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Is quantitative oestrogen receptor expression useful in the evaluation of the clinical prognosis? Analysis of a homogeneous series of 797 patients with prospective determination of the ER status using simultaneous EIA and IHC

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ABSTRACT

Objective: Oestrogen receptor (ER) determination in breast cancer (BC) is a major yardstick for the prognosis and for response to hormonal therapy (HT). As several techniques have been proposed for ER quantification, the purpose of our study was to assess whether the qualitative or quantitative analysis of ER expression might influence the prognosis and response to treatment.

Materials and methods: We analysed overall survival (OS) and disease-free survival (DFS) in 797 primary BC cases with ER determination by enzyme immunoassay (EIA) and immunohistochemistry (IHC). The clinical impact according to qualitative or quantitative analysis of ER expression was assessed. Response to HT was evaluated according to quantitative EIA-determined ER expression levels.

Results: According to the qualitative analysis of ER expression, patients with EIA-determined and IHC-determined ER-positive tumours had significantly longer OS and DFS ($p < 0.001$). The analysis stratified on quartiles of ER levels showed significantly different outcomes according to EIA- and IHC-determined subgroups. In the group of patients who received adjuvant treatment, 5-year OS was significantly different between the groups, with a clear benefit for the highest EIA-determined ER quartiles ($p < 0.001$). Comparatively, in terms of 5-year DFS, a clear separation was noted between groups for adjuvant treatment ($p < 0.001$). The group with moderate ER+ values was clearly distinct from the ER-negative population. Quantitative ER expression helped to better distinguish the beneficial or detrimental effect of HT within quartiles of ER-expressing tumours. Based on the STEPP

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analysis which showed a trend towards an ER effect on DFS as a function of HT assignment, we confirm the benefit of HT in patients with a very high EIA-determined ER level and a detrimental impact on negative and weakly positive groups.

Conclusion: Quantitative ER expression in BC helps to better discriminate heterogeneity in clinical outcome and response to HT.

1. Introduction

Among the predictive and prognostic factors in primary breast cancer (BC), oestrogen receptor (ER) determination is the strongest indicator of responsiveness to adjuvant endocrine therapy.[1,2] Patients exhibiting ER-positive (+) BC have a better prognosis since they often benefit from tailored treatment with hormonal therapy.[1,3]

However, despite its pivotal value in treatment decision-making, the most suitable method for determining ER expression remains a matter of debate, since different methods have been proposed: biochemical, immunohistological and more recently gene-expression profiling.[4–6] Several studies have compared these different techniques to assess the superiority of one over the other. Finally, the IHC procedure was implicitly proposed as the reference method by different boards and peer committees.[7,8] In addition, a binary qualitative scale of ER expression was unanimously adopted. However, it is not certain whether the debate is definitively closed because the advent of genomic and automated analysis could provide some arguments in favour of other ER determination methods.[6,9] Furthermore, the value of quantitatively determined ER expression has reappeared in recent publications.[10,11]

Interestingly, a recent study by Magné and colleagues[12] threw a spanner in the works. They suggested that the prognostic significance could differ according to the method of ER assessment. Moreover, in a recent analysis of tumour blocks from the ATAC trial,[10] the authors suggested that the efficacy of tamoxifen or aromatase inhibitors could differ across all subgroups based on quantitative expression. Consequently, it could be hypothesised that the use of a qualitative instead of a quantitative cut-off for ER positivity might underestimate the real value of these tests.

Based on these facts, the following study was undertaken (i) to evaluate the significance of the method of ER determination on the prognosis and (ii) to evaluate the impact of the quantitative analysis of ER expression on the prognosis. Our aim was to investigate whether more thorough quantitative stratification of ER expression could identify subgroups exhibiting a differential prognosis of particular interest to the efficacy of the hormonal adjuvant therapy.

2. Patients and methods

2.1. Study population

The study group was constituted from the population of patients treated for operable primary BC in the Department of Breast Surgical Oncology from 1984 to 2003 at the Conception Hospital in Marseille, France.

Clinical information was prospectively collected in a clinical database (MEDLOG) and updated periodically. The final study population consisted of 797 patients after excluding patients with an unknown ER status and incomplete data; and patients who received primary chemotherapy or endocrine therapy were not considered for this study. The following data were collected for the study: clinical tumour stage, pathological tumour size, histoprognostic grade (Scarff, Bloom and Richardson), lymph node involvement, oestrogen receptor (ER) and progesterone receptor (PR) status.

Patients underwent a breast-conserving lumpectomy with axillary lymph node dissection ($n = 638$) or a modified radical mastectomy ($n = 159$).

2.2. Routine assessment of ER status

ER was determined by enzyme immunoassay (EIA), and immunohistochemistry (IHC). For the IHC method, the tumour was considered oestrogen receptor (ER) positive if $\geqslant$10% of the neoplastic cells showed nuclear staining (anti-ER monoclonal antibodies, Abbott kits, Rungis, France). For the EIA method, according to an international consensus, ER positivity was defined as 15 fmol/mg or more of ER protein detected.[13,14] Measurements were performed with an Abbott kits (Abbott Laboratories, Chicago, USA) according to the manufacturer's instructions. The determination technique has previously been described.[5,15] ER measurements were performed on cytosols prepared according to the recommended EORTC procedure with quality control.[4,5,16,17] During the study period, all tumours underwent ER assessment by both techniques EIA and IHC which were performed by the same laboratory platform (pathology and biology).

