supplementation of 1000 mg/day vitamin C caused an increase of 88% to 95%, and the serum vitamin C reached nearly a steady-state after one month. Although the effects of several short-term feeding trials of a high fruit and vegetable diet on serum carotenoids and vitamin C have been reported [10–13], there is little research on the effects of long-term vitamin C supplementation on serum vitamin C.

After confirming the feasibility of a population-based chemoprevention study through a pilot investigation [11], we started a population-based randomized double-blinded controlled trial that was originally undertaken to evaluate the vitamin C and/or β-carotene supplementation on the ten-year cumulative incidence rate of gastric cancer. After modification of the study protocol, the study purpose was changed to evaluate the five-year change in serum pepsinogens, which we regarded as a measure of the progression of chronic atrophic gastritis. The first objective of this report was to evaluate the effect of five-year vitamin C supplementation on the serum vitamin C concentration and to identify the factors associated with change in the serum concentration. The second objective was to evaluate the effects of an intervention trial of vitamin C supplementation on dietary consumption of vegetables and fruits.

SUBJECTS AND METHODS

Subjects

Target subjects were men and women 40 to 69 years of age living in a village within the Yokote Public Health Center District in Akita Prefecture, one of the regions with the highest mortality from gastric cancer in Japan, who participated in annual screening programs for circulatory diseases conducted by each municipality under the National Health and Welfare Services Law for the Aged. We measured their serum concentration of pepsinogen (PG) I, II and calculated PG I/II ratio and asked persons diagnosed with chronic atrophic gastritis (defined as PG I <70 ng/mL and PG I/II <3.0) to take diet supplements containing vitamin C and/or β-carotene. The recruitment of study participants was conducted from June through September, 1995, in the first year. It was originally scheduled to be continued up to 1998, when at least 1,812 subjects were expected to participate. This sample size would have permitted the detection of a 40% reduction of the ten-year cumulative incidence of gastric cancer in the intervention group (from 7% to 4%) with 5% alpha-error (two-sided) and 80% power. However, in response to a NCI report [14] indicating that two β-carotene trials had shown no benefit and potential harm from the supplement on Jan. 18, 1996, we decided to modify the initial study protocol by removing the β-carotene supplementation and by stopping further recruitment of study participants. The β-carotene supplementation lasted only for three to six months. Fig. 1 shows the trial profile. Out of 439 persons initially participating in the study, 134 dropped out

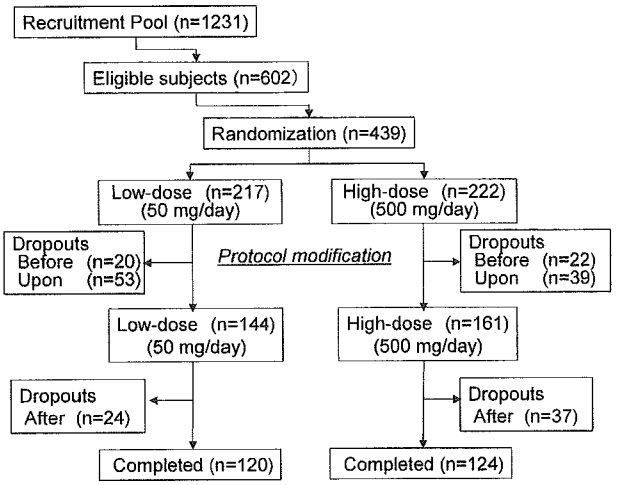

Fig. 1. Profile of a randomized controlled trial.

before and upon the study protocol modification, and these subjects were included in this study as a referent group without vitamin C supplementation. Of the 305 remaining participants, 244 completed the study. Since the purpose of this study was to examine the effect of five-year supplementation, 61 subjects (24 on low-dose and 37 on high-dose) who dropped out after the protocol modification were excluded from the analysis.

Design

The rationale, design and methods of the study, the characteristics of the participants, and the measures of compliance have been described in detail elsewhere [15]. At the outset, we prescribed capsules containing full doses of β-carotene (15 mg/day) and vitamin C (500 mg/day) to all participants for four weeks. We conducted this "run-in" procedure in order to identify and exclude at an early stage the subjects who either did not comply or showed side effects. We then randomized the remaining participants into four treatment groups using a 2×2 factorial design, whereby 0 or 15 mg/day β-carotene and 50 or 500 mg/day vitamin C were supplemented in a double-blind manner. After the study protocol was modified, β-carotene supplementation was halted, but the prescription of vitamin C was continued for five years. We obtained informed consent again from individuals willing to take part in the modified trial, and provided them with new capsules containing vitamin C only (50 mg/day or 500 mg/day). Compliance with treatment was constantly encouraged and monitored by nurses who interviewed the participants and recorded pill counts every three months. We also monitored adverse effects using a questionnaire at every visit. The study protocol was approved by our institutional ethics committee.

Demographic, Lifestyle and Medical Information

At recruitment, all participants were given a self-administered questionnaire on weight, height and demographic details,

such as marital and occupational status, education attainment, smoking status, alcohol consumption, disease history, family history of disease and general health status. Body mass index (BMI, kg/m^2) was calculated as body weight divided by the square of body height.

Dietary Intake

The dietary section was a 108-item semiquantitative food frequency questionnaire, concentrating on habitual consumption of food and beverage during one year at study entry and after supplementation (fifth year). The frequency of usual consumption of the various foods was recorded by the following categories: ≥7 times/day, 4–6 times/day, 2–3 times/day, 1 times/day, 5–6 times/week, 3–4 times/week, 1–2 times/week, 1–3 times/month and never or less than 1 time/month. The midpoint of the interval (5 times/day, 2.5 times/day, etc) was used to calculate the frequency of intake. The usual portion size was also included in the questionnaire, and subjects were asked to describe their usual portion size as small, medium or large [16]. For carrots, spinach, pumpkins, cabbage and radishes, an actual-size photograph of the vegetables was shown. Nutrient intakes were computed from the questionnaire using the Standard Food Composition Tables published by the Science and Technology Agency of Japan [17]. Data on carotenoid composition in fruit and vegetables provided by the carotenoid food-composition database were used to estimate daily intake of individual carotenoids from food intake assessed by the food-frequency questionnaire [18]. Validity of the food frequency questionnaire was assessed in a validation study. The median correlation coefficients between the intakes obtained from FFQ and those obtained from 28-day semi-weighed dietary records were 0.52 and 0.41 for 15 nutrients and 0.38 and 0.32 for 19 food groups in 102 men and 113 women, respectively. The correlation coefficients for vitamin C, vegetables and fruit intake were 0.42, 0.33 and 0.61 in men and 0.32, 0.35 and 0.50 in women (unpublished data).

Biochemical Analysis

Fasting blood samples collected upon entry in the study and at five years were analyzed for serum vitamin C and lipids. The subjects were asked not to eat or drink anything except water after nine o'clock on the day before blood sampling. The serum was sampled between nine and eleven o'clock in the morning. All samples were stored at −70°C to −85°C and were analyzed simultaneously after the completion of the five-year supplementation. Sera for ascorbic acid measurement were stabilized by addition of *meta*-phosphoric acid (Wako Pure Chemical, Osaka, Japan). Serum ascorbic acid was measured by the following method [19]. Briefly, 450 μL of the samples was sequentially mixed with 0.15 mL of 0.15% dithiothreitol (Nacalai Tesque Inc., Kyoto, Japan), 0.15 mL of 0.5% N-ethylmaleimide (Nacalai Tesque Inc., Kyoto, Japan), and 0.75 mL of trichloroacetic acid (Wako Pure Chemical, Osaka, Japan). After centrifugation, the supernatant was mixed with 0.75 mL of a chromogen (phosphate: water: 1.8% FeCl3: 4% dipyridyl = 1:1:1:2), incubated at 37°C for 30 minutes, and the optical density at 525 nm was then measured with a UV/Vis spectrophotometer (V-550, Nihon Bunko, Tokyo, Japan). The interassay coefficient of variation for vitamin C is 2.2%. All assays were conducted by persons blinded to the intervention assignment and the questionnaire data.

Statistical Analysis

Baseline characteristics, nutrient intake and food consumption were summarized in terms of the mean and standard deviation for continuous variables, frequencies and percentages for categorical variables. Comparisons of continuous variable between two supplementation groups and the dropout group were examined by one-way ANOVA followed by the Duncan test. ANCOVA was also used to adjust for possible differences due to the possible confounding variables. Comparisons of categorical variable between two supplementation groups and the dropout group were performed with the chi-square test. A p value <0.05 was considered to indicate statistical significance. The serum vitamin C variable was log-transformed to improve normality. The effect of possible confounding factors was examined by multiple regression analysis. The change in log-transformed serum vitamin C concentration between baseline and five years after vitamin C supplementation was used as the dependent variable for the model. Gender, age, smoking status, ethanol intake, change in vitamin C intake, and log-transformed serum vitamin C concentration at baseline and two dummy variables for vitamin C supplementation group were included simultaneously in the model as independent variables. The interaction between vitamin C supplementation group and alcohol drinking and smoking status (as dummy variables) was tested by adding a cross-product variable in the regression models for log-transformed serum vitamin C concentration. The Statistical Analysis System, Version 6.12 (SAS Institute Inc., Cary, NC) software package was used for data analysis.

RESULTS

General Characteristics of Study Subjects

The general characteristics of the study subjects together with their mean baseline intake of energy, dietary fiber and vitamins of the study subjects are presented in Table 1. There were no differences in any baseline characteristics between the two supplementation groups (low-dose and high-dose group) and the dropout group. Compliance in taking the vitamin capsules was 92.9% in men and 95.4% in women.

Table 1. General Characteristics of the Study Subjects at Baseline[1]

	Low-dose (50 mg/day) (n = 120)	High-dose (500 mg/day) (n = 124)	Dropout[5] (n = 134)
Age (years)	58.7 ± 6.56[2]	56.3 ± 8.7	57.2 ± 7.86
Male	41 (34.2%)[3]	41 (34.2%)	48 (35.8%)
Current smoker[4]	12 (11.3%)	12 (11.3%)	18 (19.6%)
Height (cm)	153.2 ± 9.2	154.1 ± 8.5	152.6 ± 8.5
Weight (kg)	55.0 ± 8.9	55.2 ± 8.1	56.0 ± 9.3
BMI (kg/m^2)	23.4 ± 2.9	23.2 ± 2.7	24.0 ± 3.3
Energy (kcal/day)	2086 ± 618	2078 ± 653	1947 ± 641
Fiber (g/day)	4.70 ± 2.12	4.50 ± 1.92	4.11 ± 1.83
Vitamin A (IU/day)	3059 ± 2075	2710 ± 1921	2614 ± 1867
Retinol (μg/day)	482 ± 447	410 ± 425	522 ± 803
Total carotene (μg/day)	2598 ± 2363	2390 ± 2005	2370 ± 1691
α-carotene (μg/day)	306 ± 331	270 ± 252	281 ± 251
β-carotene (μg/day)	2089 ± 2023	1940 ± 1721	1888 ± 1429
Lycopene (μg/day)	3326 ± 9992	2598 ± 3814	1731 ± 2690
Vitamin C (mg/day)	150 ± 101	143 ± 87	132 ± 81
Vegetables (g/day)	252 ± 197	246 ± 171	225 ± 178
Fruits (g/day)	207 ± 220	196 ± 168	157 ± 120
Ethanol (g/day)	11.7 ± 21.6	12.9 ± 20.6	9.7 ± 17.8

[1] There were no statistically significant differences among the three groups in any baseline characteristics listed.
[2] Values are expressed as Mean ± SD.
[3] Number of subjects and percentage in parentheses.
[4] It is based on male subjects, because no females smoked.
[5] Dropout was defined as subject who dropped out before and upon modification of the study protocol.

Effect of Long-Term Oral Vitamin C Supplementation on Serum Vitamin C Concentrations

Serum vitamin C concentrations at baseline and after one-year and five-year vitamin C supplementation, during which the subjects had received either low-dose (50 mg/day) or high-dose vitamin C (500 mg/day) or dropped out before and upon the modification of the study protocol are shown in Table 2. At baseline, the mean concentration of serum vitamin C (log-transformed and untransformed) did not differ between the low-dose group, the high-dose group and the dropout group. Serum vitamin C concentrations were markedly and significantly increased at one year and slightly decreased after five years of vitamin C supplementation. At one- and five-year follow-up, the serum concentrations significantly differed among the three groups. Changes in serum vitamin C concentrations were significantly higher in the high-dose group (21.3 μmol/L increase) than in the low-dose group (5.72 μmol/L increase), or the dropout group (0.50 μmol/L decrease) after five years of vitamin C supplementation. After adjustment for gender, age, body mass index, ethanol intake, smoking status, consumption of vegetables and fruits, a significant difference was still maintained. In the high-dose group receiving 500 mg/day, the serum vitamin C concentration increased by 38.5% after five years, which is a significantly higher increase than in the other two groups.

