

Prognostic significance of circulating P53 antibodies in patients undergoing surgery for locoregional breast cancer

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P53 mutations can lead to the production of P53 antibodies in serum of cancer patients. Here we studied the prognostic value of P53 antibodies in 353 primary breast cancer patients. P53 antibodies were detected in 42 cases (12%) and were negatively related to oestradiol and progesterone receptors. The median duration of follow-up for live patients was 5.3 years. In actuarial analyses, overall survival was worse in patients with P53 antibody ($p<0.0005$); in Cox's multivariate analysis, P53 antibody was an independent prognostic variable. Thus plasma assay of P53 antibody would be useful to select rapidly and easily a population of patients with poor prognosis.

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The tumour suppressor gene *P53* is often altered in breast carcinoma. Analysis of *P53* status by molecular or immunohistochemical methods¹ shows that the *P53* alteration correlates with tumour aggression. Patients with *P53* alterations have shorter disease-free and overall survival. These observations suggested that *P53* could be a useful marker in those patients who might require more aggressive post-surgical therapy.² Accumulation of mutant *P53* in tumour cells can lead to the production of P53 antibodies in serum. Such antibodies correlate closely with other factors that indicate poor prognosis.³ These findings prompted us to evaluate P53 antibody in breast cancer patients undergoing surgery for locoregional disease and the relation between this antibody and histopathological or clinical indices. Furthermore, we studied the prognostic significance of P53 antibody.

We included 353 unselected female breast cancer patients undergoing surgery for locoregional disease at the Centre Oscar Lambret. Selection began in April, 1986, and patients represented part of the population in a previous study.⁴ The mean age was 58 years (range 28–85), and 70% were postmenopausal. All were treated by segmentectomy when

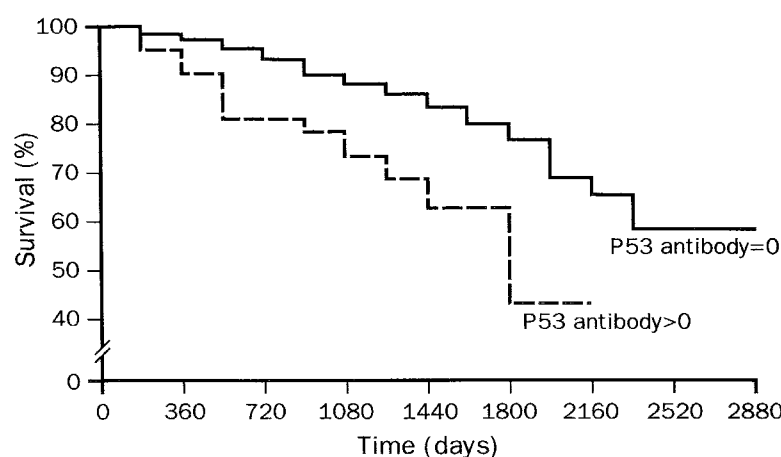


Figure: Overall survival by P53 antibody status

possible. Axillary dissection was done in all cases. Surgery was followed by radiation therapy on the chest wall after total mastectomy, on the remaining breast tissue after segmentectomy, and on adjacent nodes. Only node-positive patients received adjuvant treatment: six cycles of chemotherapy in premenopausal and tamoxifen in postmenopausal patients. After completion of treatment, the patients were followed up every 4 months for 3 years, then every 6 months for 2 years, and then yearly. Mammogram, chest radiograph, and plasma hepatic enzymes were assessed yearly; other radiographs, scans, and echographs were symptom-oriented. Mean duration of follow-up of living patients was 5.3 years. All tumours were invasive adenocarcinomas (ductal 72%, lobular 13%, other 15%). Histoprognostic grading was done according to Scarff and Bloom: 12% were grade I, 49% were grade II, and 39% were grade III ($n=328$).

Plasma was collected at the time of surgery and stored at -80°C . We devised a highly specific ELISA by testing all plasmas with two antigen preparations: the first contained the relevant P53 antigen; in the second preparation, this antigen was omitted (full details from TS). All results are expressed as the ratio between the value of the wells with P53 and the corresponding wells without P53. Studies on 200 serum samples from healthy blood donors showed a ratio of 1.1 (SD 0.4). A ratio over 2 (mean +2 SD) was judged positive. In each assay, positive serum with a low concentration of P53 antibody was used to establish a threshold for the experiment. Oestradiol and progesterone receptors were assayed by the dextran-coated charcoal method.⁴

Overall and relapse-free survival were analysed actuarially. Curves were compared with the logrank test. Cox's proportional hazards regression was used to assess prognostic significance.

42 patients (12%) had P53 antibody in serum. The relation between antibody and prognostic factors is shown in table 1. A significant negative correlation (Spearman's) was found between P53 antibody and oestrogen receptors ($p=0.0094$, $r=-0.14$) and with progesterone receptors ($p=0.0051$, $r=-0.15$). The median age of antibody-negative patients was 58 years; it was 56 years for antibody-positive patients.

Overall survival was shorter in patients with P53 antibody (total number of patients for overall survival, 348) (figure). In multivariate analysis, P53 antibody was an independent prognostic factor (table 2), as were node involvement and histoprognostic grading. In the actuarial and multivariate studies (325 patients) of relapse-free survival, P53 antibody did not have prognostic value.

