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Overall Survival of Breast Cancer Patients in Relation to Preclinically Determined Total Serum Cholesterol, Body Mass Index, Height and Cigarette Smoking: a Population-based Study

Lars J. Vatten, Olav P. Foss and Stener Kvinnsland

Mean overall 5-year survival related to preclinically determined total serum cholesterol, body mass index (BMI), height and cigarette smoking has been analysed among 242 incident cases of breast cancer aged 36–63 years that developed in a population of 24 329 Norwegian women during a mean follow-up of 12 years (range 11–14). The study factors were ascertained at least 1 year prior to diagnosis (mean = 8 years), and the cases have been followed up with respect to death for a mean time of approximately 5 years after diagnosis. Patients whose preclinical total serum cholesterol values were within the highest quartile (≥7.52 mmol/l, mean = 8.58 mmol/l) of the underlying population had a hazard ratio of dying of 2.0 (95% confidence limits, 1.1 and 3.7) compared to cases with cholesterol values in the lowest quartile (mean = 5.28 mmol/l), after adjustment for age at diagnosis, clinical stage, and body mass index. In relation to BMI (Quetelet's index: weight/height^2) patients who were obese prior to diagnosis were at higher risk of dying than those who were lean. Compared to patients in the lowest quartile of BMI (mean Quetelet = 21), the hazard ratio was 2.1 (95% confidence limits, 1.2 and 3.8) for patients in the highest quartile (mean Quetelet = 30), after adjustment for age at diagnosis, clinical stage, and total serum cholesterol. For height and for cigarette smoking, no relation with survival was observed. A potential problem of this study might be insufficient information about other well known prognostic factors, but the results suggest that preclinical total serum cholesterol and BMI are positively associated with the risk of dying among women who develop breast cancer.
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INTRODUCTION

NO OVERALL association has been shown between total serum cholesterol and the risk of developing breast cancer in women [1, 2], but there is some evidence for an inverse relation between total serum cholesterol and risk of premenopausal breast cancer [2, 3]. Once disease is manifest, however, total serum cholesterol may have an adverse influence on the prognosis of breast cancer patients [4].

Body mass index (BMI) appears to be related to the risk of breast cancer, but in opposite directions, depending on the menopausal status of the women. Whereas a high body mass may increase breast cancer risk among postmenopausal women [5], some studies have indicated that BMI is negatively associated with the risk of developing breast cancer in premenopausal women [2, 6–8]. Among patients, however, it has been suggested that increased body mass has an adverse effect on survival [4, 9–12].

The evidence suggesting that height is positively associated with the risk of developing breast cancer [7, 13, 14], has been interpreted as an effect that might be related to nutritional influences during perimenarcheal age [15, 16], an important phase for height determination and for breast tissue development. With respect to prognosis, there is little support for any relation between height and survival in breast cancer patients [12].

Cigarette smoking is not likely to be related to breast cancer risk [17], despite hypotheses suggesting a possible negative association [18]. In relation to survival, it might be of interest to examine the hypothesis that cases who smoke have a better prognosis than cases who do not smoke, possibly due to anti-oestrogenic effect of smoking [19].

This study distinctly differs from most other studies of these aspects in two important respects. Firstly, we report mean 5-year overall survival among 242 cases of breast cancer that were accrued in a fixed cohort of 24 329 Norwegian women during approximately 12 years of follow-up, making this a population-based study. Secondly, survival has been analysed in relation to

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preclinically ascertained values, since information on total serum cholesterol, body mass index, height and cigarette smoking were collected a number of years (at least one) prior to breast cancer diagnosis.

SUBJECTS AND METHODS

Study population

Between 1974 and 1978 all women aged between 35 and 49 years who were living in three separate counties in Norway were invited to participate in a health screening examination. A detailed description of the screening has previously been given [20, 21], so only a brief outline will be offered here. Among 26252 invited women, a total of 24617 (93.8%) attended the screening. They completed a self-administered questionnaire, and were subject to measurements of blood pressure, height and weight. A non-fasting blood sample was drawn from each subject.

The official 11-digit identification number of every Norwegian citizen facilitated linkage to the national Cancer Registry and identified cases of cancer in the study population [22]. Among the participating women, 288 had been diagnosed with a cancer (including breast cancer) prior to or during the calendar year of health screening, and these were excluded from further analysis, which resulted in 24329 women found eligible for analysis.

