before incorporating cytarabine in combination chemotherapy for Hodgkin's disease, the drug should be further investigated, exploring different dose-schedules in patients with slowly progressive disease and a reasonable bone marrow tolerance. Cytarabine has been incorporated in high dose schemes and claims a high response rate in previously treated Hodgkin patients [7] but there is no indication that the use of cytarabine in those schemes adds to the efficiency of these treatments.

1. Chalmer BL, Myers CF. Clinical pharmacology of cancer chemotherapy. In: De Vita Jr VT, Hellman S, Rosenberg SA, eds. *Cancer, Principles and Practice of Oncology*. Lippincott Company, 1982, 167–169.
2. Phillips GL, Reece DE. Clinical studies of autologous bone marrow transplantation in Hodgkin's disease. *Clinics Haematol* 1986, **15**, 151–166.
3. Silverman L, Jones R, Norton L, *et al.* Combination chemotherapy for refractory lymphoma with cytosine arabinoside (A), cisplatin (P) and etoposide (E). *Proc Asco C*-963, 1984.
4. Wimmer R, Weiner M, Strauss L, *et al.* Treatment of pediatric patients for relapsed Hodgkin's disease with cytosine arabinoside (A), cisplatin (P) and etoposide (E). *Proc Asco* 753, 1987.
5. Carter SK. Clinical aspects in the design and conduct of phase II trials. In: Buyse ME, Staquet MJ, Sylvester RJ, eds. *Cancer Clinical Trials, Methods and Practice*. Oxford University Press, 1984, 229.
6. WHO Handbook for reporting results of cancer treatment. World Health Organization, Geneva, 1979.
7. Vose JM, Bierman PJ, Armitage JO. Hodgkin's disease: the role of bone marrow transplantation. *Seminars in Oncology* 1990, **17**, 749–757.

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Lymphocyte Infiltrates as a Prognostic Variable in Female Breast Cancer

S. Aaltomaa, P. Lipponen, M. Eskelinen, V.-M. Kosma, S. Marin, E. Alhava and K. Syrjänen

The predictive value of lymphocyte infiltrates (LI) was studied in 489 patients with breast cancer followed-up for over 10 years. LI were positively correlated to axillary lymph-node status, tumour diameter and histological and morphometric variables ($P < 0.001$). In a multivariate analysis LI were independently related to axillary lymph-node status. LI predicted recurrence-free survival (RFS) in rapidly proliferating tumours ($P = 0.0269$). LI predicted RFS ($P = 0.08$) and breast cancer related survival (BS) ($P = 0.0164$) in rapidly proliferating, axillary lymph-node negative tumours. In a multivariate analysis LI independently predicted BS ($P = 0.08$) in rapidly proliferating tumours. LI independently predicted BS in rapidly ($P = 0.025$) and slowly ($P = 0.09$) proliferating, axillary lymph-node negative tumours. If the tumours were not categorised according to proliferation rate, LI and outcome were not significantly related. The results clearly confirm the presence of efficient immunological antitumour defence mechanisms in human breast cancer. Consequently tumour–host interactions are subject to further studies particularly in axillary lymph-node negative breast cancer.
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INTRODUCTION

BREAST TUMOURS are often surrounded by inflammatory cell infiltrates as a sign of tumour–host interactions [1–6] and the presence of lymphocyte infiltrates (LI) is considered to reflect hosts effort to resist tumour growth [3]. Although there is evidence in experimental conditions for an immunological antitumour activity against breast tumour cells [6, 7] the effectiveness of these mechanisms in clinical oncology of breast tumours is a matter of debate [8–10]. Some authors have related the presence of LI to favourable prognosis [11, 12] whereas others have found no positive correlation between prognosis and the LI [13, 14]. The presence of dense LI has also been related to high recurrence rate [15]. Previous studies have shown that LI are significantly related to malignant histological features [3, 5, 12, 15]. Therefore, the assessment of the independent role of LI is impossible without multivariate statistics and fractioning of breast tumours into comparable groups in relation to other prognostic factors. Thus, the present study was carried out to evaluate the predictive value of LI in relation to clinical, morphomerical and histological variables in 489 breast cancer patients followed up for over 10 years in one Finnish institution.