2.3. Treatment

How the decision was made to administer adjuvant treatment has previously been described.[15] Briefly, hormonal therapy was administered to all patients with oestrogen receptor (ER)-positive tumours for a period of 5 years.

2.4. Statistical analysis

The EIA-determined ER values (expressed as log(ER EIA + 1) exhibited two normal distributions as previously described[18] using maximum likelihood, assuming a constant coefficient of variation. In this study, patients were grouped and analysed according to EIA- or IHC-determined measurements, respectively. We performed qualitative and quantitative analyses of ER expression.

Thus for the quantitative analysis, EIA-determined ER data were divided into four strata: ER negative (true negative), weakly ER+ (<25th quartile), moderately positive (25th–75th quartile) and highly positive (>75th quartile). Also, given the variations previously observed in ER levels in premenopausal versus postmenopausal patients,[19] *we considered the following categories:* in the case of non-menopausal women, tumours with an ER value of <16.75 fmol/mg protein (25th percentile) were classified as weakly ER+, when the ER value was 16.75–119 fmol/mg protein they were considered moderately ER+, and highly ER+ when the ER value was >119 fmol/mg protein (75th percentile). Tumours from postmenopausal patients were considered weakly ER+ when the ER level was <20 fmol/mg protein (25th percentile), moderately ER+ when the ER level was 20–292 fmol/mg protein and highly ER+ when the ER level was >292 fmol/mg protein (75th percentile). In both pre- and post-menopausal populations, weakly ER+ corresponds to ER values below the 25th percentile, moderately ER+ corresponds to values between the 25th and 75th percentiles and highly ER+ corresponds to values exceeding the 75th percentile.

Survival rates were determined according to qualitative and quantitative ER expressions. All survival statistics were measured from the date of the diagnosis. The actuarial survival and recurrence rates were calculated using the Kaplan–Meier method, and comparisons were made using the log-rank test. A multivariate analysis using the Cox proportional hazards regression model was used to determine predictive factors for survival.

In order to explore the trends in the effect of differences in hormonal therapy according to ER expression in subgroups, we used subpopulation treatment effect pattern plot (STEPP) methodology.[20,21] STEPP involves defining several overlapping subgroups of patients based on a covariate of interest and studying the resulting pattern of the treatment effects estimated within each subgroup. For this sliding window STEPP analysis, the subpopulation contained 200 patients and slides by dropping approximately 100 patients. All tests were two-tailed and a *p*-value of less than 0.05 was considered significant. All statistical analyses were performed with SPSS® software, version 12.0 and R 2.10.1.

Table 1 – Study population characteristics.

	N = 797
Age, median (range)	57 (22–91)
Non-menopausal (%)	278 (34.9)
Menopausal (%)	519 (65.1)
Tumour size (%)	
⩽2 cm	486 (61)
>2 and smaller than or equal to 5 cm	263 (33)
>5 cm	48 (6)
Nodes (%)	
Negative	433 (54.3)
Positive	364 (45.7)
Grade (%)	
1–2	548 (68.8)
3	249 (31.2)

Table 2 – Distribution of oestrogen receptor level according to determination method.

ER score	Total (*n* = 797)
EIA	
Median, (range)	84 (0–926)
<15 fmol/mg protein	185 (23.2)
⩾15 fmol/mg protein	612 (76.8)
Distribution quartiles, median (range)	
True negative (*n* = 26)	0
Weakly ER+ (*n* = 171)	4 (1–18)
Moderately ER+ (*n* = 403)	84 (17–292)
Highly ER+ (*n* = 197)	341 (122–926)
IHC	
Median, (range)	10 (0–100)
Equal to 0	
<10%	412 (51.7)
⩾10%	385 (48.3)
Distribution quartiles *n* (%)	
Negative (*n* = 217)	217 (27.2)
Weakly ER+ (*n* = 270)	270 (33.9)
Moderately ER+ (*n* = 294)	294 (36.9)
Highly ER+ (*n* = 16)	16 (2)

3. Results

3.1. Patient characteristics

Seven hundred and ninety-seven patients were included in the present study. The demographics of the study population are reported in Table 1. Patients were menopausal in 65.1% (*n* = 519) of the 797 analysed cases. The tumours measured more than 2 cm in diameter in 311 patients (39%). Three hundred and sixty-four patients (45.7%) had positive axillary lymph nodes. The histological grade was 3 in 249 tumours (31.2%).

The distribution of ER expression according to the measurement method is presented in Table 2. According to the qualitative cut-off, ER positivity attained 76.8% (*n* = 612) and 48.3% (*n* = 385) using the EIA and IHC techniques, respectively.

EIA-determined ER quantitative distribution (log-transformed) in all patients and in non-menopausal and menopausal patients is shown in Fig. 1a–c. For the overall population, ER values were divided into two normally distributed categories, but in menopausal patients the distribution exhibited a shift towards high values in comparison with ER levels in non-menopausal patients, as previously described.[22] For quantitative EIA-determined ER data, groups were stratified according to quartiles.

