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Tumour Enzymes and Prognosis in Human Breast Cancer*

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Abstract—*The activities of 6-phosphogluconate dehydrogenase, phosphofructokinase and α-glycerolphosphate dehydrogenase were measured in primary carcinomas from a series of 333 patients with carcinoma of the breast and the usefulness of these estimations as additional prognostic parameters was evaluated. The patients in the series were followed for up to 50 months during which time 84 patients have developed recurrent disease. Life table analyses of the results showed that the probability of remaining free from recurrence was greater in women whose carcinomas had low activities of 6-phosphogluconate dehydrogenase and phosphofructokinase and high α-glycerolphosphate dehydrogenase activity. Low ratios of α-glycerolphosphate dehydrogenase to 6-phosphogluconate dehydrogenase were associated with a considerably increased risk of recurrence. These findings further indicate the usefulness of such assays as an aid to prognosis.*

INTRODUCTION

In previous communications from our group [1, 2] we have suggested that measurements of the activities of certain enzymes of carbohydrate metabolism in primary mammary carcinomas might prove useful in predicting those patients in which carcinomas were likely to recur because the enzyme activities showed significant differences in accordance with certain clinico-pathological factors associated with prognosis. Poorly differentiated carcinomas (Grade III) showed significantly higher activities of 6-phosphogluconate dehydrogenase (6PGDH), lactate dehydrogenase (LDH) and phosphohexose isomerase (PHI) than well differentiated (Grade I) tumours [1]. Tumours from patients without nodal involvement at the time of mastectomy showed significantly higher activity of α-glycerolphosphate dehydrogenase, higher α-GPDH/6PGDH ratios and lower activity of phosphofructokinase (PFK) than those in whom four or more lymph nodes were found to contain metastatic deposits [2]. Although there were very few recurrences amongst these cases at the time of our previous reports [1, 2] we postulated that the measurements of PFK, 6PGDH and α-GPDH activities and α-GPDH/6PGDH ratios might be useful in predicting the risk of recurrence. We have now extended this study and followed a series of 333 patients, in whose carcinomas we have measured the activities of these enzymes, for up to 50 months. Based on the recurrences which have occurred amongst these patients, we present further evidence which strengthens our original hypothesis.

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MATERIALS AND METHODS

Clinical

Samples of primary tumours were obtained from patients with carcinoma of the breast undergoing mastectomy. The diagnosis of carcinoma was confirmed by histological examination in each case. The tissue was wrapped in aluminium foil, frozen in solid CO_2 and transferred to the laboratory where it remained frozen in liquid nitrogen until further processing. All patients were staged pathologically by examination of axillary lymph nodes found in the mastectomy specimens. The nodes were processed routinely and sections from two levels of each lymph node block were examined in order to improve the chances of finding small metastatic deposits. In this series an average of 25 axillary lymph nodes (range 8–60) were found in modified radical mastectomy specimens. The malignancy grade

of infiltrating duct carcinomas was determined according to the procedure of Bloom and Richardson [3].

The first patient entered the series on 1 September 1975 and the results were analysed on 31 October 1979. During this period a series of 331 patients (age range 26–80) have been studied and followed for up to 50 months. There were 110 pre-menopausal and 223 menopausal or post-menopausal patients in this series. The patients were not selected on any specific clinical criteria other than suitability for surgical treatment and availability of sufficient tumour for enzyme study after tissue had been taken for histological examination. Most of the tumours utilized for biochemical study measured more than 1.5 cm in greatest diameter. In this series there were: five patients with medullary carcinoma with lymphoid stroma, four with mucoid carcinoma and 20 with infiltrating lobular carcinoma. Of the remaining patients seven had *in situ* ductal carcinoma or predominantly *in situ* carcinoma with only minimal infiltration. The remainder of the 297 patients had infiltrating ductal carcinomas without special features and these were all graded according to the procedure of Bloom and Richardson [3]. During the follow-up period, 84 of 333 patients developed recurrences. The clinical details of both recurrent and non-recurrent patients are presented in Table 1.