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PATIENTS AND METHODS

The study comprised 489 female patients with breast cancer that was treated and followed up at the University Hospital of Kuopio during 1968–1990. In these patients the case histories and preoperative biopsy specimens from the primary tumours were available for analysis. Axillary lymph node status was assessed by histological examination in 428/489 (87%) of cases and in 61 (12%) of cases a clinical judgement was available. Tumour size was the maximum tumour diameter as measured in fresh mastectomy specimen by the operating surgeon. Metastases were detected by routine chest and bone X-rays, laboratory tests reflecting bone and liver metabolism and histologically when appropriate. The follow-up was usually at 3 month intervals during the first year, at 6 month intervals during the next 2 years and annually thereafter. The determination of deaths due to breast cancer was made by critical review of necropsy reports, death certificates and case histories. The pertinent clinical data of patients are presented in Table 1.

The histopathological samples were preoperative biopsy specimens fixed in formalin (pH 7.0) immediately after removal and embedded in paraffin. 5 μ thick sections were cut in 1990 and stained with haematoxylin and eosin. The histological grading [16] and histological typing [1, 17] of tumours was completed simultaneously by two certified pathologists (V-MK, SM) using a consultation microscope in a blinded manner. The amount of tumour necrosis and nuclear pleomorphism were scored semiquantitatively as shown in Table 2.

The mitotic figures were counted with a consultation microscope by two observers (PL, SA) using an objective magnification of 40 × (field diameter 490 μ). The mitotic figures were identified as described earlier [18] from the most cellular areas of the tumour margins avoiding necrotic areas. The volume corrected mitotic index (M/V index) was estimated as described by Haapasalo *et al.* [19]. The M/V index expresses the number of mitotic figures/mm^2 of neoplastic tissue in the section.

The density of LI was graded into three categories and they were: absent and weak (Fig. 1a), moderate and dense (Fig. 1b). Only LI were scored whereas polymorphonuclear leucocytes and plasma cells were tried to be excluded in the scoring process. LI around blood vessels, in the centre and periphery of tumours and around invasive carcinoma cells were included in the scoring process. The general density of LI was important. The LI was dense when the tumour margins and stroma contained a dense lymphocyte infiltrate (Fig. 1b). LI was weak when occasional lymphocytes were encountered in the stroma and the inflammatory cell reaction around the tumour also consisted of mainly occasional cells or regionally of moderate cell reaction. Moderate LI was situated between these two extremes. Previous studies conducted in our laboratory have indicated that LI are mainly T-lymphocytes [3].

Table 1. The clinical characteristics of patients

No. patients	489
Age at diagnosis, mean (S.D.)	59.9 (13.5) years
Follow-up time, mean (S.D.)	11.7 (4.1) years
Diameter of tumour, mean (S.D.) (S.E.)	3.8 (4.1) (0.2) cm
Axillary lymph node negative/positive	265/224
Type of primary treatment	
Modified mastectomy	233
Mastectomy and adjuvant therapy	242
No mastectomy	14
Number of cases with recurrences	204
Causes of death	
Breast cancer/other	198/74
Histological grade	28/279/161
Grade 1/Grade 2/Grade 3	
Histological type	427/51/2/17
Ductal/lobular/medullary/other	
Lymphocyte infiltrate	104/145/240
Weak/moderate/dense	

Table 2. The relationship between lymphocyte infiltrates, histological type, grade, mitotic frequency, tumour necrosis, pN status and tumour size

	Lymphocyte infiltrate			
Variable	Weak	Moderate	Dense	*P* value*
Histological type				
Ductal	78	127	214	
Lobular	21	14	16	0.006
Other	4	5	10	
Histological grade‡				
Grade 1	12	9	7	
Grade 2	74	102	103	<0.001
Grade 3	13	27	121	
M/V index				
≤ 15/mm^2	77	99	91	<0.001
> 15/mm^2	27	46	149	
Tumour necrosis				
None	86	126	161	
Spotty	9	11	46	<0.001
Moderate	1	3	15	
Heavy	1	1	6	
pN status†				
Negative	49	70	100	<0.011
Positive	31	53	125	
Tumour size†				
0–2.0 cm	32	52	17	
2.1–5.0 cm	48	67	29	0.443
>5.0 cm	76	130	32	

*χ^2 test; †were not available in all cases.
‡Mainly intraductal tumours were not graded.