Based on the distribution of serum vitamin C concentrations at baseline, serum vitamin C concentrations were divided into quartiles. Mean serum vitamin C concentrations of each quartile were 54.7, 70.5, 82.2 and 101.7 μmol/L, respectively (Table 3). Fig. 2 also shows the serum vitamin C concentrations at each point by supplementation group. Table 3 indicates that the range between the mean values of serum vitamin C in the highest quartile and the lowest quartile clearly shrank after one and five years, compared with those at baseline. Thus, the mean vitamin C concentration of the subjects in the highest quartile initially declined from 101.7 μmol/L at baseline to 94.5 μmol/L after five years. Similarly, the mean vitamin C concentration of subjects in the lowest quartile initially increased from 54.7 μmol/L at baseline to 84.7 μmol/L after five years. As a result, the mean difference in serum vitamin C concentration between the highest (Q4) and lowest quartile (Q1) declined from 47.0 μmol/L at baseline to 9.8 μmol/L after five years. Consequently, the mean serum vitamin C concentrations

Table 2. Changes in Serum Vitamin C Concentrations by Supplementation Group

Group	Serum Vitamin C Concentrations				
	Baseline[1] μmol/L	1-year follow-up point[1] μmol/L	5-year follow-up point[1] μmol/L	Change[2,3] μmol/L	% change[3,4] %
Low-dose (n = 120)	78.6 ± 1.69	97.4 ± 1.87[b]	84.5 ± 1.52[b]	5.72 (2.18, 9.25)[b]	13.0 (5.09, 20.9)[b]
High-dose (n = 124)	76.8 ± 1.88	108.9 ± 2.17[a]	98.0 ± 2.41[a]	21.3 (15.8, 26.7)[a]	38.5 (27.0, 49.9)[a]
Dropout (n = 134)	77.2 ± 1.58	88.2 ± 2.01[c]	78.2 ± 1.66[c]	−0.50 (−4.36, 3.37)[c]	3.29 (−2.05, 8.63)[b]
Unadjusted *p* value	>0.05	0.0001	0.0001	0.0001	0.0001
Adjusted[5]	>0.05	0.0001	0.0001	0.0001	0.001

[1] Mean ± SE. Values in the same column with different superscript letters are significantly different by Duncan's method.
[2] Changes in serum vitamin C concentrations between baseline and after five years of vitamin C supplementation.
[3] Mean (95% CI). Values in the same column with different superscript letters are significantly different by Duncan's method.
[4] Mean change expressed as a percentage of the mean baseline concentration.
[5] Adjusted for gender, age, body mass index, ethanol intake (g/day), smoking status, consumption of vegetables and fruits.

Table 3. Changes in Serum Vitamin C Concentrations by Quartiles of Serum Vitamin C Concentrations at Baseline

Quartiles[5]	Serum Vitamin C Concentrations				
	Baseline[1] μmol/L	1-year follow-up point[1] μmol/L	5-year follow-up point[1] μmol/L	Change[2,3] μmol/L	% change[3,4] %
Q1 (n = 89)	54.7 ± 1.07[a]	95.4 ± 3.07[a]	84.7 ± 2.95[a]	30.5 (24.1, 36.8)[d]	65.2 (47.8, 82.6)[c]
Q2 (n = 98)	70.5 ± 0.72[b]	95.0 ± 2.41[a]	83.8 ± 1.94[a]	13.0 (9.32, 16.6)[c]	18.6 (13.2, 23.9)[b]
Q3 (n = 97)	82.2 ± 0.69[c]	96.2 ± 2.12[a]	84.8 ± 2.22[a]	2.22 (−2.25, 6.68)[b]	3.13 (−2.29, 8.54)[a]
Q4 (n = 94)	101.7 ± 1.16[d]	105.7 ± 2.35[b]	94.5 ± 2.27[b]	−7.17 (−11.3, −3.01)[a]	−6.97 (−11.0, −2.95)[a]
Unadjusted p value	0.0001	0.004	0.002	0.0001	0.0001
Adjusted[6]	0.0001	>0.05	0.03	0.001	0.001

[1] Mean ± SE. Values in the same column with different superscript letters are significantly different by Duncan's method.

[2] Changes in serum vitamin C concentrations between baseline and after five years of vitamin C supplementation.

[3] Mean (95% CI). Values in the same column with different superscript letters are significantly different by Duncan's method.

[4] Mean change expressed as a percentage of the mean baseline concentration.

[5] The quartiles are based on the distribution of serum vitamin C concentrations at baseline.

[6] Adjusted for gender, age, body mass index, ethanol intake (g/day), smoking status, consumption of vegetables and fruits.

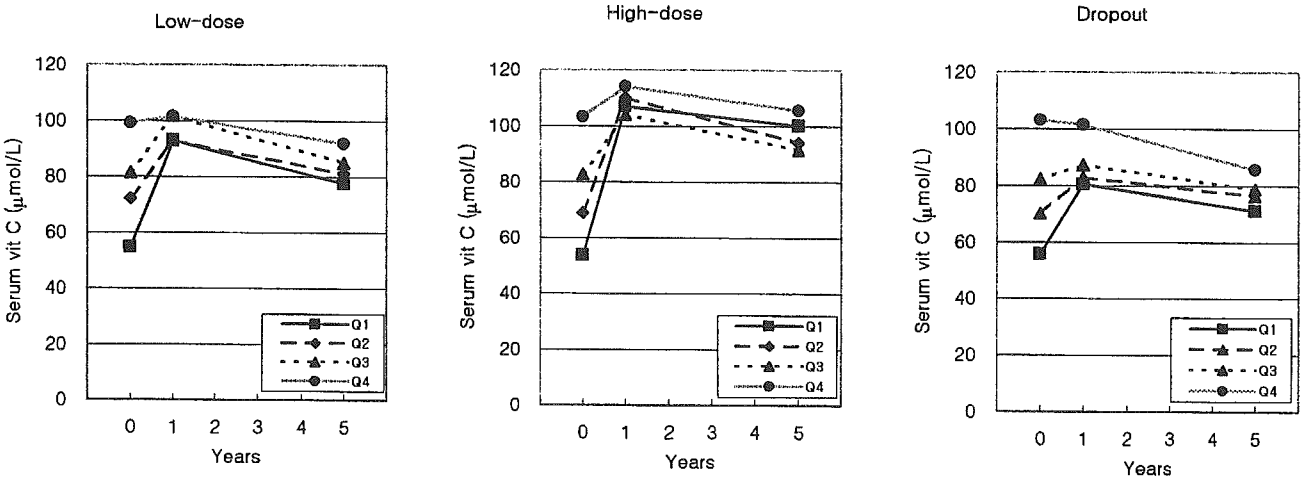

Fig. 2. Changes in serum vitamin C concentration by quartiles of serum vitamin C at baseline. The quartiles are based on the distribution of serum vitamin C concentrations at baseline by supplementation group.

became similar at one and five years after vitamin C supplementation, except in the highest quartile.

Results from the final multivariate regression models for predicting the change between baseline and five years in the serum vitamin C concentrations are shown in Table 4. In the overall subjects, the serum vitamin C concentration at baseline was significantly ($p < 0.0001$) and negatively related with changes in serum vitamin C concentrations during the supplementation period (R^2 for this model = 0.60). In addition, the high-dose ($p < 0.0001$) and low-dose ($p < 0.05$) supplementation and female gender ($p < 0.01$) were significantly and positively associated with changes in serum vitamin C concentrations. The most important variable in predicting an increase in the serum vitamin C concentration was the baseline serum vitamin C concentration (partial R^2 in this model = 0.46). Even in the two supplementation groups (high-dose and low-dose), the baseline serum vitamin C concentration (log-transformed) was found to be inversely related to change in the serum vitamin C concentration. Also, the female gender was positively related to change in concentration in the low-dose group. Overall R^2 for predicting changes in the serum vitamin C concentration was 0.66 in the low-dose group and 0.55 in the high-dose group. In the dropout group, the serum vitamin C concentration at baseline was significantly and negatively related with the change in serum vitamin C concentration during the five-year period (R^2 for this model = 0.52).

Effect of Long-Term Vitamin C Supplementation on Consumption of Vegetables and Fruits

Dietary intake of fiber, vitamin A & C and vegetables and fruits of the study subjects before and after supplementation is illustrated in Table 5. Dietary intake of vitamin C in the supplementation group was almost identical before and after its five-year supplementation (2.31 mg/day decrease, 95% CI = −15.3–10.7), while a 17.7 mg/day decrease (95% CI = −44.2–

Table 4. Multiple Regression Analysis for the Changes in Log Transformed Serum Vitamin C Concentration during the Study Period

	Total Subjects		Low-dose		High-dose		Dropout	
	b[1]	p	b	p	b	p	b	p
Supplementation (low dose = 1, other = 0)	0.071	0.03	N/A[3]		N/A		N/A	
Supplementation (high dose = 1, other = 0)	0.239	0.0001	N/A		N/A		N/A	
Log-transformed serum vitamin C at baseline	−0.904	0.0001	−0.874	0.0001	−1.008	0.0001	−0.835	0.0001
Gender (female = 1, male = 0)	0.135	0.0012	0.174	0.0004	0.078	0.43	0.029	0.78
Age	0.003	0.13	0.004	0.19	0.005	0.08	0.000	0.94
Smoking (current smoker = 1, others = 0)	−0.061	0.18	−0.097	0.11	−0.044	0.64	−0.100	0.41
Ethanol intake (g/day)	−0.000	0.84	0.000	0.72	0.000	0.82	−0.005	0.13
Change in vitamin C intake (mg/day)	−0.000	0.61	0.000	0.75	0.000	0.54	−0.000	0.24
R^2 for model[2]	0.60		0.66		0.55		0.52	

[1] Values are expressed as beta coefficients from multiple regression analysis.
[2] p for the model <0.0001.
[3] Not applicable.

8.86) was observed in the dropout group, although it did not reach statistical significance. After vitamin C supplementation, the dietary vitamin C intake of the supplementation group (144 mg/day) was significantly higher than that of the dropout group (116 mg/day). For consumption of vegetables, three subgroups were added (green/yellow vegetables, pickled vegetables and other vegetables) on the basis of the amount of vitamin content. The total vegetable intake and especially the other vegetable (Chinese cabbage, radish etc) intake of the supplementation group was significantly higher than in the dropout group five years after supplementation. Change in total vegetable intake showed a 72.7 g/day increase in the supplementation group and a 18.1 g/day increase in the dropout group.

There were no substantial adverse effects of vitamin C supplementation (high and low dose) such as pain and flatulence of abdomen, nausea and vomiting which were monitored by questionnaire at three-month intervals.

DISCUSSION

The results of the present study indicated that the serum vitamin C concentration only in the high-dose group (500 mg daily) was markedly increased at one year (31.4 μmol/L increase) and after five years (21.3 μmol/L increase) of vitamin C supplementation. The previous study [20] reported that the change in serum vitamin C was 20.1 μmol/L after two-month supplementation of vitamin C (500 mg daily). Our previous pilot study [11] with 1000 mg daily of vitamin C for three months revealed a highly significant increase in serum vitamin C (64.2 μmol/L) due to the vitamin C supplementation. The results of these two earlier studies [11,20] corroborate the similar increase observed in the present study, even though the duration of supplementation in these studies was much shorter than in our study. The EPIC-Norfolk prospective study [21] suggests that the 20 μmol/L rise in plasma ascorbic acid concentration, equivalent to about a 50 g per day increase in fruit and vegetable intake, was associated with about a 20% reduction in risk of all-cause mortality ($p < 0.0001$). A prospective cohort study [1] in China, in which the highest tertile of serum concentration of ascorbic acid at baseline was associated with a reduced risk of progression to dysplasia or gastric cancer (OR = 0.2, 0.1–0.7, p for trend = 0.006) compared with subject with the lowest tertile level. In the present study, the mean serum vitamin C concentration at five years in the high-dose group (21.3 μmol/L increase) was over 85 percentile of baseline serum vitamin C. Based on previous study [1,21], our finding suggests that the increase in serum vitamin C afforded by 500 mg vitamin C supplementation for five years might have clinical and public health implications.

