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Letters

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Prognostic Factor Clustering in Breast Cancer: Biology or Chronology?

M. Tubiana-Hulin, K. Hacène, P.M. Martin and F. Spyratos

MITTRA AND Mac Rae [1] have reported a meta-analysis of published correlations between 10 prognostic factors in operable breast cancer and found a strong correlation between various biological prognostic factors, but few correlations between lymph node status or tumour size and biological factors. They support the view that the two clinical factors reflected the age of the tumour, independently of its biological aggressiveness. This was discussed in a Lancet editorial [2] which pointed to the methodological limits of the meta-analysis and the arbitrary classification of tumour grade as a biological factor, in spite of its strong relation to other biological factors on the one hand, and lymph node status or tumour size on the other.

Nevertheless, the apparent lack of prognostic value of lymph node status for overall survival after recurrence suggests that this parameter has little to do with the biological behaviour of breast cancer, and lends weight to a chronological interpretation. The significance of tumour size is less clear, as some authors have found that the prognostic value of clinical stage persists after recurrence.

Here we report the results of a principal components analysis (PCA) [3] and a hierarchical variable clustering [4], performed on data from 319 patients with operable breast cancers who were representative of the overall patient population with primary breast cancers seen at our institution. Interim results of a multiparametric prognostic study concerning these data have already been published [5].

The parameters were menopausal status, clinical tumour size, oestrogen and progesterone receptor levels, DNA by flow cytometry, cathepsin D, urokinase plasminogen activator (uPA), thymidine kinase, number of involved nodes and tumour SBR grading replaced by a modified SBR (MSBR) that only takes into account the two nuclear components of the SBR [6]. Additional factors, such as pS2, epidermal growth factor receptor and peritumoral vascular emboli, were also analysed.

The analysis shows three clusters of prognostic factors (Figure 1). One cluster included the number of invaded nodes, tumour size and peritumoral vascular emboli. The proximity of this last factor is logical, as it is the anatomical link between the tumour and node invasion. Another cluster included factors reflecting invasiveness (proteases) and cell proliferation. The position of the nuclear grade on Figure 1 reflects its link to these categories of factors and its distance from clinical (anatomical) factors. The last cluster included biological factors reflecting

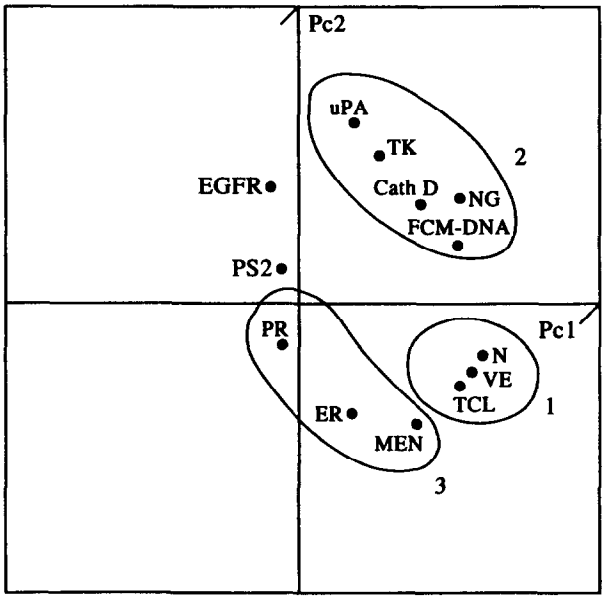

Figure 1. Principal component analysis. Scatter configuration of correlation between the 13 variables and the first two principal components (Pc1, Pc2). Abbreviations: DNA Fcm, DNA ploidy by flow cytometry; NG, nuclear grade; TK, thymidine kinase; uPA, urokinase plasminogen activator; Cath.D, cathepsin D; EGFR, EGF receptor; TS, clinical tumour size; N, number of invaded nodes; VE, vascular emboli; PR, progesterone receptor; ER, oestrogen receptor; Men, menopausal status.

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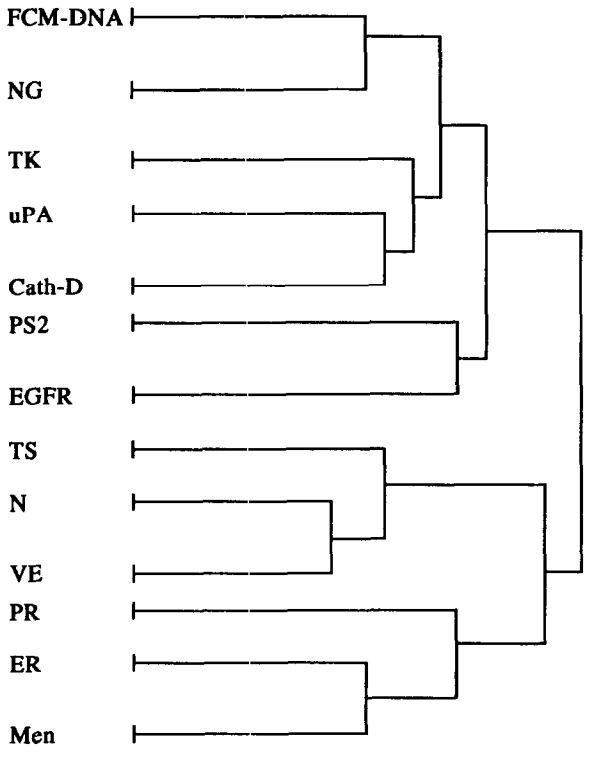