In morphometric measurements the IBAS 1 and 2 image analyser was used. The images of most atypical well preserved microscopic fields were focused on a video screen through a video camera attached to the microscope (magnification 40 ×). A mean of 75 nuclei were traced using a digitiser tablet and a mouse connected to the computer. The computer calculated mean nuclear area (NA), standard deviation of nuclear area (SDNA), nuclear perimetry (PE), standard deviation of nuclear perimetry (SDPE), largest nuclear diameter (D_{max}), shortest nuclear diameter (D_{min}) and the mean area of the 10 largest nuclei (NA10).

The basic statistics were done by using the SPSS/PC+ program package in a Toshiba T3200 computer. Univariate survival analysis was based on life table method with the statistics by Lee and Desu [20] (SPSS-X). In multivariate survival analysis the Cox's model [21] of the BMPD(2L) (BMPD Statistical Software, Department of Biomathematics, UCLA) program package was used in a VAX computer. Recurrence free survival (RFS) was defined as the time elapsed between primary surgical therapy and first verified metastasis or recurrent growth. The

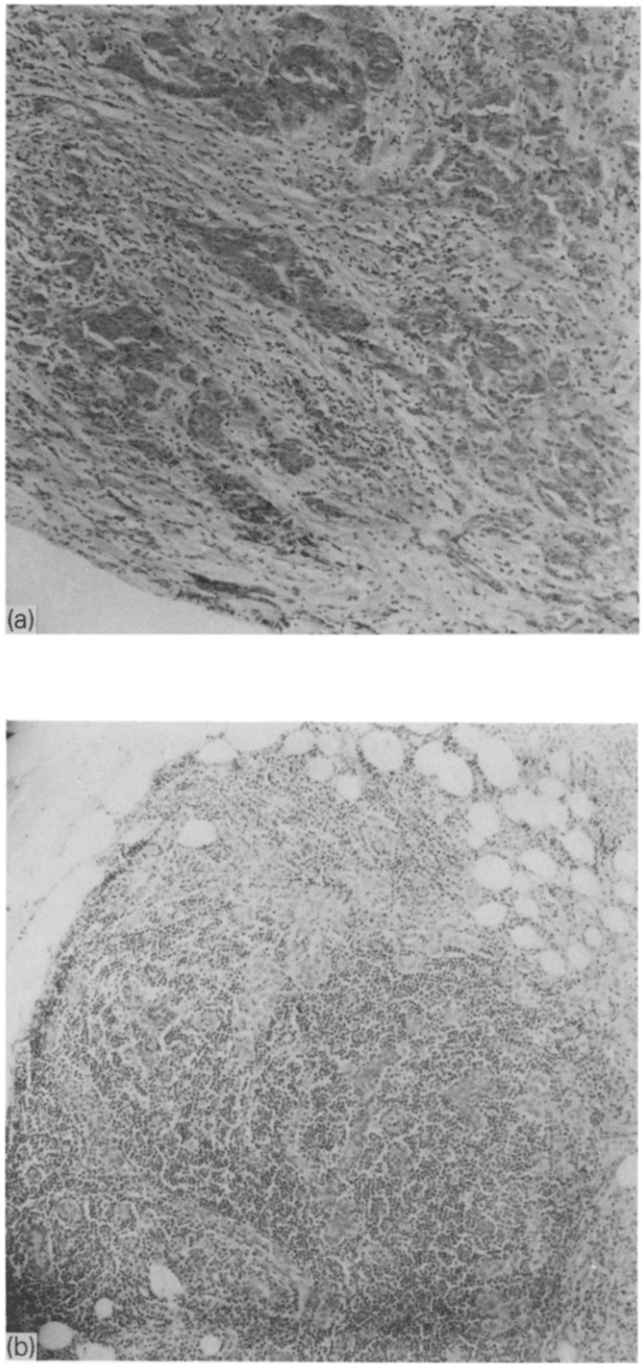

Fig. 1. Breast tumour surrounded by weak (a) and dense (b) LI (100 ×).

specific breast cancer related survival (BS) was used in all analyses. Nuclear morphometric variables, *M/V* index and tumour size were used as continuous variables in multivariate analysis. Other variables were categorised as shown in Tables 1 and 2. Multivariate analysis included only those cases with a complete set of data.