For IHC-determined quantitative ER expression, four groups were constructed in a semi-continuous manner: ER negative (absence of tumour cell staining), weakly ER+ (<40% positive cells), moderately ER+ (40–75% of tumour cells stained) and highly ER+ (>75%).

3.2. ER expression and survival rates

The median follow-up was 76 months (range, 0–231 months).

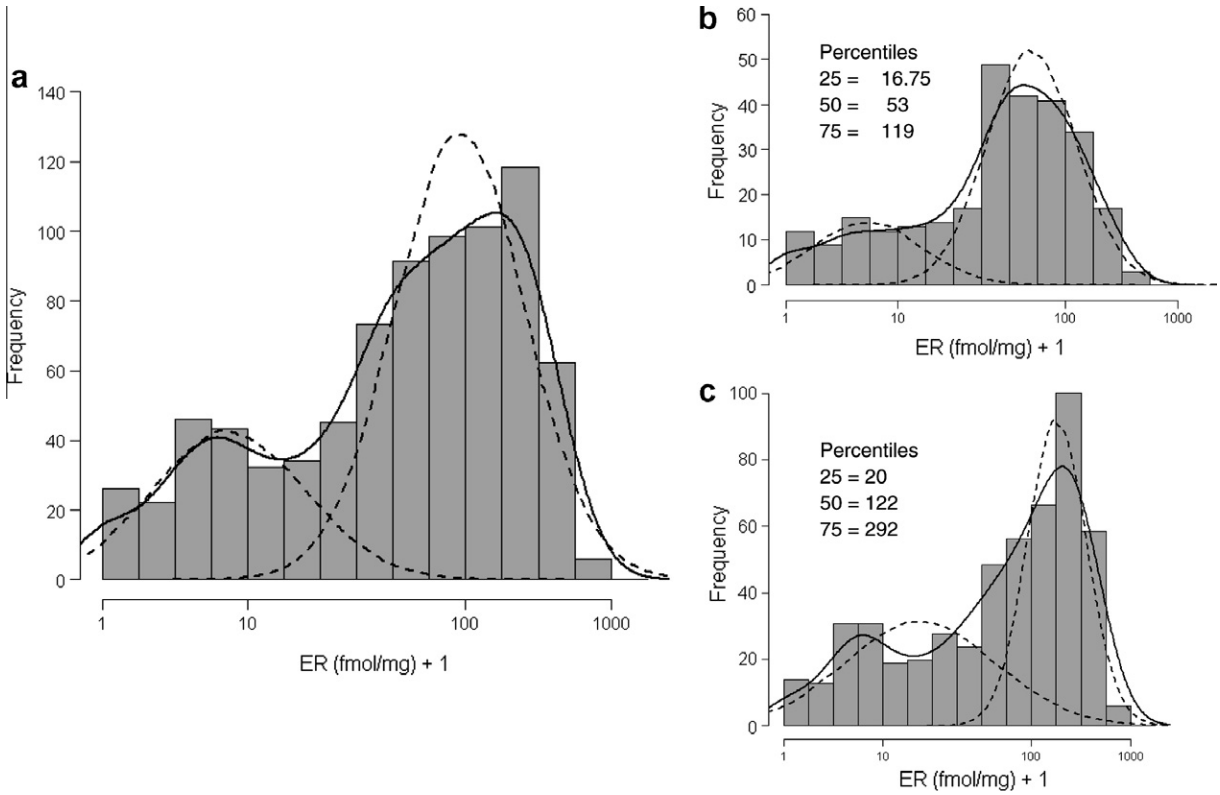

Fig. 1 – EIA-determined ER value distribution in the total population (a), in non-menopausal (b) and menopausal (c) groups. Deconvolution of ER concentrations into normal distributions. Two normal distributions (dotted curves) fitted with the frequency distribution curve (plain curve) of the logarithmically transformed ER values.

According to the qualitative analysis of ER expression, 5-year OS was 91.1% (95% confidence interval (CI), 88.3–93.3%) for EIA-determined ER+ patients and 76.9% (95% CI, 69.7–82.5%) for EIA-determined ER-negative patients ($p = 0.002$). Similarly, better 5-year OS was observed for IHC-determined ER+ (91.2%, 95% CI: 87.3–93.9%) patients compared to that for IHC-determined ER-negative patients (84.5%, 95% CI: 80.3–87.8%), $p = 0.009$.

The quantitative analysis of ER expression showed that 5-year OS differed significantly ($p = 0.001$) between the categories determined with the EIA method. However, the weakly ER+ group was more clearly distinguished from the negative and moderately to highly ER+ groups (Fig. 2A). Similarly, 5-year OS was significantly different between IHC-determined ER categories, with a more pronounced difference between negative and highly ER+ groups ($p = 0.006$) (Fig. 2B).