Unlike β-carotene [11], the concentration of ascorbic acid in the plasma and other body fluids does not increase proportionally as the daily oral dose of vitamin C is increased. Blanchard *et al.* [22] suggest that both saturable gastrointestinal absorption and nonlinear renal clearance of ascorbate act additively to produce the ceiling effect in plasma concentrations. As a consequence of this ceiling effect, the serum vitamin C concentration tends to approach an upper limit. An NIH study [23] of vitamin C depletion-repletion pharmacokinetics, in which vitamin C doses ranging from 30 to 2500 mg daily, showed a sigmoid relationship between the ascorbate dose and the steady-state plasma concentration. The plasma concentration produced by the present RDA (60 mg/day) was on the bottom third of the steep portion of the curve; the 200-mg dose was beyond the steep portion, and plateau plasma vitamin C was close to maximum at 500 mg daily. Plasma vitamin C completely saturated at the 1000 mg daily dose. In most randomized clinical trials of vitamin C, there was no apparent adverse effect of a higher dose of vitamin C (less than 1000 mg daily). The recommended daily allowance of vitamin C was 50 mg according to the Ministry of Health and Welfare of Japan at the time of designing this study [24]. As a prophylactic antioxidant we chose to administer ten times the daily dose recommended in Japan and gave 500 mg ascorbic acid in capsule form. This amount of

Table 5. Changes in Dietary Fiber and Vitamins, Vegetables and Fruits during Five-Year Vitamin C Supplementation

Dietary intake	Supplementation Group (n = 216)	Dropout (n = 53)
Dietary Fiber (g/day)		
Baseline	4.59 ± 2.00[1]	4.09 ± 1.82
After 5 years	4.42 ± 1.81	3.94 ± 1.38[2]
Change	−0.17 (−0.43–0.09)[3]	−0.16 (−0.66–0.35)
Vitamin A (IU/day)		
Baseline	2879 ± 1969	2572 ± 1673
After 5 years	2845 ± 1920	2510 ± 2320
Change	−47.3 (−340–246)	−50.4 (−644–543)
Retinol (μg/day)		
Baseline	448 ± 425	450 ± 706
After 5 years	485 ± 554	367 ± 464
Change	36.7 (−43–117)	−83.2 (−291–124)
Total carotene (μg/day)		
Baseline	2470 ± 2205	2450 ± 1831
After 5 years	2385 ± 1861	2289 ± 2290
Change	−84.6 (−366–196)	−161.1 (−840–517)
α-Carotene (μg/day)		
Baseline	285 ± 295	288 ± 252
After 5 years	288 ± 262	298 ± 434
Change	3.11 (−37–44)	10.2 (−110–130)
β-Carotene (μg/day)		
Baseline	1994 ± 1894	1970 ± 1600
After 5 years	1912 ± 1597	1810 ± 1811
Change	−82.2 (−324–160)	−160.0 (−725–405)
Lycopene (μg/day)		
Baseline	2951 ± 7815	1749 ± 3014
After 5 years	3407 ± 5155	2506 ± 3664
Change	457 (−338–1251)	757 (−461–1976)
Vitamin C (mg/day)		
Baseline	146 ± 93[1]	134 ± 91
After 5 years	144 ± 89	116 ± 61[2]
Change	−2.31 (−15.3–10.7)[3]	−17.7 (−44.2–8.86)
Total vegetable (g/day)		
Baseline	253 ± 188	239 ± 214
After 5 years	325 ± 203	257 ± 151[2]
Change	72.7 (45–101)	18.1 (−41.2–77.3)
Pickled vegetable (g/day)		
Baseline	57 ± 52	50 ± 41
After 5 years	128 ± 118	101 ± 83
Change	70.7 (55.7–85.5)	50.9 (31.3–70.4)
Green/yellow vegetable (g/day)		
Baseline	54.7 ± 55.7	53.0 ± 44.9
After 5 years	45.1 ± 38.1	42.9 ± 44.7
Change	−9.6 (−16.9–−2.33)	−10.1 (−24.6–4.45)
Others vegetable (g/day)		
Baseline	141 ± 126	136 ± 146
After 5 years	153 ± 112	114 ± 61[2]
Change	11.7 (−3.93–27.3)	−22.7 (−59.5–14.1)
Fruits (g/day)		
Baseline	197 ± 188	158 ± 115
After 5 years	112 ± 103	91 ± 73
Change	−84.8 (−107–−62)	−67.4 (−90.0–−44.8)

[1] Values are expressed as mean ± SD.

[2] Statistically significant at $p < 0.05$.

[3] Mean (95% CI). Changes in dietary intake between baseline and after five years of vitamin C supplementation.

vitamin C is half of that we tested in a pilot study [11], in which no adverse effect was observed from taking 1000 mg of vitamin C. Data from other large intervention trials, with higher doses of vitamin C, suggest that there was no information about the potential hazards of less than 1000 mg daily [11,23].

According to several cross-sectional studies, circulating concentration of vitamin C is known to be influenced by multiple dietary factors and lifestyle factors, including gender, age, smoking status, alcohol consumption and dietary intake of vitamins and fruits and vegetables [9,25,26]. Moreover, in a three-month vitamin C supplementation study [11], it was reported that vitamin C supplementation and serum vitamin C concentration at baseline were associated with a change in serum vitamin C concentration. In our multivariate regression analysis, after the adjustment for suspected confounding factors, we found that the serum vitamin C concentration at baseline, female gender, and vitamin C supplementation significantly correlated with the change in serum vitamin C concentrations. Contrary to the observational cross-sectional studies, cigarette smoking, alcohol drinking and age were not associated with the change in serum vitamin C concentrations after vitamin C supplementation.

The present result clearly showed a reduced range between the mean values of the highest and the lowest quartiles of the baseline concentration of serum vitamin C after the one- and five-year interval, respectively. These findings suggest that the observed five-year change in serum vitamin C may largely reflect the regression to the mean effect in addition to supplementation of vitamin C and the real yearly biological change. The measurement error and the physiological fluctuation may be major causes for the regression to the mean within the intra-individual changes in biomarkers during a particular time interval. Moreover, the regression to the mean effect resulting from these unintentional factors may inevitably introduce the regression dilution bias for risk assessment in a cohort study setting the biomarkers as risk factors at baseline [27]. This phenomenon has been reported in other studies regarding the serum cholesterol concentration [28] as well as in our previous pilot study [11]. Owing to the regression to the mean effect, single measurement of serum vitamin C is not a good biomarker for dietary intake of vitamin C in either a supplemented or even an unsupplemented group.

Furthermore, we investigated the effect of the long-term vitamin C intervention program on consumption of vegetables and fruits. During the trial, the supplementation group, defined as receiving either low-dose or high-dose vitamin C for five years, demonstrated a substantial increase in vegetable intake, especially pickled and other vegetable (Chinese cabbage, radish etc.), compared to the dropout group. Also, the dietary intake of vitamin C of the supplementation group was almost identical before and after five years, and a 2.31 mg/day decrease was observed in the supplementation group, against a 17.7 mg/day

decrease in the dropout group. After vitamin C supplementation, the dietary vitamin C intake of the supplementation group (144 mg/day) was significantly ($p < 0.05$) higher than that of the dropout group (116 mg/day). In general, subjects who participate in a dietary supplementation program reportedly do not increase or even decrease their dietary intake [29]. Our intervention study was conducted in a selective group of motivated individuals (participation rate 78%), just like most intervention trials. The subjects took part in an annual health screening program, in which all participants in this study were diagnosed with chronic atrophic gastritis and may have been aware that they had a higher risk of developing gastric cancer. Consequently, participants, especially in the supplementation group (not in the dropout group), may have been particularly receptive to making dietary changes compared to the general population. Such dietary change results in increases in vegetable intakes. Therefore, this result suggests that our long-term intervention program may have no negative effect on consumption of vegetables high in vitamins, as different from any other intervention study.

The possible limitation of the present study is that our study subjects were serologically diagnosed with atrophic gastritis. More than half (52%) of screening participants were matched with this criterion. The prevalence of atrophic gastritis increased with age: 37% in 40–49 years, 52% in 50–59 years and 63% in 60–69 years. Therefore chronic atrophic gastritis was not especially prevalent in this area and is considered an aging phenomenon. The prevalence of atrophic gastritis was 55.4% (866/1,564) among screening program participants 40 to 59 years of age in another area within the same Yokote Public Health Center district (unpublished data). Moreover, the prevalence ranged from 9% to 27% among randomly selected men 40 to 49 years of age in five areas across Japan [30]. The highest prevalence was observed in the Yokote Public Health Center district (26%) and even in Tokyo (27%). Although the prevalence of atrophic gastritis was relatively higher than that in other areas, our study subjects were not a specially selected group in Japan. Nevertheless, there is a possibility that the effect of vitamin C supplementation on the serum vitamin C level may be different from the normal population. However, even though gastric juice concentrations were considerably lower in patients with atrophic gastritis than in patients with normal histological assessment, the plasma and mucosal concentrations were unaffected by the presence of atrophic gastritis [31].

In conclusion, the results of this study show that a five-year oral vitamin C supplementation (500 mg daily) of a diet in subjects with atrophic gastritis induces a remarkable increase in serum vitamin C concentration and that our intervention program appears to have no effect on consumption of vegetables high in vitamins. Whether changes in the serum concentration of vitamin C have an effect on cancer risk remains to be established.

ACKNOWLEDGEMENTS

This study was supported in part by Grants-in-Aid for Cancer Research and for the Second-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labor and Welfare of Japan and by the Foundation for the Promotion of Cancer Research, Japan. We are grateful to the following persons for their painstaking efforts in initiating, maintaining, modifying and setting the two study protocols: Dr. Yoshitaka Tsubono of the Division of Epidemiology, Tohoku University Graduate School of Medicine, Ms. Kaori Masugi, Mr. Takashi Sato, Dr. Tadashi Ogiwara, and other staff members at the Hiraka General Hospital, Ms. Kumiko Sasaki, Ms. Mikiko Takahashi, Mr. Shigeru Fujita, Mr. Seiji Fujita, and Mr. Shozo Sasaki (Mayor) of Sannai Village; Ms. Shizuko Kasuga, Ms. Masako Takahashi, Mr. Hiroyasu Kikuchi, Dr. Noriyuki Suzuki (director) and Dr. Yoshimichi Miyajima (former director) and Dr. Shinsuke Nagasawa (former director) of the Yokote Public Health Center; Dr. Kiyoshi Higuchi, former president of the Hiraka Medical Association; Dr. Rokuya Ishida, former president of the Hiraka Medical Association.

REFERENCES

1. You W, Zhang L, Gail MH, Chang Y, Liu W, Ma J, Li J, Jin M, Hu Y, Yang C, Blaser MJ, Correa P, Blot WJ, Fraumeni Jr JF, Xu G: Gastric dysplasia and gastric cancer: helicobacter pylori, serum vitamin C, and other risk factors. J Natl Cancer Inst 92:1607–1612, 2000.
2. Jacobs EJ, Connell CJ, Patel AV, Chao A, Rodriguez C, Seymour J, McCullogh ML, Calle EE, Thun MJ: Vitamin C and vitamin E supplementation use and colorectal cancer mortality in a large American Cancer Society Cohort. Cancer Epidemiol Biomarkers Prev 10:17–23, 2001.
3. Patterson RE, White E, Kristal AR, Neuhouser ML, Potter JD: Vitamin supplements and cancer risk: the epidemiologic evidence. Cancer Causes Control 8:786–802, 1997.
4. Byers T, Guerrero N: Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. Am J Clin Nutr 62(Suppl):1385S–1392S, 1995.
5. Blot WJ, Li JP, Taylor PR, Guo W, Dawsey S, Wang GQ, Yang CS, Zheng SF, Gail M, Li GY, Yu Y, Liu BQ, Tangrea J, Sun YH, Liu F, Fraumeni Jr JF, Zhang YH, Li B: Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. J Natl Cancer Inst 85:1483–1492, 1993.
6. Christen WG, Gaziano JM, Hennekens CH, for the Steering Committee of Physicians' Health Study II: Design of Physicians' Health Study—A randomized trial of beta-carotene, vitamin E and C, and multivitamins, in prevention of cancer, cardiovascular disease, and eye disease, and review of results of completed trials. Ann Epidemiol 10:125–134, 2000.
7. The Alpha-tocopherol, Beta carotene Cancer Prevention Study Group: The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 330:1029–1035, 1994.

8. McEligot AJ, Rock CL, Flatt SW, Newman V, Faerber S, Pierce JP: Plasma carotenoids are biomarkers of long-term high vegetable intake in women with breast cancer. J Nutr 129:2258–2263, 1999.
9. Tsugane S, Fahey MT, Kobayashi M, Sasaki S, Tsubono Y, Akabane M, Gey F: Four frequency categories of fruit intake as a predictor of plasma ascorbic acid concentration in middle-aged Japanese men. Ann Epidemiol 8:378–383, 1998.
10. Broekmans WM, Klopping-Ketelaars IA, Schuurman CR, Verhagen H, van den Berg H, Kok FJ, van Poppel G: Fruits and vegetables increase plasma carotenoids and vitamins and decrease homocysteine in humans. J Nutr 130:1578–1583, 2000.
11. Sasaki S, Tsubono Y, Okubo S, Hayashi M, Kakizoe T, Tsugane S: Effects of three-month oral supplementation of β-carotene and vitamin C on serum concentrations of carotenoids and vitamins in middle-aged subjects: a pilot study for a randomized controlled trial to prevent gastric cancer in high-risk Japanese population. Jpn J Cancer Res 91:464–470, 2000.
12. Rock CL, Flatt SW, Wright FA, Faerber S, Newman V, Kealey S, Pierce JP: Responsiveness of carotenoids to a high vegetable diet intervention designed to prevent breast cancer recurrence. Cancer Epidemiol Biomarkers Prev 6:617–623, 1997.
13. Zino S, Skeaff M, Williams S, Mann J: Randomised controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. BMJ 314:1787–1791, 1997.
14. National Cancer Institute: Beta carotene and vitamin A halted in lung cancer prevention trial [News Release]. Bethesda, MD: Office of Cancer Communications, January 18, 1996.
15. Tsubono Y, Okubo S, Hayashi M, Kakizoe T, Tsugane S: A randomized controlled trial for chemoprevention of gastric cancer in high-risk Japanese population; study design, feasibility and protocol modification. Jpn J Cancer Res 88:344–349, 1997.
16. Tsubono Y, Takamori S, Kobayashi M, Takahashi T, Iwase Y, Iitoi Y, Akabane M, Yamaguchi M, Tsugane S: A data-based approach for designing a semiquantitative food frequency questionnaire for a population-based prospective study in Japan. J Epidemiol 6:45–53, 1995.
17. Science and Technology Agency: "Standard Tables of Food Composition in Japan" 4th Rev Ed [Japanese]. Tokyo: Printing Bureau, Ministry of Finance, 1982.
18. Takahashi Y, Sasaki S, Tsugane S: Development and validation of specific carotene food composition tables for use in nutritional epidemiologic studies for Japanese populations. J Epidemiol 11: 266–275, 2001.
19. Zannoni V, Lynch M, Goldstein S, Sato P: A rapid micromethod for the determination of ascorbic acid in plasma and tissues. Biochem Med 11:41–48, 1974.
20. Huang HY, Helzlsouer KJ, Appel LJ: The effects of vitamin C and vitamin E on oxidative DNA damage: results from a randomized controlled trial. Cancer Epidemiol Biomarkers Prev 9:647–652, 2000.
21. Khaw KT, Bingham S, Welch A, Luben R, Wareham N, Oakes S, Day N: Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. Lancet 357:657–663, 2001.
22. Blanchard J, Tozer TN, Rowland M: Pharmacokinetic perspectives on megadoses of ascorbic acid. Am J Clin Nutr 66:1165–1171, 1997.
23. Levine M, Corny-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, Park JB, Lazarev A, Graumlich JF, King J, Cantilena LR: Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci 93:3704–3709, 1996.
24. Ministry of Health and Welfare: "Recommended Dietary Allowances for the Japanese," 5th ed [Japanese]. Tokyo: Daiichi-Syuppan, 1994.
25. Brook M, Grimshow JJ: Vitamin C concentration of plasma and leukocytes as related to smoking habit, sex and age of humans. Am J Clin Nutr 21:1254–1258, 1968.
26. Drewnowski A, Rock CL, Henderson SA, Shore AB, Fischeler C, Galan P, Preziosi P, Hercberg S: Serum β-carotene and vitamin C as biomarkers of vegetable and fruit intakes in a community-based sample of French adults. Am J Clin Nutr 65:1796–1802, 1997.
27. Bland JM, Altman DG: Statistic notes: regression towards the mean. BMJ 308:1499, 1994.
28. Takashima Y, Sumiya Y, Kokaze A, Yoshida M, Ishikawa M, Sekine Y, Akamatsu S: Magnitude of the regression to the mean within one-year intra-individual changes in serum lipid concentrations among Japanese male workers. J Epidemiol 11:61–69, 2001.
29. Bonithon-Kopp C, Kronborg O, Giacosa A, Räth U, Faivre J, for the European Cancer Prevention Organisation Study Group: Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. Lancet 356: 1300–1306, 2000.
30. Kabuto M, Imai H, Tsugane S, Watanabe S. Correlation between atrophic gastritis prevalence and gastric cancer mortality among middle-aged men in 5 areas in Japan. J Epidemiol 3:35–39, 1993.
31. Waring AJ, Drake IM, Schorah CJ, White KL, Lynch DA, Axon AT, Dixon MF. Ascorbic acid and total vitamin C concentrations in plasma, gastric juice, and gastrointestinal mucosa: effects of gastritis and oral supplementation. Gut 38:171–176, 1996.

