Prognostic Value of p53 and Urokinase-type Plasminogen Activator in Node-negative Human Breast Cancers

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ABSTRACT

We measured the levels of p53 and urokinase-type plasminogen activator (uPA) in 634 tumor tissues from 634 different node-negative primary breast cancer patients who underwent locoregional surgery in the Center Oscar Lambret between July 1989 and September 1994. p53 and uPA were assayed using commercially available kits in cytosols prepared for estradiol receptor (ER) and progesterone receptor (PgR) assays. The optimum clinical thresholds were chosen for prognostic studies: 4 ng/ml for p53 and 0.5 ng/ml for uPA. p53 was elevated in 13.7% of the tumors, and uPA was elevated in 27.5% of the tumors; they were negatively related (χ² test) to ER and PgR and positively related to histoprognostic grading (HPG) and tumor diameter. uPA was negatively correlated to ER and PgR, and p53 and uPA were positively correlated to each other (P = 0.0001; Spearman test). In the prognostic studies, the 316 patients who did not receive adjuvant chemotherapy were included to avoid treatment interference; this number corresponds to all of the patients operated on between 1989 and 1992. The mean duration of follow-up of living patients was 4 years. In overall survival studies, Cox univariate analyses demonstrated a prognostic value of p53 (P = 0.011; risk ratio, 1.59), uPA (P = 0.038; risk ratio, 2.32), PgR, HPG, and tumor diameter. In Cox multivariate analyses, only HPG had a statistically significant prognostic value. In relapse-free survival studies, univariate analyses demonstrated prognostic values of uPA (P = 0.0011) and of age, and both parameters retained their prognostic value in multivariate analyses (uPA: P = 0.0004). This study demonstrates not only that p53 and uPA have prognostic value but also that these two parameters are linked to other classical clinical, histological, or biological prognostic parameters, as well as to each other. Moreover, because uPA is of prognostic value in multivariate relapse-free survival studies, uPA is an important prognostic factor in node-negative breast cancer patients.

INTRODUCTION

The results of prospective randomized clinical trials and of overview analyses suggest that systematic adjuvant therapy can benefit node-negative breast cancer patients (1). Node-negative breast cancers are heterogeneous; 75% of the patients were alive and without recurrence after 10 years. The finding of selective prognostic factors to identify patients in this group at high risk of recurrence would avoid excessive treatment morbidity and costs (2).

Among the plethora of potentially useful markers that have been identified, two appear to be of particular interest: p53 and uPA.3

Of the genetic changes found in human cancer, the alteration of the tumor suppressor gene p53 is the most common (3). In the majority of cases, these alterations correspond to point mutations localized in one allele of the gene, whereas the second wild-type allele is lost. In tumors, p53 mutations change the conformation of the protein and lead to stabilization of p53 and its accumulation in the nuclei of cancerous cells (4). Using molecular analyses of the p53 gene (sequencing), immunohistochemistry, or measurement of circulating p53 antibodies, it has been demonstrated in a number of studies that alterations to p53 are related to shorter RFS and poorer OS (Table 1). All of these technical approaches have pitfalls and/or are difficult to use for routine detection.

uPA is a serine protease that catalyzes the conversion of the inactive proenzyme plasminogen to plasmin. It was initially identified in urine and is produced by many normal and malignant cells. This protease has a role in tissue remodelling, in the degradation of extracellular matrices, and in the destruction of the basement membrane in malignant cell proliferation and metastasis (5). Overexpression of uPA is strongly related to shorter RFS and poorer OS (Ref. 6; Table 2). Consequently, p53 and uPA, which are differently implicated in the development of breast cancer, are prognostic parameters. However, no study has simultaneously evaluated, in the same patient population, the relative prognostic weight of

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3 The abbreviations used are: uPA, urokinase-type plasminogen activator; HPG, histoprognostic grading; ER, estradiol receptor; PgR, progesterone receptor; LIA, luminometric immunoassay; OS, overall survival; RFS, relapse-free survival; RR, risk ratio.
these two new biological markers compared with conventional clinico-pathological markers.

