A simple and reproducible prognostic index in luminal ER-positive breast cancers.

poor prognoses. Finally, we validated the results in an independent series of ER-positive breast cancers.

patients and methods

study design and population

A series of 543 patients diagnosed with ER-positive breast cancer between 1994 and 2005 was collected at the Breast Unit of the San Giovanni Battista-Molinette Hospital of Turin, Italy, of these, 385 patients were part of a previous recently published study [12].

Sections of the 158 newly collected tumors were reviewed. Multicore tissue microarrays (TMAs), prepared as previously described [14], were retested by immunohistochemistry (IHC) for ER-status; 48 of 543 tumors were ER-negative. These cases, confirmed as negative by IHC carried out on whole tissue sections, were excluded from the study. The medical charts of the remaining 495 patients (test series) were reviewed and updated.

A cohort of 581 ER-positive breast cancers centrally reviewed and collected in the same period from OIRM Sant'Anna Hospital of Turin, Italy, was used as 'validation series' (supplementary Table S1, available at *Annals of Oncology* online).

IHC was carried out as previously reported [12], to assess AR (mouse monoclonal antibody (mAb) clone AR441, Dako, Glostrup, Denmark), ER (rabbit mAb, clone SP1, Ventana-Diapath, Tucson, AZ) and progesterone receptor (PR) (rabbit mAb, clone 1E2, Ventana-Diapath). The proliferation index was assessed using the Ki67 mouse mAb (clone MIB-1, Dako). HER2 status was evaluated using the Herceptest™ (Kit Dako). Equivocal (score 2+) cases were investigated by FISH assay (Vysis, Inc., Downers Grove, IL).

The cut-off value for ER- and PR-positivity was set at 1%, and the same cut-off was also adopted for AR-positivity [12]. The percentage of Ki67-positive cells was counted at the section periphery.

statistical analysis and construction of the prognostic index

The follow-up time was calculated using the median observation time among all patients. The follow-up was censored at the time of death or of the last clinical investigation of the patient. DSS was calculated from the date of definitive surgery to the date of death from the disease. Patients dying from other causes were censored at the time of death. In the test series of 495 patients, DSS was studied using the Kaplan–Meier method and the Log-rank test (supplementary Figure S1, available at *Annals of Oncology* online). The clinical and pathological parameters used for univariate analysis are reported in supplementary Table S2, available at *Annals of Oncology* online. The best cut-off value of tumor size was established at 15 mm [15–20]. For the multivariable analysis, prognostic factors were selected based on their statistical significance at univariate analysis. A Cox proportional hazard model was used and the effect of single variables was expressed as hazard ratio (HR) with 95% confidence interval (95% CI). Three covariates maintained their statistical significance: tumor size, number of metastatic lymph nodes and AR status. A score was attributed to each variable according to its HR. Its weight value was approximately twofold for tumor size and AR than for number of metastatic lymph nodes (supplementary Table S3, available at *Annals of Oncology* online). Thus, a score value of 2 was given to tumors >15 mm and to tumors with AR-negative. Instead, tumors ≤15 mm and tumors with AR-positive were scored as 0. Three score values were used for lymph nodes (0: lymph nodes free of metastases; 1: from 1 to 3 metastatic lymph-nodes and 2: >3 metastatic lymph nodes). A PI for ER-positive cancers (ERPI) was created using the following formula: (tumor size score value) + (number of metastatic lymph nodes score value) + (AR score value) (Table 1). The two extremes of the ERPI were 0 and 6. Kaplan–Meier analysis was then carried out for each ERPI value (Figure 1A). Following the performance curves, we set the cut-off of the ERPI at 3: value ≤3 good prognosis (ERPI-good), value >3 poor prognosis (ERPI-poor) (Figure 1B).

Table 1. Algorithm to calculate the ERPI

	Status	Points
Number of metastatic lymph nodes	0	0
	1–3	1
	>3	2
Tumor size	<15 mm	0
	>15 mm	2
Androgen receptor	0%	2
	≥1%	0

Algorithm: (tumor size score value) + (number of metastatic lymph nodes score value) + (AR score value).

A univariate analysis was carried out to study the effect of the ERPI in the entire test population and in selected subpopulations, namely G2 tumors, luminal-A and luminal-B cancers. The distinction between luminal-A and luminal-B was defined according to the proliferation rate by Ki67 (cut-off of 14%) and to *HER2*-status [21].

To validate the results, we applied the ERPI to the case series from Sant'Anna Hospital.

Receiver-operating characteristic (ROC) curve analysis was used to evaluate the ERPI performance in predicting DSS by comparing its value with other single prognostic factors.

SPSS version 17 (SPSS, Inc., Chicago, IL) software, the R environment (www.r-project.org), SAS version 9.1 (SAS Institute, Cary, NC) and S-PLUS version 6.1 (Insightful Corp., Seattle, WA) were used for statistical analyses.

results

descriptive analyses of the test and the validation cohorts

The median follow-up time was 7.8 years. The comparison of the two cohorts of patients (supplementary Table S1, available at *Annals of Oncology* online) revealed some differences in the type of treatment (surgery, radiotherapy and chemotherapy), probably because of the differences in the size of tumors and presence of vascular invasion.