During a mean follow-up of approximately 12 years after health screening, 242 incident cases of breast cancer were identified in the study population. Mean age at diagnosis was 50 years (range 36–61 years, S.D. 5.3 years). In relation to clinical stage at diagnosis, 40% were in clinical stage I, 33% in stage II, whereas 7.5% were classified to stage III or IV. A point of concern was the fact that clinical stage of the remaining 20% (49 out of 242 cases) was unspecified. From the time of diagnosis we have followed these patients up with respect to death until December 1989. During a mean follow-up after diagnosis of approximately 5 years (range 1–14 years, S.D. 3.4 years) 61 patients had died, and the remaining 181 were still alive at the end of follow-up. Among 6 patients for whom there was no information on height or weight, 3 had died, and 3 were still alive.

Study factors

The collected non-fasting blood samples were prepared at the screening site and analysed at the Central Laboratory, Ullevaal Hospital, Oslo, where total serum cholesterol was measured according to the method used in the Lipid Research Clinics Program (LRCP) [23]. Comparisons with results obtained in this programme and participation in the Cooperative Cholesterol and Triglycerides Standardization Program of the WHO showed that the reported values of cholesterol were approximately 0.4–0.5 mmol/l greater than the expected values based upon the reference method [24]. Stability of the analyses was controlled by inserting reference serum among the actual sera. Although minor fluctuations were observed over time, no systematic changes were observed between 1974 and 1978.

BMI was computed as the measured weight (in kg) divided by the squared value of the measured height (in metres) to provide Quetelet's index [25].

Serum cholesterol, height and BMI were categorised into quartiles based on the values of the underlying population. Information on cigarette smoking was divided into three separate categories: non-smokers, those who smoked 1–9 cigarettes per day and women who smoked 10 or more cigarettes daily. Baseline measurements were performed on average 8 years before diagnosis of breast cancer.

Table 1. Age-adjusted incidence rate ratios (IRR) of breast cancer, according to quartiles of total serum cholesterol, BMI (Quetelet's index), height and categories of current cigarette smoking

	Quartiles				
	I	II	III	IV	*P*
Total serum cholesterol (mmol/l)	<5.9	5.9–6.6	6.6–7.5	≥7.5	
Age-adjusted IRR	1.0	0.9	1.0	0.7	0.05
95% confidence limits		(0.7–1.3)	(0.7–1.4)	(0.4–1.0)	
BMI (kg/m²)	<22	22–24	24–27	≥27	
Age-adjusted IRR	1.0	0.8	0.8	0.5	0.001
95% confidence limits		(0.6–1.2)	(0.5–1.1)	(0.3–0.8)	
Height (cm)	<159	159–162	163–166	≥167	
Age-adjusted IRR	1.0	1.5	1.9	2.0	<0.001
95% confidence limits		(1.0–2.2)	(1.3–2.8)	(1.3–3.0)	
Smoking (cigarettes/day)	None	1–9	≥10		
Age-adjusted IRR	1.0	1.3	1.0		0.59
95% confidence limits		(0.9–1.7)	(0.8–1.4)		

Data based on 242 cases of breast cancer that developed among 24329 Norwegian women, aged 35–51 at baseline, during 11–14 years of follow-up.

The risk of developing breast cancer in relation to each of these factors has previously been analysed [3, 8, 14, 26]. One purpose of the present study has been to contrast these associations, as summarised in Table 1, with the associations between the study factors and overall survival among those who developed breast cancer during follow-up.

Statistical analysis

First, each study factor was univariately related to the survival experience of each patient by using the Kaplan–Meier life-table technique. For each study variable the survival probability of the patients were presented as stratified Kaplan–Meier plots, and log rank χ^2 statistics were computed for testing of equality between survival curves associated with each category.

The graph of the death (hazard) rate based on all the patients indicated that a log normal distribution may appropriately fit the data. There was a relatively steep increase in the death rate for 3 years subsequent to diagnosis, after which a decrease was observed. We therefore decided to apply a log normal survival model in the multivariate analyses of overall survival probability in these data [27].