We report here on the results obtained for cytosolic uPA and p53 determinations in 634 primary breast cancer from node-negative patients using LIAs for p53 (7) and uPA (8).

PATIENTS AND METHODS

Patients. Included in this study were 634 tumor samples from 634 different node-negative breast cancer patients undergoing surgery for locoregional disease in the Centre Oscar Lambret (the Anticancer Center of the North of France, Lille, France). Inclusions were between July 1989 and September 1995. The mean age of patients was 58 years (range, 28–85); 70% of patients were postmenopausal. Clinical tumor size was less than 2 cm in 3.8% of cases, between 2 and 5 cm in 82%, and more than 5 cm in 14.2%. All patients were treated by segmentectomy if the tumor was less than 3 cm wide and by total mastectomy if the tumor was larger or was centrally located. Axillary dissection was carried out in all cases. Surgery was followed by radiation therapy on the chest wall after total mastectomy or on the remaining breast tissue after segmentectomy, and on the internal mammary, subclavicular, and supraclavicular nodes. Included in the prognostic studies were the 316 node-negative patients who had not received adjuvant treatment and who corresponded to patients seen up until 1992. After this date, all of the patients received chemotherapy and were therefore not included to avoid treatment interference. For the selected patients, the minimum duration of follow-up was 3 years, and the mean duration of follow-up was 4 years. Within this population, the number of deaths was 40, and the number of relapses was 64.

Pathology. At the time of collection, tumor samples were divided into two parts: one was frozen for receptor analysis, and the other was submitted to histological study. Tumor specimens consisted solely of invasive adenocarcinomas. Seventy-two% were ductal, 13% were lobular, and 5% were of other types.

HPG according to the method of Contesso et al. (9) was carried out in the 567 ductal carcinomas: 1.3% were grade I, 37.9% grade II, and 37% grade III.

ER and PgR Assay. Both ERs and PgRs were determined by the dextran-coated charcoal method, as described previously (10). For preparation of cytosols, the frozen tissues were weighed and then pulverized. The tissues were homogenized in 20 mm Tris, 3 mm EDTA, 1 mm DTT, 0.01% azide, 0.01 m sodium molybdate, pH 7.6. The homogenate was centrifuged at 800 × g for 10 min, and the supernatant was ultracentrifuged at 105,000 × g for 60 min. Our laboratory is affiliated with the European Organization for Research and Treatment of Cancer Receptor Study Group, which undertook the quality control of the assays (11).

Immunoluminometric Assay for the Quantification of p53 and uPA. The LIA kits were provided by Byk-France (LIA-mat p53 and LIA-mat uPA, AB Sangtec Medical,

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Table 1  p53 prognostic value in primary breast cancer