Received June 17, 2002; revision accepted November 11, 2002.

Significance of nodal metastatic tumor characteristics in nodal metastasis and prognosis of patients with invasive ductal carcinoma of the breast

Takahiro Hasebe,[1] Satoshi Sasaki,[2] Shigeru Imoto[3] and Atsushi Ochiai[1,4]

[1]Pathology Division and [2]Epidemiology and Biostatistics Division, National Cancer Center Research Institute East and [3]Department of Breast Surgery, National Cancer Center Hospital East, Chiba, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577

(Received October 11, 2002/Revised December 2, 2002/Accepted December 13, 2002)

There is no study evaluating the significance of nodal metastatic tumor characteristics in tumor progression of invasive ductal carcinomas (IDCs) of the breast. The purpose of this study was to investigate whether nodal metastatic tumor characteristics play an important role in the tumor progression of IDCs. The subjects of this study were 205 IDC patients with nodal metastases. Significant associations with increased numbers of nodal metastases, and patient outcomes were evaluated by multivariate analyses, in comparison with well-known histological parameters. The numbers of lymph nodes with extra-nodal invasion and with extra-nodal blood vessel tumor emboli, the distance of extra-nodal blood vessel tumor emboli from the nodes, and the nodal tumor dimensions significantly increased the number of nodal metastases in the multivariate analysis ($P<0.001$). Cox multivariate analyses showed that the parameters which significantly increased hazard rates (HRs) of disease-free survival (DFS), distant organ metastasis (DOM) and overall survival were 6 or more mitotic figures of nodal metastatic tumors ($P<0.05$). Six or more lymph nodes with extra-nodal invasion, and an extra-nodal blood vessel tumor emboli dimension of >0.6 mm significantly increased the HRs of DFS and DOM in multivariate analyses ($P<0.05$). The present study demonstrated the important roles of nodal metastatic tumors in the tumor progression of IDCs. (Cancer Sci 2003; 94: 181–187)

Invasive ductal carcinomas (IDCs) of the breast with lymph node metastases are composed of primary invasive tumors and nodal metastatic tumors. Among patients with IDCs with lymph node metastases, some do not show tumor recurrence or death in their clinical course after the initial operation for IDCs. This suggests the numbers of lymph node metastases and/or the histological characteristics of nodal metastatic tumors as well as the primary tumor characteristics all play an important role in tumor recurrence or death in patients with IDCs with nodal metastases.

Many studies that show prognostic significance of nodal metastasis have been reported.[1–11] Almost all emphasized that the number of nodal metastases is the most significant prognostic parameter in predicting the outcomes of patients with IDCs having nodal metastases, e.g. 3 or more lymph node metastases, and in the pathological TNM (pTNM) stage classification,[12] IDCs with nodal metastasis are classified into IDCs with 1 to 3 nodal metastases, and those with 4 or more. Among other parameters, nodal tumor dimension, the presence of extra-nodal invasion or the presence of nodal efferent lymph vessel tumor emboli have been reported as important prognostic parameters.[1–6, 13–15] However, only a small number of studies have precisely examined the prognostic significance of the nodal tumor dimension in the outcome of patients with IDCs. As for extra-nodal invasion and nodal efferent lymph vessel emboli, up to the present only their presence or absence has been investigated to predict a patient's outcome. In addition, there is no study which precisely investigates the prognostic significance of nodal metastatic tumor characteristics for tumor recurrence or death in IDC patients with nodal metastasis.

The purpose of this study was to investigate the parameters that are significantly associated with increased numbers of nodal metastases, and the outcomes of patients with IDCs involving lymph node metastasis. We found that several nodal metastatic tumor characteristics had significant effects on the numbers of nodal metastases and tumor recurrence or death in IDC patients with nodal metastasis.

Materials and Methods

Cases. Four hundred and thirty-nine IDCs of the breast were surgically treated between July 1992 and November 1998 at the National Cancer Center Hospital East, and among them, 205 consecutive IDCs with lymph node metastasis served as the subjects of this study. Clinical information was obtained from the patients' medical records. All patients were Japanese women, ranging in age from 28 to 79 years (mean, 52 years). All had a solitary lesion. One hundred and seventeen patients were pre-menopausal, and 88 were post-menopausal. Partial mastectomy was performed in 16, modified radical mastectomy in 170, and standard radical mastectomy in 19. Axillary lymph node dissection consisting of level I, II or ±III was carried out in all patients. None of the patients had received radiotherapy or chemotherapy before surgery. In 205 IDC patients with nodal metastases, 15 patients received no adjuvant therapy, and 190 patients received adjuvant therapy (Table 1). In the latter group, 34 patients received tamoxifen, 51 patients received chemotherapy (CMF (Cyclophosphamide, Methotrexate, and 5-FU), AC (Adriamycin and Cyclophosphamide), or EC (Epirubicin and Cyclophosphamide)), and 105 patients received chemotherapy plus tamoxifen. Sixteen patients treated with partial mastectomy had received radiotherapy (60 Gy). All tumors were classified according to the pTNM classification (Table 1).[12] Estrogen receptors (ERs) and progesterone receptors (PRs) in the cytosol fractions were determined by enzyme immunoassay (Table 1) (Otsuka Assay Laboratory, Tokushima). The upper cut-off values of the ER and PR assays were 13 and 10 fmol/mg protein, respectively.

For pathological examination, the surgically resected specimens were fixed in 10% formalin overnight at 4°C, and the entire tumor was cut into slices at intervals of 0.5 to 0.7 cm. The size and gross appearance of the tumors were recorded, and the former was confirmed by comparison with tumor size on histological slides. Multiple histological sections for primary tumors were taken from each tumor in order to measure the maximum tumor diameter and area. The sections were processed routinely and embedded in paraffin.

Serial sections of each primary tumor were cut from the paraffin blocks. One section was stained with hematoxylin and

[4]To whom correspondence should be addressed. E-mail: aochiai@east.ncc.go.jp

Table 1. Clinicopathological characteristics of patients with IDC

No. of patients (%)		No. of patients (%)	
Clinical characteristics			
ER and PR status		pTNM stage classification	
Negative	75 (37)	IIA	39 (19)
Positive	130 (63)	IIB	88 (43)
Age (yr)		IIIA	34 (17)
≤39	18 (9)	IIIB	44 (21)
>39	187 (91)		
Adjuvant therapy			
No	15 (7)		
Yes	190 (93)		
Primary tumor characteristics			
Invasive tumor size (mm)		No. of mitotic figures	
≤20	55 (27)	≤4	111 (54)
>20	150 (73)	>4	94 (46)
Nuclear atypia		Tumor growth feature	
Mil/mod	115 (56)	Pap/cri/solid	144 (70)
Severe	90 (44)	Strand	61 (30)
Stromal fibrosis grade		Tumor necrosis	
None/mil/mod	124 (60)	Absent	163 (80)
Severe	81 (40)	Present	42 (20)
Lymphatic invasion		Blood vessel invasion	
Absent	61 (30)	Absent	59 (29)
Present	144 (70)	Present	146 (71)
Adipose tissue invasion		Skin invasion	
Absent	18 (9)	Absent	167 (81)
Present	187 (91)	Present	38 (19)
Nodal tumor characteristics			
No. of nodal metastasis		No. of dissected lymph nodes	
<10	149 (73)	<40	192 (94)
≥10	56 (27)	≥40	13 (6)
% of metastatic nodes in LNs		Nodal tumor dimension (mm)	
<80%	178 (87)	<20	183 (89)
≥80	27 (13)	≥20	22 (11)
No. of nodes with ENI		Distance of ENI from node (mm)	
<6	164 (80)	<2	147 (72)
≥6	41 (20)	≥2	58 (28)
Width of ENI (mm)		Nodal tumor nuclear atypia	
<10	184 (90)	Mild/moderate	115 (56)
≥10	21 (10)	Severe	90 (44)
Nodal tumor growth feature		No. of mitotic figures	
Pap/cri/sol	172 (84)	<6	122 (60)
Strand	33 (16)	≥6	83 (40)
Nodal tumor stroma		No. of ENBVTE	
None/mil/mod	160 (78)	<10	176 (86)
Severe	45 (22)	≥10	29 (14)
Dimension of ENBVTE (mm)		Distance of ENBVTE from node (mm)	
≤0.6	162 (79)	<2	179 (87)
>0.6	43 (21)	≥2	26 (13)
No. of mitotic figures in ENBVTE		Extra-nodal tumor growth feature	
<3	175 (85)	None/pap/cri/sol	176 (86)
≥3	30 (15)	Strand	29 (14)
Extra-nodal tumor stroma			
None/mil/mod	155 (76)		
Severe	50 (34)		

No., number; ER, estrogen receptor; PR, progesterone receptor; ER and PR status negative, ER and PR both negative; ER and PR status positive, ER and PR either or both positive; LNs, dissected lymph nodes; ENI, extra-nodal invasion; ENBVTE, extra-nodal blood vessel tumor emboli.

eosin (HE) and examined pathologically to confirm the diagnosis, and elastica staining was also performed to assess blood vessel invasion in all cases. We examined the following histological features of primary tumors and set cut-off values that significantly increased the hazard rate (HR) of tumor recurrence or death in univariate analyses using the Cox proportional hazard regression model[16] (Table 1): 1) invasive tumor size, ≤20 vs. >20 mm; 2) number of mitotic figures, ≤4 vs. >4; 3) nuclear atypia, mild/moderate vs. severe; 4) tumor growth feature, papillary/cribriform/solid vs. strand; 5) stromal fibrosis

grade, none/mild/moderate vs. severe; 6) tumor necrosis, absent vs. present; 7) lymphatic invasion, absent vs. present; 8) blood vessel invasion, absent vs. present; 9) adipose tissue invasion, absent vs. present; and 10) skin invasion, absent vs. present. Nuclear atypia of the tumor cells was evaluated employing the classification of Elston.[17] The number of tumor cell mitotic figures was counted in 10 tumor fields in the advanced edge of invasive tumors, and the field showing the highest mitotic count was selected for comparison with the number of mitotic figures in tumor cells in the lymph nodes.

Histological examination of tumor cells in lymph nodes. Evaluation of lymph nodes for metastases was done using half of each node stained with HE.

