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Acknowledgement—This work was conducted within the framework of the National Research Council (CNR), Applied Projects "Oncology" (contract No. 87.01544.44), and "Prevention and Control of Disease Factors", and with the contribution of the Italian Association for Cancer Research and the Italian League Against Tumours, Milan and Mrs A. Marchegiano Borgomainerio. The authors wish to thank Mrs J. Baggott, Mrs M.P. Bonifacino, and the G.A. Pfeiffer Memorial Library for editorial assistance.

Eur J Cancer, Vol. 27, No. 5, pp. 586–591, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Factors Predicting Late Mortality from Breast Cancer

Sakari P. Toikkanen, Harry P. Kujari and Heikki Joensuu

Survival data of a cohort of 160 patients with breast cancer, who were still alive 10 years after the primary diagnosis, and who had been followed up for at least 22 years, were investigated to find out those factors that predict late mortality from breast cancer. The 13 factors investigated included age at diagnosis, histological type and grade, mitotic count, type of tumour margin, inflammatory cell reaction, extent of tumour necrosis, primary tumour size, axillary nodal status, DNA ploidy and index, S-phase fraction and occurrence of a second primary breast cancer. Advanced age at diagnosis (> 49 years, $P = 0.002$), occurrence of a second primary breast cancer during the follow-up ($P = 0.01$), and primary tumour size (T_{3-4}, $P = 0.03$) were significantly associated with mortality from breast cancer after the 10th year of follow-up in a multivariate analysis, and the ductal invasive histological type ($P = 0.03$) and a large DNA index (> 1.2; $P = 0.06$) in univariate analyses.
Eur J Cancer, Vol. 27, No. 5, pp. 586–591, 1991

INTRODUCTION

UNLIKE IN many other forms of human cancer, a late recurrence 10 or even 20 years after the primary diagnosis is not uncommon in breast cancer [1, 2]. Most survival studies have shorter than 10-year follow-up, and because the great majority of all deaths in breast cancer take place during the first 10 years, the most important prognostic factors found in these studies, such as the axillary nodal status, size of the primary tumour and histological grade, are those that predict relatively early mortality in breast cancer [3–5]. Surprisingly little is known about the factors that predict mortality in breast cancer among patients who have survived for more than 10 years after the primary diagnosis [1, 6, 7]. Yet, due to the worldwide increase in the incidence of breast cancer and improved treatment results, the number of such patients is increasing.

The purpose of the present study was to investigate the factors predicting mortality in breast cancer among those patients who have survived the first 10 years following the diagnosis.

MATERIALS AND METHODS

Patients

According to hospital records and data from the Finnish Cancer Registry, 461 women were diagnosed to have with a biopsy verified breast cancer in the city of Turku, South-Western Finland, during the years 1945–1965. Sufficient clinico-pathological and follow-up information was available for 439 patients. The survival analysis of this larger original material is published earlier elsewhere [8]. 279 patients died within the first 10 years and a total of 160 (36%) patients were alive after the 10th year of follow-up. These 160 patients constitute the actual study material.

These patients were followed up for at least 12 additional years (range 12–32 years, median 17) or until death. 15 (9%) of the 160 patients had Paget's disease of the breast or intraductal cancer *in situ* (none of the patients with either of these two tumour types had died during the first 10 years of follow-up). 12 of the 23 patients who developed a second primary breast cancer in the remaining breast were alive after the 10th year. The clinical data and cause of death were obtained from the hospital records, the files of the Finnish Cancer Registry, the Central Statistical Office of Finland, and from local authorities. All autopsy protocols and histological slides were reviewed.

Autopsy was performed on 10 of the 31 patients who died from breast cancer after the 10th follow-up year, and a histological and/or cytological confirmation of metastatic breast cancer was available in further 10 cases. In 6 of the remaining 11 cases dissemination of breast cancer was based on radiological evidence of distant metastases, and in 5 cases on a death certificate only. 9 patients died from cancer of another organ, and the cause of death was confirmed by autopsy in 4 cases, by a histological or

Correspondence to S.P. Toikkanen.
S.P. Toikkanen and H.P. Kujari are at the Department of Pathology and H. Joensuu is at the Department of Radiotherapy, University of Turku, Kiinamyllynk. 8–10, SF-20520, Turku, Finland.
Revised 1 Feb. 1991; accepted 8 Feb. 1991.

cytological sample in 4 cases, and by radiological evidence in 1 case.