<table>
<thead>
<tr>
<th>Reference</th>
<th>Methoda</th>
<th>Pop.</th>
<th>p53+/n (%)</th>
<th>Follow-up (months)b</th>
<th>RFS UV (P)</th>
<th>RFS MV (P)</th>
<th>OS UV (P)</th>
<th>OS MV (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ostrowski et al. (21)</td>
<td>IHC</td>
<td>32/90 (36)</td>
<td>28</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Thor et al. (18, 19)</td>
<td>IHC</td>
<td>68/295 (23)</td>
<td>72</td>
<td>0.003</td>
<td>0.0391</td>
<td>0.008</td>
<td>0.0524</td>
<td></td>
</tr>
<tr>
<td>Isola et al. (16)</td>
<td></td>
<td>N</td>
<td>31/127 (24.4)</td>
<td>0.018</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silvestrini et al. (17)</td>
<td>IHC</td>
<td>113/256 (44)</td>
<td>72</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Barnes et al. (22)</td>
<td>IHC</td>
<td>38/195 (19)</td>
<td>125</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Thorlacius et al. (23)</td>
<td>CDGE, seq.</td>
<td>18/109 (16.5)</td>
<td>32</td>
<td>NS</td>
<td>0.001</td>
<td></td>
<td></td>
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<tr>
<td>Andersen et al. (24)</td>
<td>CDGE, IHC</td>
<td>42/163 (26)</td>
<td>45.8</td>
<td>0.0002</td>
<td>0.0005</td>
<td>0.0163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alfred et al. (15)</td>
<td>IHC</td>
<td>362/700 (52)</td>
<td>54</td>
<td>0.0001</td>
<td>0.008</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stemmark et al. (25)</td>
<td>IHC</td>
<td>37/164 (22.6)</td>
<td>60&lt;0.001</td>
<td>0.002</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gasparini et al. (26)</td>
<td>IHC</td>
<td>57/254 (28)</td>
<td>62</td>
<td>0.004</td>
<td>0.0063</td>
<td>0.024</td>
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<tr>
<td>Elledge et al. (27)</td>
<td>IHC</td>
<td>76/169 (45)</td>
<td>60</td>
<td>NS</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Saitoh et al. (28)</td>
<td>Seq.</td>
<td>21/53 (39.6)</td>
<td>19</td>
<td>0.05</td>
<td>0.003</td>
<td>0.003</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td>Domagala et al. (29)</td>
<td>IHC</td>
<td>68/227 (30)</td>
<td>90&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
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<tr>
<td>Witschke et al. (30)</td>
<td>WB</td>
<td>49/105 (46.7)</td>
<td>71.3</td>
<td>NS</td>
<td>NS</td>
<td>0.02</td>
<td></td>
<td></td>
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<tr>
<td>Cunningham et al. (31)</td>
<td>IHC</td>
<td>39/247 (16)</td>
<td>78</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diploid</td>
<td>31/72 (43)</td>
<td>0.02</td>
<td>0.01</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosen et al. (20)</td>
<td>IHC</td>
<td>92/440 (21)</td>
<td>119</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<tr>
<td>Bergh et al. (32)</td>
<td>Seq.</td>
<td>69/312 (21.8)</td>
<td>60</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>36/201 (17.9)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peyrat et al. (14)</td>
<td>p53-Ab</td>
<td>42/553 (12)</td>
<td>64</td>
<td>NS</td>
<td>NS</td>
<td>0.0005</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>Borg et al. (7)</td>
<td>LIA</td>
<td>62/205 (30)</td>
<td>50</td>
<td>0.003</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>Horne et al. (33)</td>
<td>IHC</td>
<td>111/228 (46.6)</td>
<td>0.0171</td>
<td>0.0307</td>
<td></td>
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<tr>
<td>Sjroen et al. (34)</td>
<td>IHC</td>
<td>64/316 (20)</td>
<td>60</td>
<td>0.02–0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

a IHC, immunohistochemistry; CDGE, constant denaturant gel electrophoresis; Seq., sequencing; WB, Western blot; p53-Ab, circulating p53 antibodies; Pop., population; N+: node-positive; N–: node-negative patients; p53+, number of patients p53 positive; n, total number of patients; UV, univariate analysis; MV, multivariate analysis; NS, not significant.
b Mean duration of follow-up except where noted.
c Maximal duration only.
Bromma, Sweden). These assays are monoclonal two-site incubation immunoluminometric assays (sandwich principle); the immunological reaction is detected by a light reaction through the oxidation of the isoluminol bound to antibodies. The p53 assay procedure for quantitative analysis of mutant and wild-type p53 protein in tumor extracts has been described previously (7). The antibodies used bind to denaturation-resistant epitopes at the NH₂ terminus of the protein and allow the quantitative determination of both wild-type and mutant p53. The monoclonal uPA antibodies used bound to urokinase in its proenzyme form, to the active enzyme, to urokinase bound to its receptor and to urokinase bound to the inhibitor PAI-1. p53 was assayed in 634 cytosols, and uPA was assayed in 600 cytosols, prepared for ER and PgR assays. All of the assayed cytosols were preserved at −20°C, −80°C, and in liquid nitrogen, and we did not find any difference among the different storage conditions (data not shown).

Statistical Analyses. Statistical analyses were carried out using the SAS statistical software on a VAX VMS 6320. Relationships between qualitative variables were determined using the χ² test (with Yates correction when necessary). Correlations between parameters were assessed according to the Spearman nonparametric test. Moreover, to make these correlations explicit, linear correlations (Pearson test) were performed on subsets of the statistical population, as detailed in a previous paper (12). OS and RFS were studied by actuarial method analysis. Comparison between curves was carried out by the log rank test. The proportional hazards regression method of Cox (13) was used to assess the prognostic significance of parameters taken in association. No time-dependent variable was introduced. The latter analyses were performed with Dash software (Dash Software Development Group, Boston, MA).