Fig. 2C shows that the 5-year DFS (EIA-determined ER) rates exhibited different outcomes according to quartiles ($p = 0.001$) but these differences were not superimposable with 5-year DFS for IHC-determined ER expression, as shown in Fig. 2D ($p < 0.001$). Thus, in the EIA analysis, highly and moderately+ ER expressions were superimposable whereas in the IHC analysis, the population with moderately and weakly+ ER expressions had similar outcomes.

In Cox's univariate (Table 3) and multivariate analyses (Table 4), we confirmed the value of quantitative ER expression. However, this value was more pronounced in the multivariate analysis for DFS.

3.3. *Quantitative ER expression and response to hormonal therapy*

In this part of the study, we tried to determine whether quantitative ER expression was able to better discriminate subgroups in terms of response to adjuvant hormonal therapy. As the values obtained with the EIA technique could be readily expressed quantitatively, we only explored quartiles in this group, and not in the IHC categories.

Thus, in the group that did not receive adjuvant therapy, in keeping with the department guidelines, 5-year OS was not significantly different between quartiles: patients with highly+ ER expression had the same survival as those with a weakly+ or negative ER status ($p = 0.14$), as shown in Fig. 3A. A marginal difference was observed for 5-year DFS (Fig. 3B, $p = 0.04$).

More interestingly, in the group of patients who received adjuvant therapy (according to clinico-pathological characteristics), 5-year OS was clearly significantly different between groups, with a clear benefit for highly ER+ patients (Fig. 3C, $p < 0.001$). Comparatively, there was a clear distinction between subgroups in terms of 5-year DFS if they received adjuvant therapy (Fig. 3D, $p < 0.001$). In particular, the group with moderately ER+ values was clearly distinct from the ER-negative population.

In addition, we used STEPP analyses to explore the pattern of treatment effect differences in terms of 5-year OS and DFS according to continuous quantitative levels of EIA- or

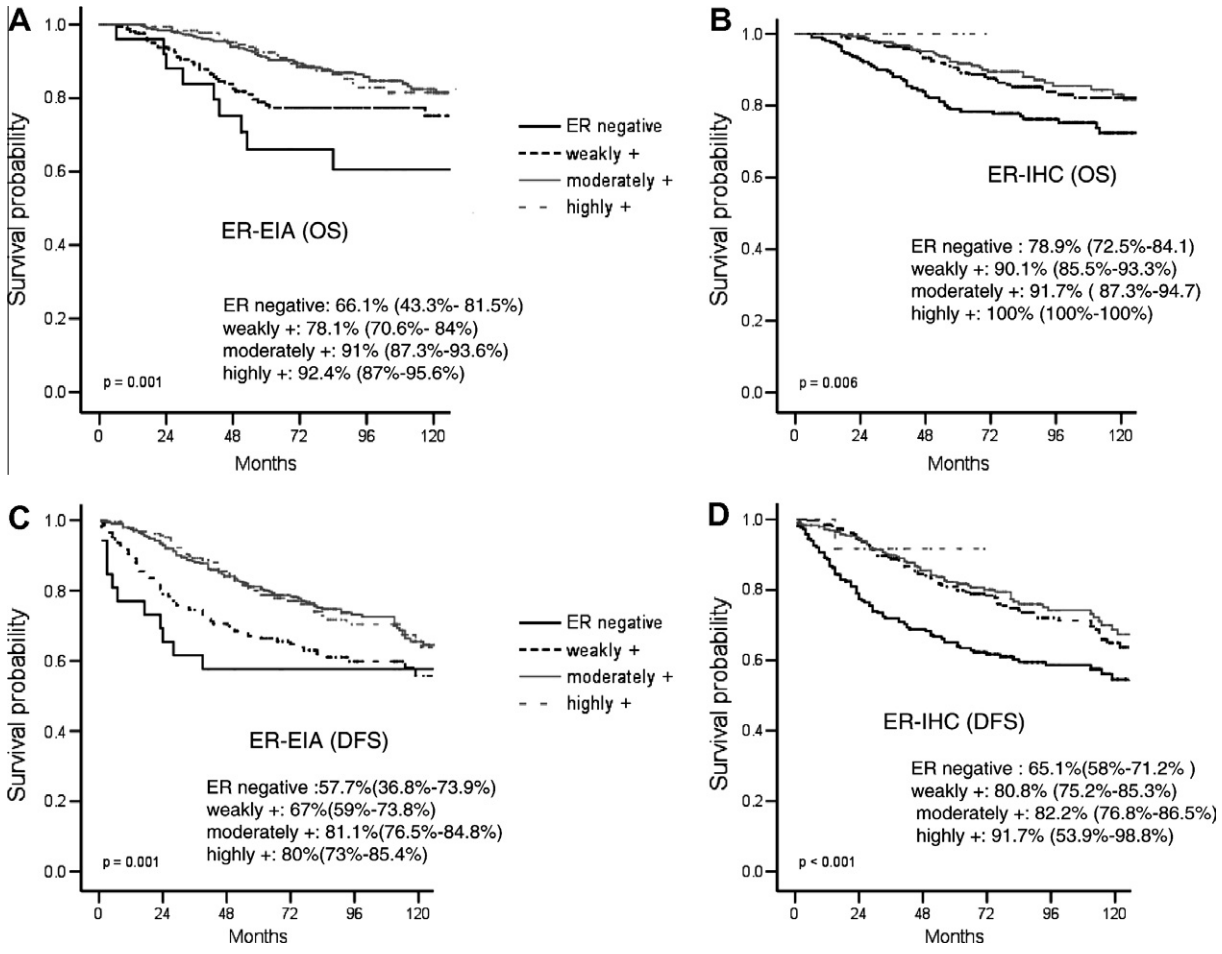

Fig. 2 – Overall survival according to EIA-determined ER expression (A) and IHC-determined ER expression (B), and disease-free survival according to EIA-determined ER expression (C) and IHC-determined ER expression (D) presented in a quantitative manner.