The mean age at diagnosis was 52 years (median 51, range, 30–79). Patients younger than 50 years at the time of the diagnosis were considered as premenopausal [9]. Staging was done according to the UICC postsurgical TNM classification [10]. 95 (59%) patients were treated by radical mastectomy, 29 (18%) by mastectomy and axillary evacuation, 28 (18%) by simple mastectomy, and 8 (5%) by tumourectomy. 115 (72%) patients received additional radiotherapy. There was no significant differences in surgical ($P = 0.72$) or radiotherapy ($P = 0.57$) between the present series and the larger original material.

Histology

New haematoxylin-eosin and van Gieson stained slides were prepared from each tissue block. The histological typing and grading of the tumours were done by slightly modifying the WHO classification [11, 12], and the tumours were subsequently grouped for the survival study into 2 types: (1) infiltrating ductal carcinoma (includes apocrine, mixed mucinous and atypical medullary types), and (2) other (special) types (includes infiltrating lobular carcinoma with variants, tubular, medullary, cribriform, papillary, metaplastic, pure mucinous and intraductal *in situ* carcinomas and Paget's disease of the breast). The other evaluated clinicopathological variables are shown in Table 1, and compared with those of the patients who died within the first 10 years ($n = 279$).

DNA flow cytometry

Paraffin-embedded biopsies were processed for flow cytometry by the method suggested by Hedley *et al.* [13] with slight modifications as described in detail earlier [8]. The histograms were interpretable in 140 cases (88%).

DNA ploidy was independently assessed by two of the authors without any knowledge of the clinicopathologic or survival data. Histograms with a symmetrical or an asymmetrical G0/G1 peak were classified as diploid. If two G0/G1 peaks were present, the histogram was classified as aneuploid, and if more than two, as multiploid. A histogram with a G0/G1 peak at 4N and a G2/M peak at 8N was classified as tetraploid. The aneuploid, tetraploid and multiploid cancers were combined in statistical analyses and formed the "non-diploid" subgroup. The DNA index (DI) was calculated by dividing the modal channel number of an aneuploid peak by the modal channel number of the diploid peak. The peak with the least DNA content was taken as the diploid peak. The coefficient of variation (CV) ranged from 2.6 to 9.8. The S-phase fraction (SPF) was calculated according to the rectilinear method of Baisch *et al.* [14]. The SPF could not be calculated in 50 (36%) cases either due to overlapping stemlines or the presence of excessive cell debris. In aneuploid cases with a large DI (> 1.3) the SPF was calculated for the aneuploid stemline only.

Statistical methods

Frequency tables were analysed with the χ^2 test; Yates corrected values were used. The survival analysis was performed with the BMDP computer program (BMDP Statistical Software, University of California, Los Angeles). The cumulative survival was estimated with the product-limit method, and comparison of survival between groups was calculated by Wilcoxon–Breslow and Mantel–Cox statistics. Both crude survival and survival corrected for intercurrent deaths were calculated. The relative importance of prognostic factors was assessed with Cox's proportional hazard model (BMDP 2L). All P values are two-tailed. In survival calculations the starting point was the moment when the patient had been followed up for 10 years from the diagnosis.

Table 1. Distribution by 9 clinicopathological variables in breast cancer. Patients who survived for more than 10 years following the diagnosis ($n = 160$) are compared with those who died within the first 10 years ($n = 279$)

Factor	Survival > 10 yr	Survival < 10 yr	P	
Tumour size				
≤ 2cm	48 (30)	16 (6)	< 0.0001	
2–5 cm	97 (60)	147 (53)		
> 5 cm	9 (6)	64 (23)		
T4	6 (4)	52 (19)		
Nodal status				
N0	133 (83)	87 (31)	< 0.0001	(< 0.0001*)
N1–3	27 (17)	192 (69)		
Histological grade				
I	67 (42)	44 (16)	< 0.0001	
II	60 (38)	113 (41)		
III	33 (20)	122 (44)		
Histological type				
Ductal invasive	91 (58)	223 (80)	< 0.0001	
Lobular	23 (14)	40 (14)		
Other special types	46 (28)	16 (6)		
Tumour margin circumscription				
Definite	45 (28)	18 (6)	< 0.0001	
Questionable	39 (24)	75 (27)		
None	76 (48)	186 (67)		
Mitotic count/HPF				
Rare	83 (52)	83 (30)	< 0.0001	
2–3	53 (33)	102 (37)		
> 3	24 (15)	94 (34)		
Necrosis				
None	113 (71)	156 (56)	0.002	(0.003*)
Spotty to severe	47 (29)	123 (44)		
Cell reaction				
Severe/moderate	36 (23)	71 (25)	0.49	(0.56*)
None/slight	124 (77)	208 (75)		
Age (years)				
≤ 49	72 (45)	80 (29)	0.0005	(0.0008*)
> 49	88 (55)	199 (71)		

