

# Contribution of vascular endothelial growth factor to the Nottingham prognostic index in node-negative breast cancer

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**Summary** The prognostic contribution of intratumour VEGF, the most important factor in tumour-induced angiogenesis, to NPI was evaluated by using flexible modelling in a series of 226 N-primary breast cancer patients in which steroid receptors and cell proliferation were also accounted for. VEGF provided an additional prognostic contribution to NPI mainly within ER-poor tumours. © 2001 Cancer Research Campaign <http://www.bjcancer.com>

**Keywords:** flexible modelling; N-negative primary breast cancer; NPI; VEGF

Despite substantial progress in early detection and treatment, breast cancer remains the second commonest malignancy in the female population. It affects approximately 1 in 10 women and is responsible for 23.7% of all cancer deaths (Breast Cancer Statistics, 2000). Comprehensive research has been conducted to identify clinically useful prognostic factors in primary breast cancer, with special efforts to identify among node-negative (N<sup>-</sup>) patients those with an aggressive phenotype who need adjuvant systemic treatments and those with indolent tumours who are likely to be cured by local-regional therapy. A unique, highly discriminating prognostic factor has not yet been proposed and, in consideration of the complex biology of the neoplasm, it is unlikely that one will be identified, although novel promising prognosticators are emerging. Moreover, sufficient and univocal evidence has not yet been provided for assessing either the prognostic strength of biological markers or their actual clinical impact. In fact, a few studies have evaluated the adjunctive contribution of novel factors to already validated scores proposed for breast cancer and based on different morphopathobiologic features of proven prognostic utility (Altman and Lyman, 1998; Goldhirsch et al, 1998; Hayes et al, 1998).

The Nottingham prognostic index (NPI) can be considered a gold standard for prognosis since it is based on morphopathologic features such as lymph node stage, tumour size and histologic grading of malignancy. It has been prospectively validated in several studies and has proved to maintain over time a valuable discriminating power in differentiating patients in low-, intermediate- and high-risk subsets (Haybittle et al, 1982; Galea et al, 1992; Balslev et al, 1994). Although lymph node stage has a pivotal role in the NPI definition, NPI is a validated prognostic discriminator also in N<sup>-</sup> patients (Saurbrei et al, 1997) and can be considered as a

baseline reference for the evaluation of the additional prognostic role of biological variables.

Experimental studies have suggested tumour-induced neoangiogenesis as an important step in the evolution of malignant tumours (Ellis and Fidler, 1996; Yosiji et al, 1996). Recent clinical evidence indicates that its evaluation, either as microvessel count or as vascular endothelial growth factor (VEGF) content, significantly contributes to identify patients with a worse prognosis, mainly within the N<sup>-</sup> subset (Gasparini, 1996; Heimann et al, 1996) but also within endocrine adjuvant setting (Linderholm et al, 2000). Since steroid receptor content is currently used in clinical practice and tumour proliferative activity is a recognized prognostic factor in N<sup>-</sup> patients (Hayes et al, 1998), the aim of the present study was to evaluate the adjunctive prognostic contribution of intratumour VEGF concentration to a widely adopted prognostic classification (NPI), taking into account the effect of steroid receptors and cell proliferation. For evaluation of the prognostic impact of VEGF concentration in the continuous scale of measurement, flexible regression and bootstrap techniques have been adopted.

## MATERIALS AND METHODS

The present study was carried out on a series of 226 N<sup>-</sup> primary breast cancer patients subjected, between May 1991 and May 1993, only to radical or conservative surgery plus radiotherapy and to complete axillary dissection until relapse and followed up for a median of 75 months (range, 4–98) at the Istituto Nazionale Tumori of Milan. Information on the cases was also available for steroid receptors, evaluated by the dextran-coated charcoal technique (Ronchi et al, 1986), and tumour proliferative activity, expressed as the thymidine labelling index (TLI) (Silvestrini et al, 1995). The NPI was determined according to Elston and Ellis (1991).