Table 3 – Results of univariate analysis for OS and DFS.

	OS HR (95% CI)	*p*-Value	DFS HR (95% CI)	*p*-Value
Age >50 years	1.02 (0.69–1.5)	0.93	0.88 (0.67–1.16)	0.38
pTumour size (⩽20 mm versus >20 mm)	0.45 (0.31–0.65)	<0.001	0.51 (0.40–0.64)	<0.001
Positive node	0.28 (0.20–0.41)	<0.001	0.45 (0.34–0.60)	<0.001
Grade (1–2 versus 3)	1.85 (1.29–2.67)	<0.001	1.55 (1.19–2.03)	<0.001
EIA-determined ER quartiles				
Ref ER negative (3.2%)	–	–		
Weakly ER+ (21.5%)	0.58 (0.28–1.21)	0.14	0.81 (0.45–1.48)	0.50
Moderately ER+ (50.6%)	0.33 (0.16–0.67)	<0.001	0.49 (0.27–0.87)	0.01
Highly ER+ (24.7%)	0.33 (0.15–0.72)	<0.001	0.49 (0.27–0.91)	<0.001
EIA-determined ER+	0.56 (0.38–0.81)	<0.001	0.59 (0.45–0.78)	<0.001
IHC-determined ER quartiles				
Ref ER negative (27.2%)	–	–		
Weakly ER+ (33.9%)	0.57 (0.37–0.88)	0.01	0.59 (0.43–0.80)	<0.001
Moderately–highly ER+ (38.9%)	0.49 (0.31–0.77)	0.002	0.50 (0.36–0.69)	<0.001
IHC-determined ER+	0.60 (041–0.88)	<0.001	0.64 (0.49–0.84)	<0.001

IHC-determined ER expression in the primary tumour (Fig. 4A–D). For these sliding window analyses, each subpopulation contained a total of 200 patients for both the no hormonotherapy and hormonotherapy subgroups and each subsequent subpopulation was formed moving from left to right by dropping about 100 patients with the lowest covariate

Table 4 – Results of multivariate analysis for OS and DFS.

	OS HR (95% CI)	*p*-Value	DFS HR (95% CI)	*p*-Value
Model with EIA-determined ER				
pTumour size (⩽20 mm versus >20 mm)	0.62 (0.42–0.92)	0.02	0.62 (0.47–0.82)	<0.001
Positive node	0.34 (0.23–0.50)	<0.001	0.53 (0.39–0.71)	<0.001
Grade (1–2 versus 3)	1.12 (0.75–1.68)	0.58	1.04 (0.77–1.39)	0.82
EIA-determined ER quartiles				
Ref ER negative	–	–		
Weakly ER+ (*n* = 171)	0.62 (0.30–1.28)	0.20	0.86 (0.47–1.57)	0.63
Moderately ER+ (*n* = 403)	0.40 (0.19–0.82)	0.01	0.55 (0.31–1)	0.05
Highly ER+ (*n* = 197)	0.43 (0.20–0.94)	0.03	0.59 (0.31–1.1)	0.10
EIA-determined ER+	0.65 (0.43–0.97)	<0.001	0.65 (0.48–0.88)	<0.001
Model with IHC-determined ER				
pTumour size (⩽20 mm versus >20 mm)	0.62 (0.42–0.92)	0.001	0.62 (0.47–0.82)	<0.001
Positive node	0.33 (0.23–0.49)	<0.001	0.53 (0.39–0.72)	<0.001
Grade (1–2 versus 3)	1.1 (0.71–1.6)	0.75	1.1 (0.80–1.4)	0.61
IHC-determined ER quartiles				
Ref ER negative	–	–		
Weakly ER+ (*n* = 270)	0.62 (0.42–0.91)	<0.001	0.60 (0.43–0.83)	<0.001
Moderately–highly ER+ (*n* = 310)	0.56 (0.35–0.91)	0.002	0.53 (0.37–0.75)	<0.001
IHC-determined ER+	0.68 (0.46–1.02)	0.06	0.70 (0.53–0.93)	0.01