n (%).
HPF = high power field.
* Yates correction used.

RESULTS

It is evident from Table 1 that the cancers of the patients who survived for more than 10 years differ from the cancers of the short-term survivors in many respects. They were smaller and usually did not present with axillary nodal metastases, they were more often well-differentiated (grade I), the histological type was less often invasive ductal carcinoma, they had more often a definite tumour margin, the mitotic count was smaller and necrosis more often absent.

Table 2. DNA flow cytometric parameters in breast cancer. Patients who survived for more than 10 years following the diagnosis (n = 160) compared with those who died within the first 10 years (n = 279)

	Survival		
	> 10 yr	< 10 r	*P*
Interpretable histograms	140 (88)	244 (87)	
DNA ploidy			
Diploid	60 (43)	63 (26)	0.0006 (0.0009†)
Non-diploid	80 (57)	181 (74)	
DNA-index*			
≤ 1.2	75 (54)	78 (32)	< 0.0001 (0.0001†)
> 1.2	65 (46)	166 (68)	
Interpretable S-phase fraction	90 (64)	152 (54)	
SPF*			
≤ 7%	54 (60)	52 (34)	0.0001 (0.0002†)
> 7%	36 (40)	100 (66)	

n (%).
* The most effective cutoff point in a univariate analysis was chosen (see Results).
† Yates correction used.

The comparison of the present series with the short-term survivors in regard to DNA flow cytometric parameters is presented in Table 2. The cancers of the patients who survived for longer than 10 years were more often diploid, they had more often a DI < 1.2 and a SPF < 7%.

The crude and corrected survival of the 160 patients after the 10th year of follow-up are shown in Fig. 1. 21 (68%) of the 31 late deaths from breast cancer took place 10–15 years after the primary diagnosis, 28 (90%) before the 20th year, and only 3 (10%) after the 20th year of follow-up.

The factors that significantly predicted mortality from breast cancer in a univariate analysis after the 10th year of follow-up are shown in Fig. 2 a–d. Advanced age at diagnosis (> 49 years, $P = 0.002$), development of a second primary breast cancer at any time during the follow-up ($P = 0.0007$), primary tumour size T_3 (> 5 cm) or T_4 ($P = 0.004$) and the histological type of invasive ductal carcinoma ($P = 0.03$) predicted significantly

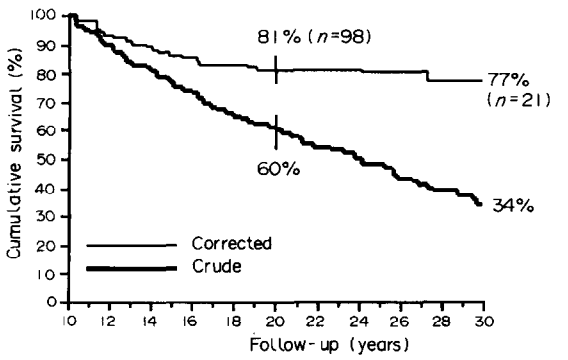

Fig. 1. Crude and corrected survival of patients with breast cancer who were alive after the 10th year of follow-up (n = 160). The number of patients at risk is given in brackets.

late deaths from breast cancer. A DNA index > 1.2 (the most effective cutoff point) had marginal significance (Fig. 2 e, $P = 0.06$), whereas DNA ploidy (Fig. 2 e, $P = 0.1$) and SPF (the most effective cutoff point 7%, $P = 0.23$) were not associated with late mortality in breast cancer, neither the type of tumour margin ($P = 0.21$), axillary nodal status ($P = 0.29$), histological grade ($P = 0.40$), the number of mitoses ($P = 0.48$), the severity of inflammatory cell reaction ($P = 0.48$), nor the extent of tumour necrosis ($P = 0.76$). The 17 patients who developed a new cancer of other organ than the breast had no late mortality from breast cancer, but 9 of them died from their new second cancer.