ERPI results on the test cohort

At univariate analysis age, treatment type, ER expression levels and *HER2*-status did not show any significant correlation with DSS. At multivariate analysis only tumor size, number of metastatic lymph nodes and AR status resulted significant for prognosis (supplementary Tables S2–S3, available at *Annals of Oncology* online). The ERPI, built on the basis of the HR, was applicable to 385 patients, 92.5% of whom were censored at follow-up.

The rate of patients censored for ERPI-good was 96.9% and 79.6% for ERPI-poor, with a highly significant difference ($\chi^2 = 40.037$, $P < 0.001$) (Figure 1B, supplementary Table S4, available at *Annals of Oncology* online). ERPI maintained its statistical significance both in patients that received hormonal

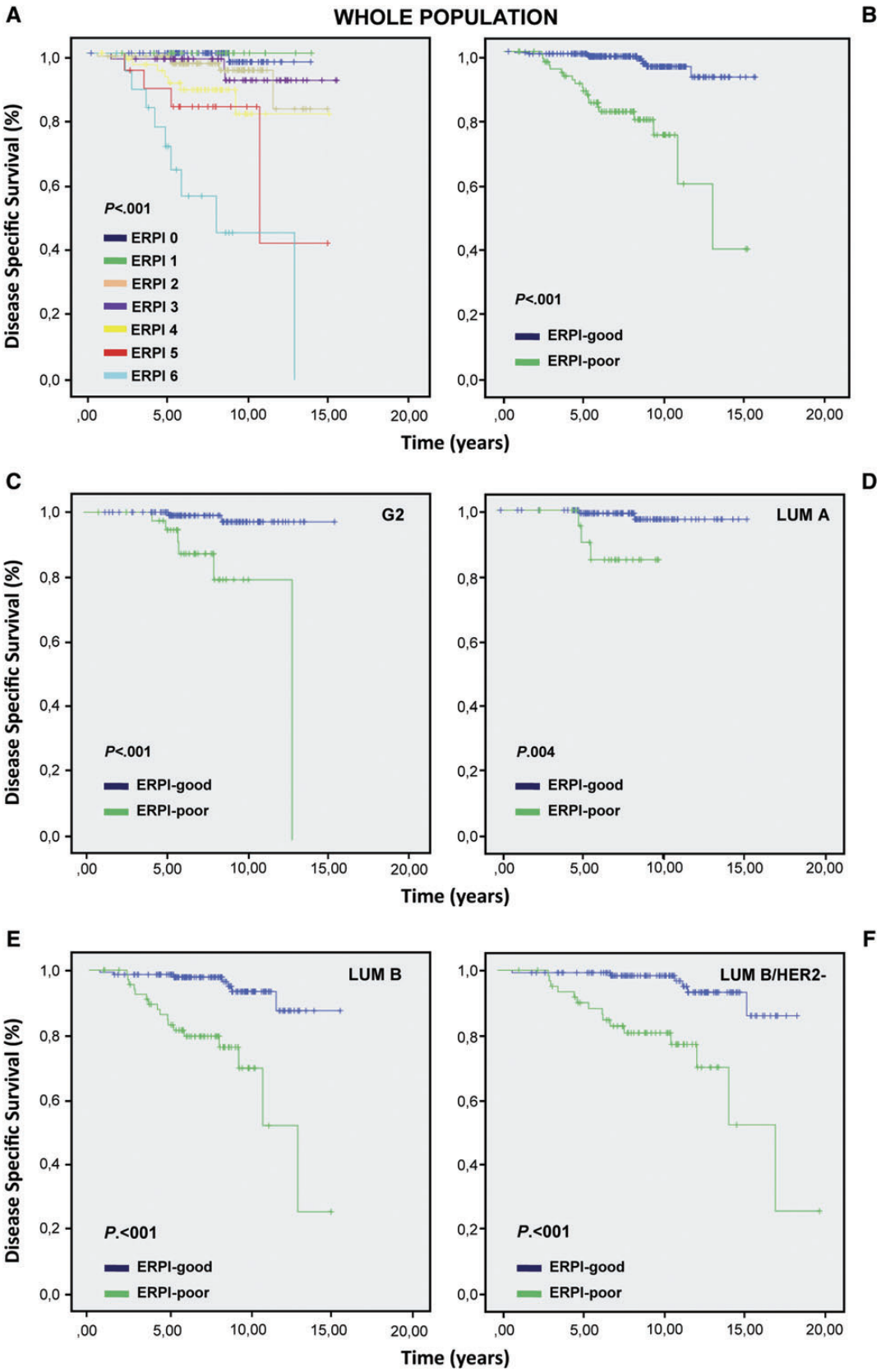

Figure 1. Kaplan–Meier analysis carried out on test cohort for each ERPI value (A). Kaplan–Meier analysis carried out for ERPI value using a cut-off of 3: a value ≤3 was considered as ERPI-good and a value >3 as ERPI-poor (B). ERPI on G2 (C), luminal-A (D) luminal-B and luminal-B HER2-negative cases (E, F).