RESULTS

Relationship between LI and other predictors

The relationship between histological variables, tumour size, axillary lymph node status and LI is shown in Table 2. The morphometric variables differed significantly among LI grades

Table 3. The relationship between lymphocyte infiltrates and morphometric variables

Variable	Lymphocyte infiltrate			
	Weak	Moderate	Dense	*P* value*
NA(μ^2)	76 (70)	81 (28)	100 (62)	0.0002
SDNA (μ^2)	19 (9)	23 (12)	30 (14)	<0.0001
NA10 (μ^2)	101 (38)	120 (46)	147 (59)	<0.0001
PE (μ)	33 (5)	36 (6)	39 (7)	<0.0001
SDPE (μ)	5 (1)	6 (8)	6 (2)	0.0190
D_{min} (μ)	7 (1)	6 (5)	9 (2)	0.0025
D_{max} (μ)	12 (2)	13 (2)	14 (2)	<0.0001
M/V index	13 (16)	14 (14)	26 (21)	<0.0001

*Analysis of variance.

Table 4. The independent predictors of pN status in a logistic multivariate analysis

	β	S.E.	*P*-value
Tumour diameter	0.0279	0.0054	<0.0001
D_{max}	0.1201	0.0463	0.0095
LI	0.3239	0.1467	0.0273

β = coefficient of the regression model; S.E. = standard error.

(Table 3). Tumours with large nuclei and high mitotic frequency showed usually also dense LI.

In a logistic multivariate regression analysis tumour size, D_{max} and LI were independently related to axillary lymph node status (Table 4). In rapidly proliferating tumours (*M/V* index ≥ 15/mm^2) a similar independent relationship was found between pN status and LI ($P = 0.03$) whereas in slowly proliferating tumours (*M/V* index < 15/mm^2) LI were not related to axillary lymph node status LI was not related to recurrence or metastatis during the follow-up.

Univariate analysis

In the entire series RFS was not related to LI ($P = 0.5$). In rapidly proliferating tumours (*M/V* index ≥ 15/mm^2) RFS was related to LI ($P = 0.0269$, Fig. 2), axillary lymph node status

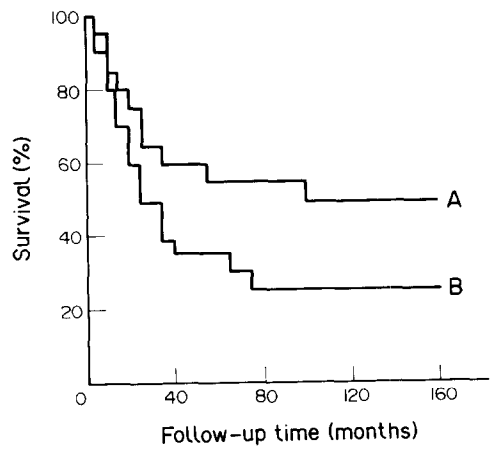

Fig. 2. The RFS of patients with *M/V* index ≥ 15/mm^2 categorised according to LI ($\chi^2 = 4.9$; $P = 0.0269$). Curve A: moderate or dense LI, $n = 27$; Curve B: weak or absent LI, $n = 192$.

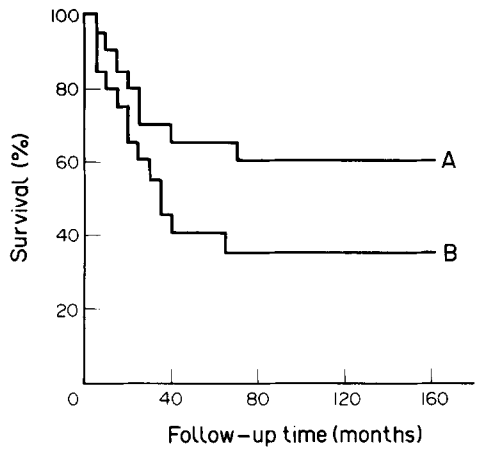

Fig. 3. RFS of axillary lymph node negative patients with *M/V* index ≥ 15/mm² categorised according to LI ($\chi^2 = 3.0$, $P = 0.08$). Curve A: moderate or dense LI, $n = 15$; Curve B: absent or weak LI, $n = 83$.