In the multivariate analysis [28] we first analysed the relation between each study factor and risk of dying over the follow-up period, only including age at diagnosis (continuous variable) as a covariate factor. Then we included clinical stage at diagnosis (four categories) as a second covariate. In the analysis of body mass index we also kept total serum cholesterol (four categories) as a covariate in the model, since this factor appeared to have a confounding effect on the result of BMI. Analogously, in the analysis of total serum cholesterol, BMI (four categories) was included in the multivariate model with age at diagnosis and clinical stage. Tables 2 and 3 therefore provide estimates of the age-adjusted death rate ratios (hazard ratios), and ratios resulting

Table 2. Adjusted hazard ratio of death for breast cancer patients, according to preclinically determined total serum cholesterol, in quartiles

	Total serum cholesterol (in mmol/l)				
	<5.85	5.85–6.63	6.64–7.51	≥7.52	
Mean	5.28	6.26	7.05	8.52	
Deaths	11	16	13	21	
Censored	53	48	55	25	
Hazard ratio*	1.0	1.2 (0.6, 2.4)	1.2 (0.6, 2.4)	3.6 (1.3, 9.1)	$\chi^2 = 4.73$ $P = 0.03$
Hazard ratio (multivariate)†	1.0	1.0 (0.5, 2.6)	1.4 (0.2, 8.6)	2.0 (1.1, 3.7)	

95% confidence limits in parentheses.
*Adjusted for age (continuous variable) at diagnosis.
†Adjusted for age at diagnosis, clinical stage at diagnosis (4 categories) and BMI (4 categories) at least 1 year prior to diagnosis.

from the multivariate analysis, where additional terms have been included in the model.

The hazard ratios have been computed by exponentiating the beta coefficient of the exposure factor, and the precision of the estimates has been presented as 95% confidence limits applying the standard error provided for each coefficient [29].

Based on the multivariate estimates, we also computed the risk of dying that can be attributed to exposure to the highest quartile of total serum cholesterol or body mass index (attributable risk percent), which indicates the proportion of deaths associated with either factor that could potentially be prevented [29].

RESULTS

The summary table of relative risks of developing breast cancer (Table 1) in the underlying population, related to total serum cholesterol, BMI, height, and cigarette smoking, shows

Table 3. Adjusted hazard ratio (HR) of death for breast cancer patients, according to preclinically determined BMI, in quartiles

	BMI (in kg/m²)				
	<22	22–24	24–27	≥27	
Mean	21	23	25	30	
Deaths	14	11	14	19	
Censored	59	52	44	23	
Hazard ratio*	1.0	0.6 (0.3, 1.3)	1.2 (0.5, 2.8)	3.0 (1.3, 6.7)	$\chi^2 = 4.24$ $P = 0.04$
Hazard ratio (multivariate)†	1.0	0.8 (0.5, 1.4)	1.2 (0.6, 2.4)	2.1 (1.2, 3.8)	

95% confidence limits in parentheses.
Data are based on 236 incident cases of breast cancer that occurred during 11 to 14 years of follow-up among 23 826 women who were 35–51 years of age in the year of cholesterol determination.
*Adjusted for age at diagnosis (continuous variable).
†Adjusted for age at diagnosis, clinical stage at diagnosis (5 categories) and total serum cholesterol (4 categories) at least one year prior to diagnosis.

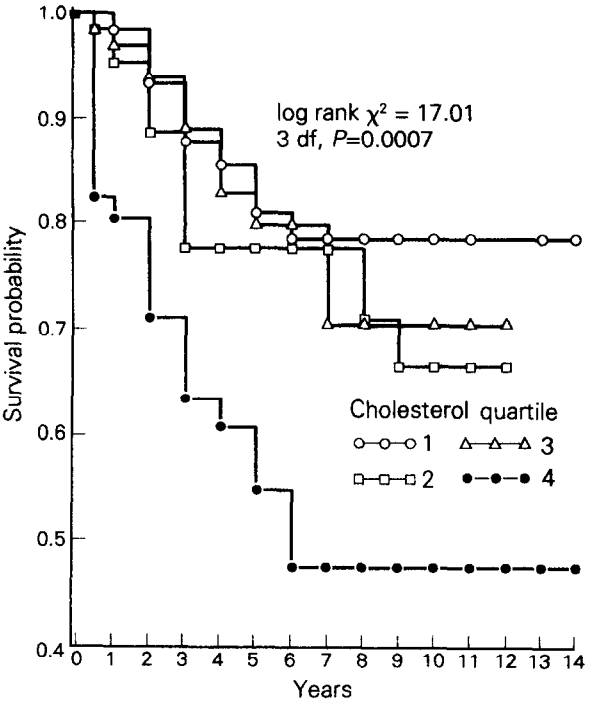

Fig. 1. Overall survival probability in breast cancer patients according to quartiles of preclinically measured total serum cholesterol. Cholesterol quartile: ○–○ = 1, □–□ = 2, △–△ = 3, ●–● = 4.

an inverse risk associated with the highest quartiles of total serum cholesterol [3] and with BMI [8], a positive association with height [14] and the absence of any relation with cigarette smoking [26].