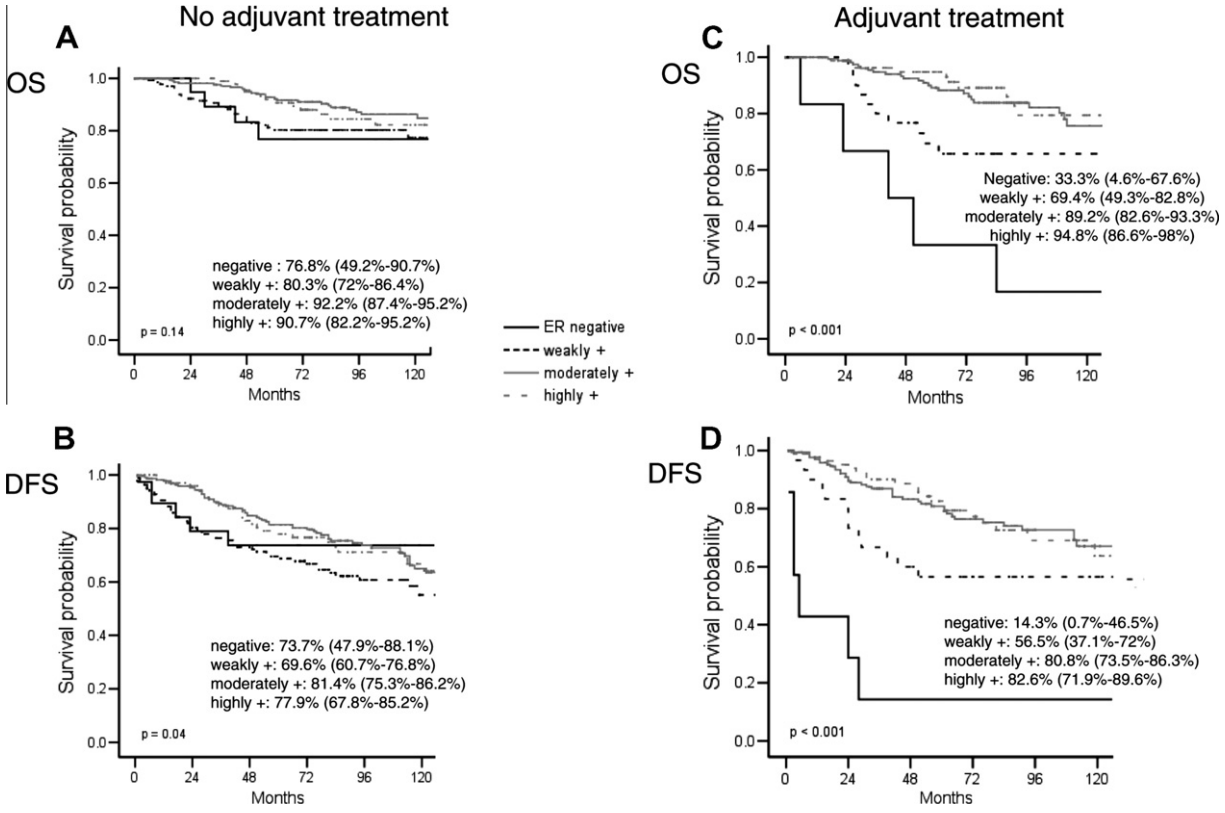

Fig. 3 – OS (A) and DFS (B) according to EIA-determined ER expression in quartile groups for patients who did not receive adjuvant treatment. OS (C) and DFS (D) according to EIA-determined ER expression in quartile groups for patients who received adjuvant treatment.

values and adding about 100 patients with the next higher covariate value. Fig. 4A shows the STEPP analysis for OS according to quantitative ER values: the detrimental effect of adding hormonotherapy when ER expression values were weakly+ or negative was found to be of borderline significance ($p = 0.078$) but was more clear-cut for DFS as seen in Fig. 4B