Table 1. Histo-pathological details of 333 patients with primary carcinoma of the breast

	Recurrent	Non-recurrent
Stage I	26	122
Stage II	58	127
Grade I	5	30
Grade II	28	118
Grade III	50	66
Involved lymph nodes		
0	26	122
1	13	45
2	9	23
3	5	7
4 or more	31	52

Numbers represent number of patients in each category. Infiltrating ductal carcinomas were graded according to the procedure of Bloom and Richardson [3] and staged by histological examination of axillary lymph nodes.

The results of the enzyme assays from 333 carcinomas were subjected to life table analyses according to the procedure described by Peto *et al.* [4]. The life tables were calculated for patients having an enzyme activity either above or below the median value for the entire group and the significance was tested using a "log-Rank test" [4].

Biochemical

The enzymes, co-enzymes and substrates used in the measurements of activities of PFK, 6PGDH and α-GPDH have been described in detail in previous communications [1, 2]. The frozen tissues were cleared of surrounding fat, weighed and cut into small pieces. They were then homogenized in a Silverson homogenizer as described by Shonk and Boxer [5] and the final concentration was adjusted to 50 mg/ml. The extracts were then centrifuged in a refrigerated centrifuge (4°C, 800 ***g***) for 20 min and the supernatants were used as the source of enzymes. The enzyme activities were measured in a Beckman Spectrophotometer (Model 25) according to the procedure of Shonk and Boxer [5]. Four carcinomas were assayed each time. The samples were assayed within a month of arrival in the laboratory.

RESULTS

The data were analysed in two stages. In the first instance, the whole series of patients were classed as a single group irrespective of pathological staging. The results of life table analyses are presented in Figs. 1–4. They clearly show that high activity of 6PGDH and/or PFK or low activity of α-GPDH and a

Table 2. Summary of the results of life table analyses on Stage II patients only

	N	R	Median (units/g weight)	Association	χ^2	P
PFK	185	58	0.040	above median	12.57	<0.005
α-GPDH	185	58	0.326	below median	6.70	<0.01
α-GPDH/6PGDH	185	58	2.19	below median	16.85	<0.005

Life tables were calculated according to the procedure of Peto *et al.* [4]. N = total number of patients; R = number of patients who are recurrent. The median value was calculated for the whole group and the association described shows increased risk of recurrence. χ^2 and P values are calculated from "Log Rank test."

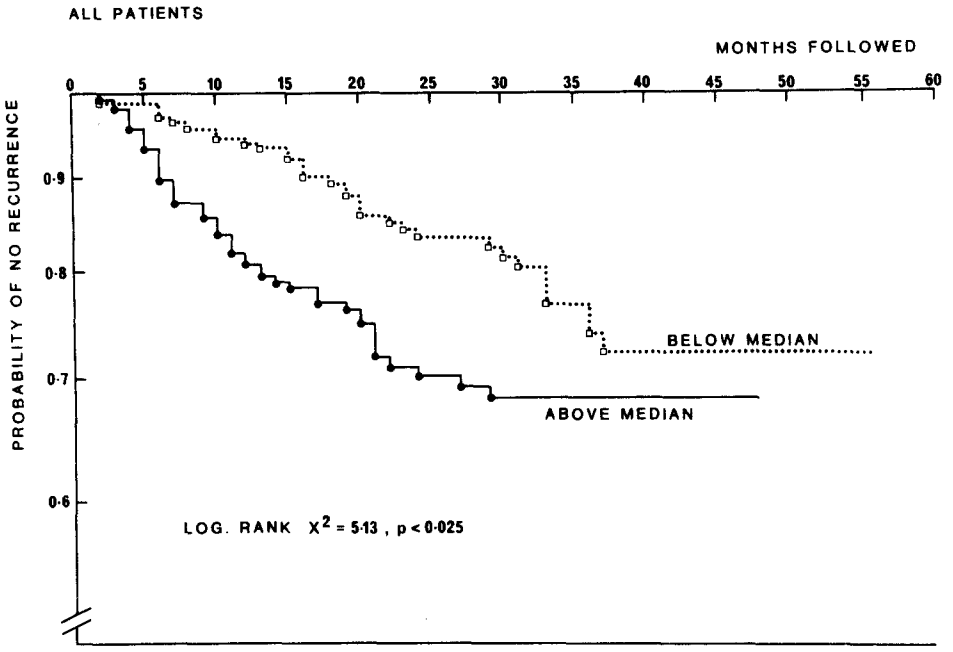

Fig. 1. *Life table for above and below the median value of* 0.147 (units/g *tumour tissue*) *of* 6*PGDH for carcinomas from all patients. Life table was calculated according to the procedure of Peto* et al. [4]. *Total number of patients* = 333, *total number of recurrences* = 84.