RESULTS

p53 and uPA Concentrations in Human Breast Cancer

The distribution of p53 concentrations in the tumors was not normal (Fig. 1). The median value was found to be 0.38 ng/mg protein, and the range was 0–108.4. The p53 concentrations were lower than 1 ng/mg protein in 77.6% of the cases and lower than 0.10 ng/mg protein in 12.4% of the cases. p53 concentrations were higher than 4 ng/mg protein in 13.7% of the cases (Table 3). The distribution of uPA concentrations is presented in Fig. 2. As for p53, the distribution was not normal. The median value was found to be 0.3 ng/mg protein, and the range was 0.01–16.3 ng/mg protein. The uPA concentrations were lower than 0.1 ng/mg protein in 14.5% of the cases and lower than 0.10 ng/mg protein in 12.4% of the cases. uPA concentrations were higher than 0.5 ng/mg protein in 27.5% of the cases (Table 3).

Relationship with Other Parameters

In this population, 70.7% of the tumors were ER positive and 62.7% were PgR positive (Table 3). The classical correla-

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**Table 2**  UPA prognostic value in primary breast cancer

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
<th>Pop.</th>
<th>uPA+/n (%)</th>
<th>Follow-up (months)</th>
<th>RFS UV (P)</th>
<th>RFS MV (P)</th>
<th>OS UV (P)</th>
<th>OS MV (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duffy et al. (36)</td>
<td>ELISA</td>
<td>N−</td>
<td>84/166 (51)</td>
<td>35</td>
<td>0.0005</td>
<td>0.0336</td>
<td>0.0004</td>
<td>0.031</td>
</tr>
<tr>
<td>Foeckens et al. (37)</td>
<td>ELISA</td>
<td>N−</td>
<td>215/671 (32)</td>
<td>48</td>
<td>&lt;0.0001</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grondahl et al. (41)</td>
<td>ELISA</td>
<td>N−</td>
<td>88/272 (32)</td>
<td>102</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Janicke et al. (35)</td>
<td>ELISA</td>
<td>N−</td>
<td>59/118 (50)</td>
<td>36/72 (50)</td>
<td>NS</td>
<td>NS</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Bouchet et al. (38)</td>
<td>ELISA</td>
<td>N−</td>
<td>89/229 (38.9)</td>
<td>30</td>
<td>0.0001</td>
<td>0.0002</td>
<td>0.0024</td>
<td>0.0041</td>
</tr>
<tr>
<td>Duggan et al. (39)</td>
<td>ELISA</td>
<td>N−</td>
<td>49/146 (33.6)</td>
<td>40/101 (39)</td>
<td>0.0098</td>
<td>0.0136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duggan et al. (39)</td>
<td>ELISA</td>
<td>N−</td>
<td>100/314 (31.8)</td>
<td>84</td>
<td>NS</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duggan et al. (39)</td>
<td>ELISA</td>
<td>N−</td>
<td>34/134 (25.4)</td>
<td>59</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferno et al. (8)</td>
<td>LIA</td>
<td>N−</td>
<td>230/688 (33)</td>
<td>42</td>
<td>0.0002</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Pop., population; EIA, enzymoimmunoassay; N−, node-negative patients; uPA+, number of patients uPA positive; n, total number of patients; UV: univariate analysis; MV, multivariate analysis.

*Mean duration of follow-up.
Table 3 Description of the studied population considering biological parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total no. of tumors</th>
<th>No. of positive tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>634</td>
<td>78</td>
</tr>
<tr>
<td>uPA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>600</td>
<td>165</td>
</tr>
<tr>
<td>ER&lt;sup&gt;c&lt;/sup&gt;</td>
<td>633</td>
<td>448</td>
</tr>
<tr>
<td>PgR&lt;sup&gt;c&lt;/sup&gt;</td>
<td>633</td>
<td>397</td>
</tr>
</tbody>
</table>

<sup>a</sup> p53 positivity threshold, 4 ng/mg protein.
<sup>b</sup> uPA positivity threshold, 0.5 ng/mg protein.
<sup>c</sup> ER and PgR positivity threshold, 10 fmol/mg protein.