VEGF expression was measured by a quantitative enzyme immunoassay technique. The role of NPI, VEGF, oestrogen and progesterone (ER, PgR) content, and TLI on disease-free survival

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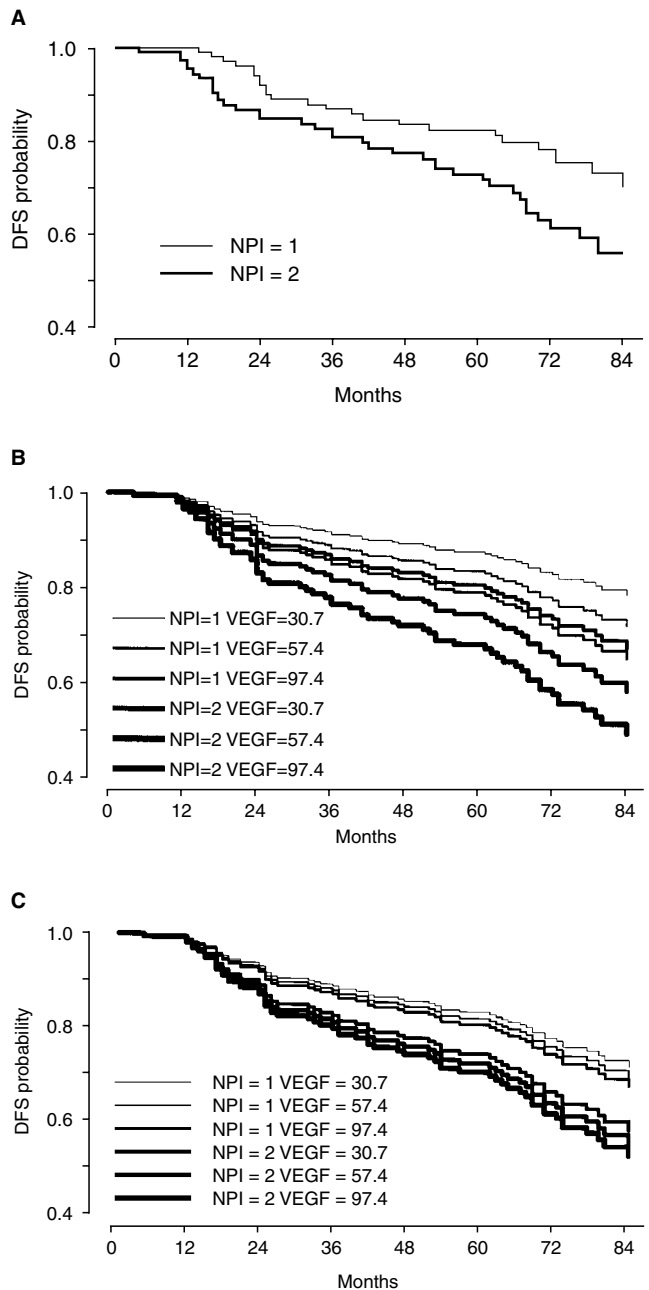
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(DFS, defined as time from surgery to local–regional recurrence, distant metastasis or contralateral tumours; 63 events) was investigated by the Cox model, once the proportional hazard assumption was verified (Grambsch and Therneau, 1994). On the basis of prior knowledge, only linear terms were considered for ER, PgR and TLI after logarithmic transformation (Silvestrini et al, 1996). For VEGF, a restricted cubic spline transformation (Durrleman and Simon, 1989) was initially considered on its logarithmic values, whereas only the linear term was finally adopted. Among pre-specified interactions (VEGF  $\times$  TLI, VEGF  $\times$  NPI, VEGF  $\times$  ER) only VEGF  $\times$  ER was selected. The additional prognostic contribution of biological variables was also evaluated by a bootstrap re-sampling technique (200 samples) applied to a backward variable selection, starting from a model including all variables and the interaction. Model comparisons were performed on the basis of Akaike Information Criterion (AIC) (Collett, 1994), considering 3 different penalty factors for the degrees of freedom (i.e. 2, 3, 4) – the first corresponding to that traditionally used for AIC and the other 2 allowing for a more conservative selection. Model predictive ability was measured by Harrell's c statistic (Harrell et al, 1996). To illustrate the effect of covariates, the expected survival curves, calculated from Cox model results, were plotted according to fixed covariate values. Selected values were the 25th, 50th or 75th percentiles for VEGF, the 25th or 75th percentiles for ER, low (1) and intermediate (2) NPI. TLI and PgR were fixed to the median values of their distributions.