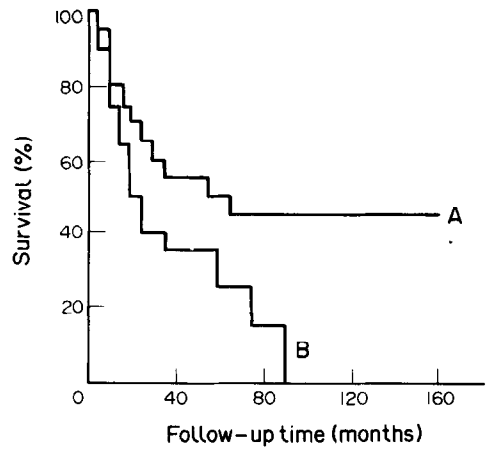

Fig. 5. RFS of axillary lymph node positive patients with *M/V* index ≥ 15/mm² categorised according to LI ($\chi^2 = 2.6$, $P = 0.10$). Curve A: moderate or dense LI, $n = 12$; Curve B: weak or absent LI, $n = 108$.

($P = 0.062$), histological grade ($P = 0.18$) and tumour size ($P = 0.33$). In slowly proliferating tumours (*M/V* index < 15/mm²) axillary lymph node status ($P = 0.0108$), tumour size ($P = 0.14$), LI ($P = 0.28$) and grade ($P = 0.7$) were related to RFS.

In the entire cohort BS was not related to LI ($P = 0.5$). In tumours with *M/V* index > 15/mm² axillary lymph node status ($P < 0.0001$), tumour size ($P < 0.0001$) and LI ($P = 0.167$) predicted BS. In tumours with *M/V* index < 15/mm² LI was not related to BS ($P = 0.6$).

In axillary lymph node negative tumours with *M/V* index > 15/mm² LI predicted RFS ($P = 0.08$, Fig. 3) and BS ($P = 0.0164$, Fig. 4). Tumour size ($P = 0.041$) predicted BS significantly as well. In axillary lymph node negative tumours with *M/V* index < 15/mm² LI, tumour size or grade were not related RFS or BS ($P > 0.3$).

In axillary lymph node positive tumours with *M/V* index ≥ 15/mm² LI predicted RFS ($P = 0.10$, Fig. 5) whereas in slowly proliferating tumours LI had no predictive value ($P = 0.4$). In survival analysis of rapidly proliferating tumours (*M/V* index ≥ 15/mm²) LI ($P = 0.7$) could not predict BS whereas in slowly proliferating tumours LI had predictive value ($P = 0.05$).

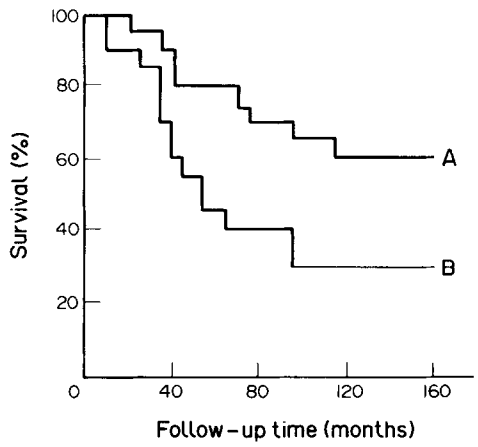

Fig. 4. BS of axillary lymph node negative patients with *M/V* index ≥ 15/mm² categorised according to LI ($\chi^2 = 5.7$, $P = 0.0164$). Curve A: moderate or dense LI, $n = 15$; Curve B: weak or absent LI, $n = 83$.

Multivariate analysis

In rapidly proliferating tumours (*M/V* index ≥ 15/mm²) LI was independently related to BS (Table 5) whereas in slowly proliferating tumours LI had no independent prognostic value. In axillary lymph node negative tumours LI had independent predictive value (Table 5) whereas in axillary lymph node positive tumours LI was not independently related to RFS or BS. LI was not independently related to RFS in axillary lymph node negative tumours or in the entire cohort.

DISCUSSION

Tumour size [22–28], axillary lymph node status [22–27], histological type [1, 17, 26], histological grade [1, 22, 23, 26], nuclear morphometric variables [22, 23, 27], mitotic frequency [22, 23, 26, 27], sex steroid receptor content [29], DNA content or S-phase fraction [24–26, 28] and oncoproteins [30] convey significant prognostic information in breast cancer. These methods are usually able to accurately predict outcome in regional breast cancer whereas they have often failed to predict outcome in small axillary lymph node negative breast tumours [14, 15, 28]. The variable results with conventional methods in

Table 5. The results of the multivariate survival analysis

Category	β(S.E.)	β/S.E.	*P*-value
Entire cohort			
M/V index ≥ 15/mm² ($n = 222$)			
pN status	0.827 (0.199)	4.152	<0.001
Tumour size	0.002 (0.001)	2.343	0.016
LI	−0.210 (0.121)	−1.742	0.088
pN(−) tumours;			
M/V index < 15/mm² ($n = 153$)			
LI	−0.418 (0.249)	−1.672	0.092
Nuclear pleomorphism		2.394	0.048
	0.835 (0.384)		
pN(−) tumours			
M/V index ≥ 15/mm² ($n = 98$)			
LI	−0.578 (0.208)	−2.782	0.025
SDPE	0.091 (0.041)	2.230	0.067

β = coefficient of the regression model; S.E. = standard error.

local tumours may be related to effective tumour–host interactions.

