

PROGNOSTIC SIGNIFICANCE OF AN ESTROGEN RECEPTOR FUNCTIONAL ASSAY WITH TAMOXIFEN IN BREAST CANCER

E. Levin, A.M. Actis, S. Caruso, R. Romero, H. Gass, N. Qualeta and R.W. Levin.
 Facultad de Medicina and Velaz Sarsfield, Tigre and Alvarez Hospitals. Buenos Aires. Argentina.

ABSTRACT

A Displacement Assay with tamoxifen based on the relative binding affinity of tamoxifen (Tam) and estradiol for the estrogen receptor (ER) in ER+ breast neoplasms, as predictor of outcome and response to hormonal treatment in 73 patients is evaluated. The Displacement Assay allowed ER+ tumors to be grouped as Displaceable (D) or Weakly Displaceable (WD) with the implication that D tumors should respond better to Tam administration. All patients received Tam associated or not with chemo or radiotherapy according to stage and evolution, but Tam was the only prolonged treatment applied. Survival and disease-free interval (DFI) curves (Kaplan-Meier method) showed highly significant differences between patients with ER+ D and ER+ WD tumors at 9 years after surgery. Seventy four % of the group with ER+ D tumors were alive in this period, versus 37% for the WD group. Similar figures were obtained for DFI ($p < 0.0001$ and $p < 0.006$ respectively). Association with axillary node number increased the significance for both outcome indicators. Progesterone receptor (PgR) measurement as another ER functional expression failed to show significant difference for survival and DFI between ER+ PgR+ and ER+ PgR- tumors, reflecting that Displacement Assay and PgR data correspond to two independent ER functional expressions. Displacement Assay results appear as reliable prognostic markers of breast cancer outcome and contribute to more appropriate treatment decisions in this pathology.

OBJECTIVE

Evaluation of an ER functional test (Displacement Assay) for mammary neoplasias based on the relative binding affinity of Tam and E₂ for the receptor.

INTRODUCTION

The outcome of 73 patients with ER+ breast tumors and 5 to 9 years evolution is presented.

Results of the Displacement Assay allowed classification of ER+ tumors in:

A) Displaceable (D): $(D_{50}E_2/D_{50}Tam) \cdot 100 = \geq 0.1$

B) Weakly Displaceable (WD): $(D_{50}E_2/D_{50}Tam) \cdot 100 = < 0.1$

D₅₀: Ligand concentration for 50% inhibition of ³H-E₂-ER binding
 PgR determination as another ER functional expression is included.

METHODS

ER and PgR determinations: D-C method

Displacement Assay: E₂ conc: 5, 10, 30, 50, 2000 nM

Tam conc: 1, 3, 5, 10, 30, 50, 50 μM

Calculations: EBDA program. Cox proportional hazards regression model. Log rank test for Kaplan Meier graphics.

Significance: $p < 0.05$

Reference for Displacement Assay technique: LEVIN E, TOMCHINSKY S, LOPEZ SJ. Displacement by tamoxifen of the estradiol-estrogen receptor binding: a functional assay for breast cancer studies.

J. Steroid Biochem. Mol. Biol. **37**, 681-686, 1990.

RESULTS

TABLE 1

Predictive value of the Displacement Assay for survival at 9 years in the 73 patients ER+ breast tumors (including all tumor stages)

Displacement	Survived	Dead	Total
D DI ≥ 0.1	34	12	46
WD DI < 0.1	10	17	27
Total	44	29	73

D: Displaceable. WD: Weakly Displaceable. DI: Displacement Index

Predictive values:

D: Positive prediction: 73.9% (34/46)

False negative: 26.1% (12/46)

WD: Negative prediction: 63.0% (17/27)

False positive: 37.0% (10/27)