To find out the relative importance and independency of the four factors with a significant prognostic value in univariate analyses (Fig. 2 a–e), they were tested in Cox's multivariate analysis. The results are shown in Table 3. The most important independent factor with an adverse influence on survival was advanced age at diagnosis ($P = 0.002$), followed by occurrence of a contralateral second primary breast cancer during the follow-up ($P = 0.01$), a large primary tumour size (T_{3-4}, $P = 0.03$), and histological type of invasive ductal carcinoma ($P = 0.07$). If the DI was included in the analysis, it did not emerge as an independent factor; nor did the SPF.

DISCUSSION

Advanced age at diagnosis (over 49 years), occurrence of a second primary breast cancer (bilaterality), and a large primary tumour size (T_{3-4}) predicted significantly late mortality from breast cancer in a multivariate analysis, and ductal invasive histological tumour type was of marginal significance. Tumour size was the only one of these three independent factors which also had significant independent prognostic value predicting early mortality [8]. On the other hand, the presence of axillary nodal metastases at the time of the primary diagnosis, the most important predictor of short-term recurrence in breast cancer [8], had no predictive power regarding late mortality. There were still 27 originally node positive patients in the series. Similarly, the other independent clinicopathological prognostic factors predicting early mortality (poor histological differentiation grade, extensive tumour necrosis, and infiltrating type of tumour margin) did not significantly predict late mortality [8]. These results are in agreement with the results of Fentiman *et al.* [1], Longlands *et al.* [3], Holmberg *et al.* [5] and Fisher [4].

The most important factor predicting late mortality, age at diagnosis, had only marginal prognostic value in predicting early mortality [8]. Age at the time of diagnosis is a disputed prognostic factor in breast cancer [1–3]. In the present study postmenopausal patients (more than 49 years old at diagnosis) had clearly more late deaths from breast cancer than the premenopausal ones (Fig. 2 a).

Postmenopausal patients are also likely to die from intercurrent diseases more frequently than premenopausal patients, and, therefore, finding the correct cause of death is of crucial importance. Special effort was made to determine the cause of death in each case, which included review of hospital records and autopsy slides, and correspondence with authorities and relatives. The difference in corrected survival between the postmenopausal and premenopausal patients was significant ($P = 0.002$). Adami *et al.* [15], Mueller *et al.* [16] and Fentiman *et al.* [1] also noticed excess mortality from breast cancer in the older age groups especially 10–15 years after the diagnosis. De la Monte *et al.* [17] found in an autopsy study that premenopausal patients with disseminated breast cancer survived significantly