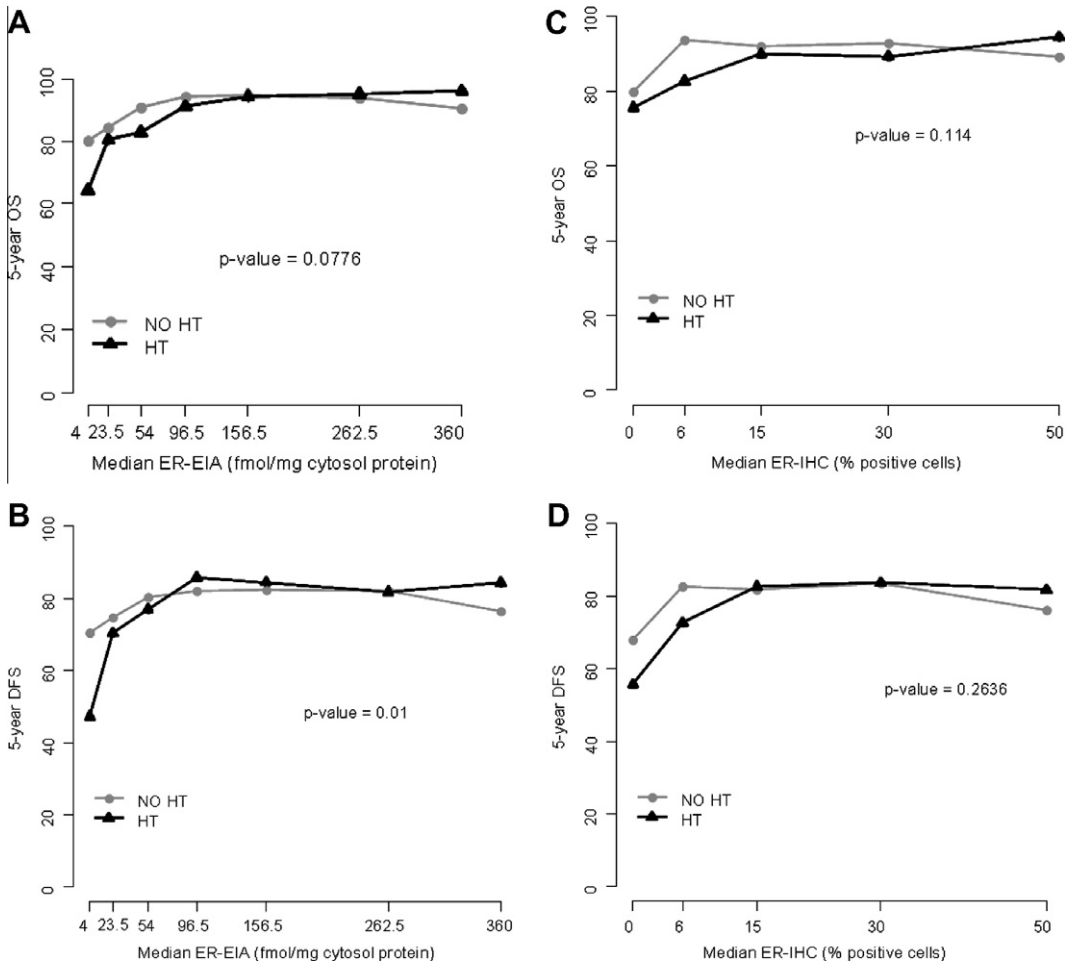

Fig. 4 – (A–D) The STEPP analyses were used to explore the pattern of hormone therapy (HT)-affected differences in terms of 5-year OS or DFS according to ER continuous values. The plot shows a trend towards differential survival according to EIA-determined ER expression in the no HT group and in the HT group.

($p = 0.01$). Interestingly, when the ER expression values were quite high (262.5 fmol/mg cytosol protein), the 5-year DFS achieved by the group without hormonotherapy was lower than that achieved by the hormonotherapy group, suggesting a benefit for adjuvant treatment in this population. Fig. 4C and D shows the STEPP analyses according to IHC quantification: the survival curves of the no hormonotherapy and hormonotherapy groups were intertwined and no difference was observed for either OS or DFS.

4. Discussion

In this study, we investigated the benefit of expressing the ER status quantitatively instead of the commonly used qualitative description, in order to assess its impact on the interpretation of the prognosis. The heterogeneity of response to hormonal therapy observed among patients with ER+ BC, could indirectly reflect unstandardised definitions and methods of quantifying ER expression among studies.[23,24] Thus, the currently used binary qualitative classification of the ER status might not be the best system for selecting patients for hormonal treatment. Moreover, unlike the EIA method,[4] the IHC method used to assess the ER status has not undergone the process of quality validation and this could explain the variation in clinical interpretation.

In our analysis, applying a standard qualitative cut-off using EIA or IHC, we showed a discrepancy between the two techniques in the frequency of ER+ tumours. Thus, whereas the frequency of ER+ patients using EIA was consistent with the rates usually reported in large studies,[10,25] in contrast the rate of ER+ tumours was almost similar to that of ER-negative tumours using the IHC technique. Intra- and inter-laboratory variations are usually observed in all biological measurements. In order to avoid inter-laboratory variations, we only retained ER determinations obtained from a single biological laboratory platform. For EIA determination of ER expression, our laboratory participated in the quality control process when the EORTC group validated the technique,[4,5] whereas no inter-laboratory standardisation has been described at a comparable level for IHC. However, this difference in the frequency of ER+ tumours did not impact on the interpretation of the respective OS and DFS rates, since patients with ER+ BC determined by qualitative EIA or IHC analysis, benefitted from a significantly longer survival duration. In contrast to a recent paper[12] which showed no difference in OS for IHC-determined ER+ versus ER-negative BC, we

showed that whatever the method of determination used, the qualitative determination of ER expression yielded the same information regarding the prognosis. The results of the above-mentioned recent study might be explained by the fact that the ER methods were not used in the same population but in a comparative historical cohort, and as noted by the authors, tumour characteristics within their groups differed over time. The advantage of our study was the prospective application of both techniques to the entire population during the study period and this strengthens the value of our results. The value of quantitative determination of ER expression was confirmed in the multivariate analysis which showed a significant relationship between moderately+ and highly+ ER values and the prognosis.

Here we showed that the quantitative analysis of ER expression levels resulted in the identification of groups with different survival rates. Since the initial studies by McGuire[13] or Heuson et al.[14] which showed the importance of quantitative ER expression in determining endocrine responsiveness, surprisingly, very few reports in the literature have presented survival data according to quantitative ER expression levels as we report herein. In a recent study analysing micro-array assessment of hormonal receptors,[26] the authors showed a wide variation in quantitative determination of ER expression by IHC in the 0–100% range. Interestingly, analysing the survival curves according to the technique used to determine ER expression (IHC and EIA) did not result in the same interpretation. Indeed, EIA-determined quartiles of ER expression more clearly distinguished weakly+ ER expression from other categories, whereas according to IHC data, this population (weakly+ ER expression) was not distinguishable from the moderately ER+ subgroup.