In contrast, the univariate Kaplan–Meier plots for each "exposure" category among those who developed breast cancer indicated an adverse effect related to survival among patients with total serum cholesterol (Fig. 1) value in the fourth quartile (mean 8.52 mmol/l, 329 mg/100 ml) of the underlying population (log rank $\chi^2 = 17.01$, 3 df, $P = 0.0007$). An analogous increased risk of death was observed among obese patients whose BMI (Fig. 2) was in the fourth quartile (mean Quetelet = 30) of the population (log-rank $\chi^2 = 16.52$, 3 df, $P = 0.0009$). For quartiles of height there was no relation with survival (log-rank $\chi^2 = 3.74$, 3 df, $P = 0.29$), and cigarette smoking (3 categories) was not associated with survival among breast cancer cases in these data (log-rank $\chi^2 = 1.04$, 2 df, $P = 0.59$).

In the multivariate analysis (Table 2) the hazard ratio of death was 2.0 (95% confidence limits, 1.1 and 3.7) among patients with cholesterol in the highest (mean 8.52 mmol/l, 329 mg/100 ml) compared to the lowest quartile (mean 5.28 mmol/l, 204 mg/100 ml) of the underlying population, after adjusting for age at diagnosis, clinical stage at diagnosis and BMI at least 1 year prior to diagnosis. The multivariate result indicated the importance of adjusting for clinical stage and BMI, since the hazard ratio only adjusting for age at diagnosis was 3.6.

For body mass index (Table 3) the corresponding hazard ratio of death was 2.1 (95% confidence limits, 1.2 and 3.8), comparing patients in the highest (mean Quetelet = 30) and the lowest quartile (mean Quetelet = 21) of BMI, after adjustment for age at diagnosis, clinical stage and total serum cholesterol. Again,

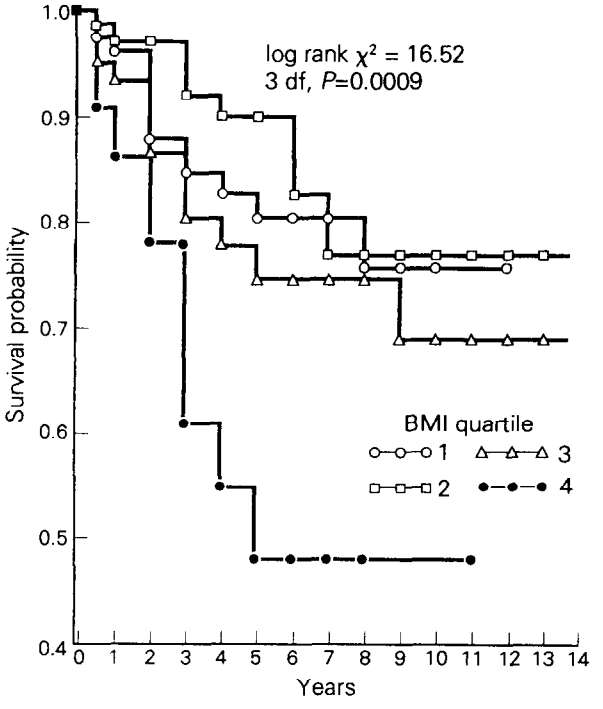

Fig. 2. Overall survival probability in breast cancer patients according to quartiles of preclinically measured BMI (Quetelet's index). BMI quartile: ○–○ = 1, □–□ = 2, △–△ = 3, ●–● = 4.

the importance of multivariate adjustments were apparent, since the hazard ratio only adjusting for age at diagnosis was 3.0.

By applying the multivariate estimates, we computed the proportion of deaths that could be attributed to the highest quartile of the population of total serum cholesterol, and of BMI. For cholesterol the attributable risk proportion was 50%, and for BMI 52%, which indicates the theoretical potential for prevention of premature deaths which may be associated with these factors.

Among patients who died and were within the highest quartile of either seum cholesterol or BMI, it should be noted that the absolute values for both factors were distributed approximately equal to those of the underlying population, suggesting that patients who died did not represent extreme values. It should also be noted that according to information obtained from the death certificates, patients in the highest category of these factors were not over-represented with respect to deaths from causes other than breast cancer (e.g. cardiovascular diseases).