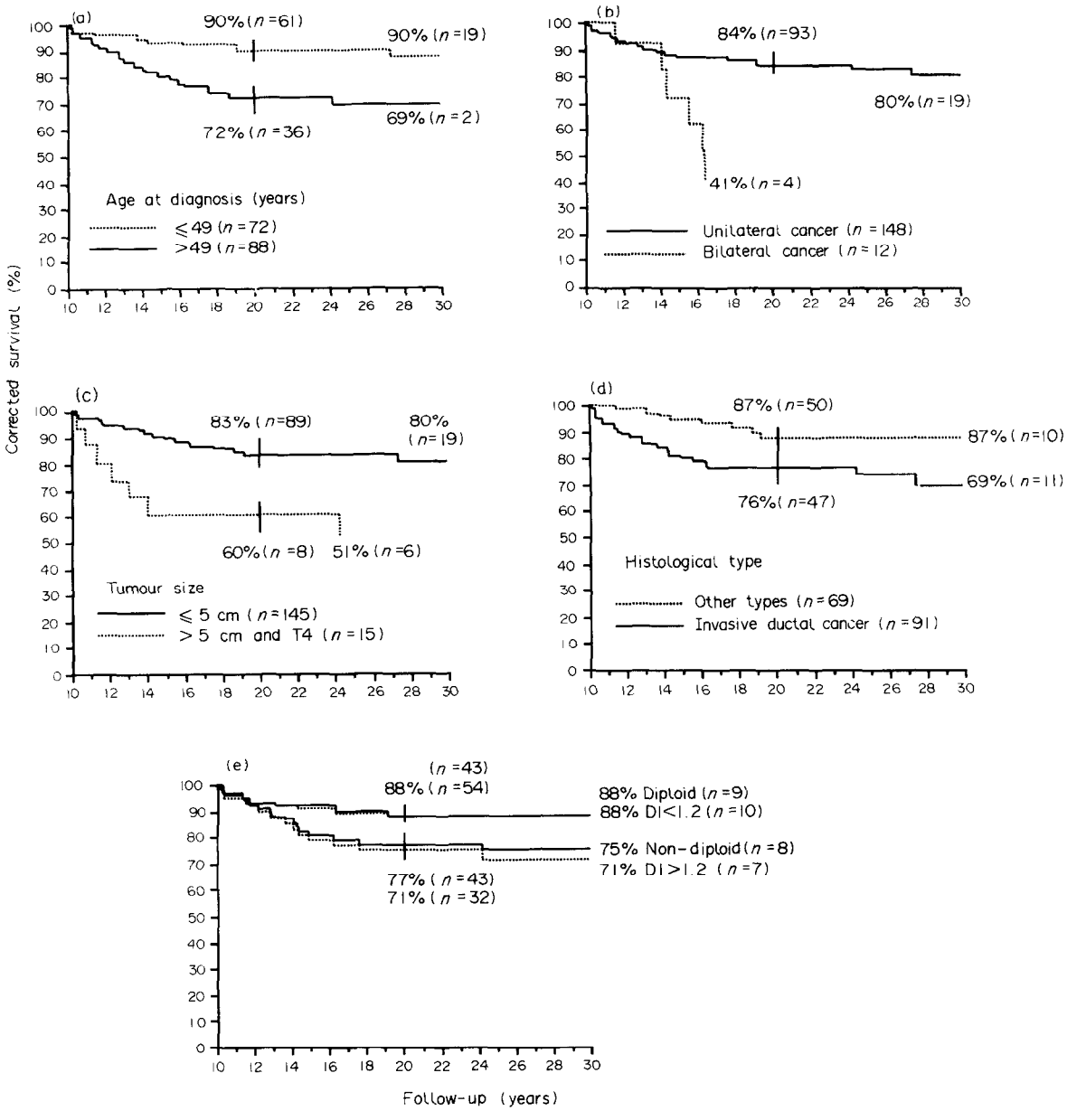

Fig. 2. Prognostic factors in breast cancer after the 10th year of follow-up: results of the univariate analyses. Wilcoxon-Breslow/Mantel-Cox tests. The number of patients at risk is given in brackets. *P* by Wilcoxon–Breslow/Mantel–Cox tests: (a) *P* = 0.006/0.002, (b) *P* = 0.008/0.0007, (c) *P* = 0.002/0.004, (d) *P* = 0.02/0.03, (e) diploid (*n* = 60) vs. non-diploid *P* = 0.1/0.1; DI < 1.2 (*n* = 75) vs. DI > 1.2 (*n* = 65) *P* = 0.07/0.0006.

Table 3. Factors that predict late mortality from breast cancer among patients who have survived for more than 10 years following the diagnosis (n = 160). Results of Cox's multivariate analysis

Factor	*P*	Step of removal	β (S.E.)	β/S.E.	Hazard rate (e^{β})
Age > 49 years	0.002	1	1.01 (0.43)	2.36	2.7 (1.2–6.5)
Bilaterality	0.01	2	1.30 (0.44)	2.96	3.7 (1.5–8.8)
Tumour size T_{3-4}	0.03	3	0.49 (0.22)	2.24	1.6 (1.1–2.5)
Histological type	0.07	4	−0.70 (0.40)	−1.73	0.5 (0.2–1.1)

The β coefficient describes how each factor contributes to the hazard, and the value of β/S.E. (standard error) describes their significance (= *z* value). The e^{β} or hazard rate for each factor with the 95% confidence interval is given.

longer than late postmenopausal patients with a similar disease, and they speculated that the process of aging may influence the metastatic behavior of breast cancer. Furthermore, Fournier *et al.* [18] have reported, based on mammographic studies, that the growth rate of breast cancers becomes slower with increasing age, which could partially explain the excess of late recurrences in older age groups.