Chi²: p < 0.005

FIGURE 1

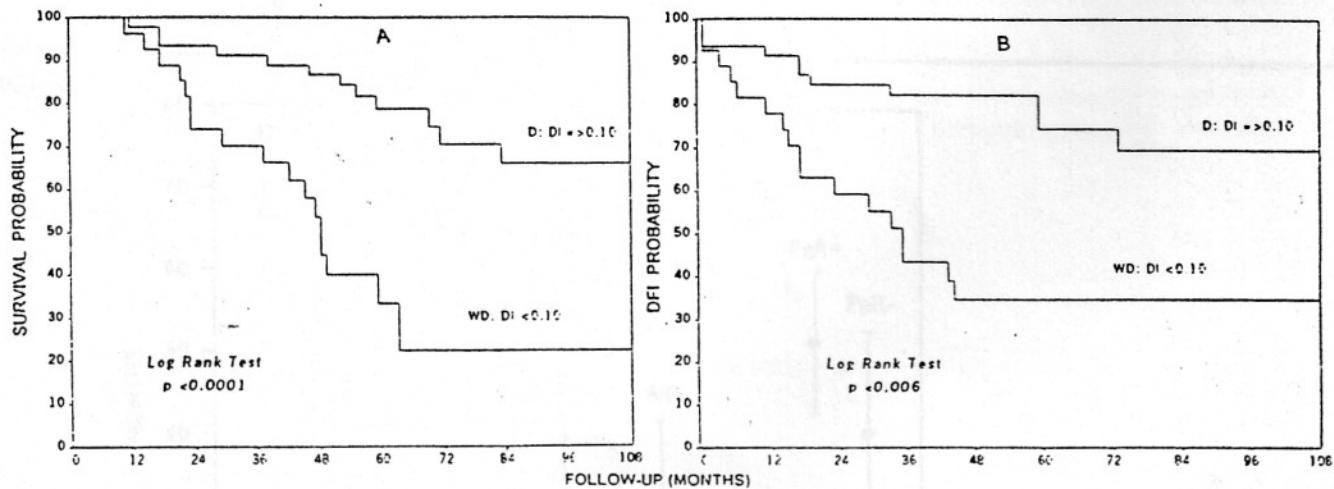


FIGURE 1. Survival (A) and DFI (B) probability curves according to Displacement Assay tumor categories (n: 73). D: Displaceable. WD: Weakly Displaceable. DI: Displacement Index.

FIGURE 2

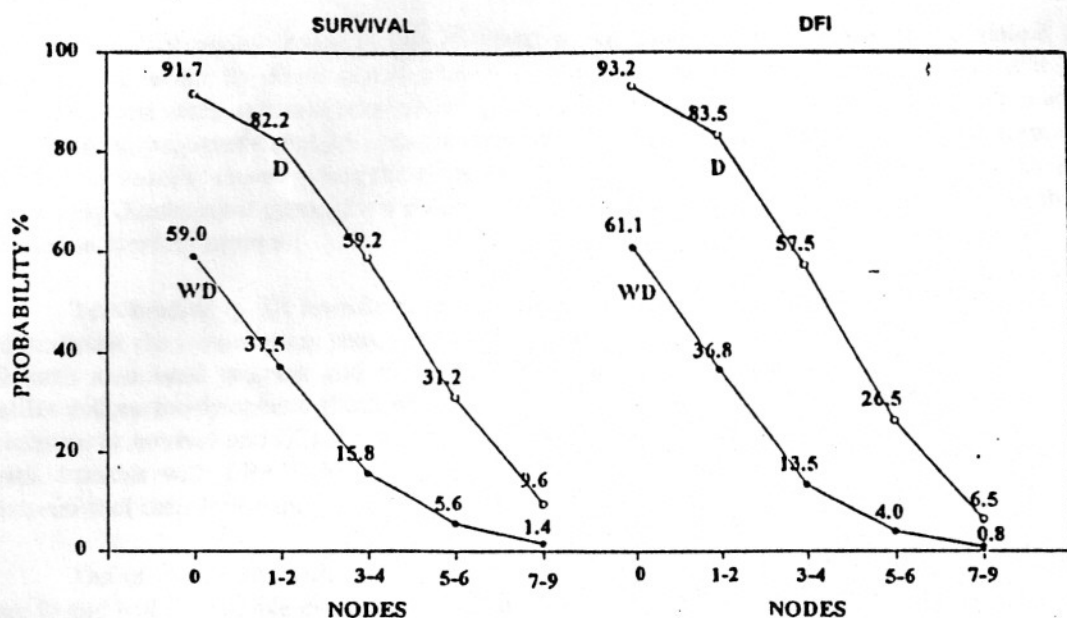


FIGURE 2. Survival and DFI probability in breast cancer patients with ER+ tumors according to Displacement Assay categories and axillary node number. Logistic regression: For survival, Displacement: $p < 0.002$, Nodes: $p < 0.0006$. For DFI, Displacement: $p < 0.002$, Nodes: $p < 0.0003$.