Differences between ER and PgR (P < 0.0001; r = 0.37), ER and age (P < 0.0001; r = 0.35), and PgR and age (0.0142; r = 0.10) were observed. The presence of p53 or uPA was inversely related using the χ² test, with the presence of ER and PgR, whereas it was positively related with HPG and tumor diameter (Table 4). uPA concentrations were inversely correlated (Spearman test) with ER (P < 0.0001; r = −0.245) and PgR (P < 0.0002; r = −0.149). Finally, there was a positive relationship between p53 and uPA (χ² = 21.58; P < 0.0001), and there was also a positive correlation between these two parameters (Spearman test; P < 0.0001; r = 0.295). The distributions of p53 and uPA were log (ln) normal, confirming what we had found for other biological parameters in breast cancers (12). A statistically significant linear positive correlation was found between ln p53 and ln uPA (Fig. 3).

Prognosis Studies

OS. A total of 313 patients were included in this study because we lost contact with 3 patients. In actuarial survival studies, the best p53 threshold for prognosis was 4 ng/mg protein; however thresholds of 3, 5, or 10 ng/mg protein also allowed us to distinguish two populations of different prognosis. For uPA, the best threshold was 0.5 ng/mg protein; thresholds of 0.2, 0.3, 0.4, 0.6, 0.7, and 0.8 ng/mg protein also allowed us to distinguish two populations of different prognosis. Shorter OS was found in patients with elevated p53 (Fig. 4) and elevated uPA (Fig. 5). HPG, PgR, (threshold, 10 fmol/mg protein), and tumor size were also prognostic factors (Table 5). In multivariate analyses, when combining the parameters that have a prognostic value in univariate studies, only HPG had a statistically significant prognostic value (RR confidence interval, 1.13–3.39; Table 5).

RFS. A total of 301 patients were included in this study. For uPA, the best threshold was 0.5 ng/mg protein; a threshold of 0.6 ng/mg protein also allowed us to distinguish two populations of different prognoses. In actuarial survival studies, shorter RFS was found in patients with high levels of uPA (Fig. 6). Age was also a prognostic factor. Neither p53 (whatever the positive threshold was), ER, HPG, PgR, nor tumor size was a prognostic factor (Table 5). In multivariate analyses with the parameters that have a prognostic value in univariate studies, uPA (RR confidence interval, 1.2–3.44) preserved its statistically significant prognostic value (Table 6).

DISCUSSION

In this study, p53 and uPA were detected with a reliable LIA. An analogous p53 assay was used by Borg et al. (7), who chose a positive clinical threshold at 0.15 ng/mg protein, with about 30% of the tumors being positive. In the present study, 40 of 281 (14.2%) cases were p53 positive, with a threshold at 0.15 ng/mg protein, whereas 30% of the tumors were positive when the threshold was 0.9 ng/mg protein; 13.7% of the tumors were p53 positive when the optimum cutoff point (4 ng/mg protein) was used. A major source of discrepancies between the two studies was that the populations studied were different: in the study of Borg et al. (7), all of the primary breast cancers were included, whereas the present study was concerned only with node-negative primary breast cancers. There was no difference in tissue preservation and cytosol preparation between the two studies. Another source of discrepancies was the difference between an in-house methodology and the standard commercially available methodology used by ourselves. In fact, we observed that the standard curve in our experiments was shifted to the left compared to the standard curve published by Borg et al. (7).