## RESULTS AND DISCUSSION

The values of VEGF ranged from 0 to 337.3 with 30.7, 57.4, 97.4, as 25th, 50th, 75th percentiles, respectively. The DFS curves according to NPI levels are shown in Figure 1A: since only N-patients were considered, the NPI classification is only a 2-level score: low and intermediate. The difference between DFS curves was statistically significant (hazard ratio (HR) for relapse: intermediate NPI vs low NPI, 1.71; 95% confidence limits (CL), 1.03–2.83;  $P = 0.039$ ). In the multivariate regression model (Table 1), using the conventional penalty factor of 2 for AIC, TLI was retained in the model in 67.5%, ER in 71.5%, VEGF in 94% and VEGF\*ER interaction in 66.5% of the bootstrap samples. Moreover, VEGF was retained in 90.5% and 76.6% of the bootstrap samples with the increased penalty terms.

The predictive ability of the model was  $c = 0.655$ , whereas considering NPI only and NPI plus steroid receptors and TLI, the c statistic dropped to 0.571 and 0.623, respectively. To provide a graphic display of model results, expected DFS curves were drawn for selected combinations of variable values (Figure 1B, ER-poor tumours; Figure 1C, ER-rich tumours). The present findings seems to suggest a discriminatory effect of VEGF in identifying patients who differ in prognosis in NPI = 1 and NPI = 2 groups (5-year probability of relapse for low vs high VEGF: 12% vs 20% and 19% vs 31%, respectively) mainly in ER-poor tumours (Figure 1B). For these patients, the estimated HR for disease for high vs low VEGF was 1.79 (95% CL, 1.15–2.79). Conversely, in ER-rich tumours, i.e., in tumours with an ER content greater than 126 fmol mg<sup>-1</sup> cytosolic protein (Figure 1C), VEGF was unable to segregate patients with different prognosis, and the HR was 1.18 (95% CL, 0.78–1.79). In ER-poor tumours angiogenesis might be at an initial stage and thus the presence of high VEGF levels could indicate the activation of such a process. Conversely, in the ER-rich subset a functional hormone control seems able to counteract



**Figure 1** Disease-free survival curves for 226 patients with node-negative resectable breast cancer. (A) Kaplan–Meier curves according to NPI. (B) Expected disease-free survival curves calculated from the Cox multiple regression model as a function of NPI (1,2) and VEGF (30.7, 57.4, 97.4 pg mg<sup>-1</sup> cytosolic protein, corresponding to the 25th, 50th and 75th percentiles of the distribution) for patients with ER-poor tumours (17 fmol mg<sup>-1</sup> cytosolic protein, corresponding to the 25th percentile of the distribution). TLI and PgR are fixed to the median values of their distributions: 2.8% and 68 fmol mg<sup>-1</sup> cytosolic protein, respectively. VEGF expression was measured by a quantitative enzyme immunoassay technique following the manufacturer's instruction (CYTimmune Science). Each sample was run in duplicate and calibration was performed according to the method proposed by O'Connell et al (1993). (C) Expected disease-free survival curves from the Cox multiple regression model as a function of NPI and VEGF (30.7, 57.4, 97.4 pg mg<sup>-1</sup> cytosolic protein, corresponding to the 25th, 50th and 75th percentiles of the distribution) for patients with ER-rich tumours (126 fmol mg<sup>-1</sup> cytosolic protein, corresponding to the 75th percentile of the distribution). TLI and PgR are fixed to the median values of their distributions: 2.8% and 68 fmol mg<sup>-1</sup> cytosol protein, respectively

angiogenesis activation in both NPI groups and, as suggested by a previous study (Coradini et al, 2000), a longer follow-up could be

**Table 1** Multivariate analysis for disease-free survival

Model terms	b (SE) <sup>a</sup>	Model effects	DF <sup>b</sup>	$\chi^2$ <sup>c</sup>	P
		Full model	6	14.44	0.0140
NPI	0.498 (0.277)	Factor	1	3.25	0.0715
ER <sup>d</sup>	0.788 (0.452)	Factor + Interaction	2	3.40	0.1823
PgR <sup>d</sup>	0.025 (0.067)	Factor	1	0.14	0.7053
TLJ <sup>d</sup>	0.407 (0.242)	Factor	1	2.84	0.0918
VEGF <sup>d</sup>	1.031 (0.430)	Factor + Interaction	2	6.76	0.0340
VEGF <sup>d</sup> *ER <sup>d</sup>	-0.183 (0.100)	Interaction	1	3.32	0.0686

<sup>a</sup>Estimated regression coefficient. In parenthesis, standard error.

<sup>b</sup>Degree of freedom.

<sup>c</sup>Wald statistics for model effects.

<sup>d</sup>Logarithmic transformation.

necessary to observe the disruption of the hormone-related growth control and angiogenesis balance.

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