Breast tumours are often surrounded by inflammatory cells as a morphological sign of host defence mechanisms [2, 3, 5]. Most of the inflammatory cells are T-lymphocytes [3] which indicates a cytotoxic response. A pronounced cell reaction has been related to both favourable [11, 12, 31] and poor prognosis [13–15, 32, 33]. Also sophisticated analyses of special inflammatory cell types as determinants of prognosis have been disappointing [4].

In this analysis LI was related to clinical, histological and morphometric variables. Tumours with axillary lymph node metastasis and large nuclei or high mitotic frequency were often surrounded by dense LI. On the other hand localised tumours with a more benign histology were usually free of LI. A similar association between tumour histology and LI has previously been reported [3, 5, 12, 15]. From the above, it follows that the immunogenity of tumours is related to their malignant behaviour and to some as yet unknown characteristics of cancer cells. Consequently, the assessment of the independent predictive value of LI is problematic due to several interrelations between established prognostic factors and LI.

In a logistic multivariate regression analysis axillary lymph node status was related to LI. The coefficient of the regression model for LI was positive (positive correlation), as if the presence of LI would promote axillary lymph node involvement. Since the LI are firmly related to several malignant features of breast cancer cells that are related to axillary lymph node metastasis, it implicitly follows that in a retrospective analysis LI are interpreted as promoters of axillary metastasis. If we were able to introduce a time scale in the analysis or exactly define the cause for the presence of LI we would probably be able to demonstrate the opposing action of LI on the axillary lymph node involvement.

LI were not related to distant metastasis during the follow-up. This is in accordance with the above since the intrinsic malignancy of cancer cells probably determines whether they become metastatic or not. Thus, the opposing action of host immune mechanisms can only be demonstrated by including a time scale in the analysis.

The relationship between RFS and LI validates the conclusions presented above. In rapidly proliferating tumours LI was able to predict RFS significantly. The difference in RFS could be demonstrated even in axillary lymph node positive tumours. The importance of the present result is emphasised by the presence of other significant predictors in the multivariate analysis like mitotic frequency [22, 23] which was a more important predictor of RFS also in the present Cox's analysis.

In survival analysis LI was a significant predictor in rapidly proliferating tumours which is in line with the results from Elston *et al.* [12] who found prognostic value for LI in grade 3 tumours. In accordance with the above, Rilke and coworkers have established prognostic value for LI [31]. In rapidly proliferating axillary lymph node negative tumours LI was the most important predictor of survival followed by nuclear morphometric variables. In slowly proliferating tumours the inflammatory cells were inferior to nuclear variables in predicting survival.

A review of the literature shows that most of analyses presented until now on the role of LI as predictors in breast cancer have used univariate methods [4, 11–14]. However, Tubiana and coworkers [32, 33] used multivariate methods and they found that dense inflammatory cell infiltrate was a sign of unfavourable prognosis. Their analysis, however, included a mixed bunch of breast tumours and no standardisation of the material was done according to proliferation rate. Most of published materials have not considered histological type, grade or proliferation rate which are all important prognostic factors in breast cancer. Due to several interrelations between LI and other prognostic variables, significant survival data related to LI may be lost without multivariate statistics. In the present series as well, inflammatory cells had no independent prognostic value if the entire cohort was analysed.

The results prompted several questions. Why is the prognostic value of LI mainly confined to rapidly proliferating tumours? These tumours may bear more tumour antigens on the cell surface than slowly proliferating tumours. Secondly, they may secrete immunogenic protein products into intercellular spaces evoking an immune response. Thirdly, they may constitute a more homogenous group of tumours than the slowly proliferating ones with respect to their pretreatment history and thus the tumour–host interaction can be more easily demonstrated in these tumours. Consequently, further experimental and clinical studies are urgently needed to clarify these questions.