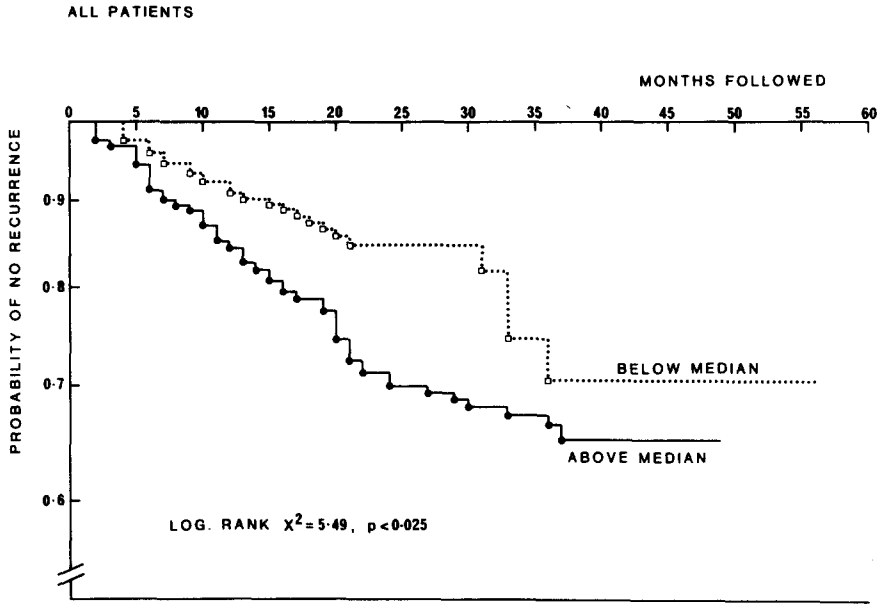

Fig. 2. *Life table for above and below the median value of* 0.051 (units/g *tumour tissue*) *of PFK for carcinomas from all patients. Life table was calculated according to the procedure of Peto* et al. [4]. *Total number of patients* = 333, *total number of recurrences* = 84.