DISCUSSION

In this population-based study of survival among 242 cases of breast cancer, obese cases (Quetelet ≥27) had a poorer prognosis with respect to overall survival than less obese patients. Similarly, cases who had total serum cholesterol values in the highest quartile of the underlying population (≥7.52 mmol/l) had a poorer prognosis than women with lower cholesterol values. The adverse effect on prognosis was confined to patients whose value of BMI or total serum cholesterol was in the highest quartile of either one of these factors, measured at least 1 year prior to diagnosis.

Few studies have analysed the relation between serum cholesterol and survival among breast cancer patients [4, 12]. However, in a 5-year follow-up of 374 breast cancer patients, Tartter *et al.* [4] detected a similar adverse effect associated with serum cholesterol to that reported in this study. Several studies have shown that obese breast cancer cases have a poorer prognosis than leaner patients [4, 9–12].

The originality of this study derives from two characteristics. Firstly, it has a population base of approximately 24 000 women where computer linkage to the national cancer registry facilitated total ascertainment of 242 breast cancer cases which occurred during 12 years of follow-up. These patients have subsequently been followed up with respect to death for a mean period of 5 years. Secondly, in a baseline health screening examination of this cohort, information was collected on total serum cholesterol, height, weight and cigarette smoking prior to breast cancer diagnosis. It seems unlikely that values of serum cholesterol and BMI are influenced by subsequent disease status. Thus, bias due to misclassification of the study variables cannot easily explain the results of this study.

Ascertainment of case status depended on the official identification number of every Norwegian citizen and on the reliability and completeness of the mandatory reporting of incident cases of cancer to the registry [22]. Selective reporting of breast cancer cases, depending on serum cholesterol or BMI, does not seem reasonable, and thus, the results can hardly be attributed to selection bias of participating patients. Nevertheless, in 20% (49 out of 242) of patients reliable information on clinical stage at diagnosis was not available. If there was a tendency for these patients to belong to the highest quartile of either serum cholesterol or BMI, and simultaneously have an advanced clinical stage of disease, the adverse effects related to overall survival associated with these two factors might be a result of differential bias, yielding exaggerated estimates of the true effects. However, within the highest quartile of both serum cholesterol and BMI, only 1 of the cases who died had an unspecified stage of disease, which may be reassuring for the validity of our results. Furthermore, a separate analysis excluding patients with unspecified clinical stage did not materially alter the results.

Although maybe all cases that developed during follow-up were included in this study, one should keep in mind that the population was between 36 and 51 years at the beginning of case accrual, and between 47 and 63 at the end of follow-up. Thus, inclusion of future cases will be necessary in order to provide a representative analysis of survival probability in older breast cancer patients.

We examined whether deaths from causes other than breast cancer occurred more often among cases in the highest quartile of serum cholesterol or BMI, since a disproportionate distribution of such deaths (e.g. from cardiovascular disease) might explain the observed survival pattern of this study. Based on individual information from death certificates, however, no such association was found.

In the analyses the difference between age-adjusted and multivariate estimates of hazard ratios demonstrates the importance of adjusting for clinical stage. Possibly, adjustment for additional known prognostic factors, for which we had insufficient or no information [30, 31], might reduce the hazard ratio further towards the null value of one. In relation to BMI there might be a differential distribution of lymph node metastases, since obese women might tend to be diagnosed at a later stage of the disease process than lean women. For total serum cholesterol,

however, there is at present no verified association with lymph nodal involvement in breast cancer patients.

Identical arguments will apply in relation to the size of the tumour [32]. Possibly, a greater proportion of obese women may be diagnosed with a large tumour than those who are lean. This might be all the more important in this study, since the classification of tumour size of the Cancer Registry does not strictly conform to the recommendations of the International Union Against Cancer (UICC), since stage I includes cancers up to 5 cm in diameter. Again however, it is difficult to reconcile this potential for confounding bias with the effect on survival associated with serum cholesterol.

A differential distribution in receptors for sex steroids in tumour tissue might represent a third potential for confounding [33]. If obese breast cancer patients, or patients with elevated serum cholesterol, on average tend to have oestrogen or progesterone receptor negative tumours more often than other patients, poorer survival experience could be a conceivable outcome. Similarly, if a higher proportion of cells with abnormal DNA content (large aneuploid cell population) and large S-phase fraction in tumour cells from these patients was observed, this would possibly indicate characteristics that might account for reduced survival [30]. In premenopausal cases, adjuvant chemotherapy has a beneficial effect on survival [34], whereas endocrine therapy favourably affects prognosis in postmenopausal cases [35].