Another important aspect of this study was the assessment of the impact of quantitative ER measurement on the evaluation of response to hormonal therapy. Here we showed that the administration of hormonal therapy clearly distinguished BC categories with different outcomes. As previously reported, patients with the highest ER values enjoyed a longer survival duration whereas, the group with a weakly+ ER status obtained a marginal or no benefit from hormonal therapy. During the first years of the study, some patients with a 'negative' ER status received tamoxifen and we confirm the detrimental impact of hormonal therapy on OS and DFS in the group with a truly negative ER status, and to a lesser extent, in patients with a weakly+ ER status. These data confirm the deleterious effect of hormonal treatment in patients with truly ER-negative BC reported in previous studies. A recent analysis of patients with ER-negative BC from the Geneva Cancer Registry,[27] showed a 1.7% HR for death among the ER-negative patients treated with tamoxifen. This observation reached the same conclusion as our previous work on the detrimental effect of hormonotherapy in ER-negative patients.[28] Subpopulation treatment effect pattern plot (STEPP) methodology is probably more accurate for better estimating the trend in the effect of tamoxifen treatment differences according to quantitative ER subgroups.[20,21] The STEPP plots helped us to estimate the pattern of differential survival rates according to continuous variable expression and the treatment received. For instance, the benefit of chemotherapy could be better estimated, as demonstrated by Thürlimann and colleagues by showing no clear benefit from chemotherapy in non-menopausal patients with low-risk, node-positive endocrine responsive tumours.[29] Interestingly, using STEPP analysis, Pagani and colleagues showed a minimal effect of chemotherapy against tumours with high levels of ER expression in menopausal patients, but a benefit in ER-negative tumours.[30] In their study, Castiglione and colleagues showed that the CMF-containing regimens yielded superior disease-free survival across all age groups for patients with ER-negative tumours, whereas the benefit of the sequential regimen for patients with ER-positive disease increased substantially as the median age of the patient subpopulation decreased.[31] In our study, given the multiplicity of chemotherapy regimens, we were unable to indicate a cut-off level of chemotherapy efficacy. However, in our study, STEPP analyses of treatment outcome according to continuous measurements of EIA- and IHC-determined ER expressions suggest that the magnitude of the benefit from hormonotherapy may differ in predictable ways for patient subgroups within our cohort. The analysis of survival curves showed that the subgroups with high EIA-determined ER expression levels obtained a greater benefit from hormonotherapy than without it. However, the difference was only observed for the EIA-determined ER groups and not for IHC-determined ER subgroups.

Finally, this report confirms the advantage of quantitative ER expression as well as the value of EIA-determined ER expression. Recent editorials[23,24] have expressed concern about potential misdiagnosis with IHC. Despite pre-analytical disparities and non-reproducibility between laboratories, the IHC technique has been widely adopted. Other new attractive methods will probably be proposed for ER analysis, such as DNA/RNA quantification as well as RT-PCR measurement. However, the process of quality validation, supervised by reference committees, should be addressed when standardising these procedures. In the meanwhile, the question of reintroducing quantitative determination procedures with quality control for ER assessment should be addressed. In order to overcome IHC heterogeneity in determining positivity, some scoring systems have been proposed: the Reiner score,[32] Remmele score[33] and the Allred score.[34] All these scoring systems combine the intensity and the number of positive cells in different ways to calculate the score. In particular, the Allred score which evaluates the proportion of positive cells and staining intensity has been shown to have an equivalent or better ability to predict response to hormonal therapy.[35] However, these methods probably need quality control assessment and a learning curve, since an Austrian inter-laboratory study showed laboratory and observer variability in estimating the intensity and percentage of receptor-related immunostaining.[36] Furthermore, the score obtained can vary according to the detection method used and a recent report showed that the scoring system based on the percentage of positive cells could have an edge over other scoring systems.[37] Unfortunately, in our study we were unable to evaluate the value of this scoring system since it has only been recently applied in our laboratory. Beyond their role in the standardisation of ER quantification, these scoring systems have been developed to improve the predictive capacity of the IHC procedure in determining response to adjuvant treatment. Interestingly, Albain and colleagues suggested that

IHC-determined quantification of ER expression could help identify patients with ER+ tumours who may benefit from adjuvant chemotherapy in addition to hormonal therapy.[38] However, some authors have argued that the scoring systems which include staining intensity do not confer an absolute advantage since they do not reflect the EIA value or protein content accurately.[39]

In conclusion, the quantitative evaluation of ER expression could be useful for the evaluation of the prognosis in breast cancer and of response to adjuvant therapy. Our data showed that quantitative analysis of ER expression highlights the heterogeneity of ER expression in both ER-negative and ER-positive breast cancer. This quantitative approach helps to better discriminate candidates for hormonal therapy.

Conflict of interest statement

All authors of the present paper declare no financial and personal relationships or conflict of interest.

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