early anticoagulation and a "pseudosteady state" after about six hours as opposed to the 10 hours (five heparin half lives) required to achieve a true steady state when a single rate infusion is given alone. Thus earlier measurement of the kaolin cephalin clotting time and dose adjustment was possible. Subsequent measurements were not made for at least 10 hours unless the kaolin cephalin clotting time was particularly long. The guidelines increased the proportion of patients with clotting times within the therapeutic range and reduced the proportion given insufficient anticoagulation and presumably more at risk of recurrence.[4]

Despite the relative precision of the guidelines there was no evidence of improved control with time. All measurements were performed in the morning, which reduced the effects of circadian variation in the response of the clotting time.[5] The apparent variation in requirements may have been related to the problems in maintaining stable intravenous infusions on a busy general ward or to normal daily variation in heparin requirements.

Although the considerable interpatient variation in response to heparin was not completely taken into account, the guidelines resulted in better heparin control. Further improvement may be achieved by careful preparation and delivery of the infusion.

We thank Mr G Backhouse for his technical help.

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(Accepted 20 November 1985)

Content of low density lipoprotein receptors in breast cancer tissue related to survival of patients

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Abstract

The content of low density lipoprotein (LDL) receptors in tissue from primary breast cancers was determined and its prognostic information compared with that of variables of established prognostic importance. Frozen tumour specimens were selected, and tissue from 72 patients (32 of whom had died) were studied. The LDL receptor content showed an inverse correlation with the survival time. Analysis by a multivariate statistical method showed that the presence of axillary metastasis, content of receptors for oestrogen and LDL, diameter of the tumour, and DNA pattern were all of prognostic value with regard to patient survival.

Improved methods of predicting survival time in patients with breast cancer may be of value in the choice of treatment for individual patients.

Introduction

Breast cancer occurs in one out of 11 women in the United States and causes 19% of all deaths from cancer among women.[1] The disease runs an unpredictable course, which makes the choice of treatment for individual patients difficult. Improved predictive methods might identify high risk patients, who could be given intensive combination treatment, while low risk patients could be spared the side effects caused by extensive treatment.

Cholesterol is an important component of cell membranes. In human plasma cholesterol is predominantly found in the low density lipoprotein (LDL) fraction. Human cells have receptors for LDL. The receptor activity is regulated according to the cellular demand for cholesterol. Thus cells that synthesise steroid hormones and rapidly growing cells have high LDL receptor activities.[2] Certain tumour cells have raised LDL receptor activity in vitro.[3,4] A high uptake of LDL, mediated by receptors, in solid tumours in vivo has been shown in animals.[5-7] Whether the high LDL receptor activity is caused by the proliferation of tumour cells or by some cellular abnormality associated with the disease is not clear. The LDL receptor activity in human solid tumours is not known. We therefore investigated whether the density of LDL receptors in breast cancer tissue could be of any prognostic importance.

In breast cancer the best prognostic factors so far identified are metastases in the axillary lymph nodes and the size of the primary tumour.[8-11] Recent observations, however, have focused interest on variables of the primary tumour such as DNA pattern, proliferative index, and contents of oestrogen receptors and retinoic acid receptors[12-18] (D Killander *et al*, third European Organisation for Research on Treatment of Cancer breast cancer working conference, Amsterdam, 1983). In the present study we assessed the density of receptors for LDL and retinoic acid in specimens of frozen primary mammary carcinomas from selected patients and compared the prognostic value of these densities with already established prognostic variables such as axillary lymph node state, DNA pattern of the tumour cells, oestrogen receptor content, diameter of the tumour, and age of the patient. For the multivariate statistical analysis of the simultaneous predictive relevance of these seven variables for the prognosis of the patients, measured as survival time after diagnosis, we used partial least squares analysis with cross validation.[19-22]

Patients and methods

PATIENTS

Frozen primary breast tumours from 72 patients who had undergone surgery during 1978-9 were selected for study; 32 of the patients had died (28 had had stage I-II disease and four stage III disease at the time of surgery) and 40 were alive (39 with stage I-II disease and one with stage III disease).

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The observation period was set to six years. The median age was 61·5 (range 25-84) years. All patients had been treated by modified radical mastectomy. Postoperative irradiation had been given to patients with positive axillary lymph nodes as determined by histopathological examination, metastasis in one or more nodes being regarded as positive.

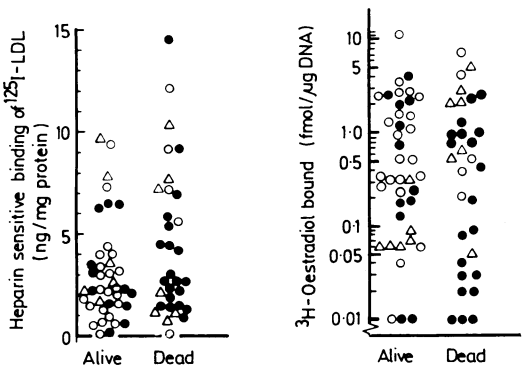

FIG 1—LDL receptor content (left) and oestrogen receptor content (right) in 40 patients still alive and 32 patients who had died.