Age at diagnosis was the most important independent prognostic factor in a multivariate analysis, i.e. its prognostic value cannot be explained by the other prognostic factors. 7 of the 12 patients who developed a second primary breast cancer and who were alive at 10 years from the diagnosis died from their disease, and 4 of these 7 were postmenopausal. 9 of the 15 patients with intraductal *in situ* carcinoma or Paget's disease were postmenopausal, and only 1 of these 15 patients died from breast cancer. No difference could be found in radicality of treatment between the premenopausal and postmenopausal patients ($P = 0.36$).

The patients who developed a second primary breast cancer had significantly poorer long-term survival than the patients with unilateral disease ($P = 0.0007$). Similar results have been published by Holmberg *et al.* [19], and by Robbins and Berg [20]. This may be of clinical importance, since mortality caused by the second primary breast cancers might be partially prevented by careful follow-up and early diagnosis. 5 of the 6 patients with a second primary breast cancer and with axillary node metastases died from breast cancer, whereas only 2 of the 6 such patients with negative axillary nodes succumbed, indicating the prognostic importance of the axillary nodal status in second breast cancer. Since the mortality caused by a second breast cancer is probably predicted by the same prognostic factors that influence early mortality in the first breast cancer, such as axillary nodal status, the second primary breast cancers could have been excluded from the Cox's multivariate analysis shown in Table 3. When this is done, the most important prognostic variable is age at diagnosis ($P = 0.002$), followed by the size of the primary tumour ($P = 0.04$), and histological type ($P = 0.07$).

However, there is no general agreement that patients who develop a second primary breast cancer have poorer survival than patients with unilateral disease [21]. This may be due to follow-up times shorter than 10 years.

A large primary tumour size is a well-established prognostic factor in breast cancer [4, 22], but its effect on survival has been reported to disappear after the 10th year, at least for patients with stage I-II disease [1, 3]. It has been suggested that large primary cancers have a short doubling time and aggressive clinical behaviour. Our results indicate that patients with T_{3-4} primary tumours had significantly greater late mortality from breast cancer than patients with smaller primary tumours ($P = 0.004$), suggesting that some of the T_{3-4} primaries have non-aggressive short-term behaviour. However, a closer analysis of the T_{3-4} tumours causing late cancer deaths revealed that only 3 of them were larger than 5 cm in diameter (T_3) and 4 were smaller, but with histological skin fixation, and, hence, were of pT_4 at presentation. Furthermore, it is evident from survival curves in Fig. 2 c that late mortality from T_{3-4} cancers was confined to the first 15 years. This is consistent with the results of Duncan and Kerr [23].

The special histological types (pure mucinous, papillary, cribriform, tubular and medullary types) were more common among the long-term survivors (Table 1). This agrees with earlier findings [6, 7]. Patients with carcinoma of invasive ductal type had more unfavourable prognosis even after the 10th year of follow-up ($P = 0.03$). Only 9 (13%) of the 69 patients with some of the special histological types died from breast cancer after the 10th year of follow-up as compared with 22 (24%) of the 91 patients with the ductal type, which suggests that a significant portion of such cancers will never give rise to distant metastases.

We were able to find only one report on the effect of nuclear DNA content on long-term survival in breast cancer. Auer *et al.* [24], judging from static cytometric histograms, that cancers of the long-term survivors have more often diploid DNA content than those of the short-term survivors. In our study the flow cytometric DNA histograms were interpretable in 140 (88%) of the 160 cases, and they were classifed without knowledge of clinicopathologic data. Diploid cancers ($P = 0.0009$), and cancers with the SPF < 7% ($P = 0.0002$), were overrepresented among the long-term survivors. The DI with the most effective cutoff point found (1.2) was of marginal prognostic value in predicting late mortality from breast cancer in a univariate analysis ($P = 0.06$), suggesting that some breast cancers with a grossly aneuploid DNA content may have a long natural history leading to late death from cancer. Among the short-term survivors [8] DNA ploidy, the DI and SPF were all significant prognostic discriminators in univariate analyses, and SPF had independent prognostic value in a multivariate analysis, but none of these flow cytometric variables had independent value in predicting late mortality from breast cancer in the present series.

