standards. A clearer picture of the interlaboratory comparibility will, however, emerge with comparison of both quality controls and distributions of steroid receptors routinely analysed in each laboratory. The reference distribution curve obtained by the seven well-trained European laboratories cooperating in this study should facilitate this quality assessment. In-house comparison of the distribution of receptors, achieved over different periods of time, is also essential for reagent quality survey.

Determination of ER and PR in breast cancers as a guide to prognosis and therapeutic selection has become standard medical practice. Classification of the patients as negative or positive is the element of receptor assays that is most widely used in clinical practice. However, postmenopausal breast cancer patients with the highest ER levels experience the greatest benefit from hormonotherapy [19]. Moreover, in postmenopausal patients not treated with adjuvant therapy, the highest levels of ER have been associated with a spontaneous poor recurrence-free survival [20]. In this study, analysis of the ER-EIA frequency distribution curve demonstrated three groups of postmenopausal patients, in agreement with previously reported data using RLA [20]. Knowledge of where a patient is situated in the distribution of receptor concentrations is thus certainly more clinically informative than actual receptor levels of the tumour tissue, and even more than classification as being either receptor positive or negative, which is in itself rather arbitrary. This strongly indicates that the accuracy of ER level is important for optimal therapeutic strategy, and that receptor level distributions should be reported by each laboratory performing these assays.

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