On the basis of this analysis the following conclusions are drawn: Inflammatory cell infiltrates (a) are related to several clinicopathological features in breast cancer, (b) predict axillary lymph node metastasis, (c) are significant predictors of RFS and BS in rapidly proliferating breast tumours, particularly in axillary lymph node negative tumours.

1. Page DL, Anderson TJ. *Diagnostic Histopathology of the Breast.* Churchill Livingstone, Edinburgh, 1987.
2. Syrjänen KJ, Hjelt LH. Tumor–host interrelationship in carcinoma of the female breast. *Surg Gynec Obstet* 1978, **147**, 43–48.
3. Naukkarinen A, Syrjänen K. Quantitative immunohistochemical analysis of mononuclear infiltrates in breast carcinomas-correlation with tumour differentation. *J Pathol* 1990, **160**, 217–222.
4. Wintzer H-O, Bohle W, von Kleist S. Study of the relationship between immunohistochemically demonstrated lymphocytes infiltrating breast carcinomas and patient's survival. *J Cancer Res Clin Oncol* 1991, **117**, 163–167.
5. Syrjänen KJ, Kosma V-M. Hormone receptor levels related to histological parameters of tumor–host relationship in female breast cancer. *J Surg Oncol* 1982, **21**, 49–53.
6. Anichini A, Fossati G, Parmiani G. Clonal analysis of the cytolytic T-cell response to human tumors. *Immunol Today* 1987, **8**, 358–389.
7. Whiteside TL, Miescher S, Hurlimann J, Moretta L, von Fliedner V. Clonal analysis and in situ characterization of lymphocytes infiltrating human breast carcinomas. *Cancer Immunol Immunother* 1986, **23**, 169–178.
8. Johnson JP, Riethmuller G, Schirrmacher V. Tumor immunology: Paul Erlich's heritage. *Immunol Today* 1989, **10**, 35–37.
9. Kripke ML. Immunoregulation of carcinogenesis: past, present, and future. *J Natl Cancer Inst* 1988, **80**, 722–727.
10. Old LJ. Structural basis for tumor cell recognition by the immune system. In: Melchers F, *et al.* eds *Progess in Immunology*, Berlin, Springer, 1035–1062.
11. Black MM, Hankey BF, Barclay THC. Intrastage prognostic heterogeneity: Implications for adjuvant chemotherapy of breast cancer. *J Natl Canc Inst* 1982, **68**, 445–447.
12. Elston CW, Gresham GA, Rao GS, *et al.* The cancer research campaign (King's/Cambridge) trial for early breast cancer. *Br J Cancer* 1982, **45**, 655–669.
13. Dawson PJ, Karrison T, Ferguson DJ. Histologic features associated with longterm survival in breast cancer. *Hum Pathol* 1986, **17**, 1015–1021.
14. Roses DF, Bell DA, Flotte TJ, Taylor R, Ratech H, Dubin N. Pathologic predictors of recurrence in stage I (T1N0M0) breast cancer. *Am J Clin Pathol* 1982, **78**, 817–820.
15. Rosen PP, Saigo PE, Braun DW, Weathers E, De Palo A. Predictors of recurrence in stage I (T1M0N0) breast carcinoma. *Ann Surg* 1981, **193**, 15–25.