The following histological parameters were examined and cut-off values were chosen that were significantly associated with tumor recurrence in univariate analyses using the Cox proportional hazard regression model[16] (Table 1): 1) number of nodal metastases (<10 vs. ≥10); 2) number of dissected lymph nodes (<40 vs. ≥40); 3) percentage of metastatic nodes in dissected lymph nodes (<80% vs. ≥80%); 4) nodal metastatic tumor dimension (<20 vs. ≥20 mm); 5) number of lymph nodes with extra-nodal invasion (<6 vs. ≥6); 6) distance of extra-nodal invasion from node (<2 vs. ≥2 mm); 7) width of extra-nodal invasion (<10 vs. ≥10 mm); 8) nuclear atypia (mild/moderate vs. severe); 9) nodal tumor growth feature (papillary/cribriform/solid vs. strand); 10) number of mitotic figures of

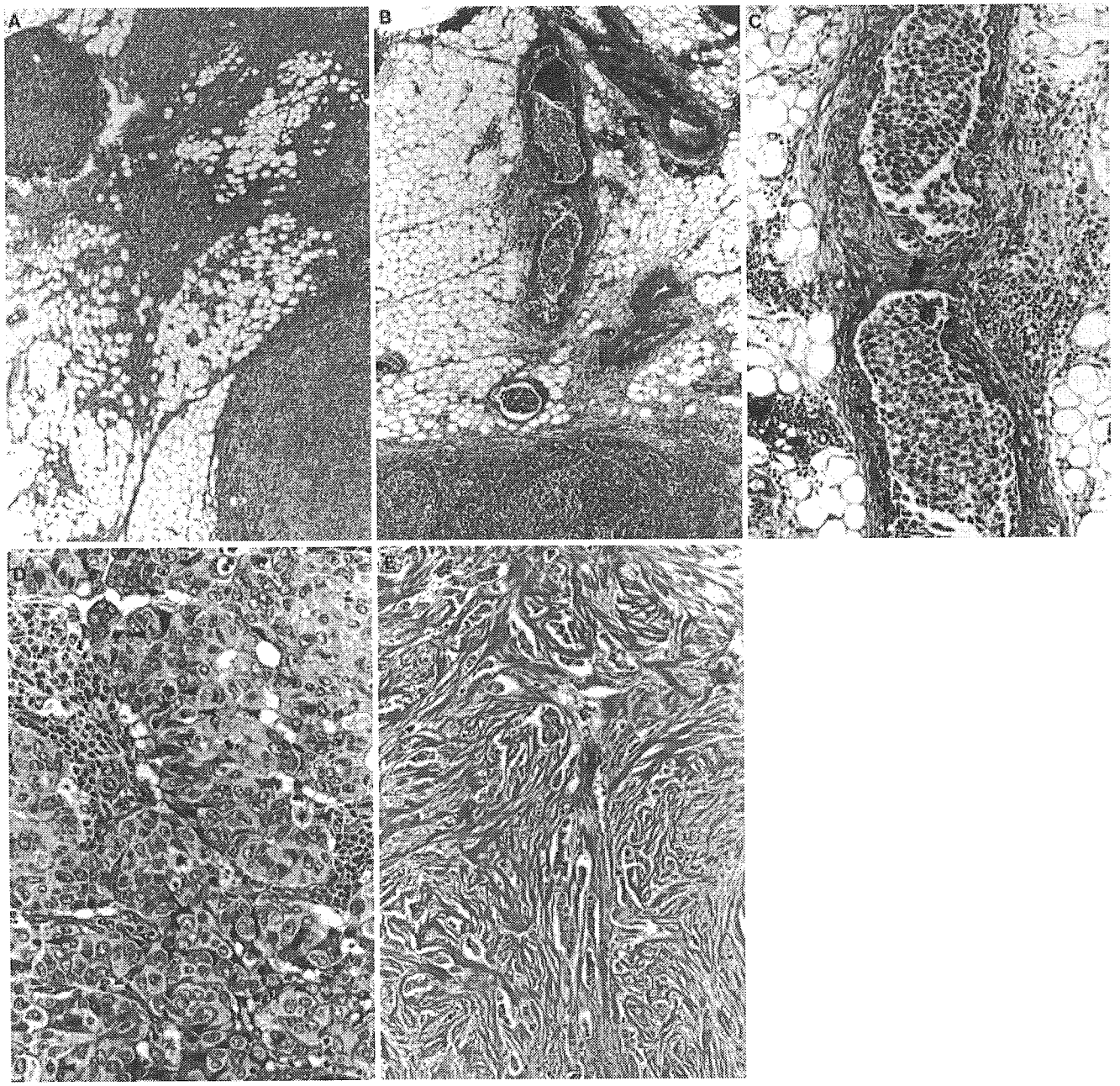

Fig. 1. Histological features of nodal metastatic tumors. (A) The tumor cells invade through the capsule of the lymph node. (B) Large extra-nodal blood vessel tumor emboli are observed. (C) An extra-nodal blood vessel tumor embolus. Tumor cell nests in vessels lined by endothelium with supporting smooth muscle. (D) Nodal tumor cells show several mitotic figures (arrowheads). (E) Tumor cells grow in a strand formation with abundant stromal fibrosis.

nodal metastatic tumor (<6 vs. ≥6) (1 high-power field); 11) nodal tumor stroma (none/mild/moderate vs. severe); 12) number of extra-nodal blood vessel tumor emboli (<10 vs. ≥10); 13) extra-nodal blood vessel tumor emboli dimension (≤0.6 vs. >0.6 mm); 14) distance of extra-nodal blood vessel tumor emboli from nodes (<2.0 vs. ≥2.0 mm); 15) number of mitotic figures of extra-nodal blood vessel tumor emboli (<3 vs. ≥3) (1 high-power field); 16) extra-nodal tumor growth feature, (extra-nodal invasion absent/papillary/cribriform/solid vs. strand); and 17) extra-nodal tumor stroma (extra-nodal invasion absent/none/mild/moderate vs. severe).

Extra-nodal invasion was defined as the extension of tumor cells through the capsule of at least one lymph node into the perinodal adipose tissue (Fig. 1A). The presence of tumor cells within the capsule or in perinodal vessels was not considered extra-nodal invasion. In this study, we noticed that all dissected lymph nodes had blood vessels with a smooth muscle-supported endothelial lining in perinodal adipose tissues, and it was very easy to assess tumor emboli in these blood vessels (Fig. 1, B and C). Therefore, we evaluated the presence of extra-nodal blood vessel tumor emboli instead of the presence of efferent lymph vessel tumor emboli. We did not evaluate the prognostic significance of efferent lymph vessel tumor emboli as reported by Hartveit *et al.*,[14, 15] because extra-nodal tumor invasive foci surrounded by perinodal fat cells showed very similar features to those of efferent lymph vessel tumor emboli.

The distance and width of extra-nodal invasion, the dimension and distance of extra-nodal blood vessel tumor emboli, and the small lymph node metastatic tumor dimensions were measured with a microscope equipped with a 10× eyepiece enclosing a graticule consisting of a grid lattice of 9 vertical and 9 horizontal grids that intersected at 81 points in each field. Most measurements were made with a 2× or 4× objective. If several foci of extra-nodal invasion were observed in one nodal metastatic tumor, the greatest length and width of each extra-nodal invasion were considered as the length and width of the extra-nodal invasion, respectively. Nuclear atypia, structural atypia, the number of mitotic figures (Fig. 1D) and the stromal fibrosis grade (Fig. 1E) of intra- and extra-nodal metastatic tumors or extra-nodal blood vessel tumor emboli were evaluated in the same manner as for the primary tumors.

One author (T.H.) assessed all characteristics of primary tumors and nodal metastatic tumors, and another author (A.O.) identified the characteristics of IDCs to confirm tumor characteristics in primary and nodal metastatic tumors examined by T.H. Whenever there was a discrepancy, both authors re-examined the slides to reach a consensus.

Outcome. The survival of patients was evaluated by follow-up to a median period of 55 months, as of November of 2000. One hundred and thirty-five patients were alive and well, 70 had tumor recurrence, 49 had initial distant organ metastasis and 36 had died of their disease. Disease-free survival, initial distant organ metastasis, and overall survival were measured from the date of surgery. Metastasis or local recurrence was taken as evidence of tumor relapse. Initial distant organ metastasis was observed in the following organs: 1) bone, 19 cases; 2) lung, 15 cases; 3) liver, 15 cases; and 4) brain, 5 cases. Only deaths due to breast cancer were considered for the purposes of this study.

Statistical analysis. Multiple regression analysis was performed to identify parameters associated with increased nodal metastasis after adjustment for parameters showing a significant correlation with nodal metastasis in univariate analyses using Spearman's correlation coefficient test.

Parameters of nodal tumors and primary tumors that were significantly associated with tumor progression in the univariate analyses were entered into the Cox proportional multivariate analyses[16] adjusted for age (≤39 vs. >39) (Table 1), ER/PR expression (both negative vs. either or both positive), and adjuvant therapy (no vs. yes) to identify the parameters that were most significantly associated with tumor recurrence and death. Survival curves were drawn by the Kaplan-Meier method.[18] All multivariate analyses were evaluated employing the step-down method until all of the remaining parameters were significant at a P value of below 0.05.

We examined by Spearman's correlation test whether there were any significant associations among parameters of nodal tumors that significantly increased the HRs of tumor recurrence or death in the multivariate analyses. We also examined whether there were any parameters significantly increasing the HR of tumor recurrence or death that were common to primary tumors and nodal tumors, e.g. nuclear atypia or mitotic figures. All analyses were performed with Statistica/Windows software (StatSoft, Tulsa, OK).

Results

Parameters significantly associated with increased number of nodal metastases. The number of lymph nodes with extra-nodal invasion, the number of extra-nodal blood vessel tumor emboli, the distance of extra-nodal blood vessel tumor emboli from the nodes, the nodal tumor dimension, and the number of lymph vessel invasion were associated with significantly increased numbers of lymph node metastases in the multivariate analysis (Table 2).

Prognostic significance of nodal metastatic tumor characteristics. The parameters which significantly increased the HRs of tumor recurrence, initial distant organ metastasis and tumor death in multivariate analyses were 6 or more mitotic figures of nodal metastatic tumors, and severe nuclear atypia of primary tumors (Table 3, Fig. 2A). Six or more lymph nodes with extra-nodal invasion, and extra-nodal blood vessel tumor emboli with a dimension of >0.6 mm significantly increased the HRs of tumor recurrence and initial distant organ metastasis in the multivariate analyses (Fig. 2, B and C). Negative status of ER/PR significantly increased the HRs of tumor recurrence and death in the multivariate analyses. The HRs of tumor recurrence were significantly increased in IDCs with severe primary tumor stroma, in those with lymph vessel invasion, in those with skin invasion, and in IDC patients of 39 years of age or younger. Strand growth feature of nodal metastatic tumors significantly increased the HR of initial distant organ metastasis, and 10 or more nodal metastases and 5 or more mitotic figures of primary tumors significantly increased the HRs of tumor death in the multivariate analyses.

Correlations among significant prognostic parameters of nodal tumors. Number of nodal metastasis, number of nodes with extra-nodal invasion and dimension of extra-nodal blood vessel tu-

Table 2. Multiple regression analyses for parameters significantly associated with increased number of nodal metastases

Parameters	RR	95% CI	P value
Increased nodal metastases			
No. of lymph nodes with ENI	1.77	1.62–1.91	<0.001
No. of ENBVTE	1.25	1.15–1.34	<0.001
Distance of ENBVTE (mm)	1.16	1.07–1.25	<0.001
Nodal tumor dimension (mm)	1.13	1.06–1.20	<0.001
No. of lymph vessel invasions	1.10	1.03–1.18	0.006

RR, relative risk; CI, confidence interval; No., number; ENI, extra-nodal invasion; ENBVTE, extra-nodal blood vessel tumor emboli; Distance of ENBVTE, distance of ENBVTE from lymph node. The multivariate analysis was performed by employing a multiple regression model adjusted for parameters showing a significant correlation with increased number of lymph node metastases in the univariate analyses. The step-down method, until all remaining parameters were significant at a P level below 0.05, was used in the multivariate analysis.