●=Patients with metastases in axillary lymph nodes; ○=patients without metastases in axillary nodes; △=patients in whom the axillary state was not determined.

(positive/negative), diameter of the tumour, DNA pattern (euploid/aneuploid), LDL receptor content, retinoic acid receptor content, and oestradiol receptor content (log transformed) was assessed by the partial least squares method as developed by Wold *et al.*[19 21 22] This method with one dependent variable resembles multiple regression. Whereas multiple regression assumes that all variables are 100% relevant to the dependent variable (survival time), however, partial least squares analysis avoids this assumption, thus allowing for an estimation of the standard deviation of the independent variable (LDL receptor content, age of patient, etc).[22] As in multiple regression, one or more linear combinations of the measured variables are calculated—for example, 0·68×LDL receptor content+0·14×age−0·37×oestrogen receptor content+...=score. The coefficients in front of the variables (weights) indicate the relative predictive importance of the variable. Thus the score for a patient is the sum of her weighted variable measures. The number of significant linear combinations is determined by cross validation,[19-22] a ratio of cross validation to standard deviation below 0·95 being considered to be significant. In the present study high positive scores are associated with a good prognosis and high negative scores with a poor prognosis.

Before the partial least squares analysis data were normalised to zero mean and unit variance. Each patient was assigned the number of months for which they had survived; those still alive at the end of the observation period (six years) were given a survival value of 72 months.

Results

The LDL receptor content in breast cancer tissue varied widely between patients (fig 1). When the LDL and oestrogen receptor contents were

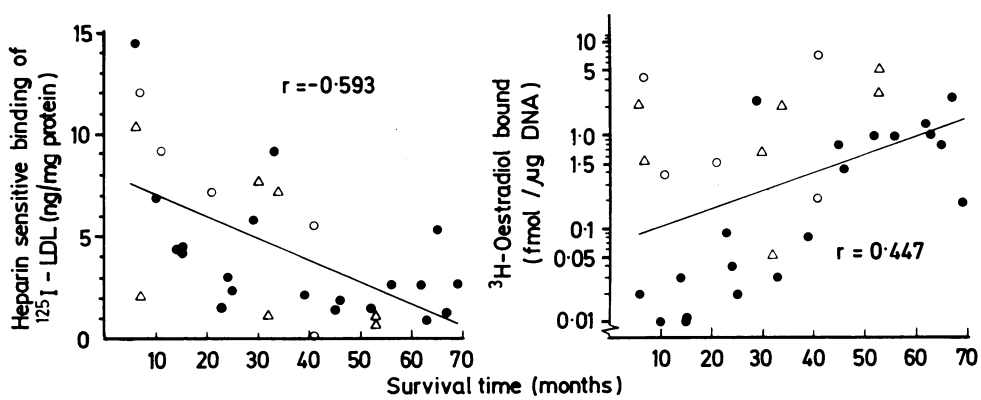

FIG 2—Relations between LDL receptor content (left) and oestrogen receptor content (right) and survival time in 32 patients who had died.

●=Patients with metastases in axillary lymph nodes; ○=patients without metastases in axillary nodes; △=patients in whom the axillary state was not determined.

TUMOURS AND ANALYSIS OF TUMOUR CELLS

The removed breast had been placed in ice cold 150 mM sodium chloride. The size of the tumour was determined (maximal diameter) and specimens stored at −80°C. The content of LDL receptors in thawed tumour specimens was determined by a binding assay[23] after incubation of tissue homogenates with 50 µg/ml LDL labelled with iodine-125 as described previously.[24] LDL (density 1·020-1·065) was isolated from serum by ultracentrifugation[25] and labelled with ^{125}I as described by Langer *et al.*[26] The binding of ^{125}I-LDL (LDL protein) was related to the protein content of the homogenate determined by the Lowry assay.[27] The content of oestrogen receptors was determined by isoelectric focusing on polyacrylamide gel as described by Wrange *et al.*[28] The content of retinoic acid receptors was determined after binding of retinoic acid labelled with tritium (supplied by Hoffman La Roche, Switzerland) to the cytosol fraction. The receptor-ligand complex sedimented in the 2 S region of a sucrose gradient as described by Ong and Chytil.[29] The contents of oestrogen receptors and retinoic acid receptors were related to the DNA content in the sample. The DNA pattern of the tumour cells was analysed by single cell DNA measurements on imprints from thawed tumours. The imprints were fixed, stained, and analysed as described by Bjelkenkrantz.[30]

STATISTICS

The simultaneous predictive relevance for survival time of the age of the patient at the time of diagnosis, metastasis in the axillary lymph nodes compared between patients who were dead and those who were alive (fig 1) the averages in the two groups were not significantly different. The retinoic acid receptor content was also similar in both groups of patients. When the survival time of the 32 patients who had died was related to the receptor contents of the tumours (fig 2), however, the LDL receptor content was negatively correlated ($p<0·001$) to survival time whereas the logarithm of the oestrogen receptor content showed a positive correlation ($p<0·02$). There was no significant correlation, however, between the LDL receptor content and the logarithm of the oestrogen receptor content in these patients ($r=-0·112$). The retinoic acid receptor content did not show any correlation with survival time.