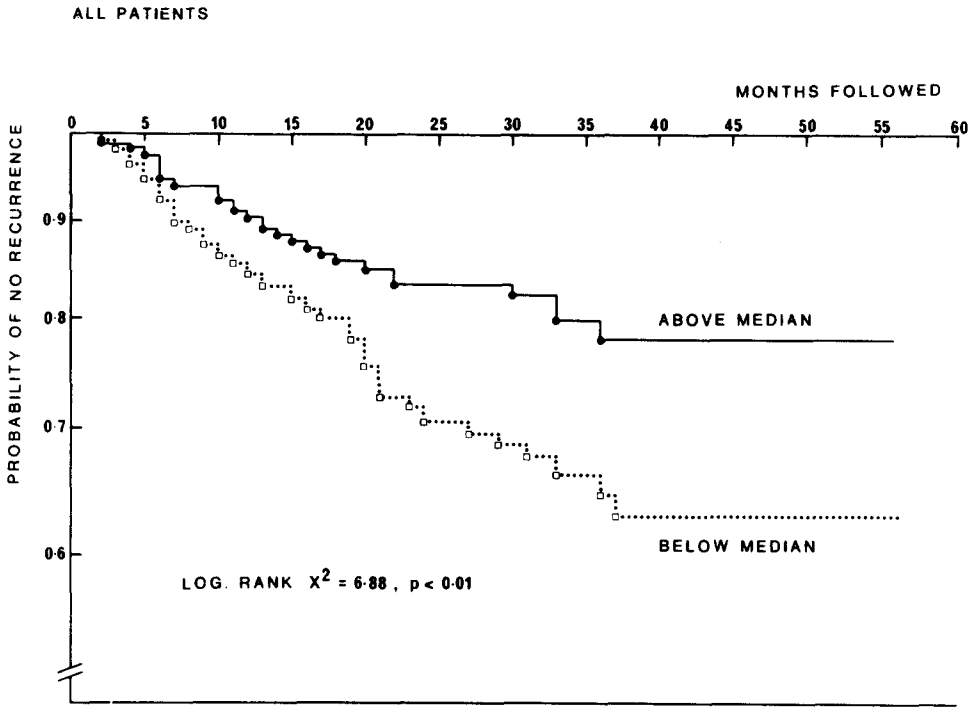

Fig. 3. Life table for above and below the median value of 0.312 (units/g *tumour tissue*) *of α-GPDH for carcinomas from all patients. Life table was calculated according to the procedure of Peto* et al. [4]. *Total number of patients* = 333, *total number of recurrences* = 84.

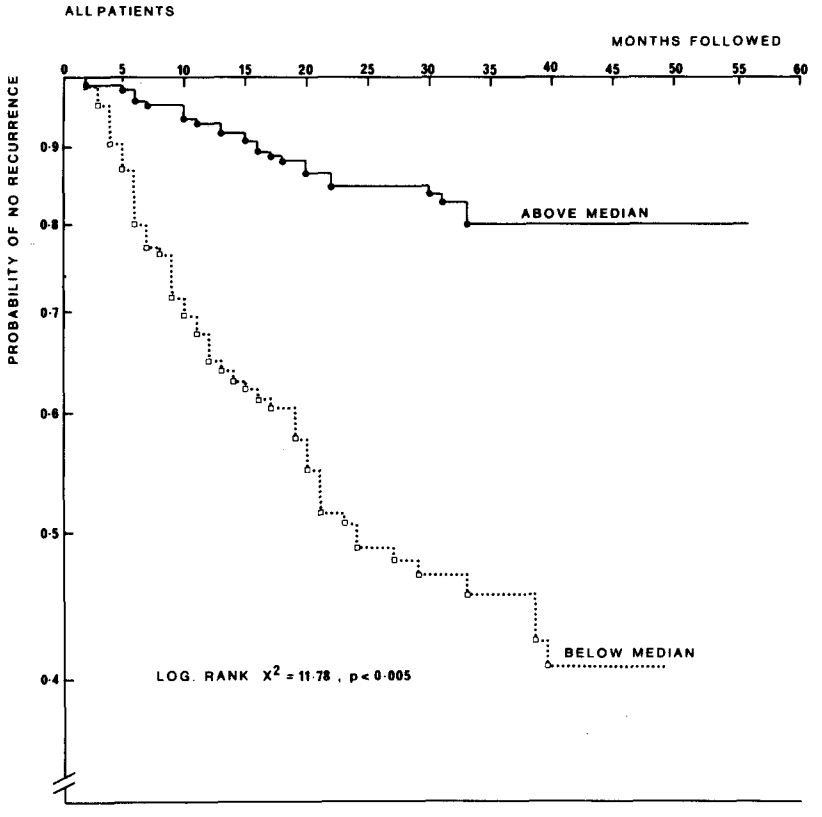

Fig. 4. Life table for above and below the median value of 2.184 *of ratio α-GPDH/6PGDH for all patients. Life table was calculated according to the procedure of Peto* et al. [4]. *Total number of patients* = 333, *total number of recurrences* = 84.

low α-GPDH/6PGDH ratio are associated with an increased risk of recurrence.

The patients were then separated on the basis of pathological staging into Stages I and II and the same life table analyses were performed on each group. Although both groups of patients showed similar phenomena to that reported in Figs. 1–4, the lines were not statistically significant for Stage I patients. The significant data obtained on Stage II patients are summarised in Table 2.