Our results agree with previous studies that reported a frequency of 12–14% for P53 antibody in the serum of patients with breast cancer,^{3,5–7} and we confirmed the relation between the presence of such antibodies and the absence of oestrogen and progesterone receptors. As shown by Barnes et al⁸ who used immunohistochemistry, we demonstrate in Cox's analysis that P53 comes after node status in the prediction of overall survival. Thor et

	P53 antibody		χ^2	p
	Negative	Positive		
Lymph nodes				
<0	125	17	0.27	0.87
1-3	75	9		
>3	99	15		
Size (diameter, cm)				
<2	6	0	0.99	0.61
2-5	239	34		
>5	51	6		
Histoprognostic grading				
I	35	4	4.9	0.08
II	148	13		
III	107	21		
Oestrogen receptors (fmol per mg protein)				
<10	84	16	2.24	0.135
>10	227	26		
Progesterone receptors (fmol per mg protein)				
<10	97	21	5.88	0.015
>10	214	21		

Table 1: Association between presence of P53 antibody and prognostic variables

	Overall survival		Relapse-free survival	
	Univariate	Multivariate (risk ratio)	Univariate	Multivariate (risk ratio)
P53 antibody	0.0005	0.018 (1.93)	0.1	0.07 (1.42)
Oestradiol receptors	0.0001	0.13 (0.66)	0.0002	0.23 (0.75)
Node status	0.0002	0.0006 (1.04)	0.0006	0.0001 (1.05)
Histoprognostic grading	0.0002	0.0093 (1.72)	0.0001	0.015 (1.54)
Progesterone receptors	0.0025	0.67 (1.13)	0.0007	0.81 (0.94)
Tumour diameter	0.0043	0.069 (1.11)	0.0017	0.08 (1.09)

Table 2: **Prognostic factors (univariate and multivariate analyses)**

al^o reported a 6-year overall survival of 69% in P53-negative patients and 46% in the P53-positive group. Our findings after 6 years of follow-up were, respectively, 68% and 40%. Thus serological analysis of P53 alterations gives similar results to immunohistochemistry.

We studied an unselected population with primary breast cancer, which included node-positive and node-negative patients. Study of a large population of node-negative patients will be of interest, since we need new prognostic factors in that subgroup. In such a population of node-negative patients the small size of the biopsy specimens would preclude molecular and immunohistochemical analyses, but serum could be analysed.

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Non-endoscopic method to obtain *Helicobacter pylori* for culture

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A sampling method to collect samples for *Helicobacter pylori* culture that is easier than endoscopy would be useful. We used a capsule attached to a highly absorbent nylon string, which is swallowed to obtain gastric secretions. Antral biopsy and string specimens, sequentially obtained from 36 adults with clinically suspected ulcer or gastric disease were cultured. 9 (25%) patients were negative in both tests; 18 (50%) were positive by both methods; 6 (17%) were biopsy positive and string-test negative; and 3 (8%) were biopsy negative and string-test positive. The new test is useful, simple, and causes little discomfort.

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The diagnosis of *Helicobacter pylori* infection usually involves invasive (endoscopic) methods, complex procedures such as the breath test, or serological detection of antibodies to *H pylori*. Culture is the gold standard for detection of a microorganism and is the only method that allows study of antimicrobial susceptibility. However, an upper gastrointestinal endoscopy is required to obtain a sample for culture of *H pylori*. Endoscopy is widely used to obtain a good sample and the best way to observe the condition of the upper gastrointestinal mucous membrane, but the method is costly and uncomfortable. Entero-test, a capsule attached to an absorbent nylon string (HDC Corp, Mountain View, California), is a tool for the recovery of fluid from the upper small bowel via the stomach.

We used the technique to collect *Giardia lamblia* trophozoites and for this purpose, the string remained in the duodenum for about 3.5 h. When we used the capsule in children with persistent dyspepsia, we thought we could extend its use to investigate the presence of *H pylori*, which we did only on endoscopic biopsy samples. 196 biopsy specimens and 72 strings were cultured from 203 patients over 3 years. Transport delays and culture conditions were similar in both types of samples. *H pylori* was cultured in 25 (12.8%) of the biopsy samples and in 9 (12.5%) of the string samples.

In a prospective study in adult patients we used the paediatric version of the test (90 cm nylon line) instead of the adult one (140 cm), and we withdrew the string after 1 h. 36 outpatients over the age of 21 with clinically suspected peptic ulcer disease or chronic gastritis (19 patients had active duodenal ulcer, 2 healed ulcer, 8 chronic gastritis, and 7 other non-ulcer dyspepsia), who agreed to take part in the study, were simultaneously evaluated by endoscopic biopsy and by the string test 1-2 days later. Before re-use, the endoscope and biopsy forceps were cleaned, disinfected, and washed with sterile water. The capsule was swallowed with water after an overnight fast.

The gastric mucosa biopsy specimen was placed in saline. The string, after withdrawal, was placed in a sterile petri dish. Both types of samples were transported to the microbiological laboratory within 1 h and processed immediately. The biopsy specimen and the end portion of the string were inoculated on brucella agar culture-plates (BBL, Becton Dickinson) supplemented with 7% haemolysed horse blood, 1% Vitox (Oxoid), and made selective with vancomycin (15 µg/mL) and trimethoprim (5 µg/mL) and on the same medium without