Table 3. Multivariate analyses for tumor recurrence and death in all IDCs with nodal metastases

Parameters	Cases	TRR (%)	HR	95% CI	*P* value
Number of mitotic figures of nodal tumor					
≤5	122	29 (24)	Referent		
>5	83	41 (50)	2.5	1.5–4.2	<0.001
Dimension of extra-nodal blood vessel tumor emboli					
≤0.6 mm	162	42 (26)	Referent		
>0.6 mm	43	28 (65)	1.8	1.1–3.1	<0.001
Primary tumor nuclear atypia					
Mild/moderate	115	28 (24)	Referent		
Severe	90	42 (47)	2.2	1.3–3.8	0.003
Number of nodes with extra-nodal invasion					
≤5	164	42 (26)	Referent		
>5	41	28 (68)	3.1	1.7–5.2	0.003
Lymph vessel invasion					
Absent	61	12 (20)	Referent		
Present	144	51 (40)	2.7	1.4–5.2	0.004
ER/PR status					
Negative	70	38 (51)	Referent		
Positive	135	32 (25)	0.5	0.3–0.8	0.004
Primary tumor stroma					
Mild/moderate	124	31 (25)	Referent		
Severe	81	39 (48)	1.9	1.2–3.2	0.008
Skin invasion					
Absent	167	52 (31)	Referent		
Present	38	18 (47)	1.8	1.0–3.2	0.035
Age					
≤39 yr.	18	10 (55)	Referent		
>39 yr.	187	60 (32)	0.5	0.2–0.9	0.040
	Cases	IDOMR (%)	HR	95% CI	*P* value
Number of nodes with extra-nodal invasion					
≤5	164	28 (17)	Referent		
>5	41	21 (51)	3.1	1.6–6.1	<0.001
Dimension of extra-nodal blood vessel tumor emboli					
≤0.6 mm	162	27 (17)	Referent		
>0.6 mm	43	22 (45)	2.8	1.5–5.0	0.001
Primary tumor nuclear atypia					
Mild/moderate	115	17 (15)	Referent		
Severe	90	32 (36)	2.7	1.5–4.9	0.002
Nodal tumor growth feature					
Non-strand	172	33 (19)	Referent		
Strand	33	16 (48)	2.7	1.4–5.3	0.003
Number of mitotic figures of nodal tumor					
≤5	122	21 (17)	Referent		
>5	83	28 (34)	2.3	1.2–4.0	0.007
	Cases	MR (%)	HR	95% CI	*P* value
ER/PR status					
Negative	70	24 (32)	Referent		
Positive	135	12 (9)	0.2	0.1–0.5	<0.001
Primary tumor nuclear atypia					
Mild/moderate	115	9 (7)	Referent		
Severe	90	27 (30)	3.3	1.5–7.3	0.003
Number of mitotic figures of nodal tumor					
≤5	122	12 (10)	Referent		
>5	83	24 (29)	2.3	1.1–4.8	0.023
Number of nodal metastases					
≤9	149	15 (10)	Referent		
>9	56	21 (38)	2.3	1.0–5.1	0.047
Number of mitotic figures of primary tumors					
≤4	111	9 (8)	Referent		
>4	94	27 (29)	2.3	1.0–5.1	0.047

DFS, disease-free survival; IDOM, initial distant organ metastasis; OS, overall survival; HR, hazard rate; CI, confidence interval; TRR, tumor recurrence rate; IDOMR, DOM rate; MR, mortality rate; ER, estrogen receptor; PR, progesterone receptor. The multivariate analyses for TRR, IDOMR, and MR were performed using the Cox proportional hazard regression model adjusted for parameters significantly associated with TRR, IDOMR, and MR in the univariate analyses. The step-down method, until all the remaining parameters were significant at a *P* level below 0.05, was used for the multivariate analysis.

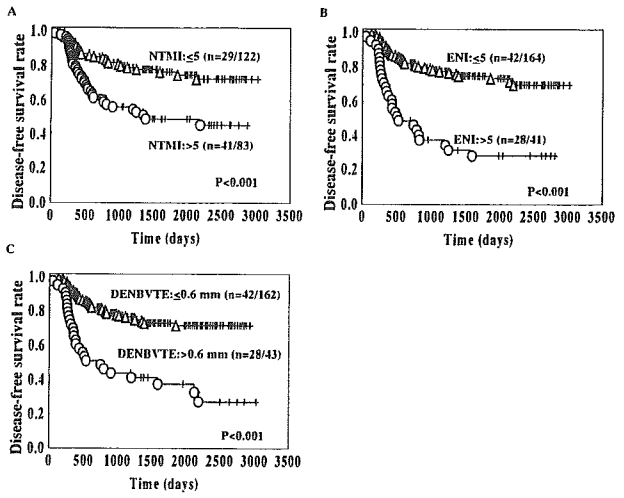

Fig. 2. Disease-free survival of IDC patients. (A) Cases with nodal metastatic tumors with mitotic figures >5 have significantly shorter disease-free survival period than those with nodal metastatic tumors with mitotic figures ≤5 ($P<0.001$). NTMI, nodal tumor mitotic figures. (B) Cases with >5 lymph nodes with extra-nodal invasion have significantly shorter disease-free survival period than those with ≤5 lymph nodes with extra-nodal invasion ($P<0.001$). (C) Cases with lymph nodes with extra-nodal blood vessel tumor emboli >0.6 mm distant from the node show significantly shorter disease-free survival period than those with lymph nodes with extra-nodal blood vessel tumor emboli ≤0.6 mm distant from the node ($P<0.001$). ENI, extra-nodal invasion; DENBVTE, distance of extra-nodal blood vessel tumor emboli.

Table 4. Correlations between parameters of nodal tumors

	Parameters of nodal tumors				
	No.of LNM	No. of ENI	MF	GF	Dim of ENBVTE
	rv	rv	rv	rv	rv
	Pv	Pv	Pv	Pv	Pv
No. of LN		0.830	0.100	0.322	0.520
		<0.001	0.151	<0.001	<0.001
	ENI		0.116	0.360	0.707
			0.098	<0.001	<0.001
		Mit		−0.054	0.129
				0.439	0.219
			GF		0.073
					0.487

No., number; LNM, lymph node metastasis; ENI, nodes with extra-nodal invasion; MF, no. of mitotic figures (/1 high-power field); GF, growth feature of nodal tumors; Dim, maximum dimension; ENBVTE, extra-nodal blood vessel tumor emboli.

Table 5. Correlation values of parameters that are common to primary-invasive tumors and nodal tumors

Parameters	*R* value	*P* value
Nuclear atypia	0.573	<0.001
Number of mitotic figures	0.568	<0.001
Growth feature	0.380	<0.001
Tumor stroma	0.321	<0.001

mor emboli were significantly associated with each other (Table 4). Growth feature of nodal tumors was significantly associated with number of nodal metastases and number of nodes with extra-nodal invasion. Number of mitotic figures in nodal tumors, however, had no significant association with other parameters.

Values of correlations between parameters that are common to primary tumors and nodal tumors. Nuclear atypia, number of mitotic figures, tumor growth feature, and tumor stroma, which are common histological features to primary tumors and nodal tumors, significantly increased the HR of tumor recurrence or death in the multivariate analyses (Table 3). Significant mutual associations were observed among the parameters that were common to primary tumors and nodal tumors (Table 5).

Discussion

The current study clearly showed that the following five parameters associated with increased nodal metastasis are very important; 1) the number of lymph nodes with extra-nodal invasion, 2) the number of extra-nodal blood vessel tumor emboli, 3) the distance of extra-nodal blood vessel tumor emboli from node, 4) the nodal tumor dimensions and 5) the number of lymphatic invasions in primary tumors. Out of these five parameters, four are associated with nodal metastatic tumors, which strongly suggests that nodal metastatic tumors have a more significant effect on increased numbers of nodal metastases than primary tumors. Thus, nodal metastatic tumors that have superior abilities of extra-nodal invasion, extra-nodal blood vessel invasion, several extra-nodal blood vessel spreading and proliferative activity most likely develop increased numbers of nodal metastases.

The current study also showed that several nodal metastatic tumor characteristics play very important roles in the outcomes of IDC patients with nodal metastases. Among them, the number of mitotic figures of nodal metastatic tumors showed a significant association with poor outcome of patients, independently of the status of the nodes. The proliferative activity of primary tumor cells is a very significant factor in the prediction of outcomes in IDC patients.[19,20] In this study, there was a significant correlation in the number of mitotic figures between primary tumors and nodal tumors, and the number of mitotic figures of the latter had a more significant power to predict outcomes of patients with IDC than the number of mitotic figures of the former. Thus, high mitotic ability of nodal metastatic tumor cells, but not that of primary tumor cells, most likely defines the true malignant potential of IDCs with nodal metastases. This strongly suggests that primary tumor cells having arrived in a lymph node acquire a high proliferative ability through the overexpression of factors which accelerate the cell cycle.[21,22] Therefore, we conclude that the number of mitotic figures associated with nodal metastatic tumors is the best parameter to allow accurate prediction of the outcome of IDC patients with nodal metastases.

In addition to the number of mitotic figures, nuclear atypia, tumor growth feature, and tumor stroma are common histological features to primary tumors and nodal tumors, and among them tumor growth feature of nodal tumors was superior to that of primary tumors for prediction of the outcomes of patients with nodal metastasis in this study. Thus, our results strongly suggest that we have to examine histological features of nodal tumors in addition to those of primary tumors in order to accurately predict outcomes of patients with IDC with nodal metastasis.

Among other parameters peculiar to nodal tumors, the number of extra-nodal invasions, and the dimensions of the extra-nodal blood vessel tumor emboli were seen to be significant key parameters showing the true malignant potential of IDCs with nodal metastases in this study, and in addition played important roles in increasing the number of nodal metastases. Thus, we conclude that nodal metastatic tumor cells that have superior abilities of proliferation, extra-capsular invasion, and intra-vascular spread have a very significant influence on the outcomes of IDC patients with nodal metastases, as well as on the number of nodal metastases.

In conclusion, this is the first study to clearly demonstrate the significant role of nodal metastatic tumor cells in the tumor progression of breast IDCs. Thus, we plan to investigate the following items to further clarify the tumor metastatic pathway; 1) the functions of nodal metastatic tumor cells, and tumor cells in extra-nodal tissue or extra-nodal blood vessels, and 2) factors accelerating the abilities of proliferation, extra-nodal invasion, and extra-nodal blood vessel invasion in nodal metastatic tumors. This information is anticipated to provide valuable insights into tumor progression via the lymph nodes, and thereby to allow the design and implementation of effective treatment and preventive strategies.

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare and by a Grant-in-Aid for the 2nd Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan.

1. Fisher ER, Palekar A, Rockette H, Redmond C, Fisher B. Pathologic findings from the national surgical adjuvant breast project. (Protocol No. 4) V. Significance of axillary nodular micro- and macrometastases. *Cancer* 1978; **42**: 2032–8.
2. Rosen PP, Saigo PE, Braun DW, Weathers E, Fracchia A, Kinne DW. Axillary micro- and macrometastases in breast cancer. Prognostic significance of tumor size. *Ann Surg* 1981; **194**: 585–91.
3. Fisher ER, Gregorio RM, Redmond C, Kim WS, Fisher B. Pathologic findings from the national surgical adjuvant breast project. (Protocol No. 4) The significance of extranodal extension of axillary metastases. *Am J Clin Pathol* 1976; **65**: 439–45.
4. Mignano JE, Zahurak ML, Chakaravarthy A, Piantadosi S, Dooley WC, Gage I. Significance of axillary lymph node extranodal soft tissue extension and indicators for postmastectomy irradiation. *Cancer* 1999; **86**: 1258–62.
5. Donegan WL, Stine SB, Samter TG. Implications of extracapsular nodal metastases for treatment and prognosis of breast cancer. *Cancer* 1993; **72**: 778–82.
6. Mambo NC, ChB MB, Gallager HS. Carcinoma of the breast. The prognostic significance of extranodal extension of axillary disease. *Cancer* 1977; **39**: 2280–5.
7. Smith JA, Gamez-Araujo J, Gallager HS, White EC, McBride CM. Carcinoma of the breast. Analysis of total lymph node involvement versus level of metastasis. *Cancer* 1977; **39**: 527–32.
8. Fisher B, Slack NH. Number of lymph nodes examined and the prognosis of breast carcinoma. *Surg Gynecol Obstet* 1970; **131**: 79–88.
9. Quiet CA, Ferguson DJ, Weichselbaum RR, Hellman S. Natural history of node-positive breast cancer: the curability of small cancers with a limited number of positive nodes. *J Clin Oncol* 1996; **14**: 3105–11.
10. Jatoi I, Hilsenbeck SG, Clark GM, Osborne CK. Significance of axillary lymph node metastasis in primary breast cancer. *J Clin Oncol* 1999; **17**: 2334–40.
11. Fisher B, Bauer M, Wickerham L, Redmond CK, Fisher ER. Relation of number of positive axillary nodes to the prognosis of patients with primary breast cancer. An NSABP Update. *Cancer* 1983; **52**: 1551–7.
12. Sobin LH, Witteklnd CH. TNM Classification of malignant tumors. 5th ed. New York: Wiley-Liss Inc; 1997. p. 123–30.
13. Fisher BJ, Perera FE, Cooke AL, Opeitum A, Dar, AR, Venkatesan VM, Stitt L, Radwan JS. Extracapsular axillary node extension in patients receiving adjuvant systemic therapy: an indication for radiotherapy? *Int J Radiat Oncol Biol Phys* 1997; **38**: 551–9.
14. Hartveit F, Skjaerven R, Maehle BO. Prognosis in breast cancer patients with tumour cells in the efferent vessels of their axillary nodes. *J Pathol* 1983; **139**: 379–82.
15. Hartveit F. Paranodal tumour in breast cancer: extranodal extension versus vascular spread. *J Pathol* 1984; **144**: 253–6.
16. Cox DR. Regression models and life-tables. *J R Stat Soc* 1972; **34**: 187–220.
17. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991; **19**: 403–10.
18. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53**: 457–81.
19. Cooper LS, Gillett CE, Smith P, Fentiman IS, Barnes DM. Cell proliferation measured by MIB1 and timing of surgery for breast cancer. *Br J Cancer* 1998; **77**: 1502–7.
20. Rudolph P, Alm P, Heidebrecht HJ, Bolte H, Ratjen V, Bldetorp H, Fermo M, Olsson H, Parwaresch R. Immunologic proliferation marker Ki-S2 as prognostic indicator for lymph node-negative breast cancer. *J Natl Cancer Inst* 1999; **91**: 271–8.
21. Milde-Langosch K, Bamberger AM, Methner C, Rieck G, Loning T. Expression of cell cycle-regulatory proteins Rb, p16/MTS1, p27/KIP1, p21/WAF1, cyclin D1 and cyclin E in breast cancer: correlations with expression of activating protein-1 family members. *Int J Cancer* 2000; **97**: 468–72.
22. Crowe DL, Brown TN, Kim R, Smith SM, Lee MK. A c-fos/estrogen receptor fusion protein promotes cell cycle progression and proliferation of human cancer cell lines. *Mol Cell Biol Res Commun* 2000; **3**: 243–8.