It is concluded that late deaths from breast cancer are more frequently seen in postmenopausal women and in patients who develop a second primary breast cancer than in premenopausal women or patients with unilateral disease. The few patients with a T_{3-4} primary cancer who are alive 10 years after the diagnosis still have a considerable risk of dying from their disease. Cancers of a histological type other than the invasive ductal carcinoma caused only rarely late mortality, and axillary nodal status, histologic grade, type of tumour margin, extent of tumour necrosis, DNA ploidy or SPF had no significant influence on late mortality.

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Acknowledgements—The authors thank the staff at the Finnish Cancer Registry and local authorities for providing the survival data.

This study was financially supported by the Finnish Foundation for Cancer Research.

Eur J Cancer, Vol. 27, No. 5, pp. 591–594, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Indomethacin Modulation of Monocyte Cytokine Release following Pelvic Irradiation for Cancer

B. Petrini, G. Wolk, J. Wasserman, I. Vedin, Ö. Strannegård, H. Blomgren and P.-L. Esposti

Pelvic irradiation for urogenital cancer reduced monocyte release of tumour necrosis factor alpha (TNF-α). Addition of indomethacin to monocyte cultures increased TNF-α production after but not before irradiation. *E. coli* lipopolysaccharide (LPS) increased TNF-α release before as well as after radiation therapy and addition of indomethacin to LPS-stimulated monocytes further increased TNF-α production following radiotherapy. Spontaneous interleukin-1 (IL-1) release was increased in the cancer patients and was not significantly affected by radiation therapy. LPS increased IL-1 release before as well as after irradiation, but indomethacin did not further change IL-1 secretion. These findings suggest that prostaglandins differentially regulate TNF-α and IL-1 release. Administration of cyclo-oxygenase inhibitors during radiation therapy might increase TNF-α release *in vivo* and thereby enhance the host defence against tumours.
Eur J Cancer, Vol. 27, No. 5, pp. 591–594, 1991

INTRODUCTION

TUMOUR NECROSIS factor alpha (TNF-α) is a cytokine released by monocytes/macrophages when stimulated, e.g. by *Escherichia coli* lipopolysaccharide (LPS), and is generally considered to be a mediator of inflammation and cytotoxicity against tumour cells [1, 2]. Interleukin-1 (IL-1) is a cytokine with effects partly overlapping those of TNF-α [3], but also with important macrophage and lymphocyte activating properties [4]. It is known that TNF-α stimulates the release of prostaglandins and IL-1 [5] and that prostaglandin E_2 reduces LPS-triggered release of TNF-α [6, 7].

We have shown that radiation therapy for cancer may activate monocytes to prostaglandin-mediated suppression of immune reactivity [8] and that inhibitors of cyclo-oxygenase, which is important in biosynthesis of prostaglandins, could partly revert radiation-induced immunosuppression *in vitro* as determined by lymphocyte mitogen reactivity [9]. It is well known that cytokines play an important role in lymphocyte reactivity and proliferation [1–4]. Therefore we performed *in vitro* experiments to investigate whether radiation therapy affects cellular release of TNF-α and IL-1 and whether this process might be modulated by a cyclo-oxygenase inhibitor.

Correspondence to B. Petrini.
B. Petrini, G. Wolk, J. Wasserman, I. Vedin and Ö. Strannegard are at the Central Microbiological Laboratory of Stockholm County Council, Box 70470, S-10726 Stockholm; and H. Blomgren and P.-L. Esposti are at the Radiumhemmet, Karolinska Hospital, Stockholm, Sweden.
Revised 17 Sep. 1990; accepted 1 Feb. 1991.

MATERIALS AND METHODS

Patients and controls

14 patients, 13 men and 1 woman aged 59–76 years (mean 68) were examined. None of them had previously received radiation therapy or treatment with cytotoxic drugs. All men had moderately or poorly differentiated prostatic cancer without evidence of metastatic spread. The female patient had a poorly differentiated localised bladder cancer of urothelial differentiation. 10 female and 6 male laboratory staff members, aged 22–57 years (mean 40) served as controls for intertest variability.

Radiation therapy

All the patients received 8 MV radiation therapy from a linear accelerator using an anterior open beam and two oblique wedge filter beams from the back. The 100% isodose included most of the false pelvis. Radiation therapy was given 5 days a week with a cycle dose of 1.8 Gy, up to 63 Gy (prostate cancer) and 64.4 Gy (bladder cancer).