Figure 2. Hierarchical variable clustering. Representation of the 13 variables by a hierarchical tree or dendogram which provides homogeneous partitions of the variables. Abbreviations: see Figure 1.

hormone sensitivity, whereas EGFR and pS2 appeared relatively isolated. Figure 2 confirms the variable clustering obtained by the PCA.

This study shows the strong correlation between tumour size, node invasion and vascular emboli, and a clear separation between this group of factors and all other biological factors, including nuclear grade. These results are in keeping with Mittra and Mac Rae's meta-analysis, in which vascular emboli were not studied. They also emphasize the fact that so-called clinical factors differ in kind from other factors, although our data do not support the chronological/biological interpretation.

1. Mittra I, Mac Rae KD. A meta-analysis of reported correlations between prognostic factors in breast cancer: does axillary lymph node metasasis represent biology or chronology? *Eur J Cancer* 1991, **27**, 1574–1583.
2. Editorial. *Lancet* 1992, **340**.
3. Anderson TW. *An Introduction to Multivariate Statistical Analysis*, 2nd edn. New York, John Wiley, 1984.
4. JC Gower, GJS Ross. Minimum spanning trees and single linkage cluster analysis. *J R Stat Soc* 1969, **18**, 54–64.
5. Spyratos F, Martin PM, Hacène K, *et al.* Multiparametric prognostic evaluation of biological factors in primary breast cancer. *J Nat Cancer Inst* 1992, **84**, 1266–1272.
6. Le Doussal V, Tubiana-Hulin M, Friedman S, Hacène K, Spyratos F, Brunet M. Prognostic value of histologic grade nuclear components of Scarff-Bloom-Richardson (SBR). *Cancer* 1989, **64**, 1914–1921.

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Circumvention of Doxorubicin-resistance in Tumours by Albumin-conjugated Doxorubicin

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DOXORUBICIN (DOX) is a clinically important antineoplastic agent, but chemoresistance often prevents successful treatment of cancer patients. Although a panel of compounds (chemosensitisers) have been found to reverse drug-resistance *in vitro* [1], the results of most of the clinical studies have been inconclusive and rather disappointing [2]. Therefore, as a new approach, we analysed the reversing effect of bovine serum albumin-conjugated DOX on DOX-resistant tumours *in vivo*. Protein–drug conjugates have been shown to have a prolonged biological half-life and an increased tumour uptake [3].

The DOX-resistant tumours, murine L1210, revealed a 30-fold resistance to DOX [4]. Solid L1210 tumours were generated by subcutaneous inoculation of ascites tumour cells into mice (1.5×10^7 tumour cells/mouse). Coupling of DOX to bovine serum albumin was carried out using the method described previously [3].

Female NMRI mice (Breeding Centre, Hannover, Germany), 6–8 weeks old, bearing DOX-resistant tumours were treated with a single intraperitoneal (i.p.) injection of DOX (8 mg/kg), bovine serum albumin-conjugated DOX (conjugate at equivalent doses of DOX) or 0.9% NaCl solution (each group 10 mice; $n = 30$ mice). Tumour growth was measured daily using calipers and the tumour volume was calculated by the formula $(a^2 \times b)/2$ where b was the largest diameter and a was the diameter perpendicular to b.

The *in vitro* effects (increase/decrease on 4th day after treatment) of DOX and bovine serum albumin-conjugated DOX (BSA-DOX) on growth of DOX-resistant solid L1210-tumours of mice compared to untreated tumours (control) is shown in Figure 1. Mice treated with the protein–DOX conjugate had a significant reduction in tumour volume compared to mice treated with DOX alone ($p = 0.023$, Kruskal Wallis test; $p = 0.029$, Wilcoxon rank sum test). Mice treated with doxorubicin alone had no significant changes in tumour volume compared to tumours of control mice. In contrast to unconjugated DOX, the conjugate was well tolerated and no loss of body weight could be found in the conjugate-treated mice (data not shown).

There is increasing evidence that several mechanisms are operational in DOX resistance [5, 6]. One of the most important resistance mechanisms is due to the expression of a 170 kDa membrane glycoprotein which functions as an energy-dependent efflux-pump [7], but glutathione S-transferase-π and topoisomerase II may be additionally involved [8]. However, in the

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