TABLE 2

Probability of metastasis or relapse at 9 years according to Displacement tumor categories and axillary node number

Nodes	Displaceable	Weakly Displaceable
0	6.8 %	38.9 %
1 - 2	16.5 %	63.2 %
3 - 4	42.5 %	86.5 %
5 - 6	73.5 %	96.0 %
7 - 9	93.5 %	99.2 %

FIGURE 3

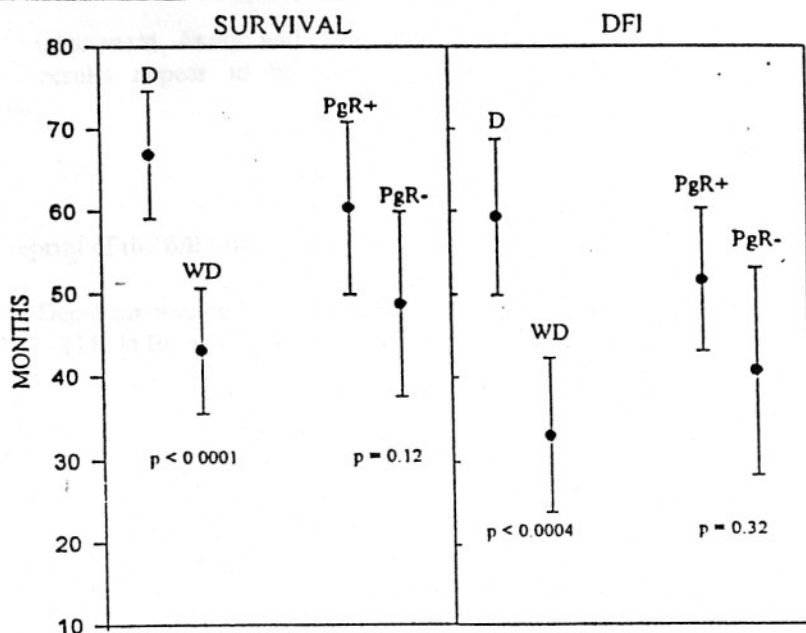


FIGURE 3. Survival and disease-free interval in patients with ER+ breast tumors ($n = 73$). Data for Displacement Assay categories and PgR expression. Mean \pm 2 SE. (Mann-Whitney U-test).

DISCUSSION

The Displacement Assay proposed, based on the competition of Tam with the natural ER ligand in each tumor, should reflect the main antineoplastic capability of the drug. However, resistance to hormonal therapy develops in most cases, specially after prolonged treatments even when associated with chemo and/or radiotherapy. In this respect, treatments applied in the current study, although not following a unified protocol, used Tam for D and for WD tumors, showing that the response was more favorable and prolonged in the Displaceable category. The Weakly Displaceable group, even under Tam administration, had a poorer response, as if the tumors were ER- as stated in previous reports.

Tam binding to ER introduces changes in the receptor protein reflected in pleiotropic routes that convey and modulate the transcription message: binding to the cognate DNA response elements, dimers formation, cross-talk with associated proteins and members of other transduction pathways, phosphorylation patterns, isoform profiles and nucleo-cytoplasm shuttling as well as others not yet sufficiently explored. Displacement and PgR data in relation to survival and DFI showed that PgR expression was not a reliable prognosis indicator in this 73 patient series. Patients with ER+ PgR+ D tumors should have a better outcome than those with ER+ WD tumors irrespective of their PgR expression.

Out of 16 patients with tumors nuclear grade 3 and mitotic index higher than 6, 11 were WD. The other 5 were D and had a favorable evolution according to survival: one tumor, stage II, 102 months; 3 tumors, stage III, 56, 70 and 84 months; and one tumor, stage IV, 46 months survival. To sum up, the Displacement Assay with Tam, as an ER functional expression, appears to be a reliable prognostic indicator in ER+ breast neoplasias by providing a further categorization into Displaceable and Weakly Displaceable tumors. Data from the Displacement Assay associated with other prognostic features help to make more appropriate treatment decisions, even for some cases where discrepancies with other prognostic factors are apparent.

CONCLUSIONS

1. Displacement Assay data (D and WD) allowed classification of ER+ breast tumors into two categories according to the relative binding affinity of E_2 and Tam for ER.
2. Survival and disease free interval (DFI) results show that at 9 years after surgery, patients with ER+ D tumors have a favorable evolution (74% alive; 80% DFI). Those with ER+ WD tumors have a worse evolution (63% dead; 63% with metastasis/relapse).
3. Association of Displacement results (D or WD) with axillary node number increases the significance of each of these outcome indicators.
4. Displacement Assay and PgR data correspond to two independent ER functional expressions. Displacement results appear to be more reliable prognosis indicators for Survival and DFI than PgR determinations.

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Dr. E. Levin - Departamento de Bioquímica Humana - Facultad de Medicina
Paraguay 2155 - (1121) Buenos Aires - Argentina