Alcohol consumption and the risk of cancer in Japanese men: the Miyagi cohort study

N Nakaya[1,2], Y Tsubono[1], S Kuriyama[1], A Hozawa[1], T Shimazu[1], K Kurashima[1,3], S Fukudo[3], D Shibuya[4] and I Tsuji[1]

The objective of this study was to investigate the association between alcohol consumption and the risk of total cancer, and to estimate the proportion of total cancer attributable to drinking habit in Japanese men. From June through August 1990, a total of 21 201 Japanese men completed a self-administered questionnaire on various health habits, including alcohol consumption. During 153 389 person-years of follow-up through December 1997, we identified a total of 882 cases of cancer. We used Cox proportional hazards regression to estimate the relative risk of total cancer according to categories of alcohol consumption. The risk for total cancer was significantly higher in ex-drinkers than never-drinkers. There was a dose–response relationship between the amount of alcohol consumed and the risk of total cancer among current drinkers: multivariate RRs in reference to never-drinkers (95% confidence intervals (CI)) were 1.1 (0.8–1.3), 1.3 (1.0–1.7), and 1.3 (1.1–1.7) in current drinkers who consumed less than 22.8 g, 22.8–45.5 g, 45.6 g or more alcohol per day, respectively (*P* for trend <0.001). Estimated 17.9% (95% CI 3.1–30.5) of total cancer risk was attributable to drinking habit. In our findings, approximately 20% of the total cancer cases in Japanese men may be prevented by alcohol control. *European Journal of Cancer Prevention* 14:169–174 © 2005 Lippincott Williams & Wilkins.

European Journal of Cancer Prevention 2005, 14:169–174

Keywords: Alcohol consumption, cancer, Japanese, population attributable fraction, prospective cohort study

[1]Division of Epidemiology, Department of Public Health and Forensic Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan, [2]Psycho-Oncology Division, National Cancer Center Research Institute East, Kashiwanoha 6–5–1, Kashiwa, Japan, [3]Department of Behavioral Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan and [4]Miyagi Cancer Society, Sendai, Japan.

Correspondence to: N Nakaya, Psycho-Oncology Division, National Cancer Center Research Institute East, Kashiwanoha, Kashiwa 277–8577, Japan. Fax: (+81) 4 7134 7026; e-mail: nakaya-thk@umin.ac.jp

Received 14 April 2004 Accepted 24 June 2004

Introduction

Cancer is the leading cause of death among Japanese men, and it accounted for 34.3% of mortality (181 393 deaths) from all causes in 2001. Various lifestyle factors, such as diet, smoking and drinking, have been shown to play a role in the causation of cancer (Williams *et al.*, 1999).

Alcohol consumption has been associated with increased risk of cancer in several organs, and a panel of experts commissioned by the World Cancer Research Fund and the American Institute for Cancer Research in 1997 concluded that there is 'convincing' evidence that drinking increases the risk of cancer of the oral cavity, pharynx, oesophagus and liver, and 'probable' evidence that drinking increases the risk of cancer of the colon, rectum and breast (World Cancer Research Fund/American Institute for Cancer Research, 1997).

Several studies have shown a statistically significant association between excessive drinking and increased risk of cancer of the stomach (Hirayama *et al.*, 1989; Kato *et al.*, 1992), colon (Hirayama *et al.*, 1989; Otani *et al.*, 2003; Shimizu *et al.*, 2003), rectum (Hirayama *et al.*, 1989; Otani *et al.*, 2003; Shimizu *et al.*, 2003), liver (Kono *et al.*, 1986), oesophagus (Hirayama *et al.*, 1989; Kinjyo *et al.*, 1998), oral cavity (Hirayama *et al.*, 1989), and pharynx (Hirayama *et al.*, 1989) in the Japanese population. Three prospective studies have examined the association between alcohol drinking and the risk of total cancer, and found that excessive drinking consistently increased the risk of total cancer in the Japanese population (Kono *et al.*, 1986; Takezaki *et al.*, 1999; Tsugane *et al.*, 1999). However, these studies had several limitations: all of them used cancer mortality as endpoint (Kono *et al.*, 1986; Takezaki *et al.*, 1999; Tsugane *et al.*, 1999), two studies only controlled for age (Kono *et al.*, 1986; Takezaki *et al.*, 1999) and smoking habits (Kono *et al.*, 1986), and two studies have not been confirmed the validity and reliability of questionnaire assessment of alcohol consumption (Kono *et al.*, 1986; Takezaki *et al.*, 1999).

The objective of this study was to investigate the association between alcohol consumption and the risk of total cancer in Japanese men, and to estimate the proportion of total cancer attributable to drinking habit.

0959-8278

Methods

Study cohort

We have reported the design of this prospective cohort study in detail elsewhere (Fukao *et al.*, 1995; Tsubono *et al.*, 2001; Nakaya *et al.*, 2003). Briefly, from June through August 1990, we delivered a self-administered questionnaire on various health habits to 25 279 men who were 40–64 years of age and lived in 14 municipalities of Miyagi Prefecture in Northern Japan. The questionnaires were delivered and collected at the subjects' residences by members of health promotion committees appointed by the municipal governments. Usable questionnaires were returned from 22 836 men, yielding a 91.9% response rate. The study protocol was approved by the institutional review board of the Tohoku University Graduate School of Medicine. We considered the return of a self-administered questionnaire signed by a subject to imply his consent to participate in the study.

Exposure data

The questionnaire assessed alcohol consumption by first asking if the subject was a never-, ex- or current drinker. Current drinkers were then asked about frequency of drinking (less than once per week, once or twice per week, 3 or 4 times per week, or 5 times or more per week), beverage type usually consumed (sake, spirits, beer, whisky, wine or other), and the amount at one occasion. We calculated from these data the amount of alcohol consumed per day in grams. The subjects were classified into five categories; never-drinkers, ex-drinkers, current drinkers who consumed less than 22.8 g alcohol per day, 22.8–45.5 g alcohol per day and 45.6 g or more alcohol per day. The traditional unit of sake, 1 *go* (180 ml) is the same as 22.8 g of alcohol, which also approximates two glasses of wine (200 ml) or two measures of spirits (50 ml) in terms of alcohol contents. We conducted a validation study for the questionnaire assessment of alcohol consumption in which 113 subjects in the study district provided four 3-day diet records in one year and then responded to the questionnaire (Ogawa *et al.*, 2003). Spearman's coefficient of correlation between the amounts of alcohol consumption consumed according to the questionnaire and the amounts consumed according to the diet records was 0.61, and the correlation between consumption measured by the two questionnaires administrated 12 months apart was 0.81.

Follow-up

We used population registries in the 14 municipalities to ascertain vital and residential status of the subjects from 1 June 1990 to 31 December 1997. We identified incident cases of cancer by means of computerized record linkage with the Miyagi Prefectural Cancer Registry covering the study area (Takano and Okuno, 1997).

Out of the 22 836 subjects who responded to the questionnaire, we excluded subjects who had incomplete responses in alcohol information ($n = 1243$). We also excluded 392 subjects who had had prevalent cancer according to self-reports on the questionnaire or records of the cancer registry. Consequently, 21 201 men with 882 incident cases of cancer remained for this analysis.

We counted person-years of follow-up for each subject from 1 June 1990, until the date of diagnosis of cancer, date of moving to outside the study municipalities, date of death, or the end of the study period (31 December 1997), whichever occurred first. A total of 153 389 person-years accrued. Follow-up of subjects who moved from the study municipalities was discontinued because of logistical limitations, and 737 subjects (3.5% of the analytic cohort) were lost to follow-up during the study period.

Statistical analysis

The Cox proportional-hazards regression was used to estimate relative risk of cancer and to adjust for potentially confounding variables, using the SAS PHREG procedure on the SAS version 8.2 statistical software package (SAS, Cary, NC, USA). Several cancer endpoints were used, including total cancer (882 cases), alcohol-associated cancers (308), and cancer at sites unassociated with drinking (567). Cancers of the colon (106), rectum (67), oesophagus (52), liver (48), oral cavity (19), and larynx (16) were regarded as being associated with alcohol consumption (World Cancer Research Fund/American Institute for Cancer Research, 1997). We also used as the endpoints the six individual cancer sites in which more than 40 incident cases were identified among the analytic cohort during the follow-up period, namely, the stomach (247 cases), lung (119), colon (106), rectum (67), oesophagus (52) and liver (48).

We considered the following variables as potential confounders: age in years, cigarette smoking (never smoked, smoked in the past, currently smoking 1–19 cigarettes per day, currently smoking 20–29 cigarettes per day, or currently smoking at least 30 cigarettes per day), education (in school until 15 years of age, 16–18, or 19 years or older); and consumption frequencies of spinach, carrot or pumpkin, tomato, orange, other fruits, and juice (less than 1 day per week, 1 or 2 days per week, 3 or 4 days per week, or daily). Because we observed similar results whether we used the categorical variables of smoking, the number of pack-years of smoking, or the number of the cigarettes currently smoked per day, we present the results with the categorical variables of smoking. We repeated all analyses after excluding each cancer cases diagnosed in the first 3 years of follow-up. *P*-values to test for linear trends were estimated using grams of alcohol consumed per day as a continuous variable with ex-drinkers excluded. All *P*-values were two-tailed.

The population attributable fraction (PAF) was calculated as pd × {RR − 1}/RR, where pd is the proportion of cases exposed to the risk factor, i.e. the proportion of ex- and current drinkers combined (Rockhill *et al.*, 1998). The 95% confidence intervals (CIs) of the PAFs were calculated by the formula of Greenland (Greenland, 1999). This formula is known to be more valid than the popular formula (RR − 1) × Pe/{1 + (RR − 1) × Pe}, where Pe is the proportion of source population exposed to the risk factor, when confounding variables exist (Rockhill *et al.*, 1998).

Results

The proportions of never-drinkers, ex-drinkers, current drinkers who consumed less than 22. 8 g, 22.8–45.5 g and 45.6 g or more alcohol per day were 16%, 8%, 23%, 18%, 35%, respectively. Table 1 compares the characteristics of the subjects according to drinking categories. Compared with never-drinkers, current drinkers were younger, more likely to be current smokers, less likely to consume daily oranges, other fruits, juice, carrot or pumpkin or tomato. Compared with never-drinkers, ex-drinkers were also older, more likely to be ex-smokers and less likely to consume oranges daily.

Table 2 shows the association between alcohol drinking and the risk of total cancer, alcohol-associated cancer and cancer at sites unassociated with drinking. The age-adjusted relative analysis showed that the risk of total cancer was significantly higher in current drinkers than in never-drinkers, and that the risk increased linearly with the amount of alcohol consumed. This finding remained basically unchanged after multivariate adjustment or exclusion of the subjects diagnosed with cancer during the first three years of follow-up. The multivariate-adjusted relative analysis showed significantly higher risk of alcohol-associated cancers in current drinkers than in never-drinkers, and that the risk increased linearly with the amount of alcohol consumed. The risk was 1.9-fold higher in current drinkers who consumed 45.6 g or more alcohol per day than in never-drinkers. Consumption of a moderate amount of alcohol (< 22.8 g/day) was not associated with a lower risk of alcohol-associated cancers. The linear increase in risk was more evident after exclusion of cancer cases diagnosed within the first 3 years of follow-up, when the risk for current drinkers who consumed less than 22.8 g alcohol per day was 1.7-fold higher than for never-drinkers. Alcohol consumption showed no significant association with the risk of cancer at sites unassociated with drinking.

Table 3 shows multivariate relative risks for the six major individual cancer sites according to drinking categories. Higher, but not significantly higher risk of alcohol-associated cancers (rectum, colon, oesophagus and liver) in current drinkers than never-drinkers was found (1.4–2.7). Remarkably, ex-drinkers had a higher risk of liver cancer and lung cancer than never-drinkers.

An estimated 17.9% (95% CI 3.1–30.5) of total cancer risk was attributable to drinking habit. Furthermore, an estimated 35.6% (95% CI 10.9–53.4) of alcohol-associated cancers risk was attributable to drinking habit.

We conducted stratified analyses according to 5-year age class, cigarette smoking, education and frequency of consumption of food items. However, the associations between alcohol consumption and the risk of total cancer were not remarkably modified by these variables (data not shown).

Discussion

This prospective cohort study investigated the association between alcohol consumption and the risk of cancer in Japanese men. The results showed that (1) the risk of total cancer in current drinkers was higher than in never-drinkers; (2) the risk of total cancer in ex-drinkers was higher than in never-drinkers; (3) total cancer risk

Table 1 Characteristics of the subjects according to alcohol consumption

Characteristics	Ex-drinkers	Never-drinkers	Current drinkers (g alcohol/day)			
			All	<22.8	22.8–45.5	≥ 45.6
No. of subjects	1594	3349	16 258	4915	3907	7436
Age, years, mean ± SD	54.9 ± 7.2	52.5 ± 7.7	51.1 ± 7.5	50.7 ± 7.6	51.6 ± 7.7	51.2 ± 7.4
Cigarette smoking[a] (%)						
Never	13.2	27.4	17.4	25.7	18.0	11.6
Past	31.6	15.3	19.6	20.9	21.4	17.8
Current	55.2	57.4	63.0	53.3	60.6	70.6
Education, in school 19 years of age or older (%)	11.5	13.1	14.8	15.7	14.6	13.6
Daily dietary consumption (%)						
Orange	18.6	23.2	16.5	20.7	17.3	13.4
Other fruits	26.9	26.9	19.9	24.4	20.5	16.4
Juice	7.4	8.6	5.9	6.5	6.2	5.4
Spinach	21.7	19.8	20.2	20.7	21.0	19.4
Carrot or pumpkin	12.8	10.2	8.9	9.8	8.8	8.4
Tomato	16.5	14.0	12.7	14.1	13.1	11.6

[a]Because of rounding, not all percentages add to 100.