In the literature, the percentage of p53-positive tumors ranged from 12% (14) to 52% (Ref. 15; Table 1). In most of the studies published (15 of 21 cases; shown in Table 1), the method used was immunohistochemistry. This method is not standardized: comparison of the published studies is hampered by the diversity of procedures and reagents used by different investigators. There are differences in the antibodies used and the preservation of tissues. Another important difference among reported studies is the lack of standardized criteria to classify a carcinoma as p53 positive. In most cases, staining was evaluated by a semi-quantitative method estimating the percentage of positive cells. The percentage of positive tumors varies from 14% (16) to 52% (15). Even in studies that used the same monoclonal antibody, Pab1801, Silvestrini et al. (17) required that more than 5% of nuclei be immunoreactive and found 44% of the tumors to be positive; Thor et al. (18, 19) and Allred et al. (15) classified carcinomas as p53 positive if any of the cells...
showed nuclear staining, and they found 22% (Refs. 18 and 19) and 52% (Ref. 15) of the tumors to be positive; Rosen et al. (20) chose 10% reactivity to define positivity and found that 21% of the tumors were positive. These results demonstrate the necessity for the development of a reliable quantitative measurement of p53 expression.

The present results, demonstrating the negative correlations between p53, ER, and PgR and the positive correlations between p53, HPG, and tumor diameter, are in agreement with those we obtained by measuring serum p53 antibodies (14) and those found in the literature (7, 15–34). In the present study, the best p53 threshold to predict OS was 4 ng/mg protein, but p53 had no prognostic value in RFS studies. The reason that p53 does not appear to be a prognostic indicator in RFS is not clear. It must be stressed that information concerning relapse was available for only 301 patients. This result is in keeping with our previous demonstration that in the general population, circulating p53 antibodies have a particularly strong prognostic value in overall prognostic studies (14); this relationship has been found by other groups (27, 28, 30). In most studies, it has been demonstrated that p53 alterations are indicators of prognosis (18 of 21 studies, shown in Table 1), often on both RFS and OS (7, 17–19, 22, 24, 26, 31, 32), and in most of the cases, p53 was independent of other prognostic factors. In node-negative patients, the prognostic value of p53 was frequently confirmed (16, 17, 18, 19, 22, 23, 27, 31). However, this finding was not reported by some authors using either complete sequencing (32) or immunohistochemistry (20, 29); a detailed analysis of these works did not allow us to define clearly the reason for these results. But in the latter studies (20, 29), the absence of standardization of the immunohistochemistry method is certainly implicated. In fact, although in immunohistochemistry studies, a subgroup of 10–20% manifestly p53-positive tumors was found to be associated

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Relation (χ² test) between the presence of cytosolic p53 (n = 634; positivity threshold, 4 ng/mg protein) or cytosolic uPA (n = 600; positivity threshold, 0.5 ng/mg protein) and clinical, histological, or biological parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p53−</td>
</tr>
<tr>
<td>Tumor diameter</td>
<td></td>
</tr>
<tr>
<td>&lt;2 cm</td>
<td>221</td>
</tr>
<tr>
<td>2 to 5 cm</td>
<td>246</td>
</tr>
<tr>
<td>&gt;5 cm</td>
<td>11</td>
</tr>
<tr>
<td>Histoprognostic grading</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>63</td>
</tr>
<tr>
<td>II</td>
<td>275</td>
</tr>
<tr>
<td>III</td>
<td>153</td>
</tr>
<tr>
<td>ER &lt;10 fmol/mg protein</td>
<td>134</td>
</tr>
<tr>
<td>≥10 fmol/mg protein</td>
<td>416</td>
</tr>
<tr>
<td>PgR &lt;10 fmol/mg protein</td>
<td>182</td>
</tr>
<tr>
<td>≥10 fmol/mg protein</td>
<td>368</td>
</tr>
</tbody>
</table>
with worse prognosis (7), without a standardized method, an optimal clinical cutoff was never looked for. The present LIA allows the quantification of p53 and the definition of an optimal clinical cutoff point.