PARTIAL LEAST SQUARES ANALYSIS

One significant dimension in the partial least squares analysis (cross validation: standard deviation=0·912) explaining 28·9% of the variance in survival time was found. The predictive relevance (weight) of each of the seven variables included in the analysis was: age of patient at diagnosis, −0·14; metastasis in axillary lymph nodes, −0·44; diameter of tumour, −0·32; DNA pattern of tumour cells, −0·37; retinoic acid receptor content, −0·12; oestrogen receptor content, +0·37; and LDL receptor content, −0·68. Thus the LDL receptor content was the best predictor of survival; the age of the patient and retinoic acid receptor content were weak predictors, and the four other variables all had a similar predictive value.

Patients with a survival time below 35 months had the lowest scores—that is, they contributed most to the partial least squares prediction (fig 3). When the LDL receptor content was excluded from the analysis the correlation coefficient between the scores and the survival time decreased from 0·54 to 0·41 and, according to cross validation, the partial least squares dimension was not significant (cross validation: standard deviation = 0·979).

Discussion

In this study partial least squares analysis yielded a score for each patient, which could be used to relate the seven variables measured to survival time (fig 3). The content of LDL receptors in the tumours was a significant predictor, which in this limited series seemed to be independent of axillary lymph node metastases, DNA pattern, tumour diameter, and oestrogen receptor content, four factors of established prognostic importance in breast cancer.[8-11 14-18] In addition, the inclusion of LDL receptor content into the partial least squares analysis substantially improved the correlation between the scores and survival time. As figure 3 shows, the scores were low mainly in patients who survived for less than three years—that is, the variables measured have prognostic relevance for a limited period of time. This may be due to changes in properties of the tumours over time.

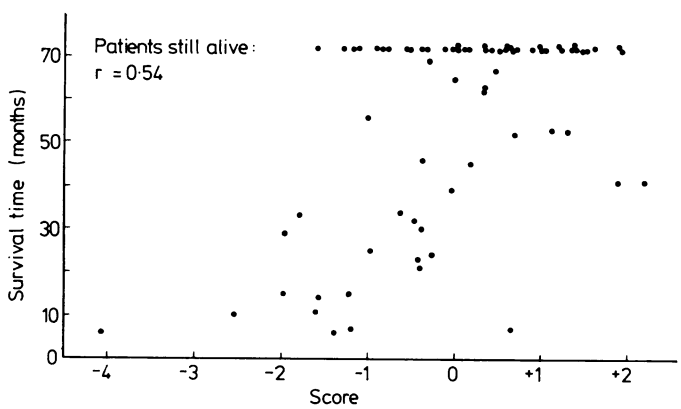

FIG 3—Survival time as function of each patient's predictive score (the 40 patients still alive were assigned the observation period of six years). This dimension explained 28·9% of the variance in survival time.

The LDL receptor content in solid tumours is of interest for several reasons. It has been shown that plasma cholesterol concentration is partly regulated by LDL receptors.[31] Furthermore, epidemiological studies have shown increased mortality from cancer among patients with low serum cholesterol concentrations.[32 33] The cause-effect relation is not clear. One explanation might be that the hypocholesterolaemia is caused by a high content of LDL receptors in a subclinical tumour present at the time of venepuncture. Recent evidence strongly suggests that hypocholesterolaemia in acute leukaemia is caused by high uptake of LDL, mediated by the receptors, by the leukaemic cells.[34] Our present findings, that a high LDL receptor content in breast cancer tissue seems to indicate a poor prognosis, suggest that breast tumours rich in LDL receptors may grow rapidly in vivo.

Studies are now in progress to evaluate further the role of LDL receptors in solid tumours. It is too early to demand determination of LDL receptor content after surgery to assess the prognosis of the patient as the results in this are based on a limited number of selected patients. In breast cancer predictive information obtained from the primary tumour is of particular interest. These tumours are now often diagnosed at an early stage so that fewer patients present with spread to the axillary nodes, which previously was an important prognostic factor. LDL receptor activity in solid tumours is also of interest with regard to treatment as LDL may be used as a carrier for cytotoxic drugs to cells with raised LDL receptor activity.[25 35-38]

We thank Miss Kristina Söderberg for excellent technical help with the LDL receptor determinations and Dr Svante Wold, University of Umeå, for his guidance and advice on our work with the partial least squares method. We gratefully acknowledge the computer facilities provided by Labcomp AB. This study was supported by grants from Robert Lundberg's Foundation, the Swedish Society for Medical Research, the Swedish Cancer Society, and the Swedish Medical Research Council (B84-14X-05964-04C).

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(Accepted 10 December 1985)