DISCUSSION

With the increasing tendency to use both endocrine and/or chemotherapy as adjuvant therapies following mastectomy for primary carcinoma of the breast, there is an urgent requirement for suitable parameters which will predict the likelihood of recurrence with reasonable accuracy [6–8]. With this in view, many attempts have been made to ascertain whether measurements of hormones, tumour antigens, proteins, enzymes or hormone receptors might act as prognostic indices in early breast cancer [9–16]. However, with the possible exception of hormone receptors [13, 16], none of these parameters appear to be accurate enough for routine clinical use and even the data on hormone receptors have been challenged by at least one group [17]. Thus there is total reliance on pathological staging in the selection of patients for adjuvant therapies. However, selection based on such a gross classification inevitably leads to some patients receiving treatments which they do not necessarily require and others who might have benefited from such treatments being rejected because of lack of nodal involvement at the time of mastectomy. Thus it is in this area that additional parameters will give further assistance to clinicians in selecting patients for adjuvant therapies.

Since all neoplastic tissues are characterized by a higher production of lactate than their tissue of origin, due to the increased activity of lactate dehydrogenase [18], various attempts have been made to investigate the use of LDH and other enzymes of glucose metabolism in prognosis. Hilf and his colleagues [19, 20] have shown that high levels of LDH and other enzymes in the primary tumour are useful predictors of response to cytotoxic chemotherapeutic drugs. Smith *et al.* [21] reported that the activities of LDH, 6PGDH and PHI in primary carcinomas are related to the interval between mastectomy and recurrence. However, both the number of recurrent and non-recurrent cases was small. Since all patients with primary carcinomas are at risk of recurrence, it would be inaccurate either to compare recurrent with non-recurrent cases or to use an arbitrarily fixed time interval to investigate the usefulness of biochemical parameters in prognosis. Furthermore, in any clinical trial, patients entering the trial at the beginning are automatically followed up for longer periods than those who have entered at the end. The life table techniques discussed by Peto *et al.* [4] takes these discrepancies into account and compares recurrent and non-recurrent cases on a continuous interval basis irrespective of the time of entry of a patient into the trial. Therefore we have analysed our data in two stages, both using his model. In the first instance we have classed all patients in the trial as a single population. This analysis was performed in order to test whether enzyme activities can predict the risk of recurrence irrespective of pathological parameters. Secondly, we separated the patients according to the pathological stage of the disease to investigate whether our parameters were capable of predicting the risk of recurrence in separate populations. None of the enzyme activities showed any significant differences in life table analyses in tumours from patients without nodal involvement (Stage I) in whom there have, as yet, been few recurrences. Analyses either treating the entire group as one population or considering Stage II patients separately, presented in Figs. 1–4 and Table 2, however, clearly indicate that more patients whose primary carcinomas have high activities of PFK and 6PGDH and low activity of α-GPDH are likely to recur than those in whom the phenomena are reversed. Since low α-GPDH and high 6PGDH activities showed strong association with the risk of recurrence, we felt that using the ratio of these two enzymes would improve prediction still further. The data presented in Fig. 4 clearly show that the ratio is the best predictor of recurrence so far in our analyses.

In conclusion, the measurement of these enzymes of carbohydrate metabolism appear to show considerable promise as an aid to prognosis. It is not known whether these enzymes are better in selecting likely recurrence than other established factors in current use.