Table 2 Relative risk of total cancer, cancer sites associated with drinking, and cancer at sites unassociated with drinking by alcohol consumption[a]

	Ex-drinkers	Never-drinkers	Current drinkers (g alcohol/day)				*P* for trend
			All	<22.8	22.8–45.5	≥ 45.6	
Person-years	11 234	24 370	117 785	35 712	28 193	53 880	
Total cancer (882 cases)							
No. of cases	92	122	668	158	175	335	
Age-adjusted RR1	1.4 (1.0–1.8)	1.0	1.3 (1.1–1.6)	1.0 (0.8–1.3)	1.4 (1.1–1.7)	1.4 (1.2–1.8)	<0.001
Multivariate RR1	1.3 (1.0–1.8)	1.0	1.3 (1.0–1.5)	1.1 (0.8–1.3)	1.3 (1.0–1.7)	1.3 (1.1–1.7)	0.001
Multivariate RR2	1.5 (1.0–2.1)	1.0	1.3 (1.0–1.7)	1.1 (0.8–1.5)	1.3 (1.0–1.8)	1.4 (1.1–1.9)	0.003
Alcohol-associated cancers (308 cases)							
No. of cases	32	33	243	53	62	128	
Age-adjusted RR1	1.8 (1.1–2.9)	1.0	1.7 (1.2–2.5)	1.3 (0.8–2.0)	1.8 (1.2–2.7)	2.0 (1.4–3.0)	<0.001
Multivariate RR1	1.8 (1.1–2.9)	1.0	1.7 (1.1–2.4)	1.3 (0.8–2.0)	1.7 (1.1–2.6)	1.9 (1.3–2.8)	<0.001
Multivariate RR2	1.9 (1.0–3.6)	1.0	2.1 (1.3–3.3)	1.7 (1.0–2.9)	1.9 (1.1–3.3)	2.4 (1.5–4.0)	<0.001
Cancer at sites unassociated with drinking (563 cases)							
No. of cases	58	88	417	105	109	203	
Age-adjusted RR1	1.2 (0.9–1.7)	1.0	1.1 (0.9–1.4)	1.0 (0.7–1.3)	1.2 (0.9–1.6)	1.2 (0.9–1.6)	0.045
Multivariate RR1	1.2 (0.8–1.6)	1.0	1.1 (0.9–1.4)	1.0 (0.7–1.3)	1.2 (0.9–1.5)	1.1 (0.9–1.5)	0.23
Multivariate RR2	1.3 (0.9–1.9)	1.0	1.0 (0.8–1.4)	0.9 (0.7–1.3)	1.1 (0.8–1.5)	1.1 (0.8–1.5)	0.43

[a]The multivariate relative risk (RR) has been adjusted for age (in years); cigarettes smoking (never smoked, smoked in the past, currently smoking 1–19 cigarettes per day, or currently smoking 20–29, or 30 or more cigarettes per day); education (in school until age 15 years or younger, 16–18, or 19 years or older); daily consumption of orange, other fruits, juice, spinach, carrot or pumpkin, and tomato (less than 1 day per week, 1 or 2 days per week, 3 or 4 days per week, or daily). RR1 denoted the relative risk with all cases of cancer induced in the multivariate analysis, RR2 the relative risk with cases diagnosed in the first three years of follow-up excluded from the analysis. Alcohol-associated cancers include colon (106 cases), rectum (67), oesophagus (52), liver (48), oral cavity (19), and larynx (16). Values in parentheses are 95% confidence intervals.

Table 3 Multivariate relative risk of individual cancer sites according to alcohol consumption[a]

	Ex-drinkers	Never-drinkers	Current drinkers (g alcohol/day)			*P* for trend
			All	<22.8	≥ 22.8	
Stomach (247 cases)						
No. of cases	21	42	184	49	135	
Multivariate RR	0.9 (0.5–1.5)	1.0	1.0 (0.7–1.4)	1.0 (0.6–1.5)	1.0 (0.7–1.5)	0.83
Lung (119 cases)						
No. of cases	21	16	82	17	65	
Multivariate RR	2.3 (1.2–4.4)	1.0	1.2 (0.7–2.1)	1.0 (0.5–2.0)	1.3 (0.8–2.3)	0.30
Colon (106 cases)						
No. of cases	10	11	85	19	66	
RR	1.6 (0.7–3.8)	1.0	1.7 (0.9–3.3)	1.3 (0.6–2.8)	1.9 (1.2–3.7)	0.03
Rectum (67 cases)						
No. of cases	3	9	55	13	42	
Multivariate RR	0.6 (0.2–2.3)	1.0	1.4 (0.7–2.9)	1.2 (0.5–2.8)	1.5 (0.7–3.1)	0.23
Oesophagus (52 cases)						
No. of cases	4	4	44	4	40	
Multivariate RR	1.8 (0.4–7.1)	1.0	2.5 (0.9–7.1)	0.9 (0.2–3.5)	3.2 (1.1–8.9)	0.004
Liver (48 cases)						
No. of cases	10	3	35	11	24	
Multivariate RR	6.6 (1.8–24.2)	1.0	2.7 (0.8–8.9)	2.8 (0.8–10.1)	2.7 (0.8–8.9)	0.21

[a]The multivariate relative risk (RR) has been adjusted for age (in years); cigarette smoking (never smoked, smoked in the past, currently smoking 1–19 cigarettes per day, or currently smoking 20–29, or 30 or more cigarettes per day); education (in school until age 15 years or younger, 16–18, or 19 years or older); daily consumption of orange and other fruit juice, spinach, carrot or pumpkin, and tomato (less than 1 day per week, 1 or 2 days per week, 3 or 4 days per week, or daily). Values in parentheses are 95% confidence intervals.

increased linearly as the amount of alcohol consumption increased; (4) 17.9% of total cancer risk was attributable to drinking habit.

The results of this study showed that total cancer risk increased linearly with the amount of alcohol consumed, and that the risk of total cancer was significantly higher in excessive drinkers (45.6 g or more alcohol per day) than in never-drinkers. Three earlier studies examined the association between alcohol consumption and the risk of total cancer in Japanese men and consistently found that excessive drinking increased risk of total cancer (Kono *et al.*, 1986; Takezaki *et al.*, 1999; Tsugane *et al.*, 1999). A dose–response relationship between alcohol consumption and the risk of total cancer were inconsistent findings. More specifically, Kono *et al.* (1986) showed a linear association between alcohol consumption and the risk of total cancer. Tsugane *et al.* (1999) reported a J-shaped association between alcohol consumption and the risk of total cancer. Takezaki *et al.* (1999) showed linear association between alcohol consumption and the risk of total cancer.

Seven prospective cohort studies of the association between alcohol consumption and the risk of total cancer or alcohol-associated cancers have been conducted in the world excluding Japanese populations. Of these two studies found a linear association between alcohol consumption and the risk, and indicated that besides, moderate current drinkers began to increase in risk compared with the non drinkers (Thun *et al.*, 1997; Grønbæk *et al.*, 2000). Five studies found J-shaped or U-shaped associations, because of moderate current drinkers found decrease in risk compared with the non-drinkers (Farchi *et al.*, 1992; Goldberg *et al.*, 1994; Fuchs *et al.*, 1995; Maskarinec *et al.*, 1998; Renaud *et al.*, 1998).

The discrepancy between the present results and those of most previous studies showing a J-shaped or U-shaped association may be partly explained by the exclusion in this study of ex-drinkers from the reference category. In most previous studies, 'non-drinkers' comprised both never drinkers and ex-drinkers. In the present analysis, we considered ex-drinkers and never drinkers separately, and found that ex-drinkers had markedly higher risk of total cancer or alcohol-related cancer risk compared with never drinkers. Higher cancer incidence among ex-drinkers may be due to ill-health that had led them to quit drinking (Goodman *et al.*, 1995; Tsubono *et al.*, 2001). Studies of alcohol consumption and the risk of cancer may overestimate the lower risk in moderate drinkers if they did not separate never drinkers and ex-drinkers in the referent group.

Our study had several methodological advantages over previous studies examining the association between alcohol consumption and the risk of total cancer in Japan. First, our study used cancer incidence, rather than mortality, as an endpoint, which made it possible to distinguish whether alcohol consumption was related to cancer incidence, cancer survival, or both. Second, we controlled extensively for potentially confounding variables, such as smoking, education (a measure of socio-economic status), and diet (consumption of vegetables and fruits). Third, we established the validity and reliability for questionnaire assessment of alcohol consumption (Ogawa *et al.*, 2003).

Several studies have shown a statistically significant association between excessive drinking and increased risk of cancer of the stomach (Hirayama *et al.*, 1989; Kato *et al.*, 1992), colon (Hirayama *et al.*, 1989; Otani *et al.*, 2003; Shimizu *et al.*, 2003), rectum (Hirayama *et al.*, 1989; Otani *et al.*, 2003; Shimizu *et al.*, 2003), liver (Kono *et al.*, 1986), oesophagus (Hirayama *et al.*, 1989; Kinjyo *et al.*, 1998), oral cavity (Hirayama *et al.*, 1989) and pharynx (Hirayama *et al.*, 1989) in the Japanese population. No significantly higher risk of alcohol-associated cancers (rectum, colon, oesophagus and liver) in current drinkers than never-drinkers was found in our study (1.4–2.7), but our study may not have sufficient statistical power to detect small increases or decreases in risk of cancer at individual sites associated with alcohol consumption, because the number of cases of cancer sites was only modest to small (48–247 cases). Thus, our follow-up period and the number of cases of cancer were probably insufficient to evaluate the association between alcohol consumption and the risk of total cancer, alcohol-associated cancers, or major cancer sites, and we need further to estimate the associations in a future.

We concluded that the risk of total cancer was higher in ex- and current drinkers than in never-drinkers and that the risk increased linearly with the amount of alcohol consumed. Approximately 20% of the total cancer cases in Japanese men could be prevented by alcohol control.

Acknowledgement

This study attribute to Awardee of Research Resident Fellowship from the Foundation for Promotion of Cancer Research (Japan) for the 3rd Term Comprehensive 10-year-Strategy for Control. We thank Dr Yosuke Uchitomi of the Psycho-Oncology Division, National Cancer Centre Research Institute East, Kashiwa, Japan, for this study advice. We also thank Dr Tetsuya Otani of the Epidemiology and Prevention Division, Research Centre for Cancer Prevention and Screening, National Cancer Centre, Tokyo, Japan, for statistical advice in this study.

References

Farchi G, Fidanza F, Mariotti G, *et al.* (1992). Alcohol and mortality in the Italian rural cohorts of the Seven Countries Study. *Int J Epidemiol* **21**: 74–82.

Fuchs CS, Stampfer MJ, Colditz GA, *et al.* (1995). Alcohol consumption and mortality among women. *N Engl J Med* **332**: 1245–1250.

Fukao A, Tsubono Y, Komatsu S, *et al.* (1995). Cohort study on the relation of lifestyle, personality and biologic markers to cancer in Miyagi, Japan: Study design, response rate and profiles of the cohort subjects. *J Epidemiol* **5**: 153–157.

Goldberg RJ, Burchfiel CM, Reed DM, *et al.* (1994). A prospective study of the health effects of alcohol consumption in middle-aged and elderly men: The Honolulu Heart Program. *Circulation* **89**: 651–659.

Goodman MT, Moriwaki H, Vaeth M, *et al.* (1995). Prospective cohort study of risk factors for primary liver cancer in Hiroshima and Nagasaki, Japan. *Epidemiology* **6**: 36–41.

Greenland S (1999). Letter to the editor – Confidence limits made easy: interval estimation using a substitution method. *Am J Epidemiol* **149**: 884.

Grønbæk M, Becker U, Johansen D, *et al.* (2000). Type of alcohol consumed and mortality from all cause, coronary heart disease, and cancer. *Ann Intern Med* **133**: 411–419.

Hirayama T (1989). Association between alcohol consumption and cancer of the sigmoid colon: Observations from a Japanese cohort study. *Lancet* **23**: 725–727.

Kato I, Tominaga S, Ito Y, *et al.* (1992). A prospective study of atrophic gastritis and stomach cancer risk. *Jpn J Cancer Res* **83**: 1137–1142.

Kinjyo Y, Cui Y, Akiba S, *et al.* (1998). Mortality risks of oesophageal cancer associated with hot tea, alcohol, tobacco and diet in Japan. *J Epidemiol* **8**: 235–243.

Kono S, Ikeda M, Tokudome S, *et al.* (1986). Alcohol and mortality: a cohort study of male Japanese physicians. *Int J Epidemiol* **15**: 527–532.

Maskarinec G, Meng L, Kolonel LN (1998). Alcohol intake, body weight, and mortality in a multiethnic prospective cohort. *Epidemiology* **9**: 654–661.

Nakaya N, Tsubono Y, Hosokawa T, *et al.* (2003). Personality and the risk of cancer. *J Natl Cancer Inst* **95**: 799–805.