Thus, the possibility of confounding with any one of these prognostic factors cannot be excluded. It is unclear, however, whether the prognostic strength of any one of them could account for the difference in overall survival observed in this study, or whether a cumulative, combined effect of several extraneous factors might represent an underlying explanation.

In relation to the risk of developing breast cancer in the underlying cohort of this study, total serum cholesterol and BMI were inversely associated with the risk of disease among women 50 years and younger, whereas no clear pattern was observed in older cases [3, 8]. Contrasted with the results on prognosis, this may seem paradoxical, since belonging within the highest quartile of either serum cholesterol or BMI appears to have a strong adverse effect on overall survival once disease has become manifest.

For the two factors, total serum cholesterol and BMI, the results of this study consequently suggest a negative association with the risk of developing breast cancer in relatively young women, and simultaneously may signal a poor prognosis among those who develop the disease. The observed positive association between height and breast cancer risk has been ascribed to a possible effect related to adolescent growth [14–16]. In relation to prognosis, however, there was no association with height. Also, there was no relation between cigarette smoking and breast cancer in these data, for either risk of disease [26] or for survival.

The estimated attributable risk proportions suggested that 50% of deaths could be attributed to high serum cholesterol, and analogously, 52% could be attributed to elevated body mass. Typically, this measure is interpreted as the proportion which could have potentially been prevented [28], and thus, the estimates suggest the proportion of cases who might have suffered a premature death. Possibly, a greater proportion of patients in the highest quartile of either one of these two variables may have biological characteristics that determine their allocation to the 30% fraction in whom a rapid course from diagnosis until death can be expected [32].

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Cell Mediated Cytotoxicity and Cytokine Production in Peripheral Blood Mononuclear Cells of Glioma Patients

Clara M. Ausiello, Carla Palma, Alberto Maleci, Giulio C. Spagnoli, Carla Amici, Guido Antonelli, Carlo U. Casciani and Antonio Cassone

A mannoprotein preparation (MP) from *Candida albicans* induced MHC-unrestricted cytotoxicity in peripheral blood mononuclear cells (PBMC) from healthy subjects, but not in those from glioma-bearing subjects. The two groups of subjects did not significantly differ in the number of cells bearing typical natural killer (NK) markers (both in resting and MP stimulated PBMC) and NK activity. However, interferon gamma (IFN-γ) production was in tumour patients minimal or significantly reduced, as compared to healthy subjects, following PBMC stimulation by MP or phytohaemoagglutinin, respectively. In addition, minimal, if any, stimulation of interleukin-2 (IL-2) production was achieved in MP stimulated PBMC from glioma patients. Considering the pivotal role of the above cytokines in immune reponses, particularly in those concerning generation of antitumour effectors, our results consistently suggest that defective cytokine production is one possible mechanism of immunological impairment in glioma patients. They also provide indirect support for a possible clinical use of IFN-γ as an immunopotentiating agent in gliomatous subjects.
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INTRODUCTION

WE HAVE previously shown that *in vitro* stimulation of peripheral blood mononuclear cells (PBMC) of healthy subjects with MP, a mannoprotein preparation derived from the human commensal microorganism *C. albicans*, induces lymphoproliferation, lymphokine production and activation of cytotoxic effector cells active against both NK sensitive and NK resistant targets, including uncultured neoplastic cells [1–3]. Potent immunomodulatory activities of candidal constituents have also been observed in normal and *Candida*-primed mice [4, 5].

To further explore the immunomodulatory potential of *C. albicans* in tumour-bearing subjects, we focused on the activity of MP in cultured PBMC from glioma patients, which has been reported to present distinct impairments of cell mediated immune responses [6–8]. In a preliminary report [9], we showed that MP-stimulated PBMC from glioma patients proliferate, but produce less interferon-gamma (IFN-γ) than PBMC from healthy controls. MP-induced generation of cytotoxic cells, but not generation of lymphokine-activated killer (LAK) cells [10, 11], was also greatly reduced in these patients [9]. We have now extended this investigation to examine number and function of natural killer (NK) precursors, as well as interleukin-2 and IFN-γ production by cultured PBMC of glioma patients, in response to MP or phytohaemoglutinin (PHA).

PATIENTS, MATERIALS AND METHODS

Patients

Table 1 reports clinical data concerning the 15 glioma patients enrolled in this study. All had a primary malignant glioma, and their Karnofsky performance status ranged from 80 to 100. All

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