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REFERENCES

1. N. Deshpande, I. Mitchell and R. Millis, Enzyme studies in human breast tumours. *Europ. J. Cancer* **13,** 1261 (1977).
2. N. Deshpande, I. Mitchell and R. Millis, Enzymes, lymph node involvement and possible prognosis in human breast cancer. *Europ. J. Cancer* **14,** 183 (1978).
3. H. J. G. Bloom and W. W. Richardson, Histological grading and prognosis in breast cancer. *Brit. J. Cancer* **11,** 359 (1957).
4. R. Peto, M. C. Pike, P. Armitage, N. E. Breslow, D. R. Cox, S. V. Howard, N. Mantel, K. McPherson, J. Peto and P. G. Smith, Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *Brit. J. Cancer* **35,** 1 (1977).
5. C. E. Shonk and G. E. Boxer, Enzyme patterns in human tissues. I. Methods for the determination of glycolytic enzymes. *Cancer Res.* **24,** 709 (1964).
6. J. W. Meakin, W. E. Allt, F. A. Beagle, T. C. Brown, R. S. Bush, R. M. Clark, P. J. Fitzpatric, N. V. Hawkins, R. D. T. Jenkin, J. F. Pringle, J. G. Read, W. D. Rider, J. L. Hayward and R. D. Bulbrook, Ovarian irradiation and prednisone therapy following surgery and radiotherapy for carcinoma of the breast. *Canad. med. Ass.* **120,** 1221 (1979).
7. B. Fisher and N. Wolmark, Systemic adjuvant (combined modality) therapy in the treatment of primary breast cancer. In *Breast Cancer* (Edited by W. L. McGuire), p. 125. Churchill Livingstone, Edinburgh (1977).
8. G. Bonadonna, E. Brusamalino, P. Valagussa, A. Rossi, L. Brugnatelli, C. Brambilla, M. DeLena, G. Tancini, E. Baietta, R. Musumeci and U. Veronesi, Combination chemotherapy as an adjuvant treatment in operable breast cancer. *New Engl. J. Med.* **294,** 406 (1976).
9. D. Y. Wang and M. C. Swain, Hormones and breast cancer. In *Biochemistry of Women, Methods in Clinical Investigations.* (Edited by A. S. Currie and J. V. Hewitt), p. 191. C.R.C. Press, Cleveland (1974).
10. D. Y. Wang, R. D. Bulbrook, J. L. Hayward, J. C. Hendrick and P. Franchimont, Relationship between plasma carcino-embryonic antigen and prognosis in women with breast cancer. *Europ. J. Cancer* **11,** 615 (1975).
11. D. C. Tormey, P. Waalkes, D. Ahmann, C. W. Gehrke, R. W. Zumwatt, J. Snyder and H. Hansen, Biological markers in breast carcinoma: I. Incidence of abnormalities of CEA, HCG, three polyamines and three minor nucleosides. *Cancer (Philad.)* **35,** 1095 (1975).
12. D. Kessel and J. Allen, Elevated plasma sialyltransferase in cancer patients. *Cancer Res.* **35,** 670 (1975).
13. W. A. Knight, R. B. Livingston, E. J. Gregory and W. L. McGuire, Estrogen receptors as an independent prognostic factor for early recurrence in breast cancer. *Cancer Res.* **37,** 4669 (1977).
14. R. C. Coombes, T. J. Powels and A. M. Neville, Evaluation of biochemical markers in breast cancer. *Proc. roy. Soc. Med.* **70,** 843 (1977).
15. R. C. Coombes, J. C. Gazet, H. T. Ford, T. J. Powels, A. G. Nash, A. McKinna and A. M. Neville, Assessment of biochemical tests to screen for metastases in patients with breast cancer. *Lancet* **i,** 296 (1980).
16. T. Cooke, D. George, R. Shields, P. Maynard and K. Griffiths, Oestrogen receptors and prognosis in early breast cancer. *Lancet* **i,** 995 (1979).
17. G. Bonadonna, P. Valagussa, G. Tancini and G. DiFronzo, Estrogen receptor status and response to chemotherapy in early and advanced breast cancer. *Cancer Chemother. Pharmacol.* **4,** 37 (1980).
18. W. E. Knox, The enzymic pattern of neoplastic tissue. *Advanc. Cancer Res.* **10,** 117 (1961).
19. M. L. Feldstein, E. D. Savlov and R. Hilf, A statistical model for predicting response of breast cancer patients to cytotoxic chemotherapy. *Cancer Res.* **38,** 2544 (1978).
20. E. D. Savlov, J. L. Wittliff and R. Hilf, Further studies of biochemical predictive tests in breast cancer. *Cancer (Philad.)* **39,** 539 (1977).
21. J. A. Smith, R. J. B. King, B. F. Meggitt and L. N. Allen, Enzyme activity, acidic nuclear proteins and prognosis in human breast cancer. *Brit. med. J.* **2,** 698 (1970).