16. Bloom HJG, Richardson WW. Histological grading and prognosis in breast cancer. *Br J Cancer* 1957, **11**, 359–377.
17. WHO. Histological typing of breast tumors. International histological classification of tumors No 2. 2nd ed. Geneva World Health Organisation, 1981.
18. Baak JPA, Oort J. Obtaining quantitative data. In: Baak JPA, Oort J, eds. *A Manual of Morphometry in Diagnostic Pathology*. Berlin, Springer, 1983, 15–26.
19. Haapasalo H, Collan Y, Pesonen E. Volume-corrected mitotic index (M/V index) – the standard of mitotic activity in neoplasms. *Path Res Pract* 1989, **185**, 551–554.
20. Lee E, Desu M. A computer program for comparing k samples with right censored data. *Comput Programs Biomed* 1972, **2**, 315–318.
21. Cox DR. Regression models and life tables with discussion. *J R. Stat Soc B* 1972, **34**, 187–192.
22. Aaltomaa S, Lipponen P, Eskelinen M, Alhava E, Syrjänen K. Nuclear morphometry and mitotic indexes as prognostic factors in breast cancer. *Eur J Surg* 1991, **157**, 319–324.
23. Baak JPA, Van Dop H, Hermans J. The value of morphometry to classic prognosticators in breast cancer. *Cancer* 1985, **56**, 374–382.
24. Eskelinen M, Collan Y, Pajarinen P, Pesonen E, Kettunen K, Nordling S. An improved prognostic index of axillary node involvement in breast cancer incorporating DNA ploidy and tumour size. *Acta Chir Scand* 1990, **156**, 521–527.
25. Eskelinen MJ, Pajarinen P, Collan Y, Pesonen E, Alhava E, Kettunen K, Nordling S. Relationship between DNA ploidy and survival in patients with primary breast cancer. *Br J Surg* 1989, **76**, 830–834.
26. Toikkanen S, Joensuu H, Klemi P. Prognostic significance of nuclear DNA content in invasive breast cancer—a study with long-term follow-up. *Br J Cancer* 1989, **60**, 693–700.
27. Uyterlinde AM, Baak JPA, Schipper NW, Peterse H, Matze E, Meijer CJ. Further evaluation of the prognostic value of morphometric and flow cytometric parameters in breast cancer patients with long follow-up. *Int J Cancer* 1990, **45**, 1–7.
28. Reilly SM, Camplejohn RS, Barnes DM, Millis RR, Rubens RD, Richards MA. Node negative breast cancer: prognostic subgroups defined by tumor size and flow cytometry. *J Clin Oncol* 1990, **8**, 2040–2046.
29. Alanko A, Heinonen E, Scheinin T, Tolppanen E-M, Vihko R. Significance of estrogen and progesterone receptors, disease-free interval, and site of first metastasis on survival of breast cancer patients. *Cancer* 1985, **56**, 1696–1700.
30. Dykins R, Corbett IP, Henry JA, *et al.* Long-term survival in breast cancer related to overexpression of the c-*erb* B-2 oncoprotein: an immunohistochemical study using monoclinal antibody NCL-CB11. *J Pathol* 1991, **163**, 105–110.
31. Rilke F, Colnaghi MI, Cascinelli N, *et al.* Prognostic significance of HER-2/NEU expression in breast cancer and it's relationship to other prognostic factors. *Int J Cancer* 1991, **49**, 44–49.
32. Tubiana M, Pejovic MH. Chavaudra N, Contesso G, Malaise EP. The long term prognostic significance of the thymidine labeling index in breast cancer. *Int J Cancer* 1984, **33**, 441–445.
33. Tubiana M, Pejovic MJ, Contesso G, Chavaudra N, Contesso G, Malaise EP. Kinetic parameters and the course of the disease in breast cancer. *Cancer* 1981, **47**, 937–943.

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Suramin in Advanced Platinum-resistant Ovarian Cancer

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10 patients with ovarian cancer, whose disease had progressed while receiving platinum-based therapy, were entered onto a phase II clinical trial of the antiproliferative agent suramin. Suramin was administered in a fashion that is associated with durable objective disease response in patients with hormonally resistant metastatic prostate cancer. No individual had an objective response to therapy in this study, but 3 of 9 evaluable patients (33%) experienced disease stabilisation and subjective clinical improvement for periods ranging from 2 to 5 months. Disease stabilisation was associated with prolonged periods of comparatively high plasma levels of drug, which appeared to be determined primarily by reduced drug clearance. We conclude that suramin has potential activity in platinum-resistant ovarian cancer, and we have initiated a second clinical trial using pharmacological information derived from this study.
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INTRODUCTION

A NUMBER of recent clinical trials have attempted to identify chemotherapeutic agents that might cause disease regression in recurrent ovarian cancer that is resistant to cisplatin. Taxol [1], hexamethylmelamine [2], 5-fluorouracil and mitomycin C [3], and ifosfamide [4] are among the regimens reported to have activity in platinum-resistant disease. With the exception of taxol, the author's precise definition of platinum-resistance cannot be clearly discerned in any of these studies. Further, these cytotoxic agents have profound toxicity and are usually associated with disease responses of short duration.

Recent clinical studies show that suramin, a novel anticancer agent which appears to act primarily through antiproliferative mechanisms, is active against advanced stage prostate cancer, adrenocortical cancer, and some types of refractory lymphomas [5–7]. In addition, preclinical data from our group and from the