The percentage of high-uPA tumors (27.5%) remained within the range of previous studies (25.4–51%; Table 2). In all of the studies but one (35), thanks to quantitative assays, the thresholds were chosen as optimal in prognostic studies. Fernö et al. (8), using the same method as us, found a threshold (0.62 ng/mg protein) very close to ours (0.5 ng/mg protein). In the other studies, the optimum clinical cutoff points differed significantly: 10 ng/mg protein (36), 0.15 ng/mg protein (37), 2.97 ng/mg protein (35), 0.052 ng/mg protein (38), and 0.81 ng/mg protein (39); the results certainly depended on the method of detection used (40). In the present study, negative correlations were found between uPA and steroid receptors, and positive correlations were found between uPA and HPG or tumor diameter. Such results concur with most published studies (8, 35, 37, 38, 41). In univariate analyses, strong uPA prognostic values were observed, in terms of both OS and RFS. The uPA prognostic value appeared in all of the previous reports (Table 1). It is important to note that conversely to the studies on the prognostic value of p53, the studies on uPA were performed with quantitative assays and that the optimum clinical cutoff points (8, 35, 36, 37, 39), although different in absolute level, led to similar results in term of prognostic value. As far as we know, the largest population of node-negative breast cancer patients in which uPA level have been measured previously included 272 cases (41). Our results confirm the high prognostic value of uPA, using LIA, in a population of 313 cases.

The aim of the present study was to determine the relative prognostic importance of two new and strong prognostic parameters, p53 and uPA, in node-negative breast cancer patients. The positive correlation observed between p53 and uPA, and the correlations between uPA, p53, and the classical prognostic parameters ER, PgR, HPG, and tumor diameter underline the importance of multivariate analyses. In node-negative breast cancers, p53 was described as an independent prognostic factor (Table 1) on OS (17) and on RFS (15, 17, 31), and uPA was described as an independent prognostic factor (Table 2) on RFS (8, 35, 37). In OS analyses, when we included all of the

### Table 5 Prognostic factors in Cox univariate analyses

<table>
<thead>
<tr>
<th>Factor</th>
<th>OS (P)</th>
<th>RFS (P)</th>
<th>OS (RR)</th>
<th>RFS (RR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 (&lt;4; ≥4 ng/mg protein)</td>
<td>0.11</td>
<td>0.94</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>uPA (&lt;0.5; ≥0.5 ng/mg protein)</td>
<td>0.038</td>
<td>2.32</td>
<td>0.0008</td>
<td>2.28</td>
</tr>
<tr>
<td>PgR (&lt;10; ≥10 fmol/mg protein)</td>
<td>0.0194</td>
<td>0.48</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Histoprognostic grading (I, II, III)</td>
<td>0.004</td>
<td>2.42</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Tumor diameter (&lt;2; 2–5; &gt;5 cm)</td>
<td>0.026</td>
<td>1.97</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td>NS</td>
<td>0.0016</td>
<td>0.97</td>
<td>0.97</td>
</tr>
</tbody>
</table>

### Table 6 Prognostic factors in Cox multivariate analyses

<table>
<thead>
<tr>
<th>Factor</th>
<th>OS (P)</th>
<th>RFS (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 (&lt;4; ≥4 ng/mg protein)</td>
<td>0.26</td>
<td>1.59</td>
</tr>
<tr>
<td>uPA (&lt;0.5; ≥0.5 ng/mg protein)</td>
<td>0.23</td>
<td>1.52</td>
</tr>
<tr>
<td>PgR (&lt;10; ≥10 fmol/mg protein)</td>
<td>0.58</td>
<td>0.81</td>
</tr>
<tr>
<td>Histoprognostic grading (I, II, III)</td>
<td>0.0104</td>
<td>2.08</td>
</tr>
<tr>
<td>Tumor diameter (&lt;2; 2–5; &gt;5 cm)</td>
<td>0.16</td>
<td>1.67</td>
</tr>
<tr>
<td>Age</td>
<td>0.0176</td>
<td>0.97</td>
</tr>
</tbody>
</table>

*The parameters that had a prognostic value in the univariate analyses were entered in this study.*
prognostic parameters together, HPG was the strongest prog-
nostic parameter, and neither p53 nor uPA had a significant
prognostic value. In RFS analyses, two parameters (uPA and
age) were of significant value, but the RR for uPA was 2.21,
whereas for age, it was 0.97.

In conclusion, these results show that uPA and p53 are
prognostic parameters. Considering its value in multivariate
RFS studies, uPA is clearly an important prognostic parameter
in node-negative breast cancer patients that may be of use in the
clinic.

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